

Table 2. Summary of alignments for the ALLERGENSEARCH and FASTA searches of the allergen sequence database (AD6) using putative polypeptide sequences encoded by the genomic DNA-inserted DNA 5' junction in MON 89034.

AD6 Sequence Database									
Appendix	Polypeptide	ALLERGENSEARCH			FASTA search				
		# Hits	GI #	# Hits	Description	E score	% Identity	aa Overlap	
2	5_1	0	27465565	5	Niemann Pick type C2	3.8	33.3	27	
3	5_3	0	6492307	10	allergen M-177 precursor	0.014	50.0	24	
4	5_4	0	5689675	12	<i>Coprinus comatus</i> Cop c7	0.11	75.0	8	
5	5_5	0	4368136	19	major allergen OLE5c	0.82	27.3	22	
6	5_6	0	27462838	10	cytochrome oxidase subunit I	1.7	40.9	22	

Table 3. Summary of alignments for the FASTA searches of the toxin sequence database (TOXIN5) using putative polypeptide sequences encoded by the genomic DNA-inserted DNA 5' junction in MON 89034.

FASTA search of TOXIN5 Sequence Database									
Appendix	Polypeptide	# Hits	Accession #	Description	E score	% Identity	aa Overlap		
2	5_1	4	Q9BPG2	<i>Conus ventricosus</i> conotoxin scaffold ix precursor	1.8	44.4	27		
3	5_3	23	BAB36715	<i>E. coli</i> o157:h7 cysteine synthase	0.47	45.0	20		
4	5_4	33	O32587	<i>E. coli</i> cytolethal distending toxin-iiic O32001	0.73	60.0	10		
5	5_5	19	BAB36242	<i>E. coli</i> o157:h7. his operon leader peptide	0.22	71.4	7		
6	5_6	6	BAB35743	<i>E. coli</i> o157:h7. hypothetical 54.7 kDa protein	0.44	35.5	31		

Table 4. Summary of alignments for the FASTA searches of the ALLPEPTIDES database using putative polypeptide sequences encoded by the genomic DNA-inserted DNA 5' junction in MON 89034.

FASTA search of ALLPEPTIDES Sequence Database									
Appendix	Polypeptide	# Hits	GI #	Description	E score	% Identity	aa Overlap		
2	5_1	-	-	-	-	-	-	-	-
3	5_3	2	39597103	Hypothetical protein CBBG02672	5.4	50.0	20		
4	5_4	1	74192645	<i>Mus musculus</i> unnamed protein product	8.8	63.6	11		
5	5_5	1	699353	<i>Mycobacterium leprae</i> u296v	5.3	52.9	17		
6	5_6	-	-	-	-	-	-	-	-

Table 5. Summary of alignments for the ALLERGENSEARCH and FASTA searches of the allergen sequence database (AD6) using putative polypeptide sequences encoded by the inserted DNA-genomic DNA 3' junction in MON 89034.

Appendix	Polypeptide _e	AD6 Sequence Database					
		ALLERGENSEARCH			FASTA search		
		# Hits	# Hits	GI #	Description	E score	% Identity
7	3_1	0	36	1580792	<i>Periplaneta americana</i> allergen	0.12	46.2
8	3_2	0	33	27462834	cathepsin L-like protease	0.85	47.6
9	3_3	0	14	118216	Aed a 2D7 protein precursor	0.98	29.6
10	3_5	0	28	807698	pre-pro-cucumis	0.17	77.8
11	3_6	0	14	4099919	pollen allergen homolog	2.9	30.2
							43

Table 6. Summary of alignments for the FASTA searches of the toxin sequence database (TOXIN5) using putative polypeptide sequences encoded by the inserted DNA-genomic DNA 3' junction in MON 89034.

Appendix	Polypeptide _e	TOXIN5 Sequence Database					
		# Hits	Accession #	Description	E score	% Identity	aa Overlap
7	3_1	32	Q9Z4R7	<i>Eikenella corrodens</i> . lysine decarboxylase	1.3	35.7	28
8	3_2	32	BAB34841	<i>E. coli</i> o157:h7. hypothetical 9.1 kda protein.	3.7	50.0	12
9	3_3	8	Q9P4U8	<i>alternaria alternata</i> . aktir-2.	3.8	36.2	47
10	3_5	46	BAB37562	<i>E. coli</i> o157:h7. putative integral	0.056	57.1	14
11	3_6	6	U32175_1	<i>Haemophilus ducreyi</i> HhdA and HhdB precursors	1.7	36.7	49

Table 7. Summary of alignments for the FASTA searches of the ALLPEPTIDES database using putative polypeptide sequences encoded by the inserted DNA-genomic DNA 3' junction in MON 89034.

Appendix	Polypeptide _e	ALLPEPTIDES Sequence Database					
		# Hits	GI #	Description	E score	% Identity	aa Overlap
7	3_1	-	-	-	-	-	-
8	3_2	7	29840953	Putative CD9/CD37/CD63 antigens	1.3	34.6	26
9	3_3	1	71663172	<i>Trypanosoma cruzi</i> hypothetical protein	7.5	38.3	47
10	3_5	6	47183367	<i>Tetraodon nigroviridis</i> unnamed protein product	5.5	44.4	18
11	3_6	2	14578255	<i>C. elegans</i> Hypothetical protein	2.4	37.5	48

Appendix 1. Allergen, gliadin, and glutenin protein sequence database (AD6)

Venoms

Species	Comments	GI #	AA
<i>Aedes aegypti</i>	D7 protein precursor (Allergen Aed a 2).	116276	321
<i>Aedes aegypti</i>	putative 30 kDa allergen-like protein [<i>Aedes aegypti</i>].	18568322	215
<i>Aedes aegypti</i>	putative 18.2 kDa secreted protein [<i>Aedes aegypti</i>].	18568332	158
<i>Aedes aegypti</i>	30 kDa salivary gland allergen Aed a 3 [<i>Aedes aegypti</i>].	2114497	253
<i>Aedes aegypti</i>	apyrase.	556272	562
<i>Aedes albopictus</i>	putative 30 kDa allergen-like protein 30k-1 [<i>Aedes albopictus</i>].	56417500	210
<i>Aedes albopictus</i>	30 kDa salivary gland allergen 30k-2 [<i>Aedes albopictus</i>].	56417502	210
<i>Aedes albopictus</i>	30 kDa salivary gland allergen 30k-3 [<i>Aedes albopictus</i>].	56417504	271
<i>Aedes albopictus</i>	GE-rich salivary protein 30k-4 [<i>Aedes albopictus</i>].	56417506	266
<i>Aedes albopictus</i>	putative salivary secreted 30 kDa allergen-like protein 30k-5	56417508	204
<i>Anopheles gambiae</i>	30 kDa protein [<i>Anopheles gambiae</i>].	18389879	182
<i>Apis dorsata</i>	Melittin.	126955	26
<i>Apis mellifera</i>	venom protease precursor [<i>Apis mellifera</i>].	22724911	405
<i>Apis mellifera</i>	Phospholipase A2 precursor (Phosphatidylcholine 2-acylhydrolase)	24418862	167
<i>Apis cerana</i>	Phospholipase A2 (Phosphatidylcholine 2-acylhydrolase).	24638082	134
<i>Apis mellifera</i>	Allergen Api m 6.	34921475	71
<i>Apis dorsata</i>	Phospholipase A2 (Phosphatidylcholine 2-acylhydrolase).	47117012	134
<i>Apis mellifera</i>	prepromelittin [<i>Apis mellifera</i>].	5622	70
<i>Apis mellifera</i>	Hyaluronoglucosaminidase precursor (Hyaluronidase) (Hya) (Allergen	585279	382
<i>Apis mellifera</i>	profilin [<i>Apis mellifera</i>].	58585250	126
<i>Apis mellifera</i>	venom acid phosphatase precursor [<i>Apis mellifera</i>].	61656214	388
<i>Apis mellifera</i>	PREDICTED: similar to allergen Bla g 5, partial [<i>Apis mellifera</i>].	66534655	157
<i>Apis mellifera</i>	melittin, minor - honeybee.	69552	27
<i>Apis cerana</i>	phospholipase A2 (EC 3.1.1.4), venom - Indian honeybee.	7435005	134
<i>Bombus terrestris</i>	Phospholipase A2 (Phosphatidylcholine 2-acylhydrolase) (Allergen	14423832	136
<i>Crotalus durissus</i>	phospholipase A2 inhibitor [<i>Crotalus durissus terrificus</i>].	501050	200
<i>Ctenocephalides felis</i>	FS-H precursor.	1575479	98
<i>Ctenocephalides felis</i>	salivary antigen 1 precursor [<i>Ctenocephalides felis</i>].	4336703	176
<i>Ctenocephalides felis</i>	salivary allergen 2 [<i>Ctenocephalides felis</i>].	7638032	264
<i>Dolichovespula maculata</i>	Hyaluronoglucosaminidase (Hyaluronidase) (Allergen Dol m 2) (Dol m	1346322	331
<i>Dolichovespula maculata</i>	Venom allergen 5.01 precursor (Antigen 5 form 2) (Ag5-2) (Allergen	137395	227
<i>Dolichovespula maculata</i>	Phospholipase A1 2 (Allergen Dol m 1.02) (Dol m 1).	1709542	303
<i>Dolichovespula arenaria</i>	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Dol a 5) (Dol a V).	465052	203
<i>Dolichovespula maculata</i>	Phospholipase A1 1 precursor (Allergen Dol m 1.01) (Dol m 1).	548449	317
<i>Dolichovespula maculata</i>	Venom allergen 5.02 precursor (Antigen 5 form 3) (Ag5-3) (Allergen	549186	215
<i>Myrmecia pilosula</i>	major allergen Myr p II.	1587177	75
<i>Myrmecia pilosula</i>	Pilosulin-2 precursor (Allergen Myr p 2) (Myr p II).	2498604	75
<i>Myrmecia pilosula</i>	major allergen [<i>Myrmecia pilosula</i>].	312284	112
<i>Myrmecia pilosula</i>	Phosulin-1 precursor (Major allergen Myr p 1) (Myr p I) [Contains:	730091	112
<i>Phlebotomus papatasi</i>	28 kDa salivary protein precursor [<i>Phlebotomus papatasi</i>].	15963511	254
<i>Phlebotomus papatasi</i>	30 kDa salivary protein precursor [<i>Phlebotomus papatasi</i>].	15963513	253
<i>Polistes annularis</i>	Hyaluronoglucosaminidase precursor (Hyaluronidase) (Allergen Pol a	14423735	367
<i>Polistes annularis</i>	Phospholipase A1 (Allergen Pol a 1).	14423833	301
<i>Polistes annularis</i>	allergen 5.	160780	209
<i>Polistes gallicus</i>	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Pol g 5).	25091511	206
<i>Polistes dominulus</i>	venom serine protease precursor [<i>Polistes dominulus</i>].	30909091	277
<i>Polistes gallicus</i>	Phospholipase A1 (Allergen Pol g 1).	41017429	42
<i>Polistes dominulus</i>	venom phospholipase A1 1 precursor [<i>Polistes dominulus</i>].	45510887	337
<i>Polistes dominulus</i>	venom phospholipase A1 2 precursor [<i>Polistes dominulus</i>].	45510889	316
<i>Polistes dominulus</i>	venom phospholipase A1 3 precursor [<i>Polistes dominulus</i>].	45510891	316
<i>Polistes dominulus</i>	venom phospholipase A1 4 precursor [<i>Polistes dominulus</i>].	45510893	316
<i>Polistes exclamans</i>	allergen Pol e 5 precursor [<i>Polistes exclamans</i>].	51093375	226
<i>Polistes dominulus</i>	allergen Pol d 5 precursor [<i>Polistes dominulus</i>].	51093377	227
<i>Polistes exclamans</i>	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Pol e 5) (Pol e V).	549187	205
<i>Polistes fuscatus</i>	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Pol f 5) (Pol f V).	549188	205
<i>Polybia scutellaris</i>	venom allergen 5 [<i>Polybia scutellaris</i>].	31747352	206

Polybia scutellaris	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Pol s V).	47117356	207
Psoroptes ovis	allergen A precursor [Psoroptes ovis].	14388619	143
Psoroptes ovis	Derp1 antigen [Psoroptes ovis].	27450737	263
Psoroptes ovis	glutathione S-transferase [Psoroptes ovis].	6625558	219
Sarcoptes scabiei	cathepsin L-like protease [Sarcoptes scabiei type hominis].	27462834	245
Sarcoptes scabiei	glutathione S-transferase [Sarcoptes scabiei type hominis].	27462836	219
mitochondrion Sarcoptes	cytochrome oxidase subunit I [Sarcoptes scabiei type hominis].	27462838	275
mitochondrion Sarcoptes	cytochrome oxidase subunit II [Sarcoptes scabiei type hominis].	27462840	157
Sarcoptes scabiei	cytochrome Bc1 complex chain B-like protein [Sarcoptes scabiei type hominis].	27462842	131
Sarcoptes scabiei	vitellogenin-like protein [Sarcoptes scabiei type hominis].	27462844	174
Sarcoptes scabiei	paramyosin [Sarcoptes scabiei type hominis].	27462846	638
Sarcoptes scabiei	major allergen 1 [Sarcoptes scabiei type hominis].	27462848	330
Sarcoptes scabiei	Sar s 3 allergen Yv7016G03 [Sarcoptes scabiei type hominis].	38202313	260
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv4005B08 [Sarcoptes scabiei type hominis].	38202315	271
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv4005G12 [Sarcoptes scabiei type hominis].	38202317	251
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv4031D03 [Sarcoptes scabiei type hominis].	38202319	264
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv5004E08 [Sarcoptes scabiei type hominis].	38202321	256
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv5018H10 [Sarcoptes scabiei type hominis].	38202323	251
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv5026E07 [Sarcoptes scabiei type hominis].	38202325	242
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv5027C11 [Sarcoptes scabiei type hominis].	38202327	259
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv6017G11 [Sarcoptes scabiei type hominis].	38202329	248
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv6018H06 [Sarcoptes scabiei type hominis].	38202331	262
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv6023A04 [Sarcoptes scabiei type hominis].	38202333	257
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv6028G11 [Sarcoptes scabiei type hominis].	38202335	250
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv7016C10 [Sarcoptes scabiei type hominis].	38202337	259
Sarcoptes scabiei	group 3 allergen SMIPP-S YvT003F10 [Sarcoptes scabiei type hominis].	38202339	259
Sarcoptes scabiei	group 3 allergen SMIPP-S YvT004A06 [Sarcoptes scabiei type hominis].	38202341	263
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv5001A04 [Sarcoptes scabiei type hominis].	38202343	233
Sarcoptes scabiei	group 3 allergen SMIPP-S Yv9017F05 [Sarcoptes scabiei type hominis].	38202345	261
Solenopsis invicta	Sol i 1=antigen {N-terminal} [Solenopsis invicta=imported fire ant venom].	1336809	58
Solenopsis invicta	Sol i 1=antigen {N-terminal} [Solenopsis invicta=imported fire ant venom].	1336811	25
Solenopsis invicta	Sol i 1=antigen {N-terminal} [Solenopsis invicta=imported fire ant venom].	1336812	26
Solenopsis invicta	Sol i 1=antigen {N-terminal} [Solenopsis invicta=imported fire ant venom].	1336813	26
Solenopsis richteri	phospholipase (EC 3.1.1.-), venom - black imported fire ant venom.	321043	20
Solenopsis invicta	venom allergen Sol i 4.02 precursor [Solenopsis invicta].	4038411	137
Solenopsis invicta	allergen Sol i 1 precursor [Solenopsis invicta].	51093373	346
Solenopsis invicta	Venom allergen II precursor (Allergen Sol i 2) (Sol i II).	549179	138
Solenopsis richteri	venom allergen Sol r 2 - black imported fire ant venom.	7512067	119
Solenopsis geminata	venom allergen Sol g 4.01 precursor [Solenopsis geminata].	7638028	137
Solenopsis geminata	venom allergen Sol g 4.02 precursor [Solenopsis geminata].	7638030	137
Solenopsis invicta	Venom allergen IV precursor (Allergen Sol i 4) (Sol i IV).	14424465	137
Solenopsis invicta	Venom allergen 3 precursor (Venom allergen III) (Allergen Sol i 3).	14424466	234
Solenopsis richteri	Venom allergen 3 (Venom allergen III) (Allergen Sol r 3) (Sol r 3).	6136163	211
Tityus serrulatus	Tityustoxin-6 (Tityustoxin VI) (TsTX-VI) (TsTXVI) (Toxin VI) (TsTX).	1173399	62
Triatoma protracta	procalin [Triatoma protracta].	15426413	169
Vespa crabro	Venom allergen 5.01 (Antigen 5-1) (Ag5-1) (Allergen Vesp c 5.01).	549184	202
Vespa crabro	Venom allergen 5.02 (Antigen 5-2) (Ag5-2) (Allergen Vesp c 5.02).	549185	202
Vespa mandarinia	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Vesp m 5).	6136165	202
Vespula vulgaris	Chain A, Ves V 5, An Allergen From Vespula Vulgaris Venom.	11514279	209
Vespula vulgaris	Hyaluronoglucosaminidase (Hyaluronidase) (Allergen Ves v 2) (Ves v 2).	1346323	331
Vespula vulgaris	allergen 5.	162551	227
Vespula maculifrons	phospholipase A1 (EC 3.1.1.32), allergen Ves m 1 - eastern fire ant venom.	482382	300
Vespula vulgaris	allergen 5; antigen 5 [Vespula vulgaris].	4826574	204
Vespula flavopilosa	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Ves f 5) (Ves f V).	549189	204
Vespula germanica	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Ves g 5) (Ves g V).	549190	204
Vespula maculifrons	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Ves m 5) (Ves m V).	549191	204
Vespula pensylvanica	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Ves p 5) (Ves p V).	549192	204
Vespula squamosa	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Ves s 5) (Ves s V).	549193	205
Vespula vidua	Venom allergen 5 (Antigen 5) (Ag5) (Allergen Ves vi 5) (Ves vi V).	549194	206
Vespula vulgaris	hyaluronidase b [Vespula vulgaris].	62147665	340
Vespula vulgaris	allergen and phospholipase A1.	897647	336

Aeroallergens animals

Species

Canis familiaris
Canis familiaris
Canis familiaris
Canis familiaris
Canis familiaris
Canis familiaris
Canis familiaris
Canis familiaris
Cavia porcellus
Cavia porcellus
Cavia porcellus
Felis catus
Felis catus
Felis catus
Felis catus
Felis catus
Felis catus
Felis catus
Felis catus
Felis catus
Felis catus
Felis catus
Macaca mulatta
Mus musculus
Mus sp.
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Mus musculus
Rattus norvegicus
Rattus norvegicus
Rattus norvegicus
Rattus norvegicus
Thaumetopoea pityocampa

Comments

precursor Can f II [Canis familiaris].
precursor Can f II [Canis familiaris].
Major allergen Can f 1 precursor (Allergen Dog 1).
Minor allergen Can f 2 precursor (Allergen Dog 2).
albumin [Canis familiaris].
epithelial dog allergen - Canis familiaris (fragment).
albumin [Canis familiaris].
albumin [Canis familiaris].
Eosinophil granule major basic protein 1 precursor (MBP-1).
Major allergen Cav p 2.
Major urinary protein (MUP) (Allergen Cav p 1).
Eosinophil granule major basic protein 2 precursor (MBP-2).
Major allergen I polypeptide chain 1 precursor (Allergen Fel d 1-A).
fel d I chain 1 precursor with leader B [Felis catus].
fel d I chain 1 precursor with leader A [Felis catus].
major allergen I.
major allergen I.
prostaglandin FP receptor [Felis catus].
cystatin [Felis catus].
Chain A, Crystal Structure Of Fel d 1: The Major Cat Allergen.
Fel d 4 allergen [Felis catus].
albumin precursor [Felis catus].
Major allergen I polypeptide chain 2 precursor (Allergen Fel d 1-B).
encephalitogenic peptide M.
androgen-binding protein eta [Mus musculus].
DEC-205=205 kDa protein allergen {N-terminal} [mice, outbred CD-1].
Major urinary protein 6 precursor (MUP 6) (Alpha-2U-globulin).
TPA_exp: allergen dI chain C2A [Mus musculus].
TPA_exp: allergen dI chain C2C [Mus musculus].
TPA_exp: allergen dI chain C2D [Mus musculus].
TPA_exp: allergen dI chain C2Y [Mus musculus].
androgen binding protein zeta [Mus musculus].
androgen binding protein gamma [Mus musculus].
androgen binding protein gamma [Mus musculus].
androgen binding protein beta [Mus musculus].
cysteine-rich secretory protein [Mus musculus].
lacrimal androgen-binding protein delta [Mus musculus].
Major urinary protein precursor (MUP) (Alpha-2u-globulin).
Niemann-Pick type C2 [Rattus norvegicus].
PREDICTED: similar to Major urinary protein precursor (MUP).
Alpha-2u globulin.
Tha p 1.

GI

29292272
29292274
3121745
3121746
3319897
60729635
633938
6687188
119238
32363133
32469617
544241
1169665
1364212
1364213
163823
163827
17224444
17939981
38492847
45775300
886485
232086
229507
10181188
1174278
20178291
28476845
28476849
28476851
28476853
30315676
30315678
45331198
45331208
56694673
8926324
127533
27465565
62649086
81890324
74798355

AA

177
179
174
180
585
13
265
608
233
15
15
234
92
88
92
109
88
366
98
170
186
608
109
14
93
25
180
112
108
112
46
112
112
112
112
250
112
181
149
181
181
18

Aeroallergens fungi

Species

Alternaria alternata
Alternaria alternata
Alternaria alternata
Alternaria alternata
Alternaria alternata
Alternaria alternata
Alternaria alternata
Alternaria alternata
Alternaria brassicicola
Alternaria alternata
Alternaria alternata

Comments

protein disulfide isomerase [Alternaria alternata].
Aldehyde dehydrogenase (ALDDH) (Allergen Alt a 10) (Alt a X).
60S acidic ribosomal protein P1 (Allergen Alt a 12) (Alt a XII).
Alt a 1 [Alternaria alternata].
Enolase (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate).
Heat shock 70 kDa protein (Allergen Alt a 3).
major allergen Alt a 1 subunit [Alternaria alternata].
ribosomal P2 phosphoprotein [Alternaria alternata].
major allergen-like protein precursor [Alternaria brassicicola].
putative nuclear transport factor 2 [Alternaria alternata].
major allergen alt a1 [Alternaria alternata].

GI

1006624
1169290
1350779
1421808
14423684
14423730
1842045
1850540
20279107
21748153
21913174

AA

433
495
110
135
438
152
157
113
158
124
115

Alternaria alternata	allergen r Alt a 2 [Alternaria alternata].	4097481	190
Alternaria alternata	major allergen Alt a 1 subunit [Alternaria alternata].	45680856	157
Alternaria alternata	minor allergen, ribosomal protein [Alternaria alternata].	467617	113
Alternaria alternata	minor allergen [Alternaria alternata].	467619	204
Alternaria cetera	major allergen alt a1 [Alternaria cetera].	49476471	138
Alternaria mouchaccaee	major allergen alt a1 [Alternaria mouchaccaee].	49476473	138
Alternaria argyranthemii	major allergen alt a1 [Alternaria argyranthemii].	49476475	138
Alternaria conjuncta	major allergen alt a1 [Alternaria conjuncta].	49476477	138
Alternaria photistica	major allergen alt a1 [Alternaria photistica].	49476479	138
Alternaria oregonensis	major allergen alt a1 [Alternaria oregonensis].	49476481	128
Alternaria metachromatica	major allergen alt a1 [Alternaria metachromatica].	49476485	137
Alternaria radicina	major allergen alt a1 [Alternaria radicina].	49476487	137
Alternaria carotincultae	major allergen alt a1 [Alternaria carotincultae].	49476489	137
Alternaria petroselini	major allergen alt a1 [Alternaria petroselini].	49476491	137
Alternaria smyrnii	major allergen alt a1 [Alternaria smyrnii].	49476493	137
Alternaria cheiranthi	major allergen alt a1 [Alternaria cheiranthi].	49476495	137
Alternaria blumeae	major allergen alt a1 [Alternaria blumeae].	49476497	137
Alternaria dauci	major allergen alt a1 [Alternaria dauci].	49476499	137
Alternaria crassa	major allergen alt a1 [Alternaria crassa].	49476501	137
Alternaria macrospora	major allergen alt a1 [Alternaria macrospora].	49476503	137
Alternaria pseudorostrata	major allergen alt a1 [Alternaria pseudorostrata].	49476505	137
Alternaria porri	major allergen alt a1 [Alternaria porri].	49476507	137
Alternaria tagetica	major allergen alt a1 [Alternaria tagetica].	49476509	137
Alternaria capsici	major allergen alt a1 [Alternaria capsici].	49476511	137
Alternaria solani	major allergen alt a1 [Alternaria solani].	49476513	137
Alternaria cucumerina	major allergen alt a1 [Alternaria cucumerina].	49476515	137
Alternaria tenuissima	major allergen alt a1 [Alternaria tenuissima].	49476519	137
Alternaria arborescens	major allergen alt a1 [Alternaria arborescens].	49476521	137
Alternaria longipes	major allergen alt a1 [Alternaria longipes].	49476523	137
Alternaria dumosa	major allergen alt a1 [Alternaria dumosa].	49476525	137
Alternaria limoniasperae	major allergen alt a1 [Alternaria limoniasperae].	49476527	137
Alternaria sonchi	major allergen alt a1 [Alternaria sonchi].	49476529	138
Alternaria cinerariae	major allergen alt a1 [Alternaria cinerariae].	49476531	137
Alternaria brassicae	major allergen alt a1 [Alternaria brassicae].	49476533	137
Alternaria mimicola	major allergen alt a1 [Alternaria mimicola].	49476535	137
Alternaria japonica	major allergen alt a1 [Alternaria japonica].	49476539	137
Alternaria eryngii	major allergen alt a1 [Alternaria eryngii].	49476541	137
Alternaria euphorbiicola	major allergen alt a1 [Alternaria euphorbiicola].	49476543	137
Arthroderma benhamiae	tri m 4 allergen [Arthroderma benhamiae].	23894232	726
Arthroderma benhamiae	tri m 2 allergen [Arthroderma benhamiae].	23894240	292
Arthroderma benhamiae	tri m 2 allergen [Arthroderma benhamiae].	23894244	404
Arthroderma benhamiae	tri m 2 allergen [Arthroderma benhamiae].	23894248	405
Aspergillus oryzae	Alpha-amylase A precursor (Taka-amylase A) (TAA)	113779	499
Aspergillus oryzae	Oryzin precursor (Alkaline proteinase) (ALP) (Aspergillus	129235	403
Aspergillus fumigatus	enolase [Aspergillus fumigatus].	13925873	438
Aspergillus fumigatus	manganese superoxide dismutase [Aspergillus fumigatus].	1648970	221
Aspergillus fumigatus	Possible pathogenesis-related protein precursor [Aspergillus	19309414	164
Aspergillus fumigatus	large subunit ribosomal protein L3 [Aspergillus fumigatus].	21215170	392
Aspergillus fumigatus	cellular serine proteinase [Aspergillus fumigatus].	2143220	495
Aspergillus niger	xylosidase [Aspergillus niger].	2181180	804
Aspergillus fumigatus	peroxisomal-like protein [Aspergillus fumigatus].	2769700	168
Aspergillus fumigatus	rAsp f 7 [Aspergillus fumigatus].	2879888	112
Aspergillus fumigatus	rAsp f 9 [Aspergillus fumigatus].	2879890	302
Aspergillus niger	serine protease.	289172	533
Aspergillus fumigatus	IgE-binding protein [Aspergillus fumigatus].	2980819	197
Aspergillus fumigatus	rAsp f 4 [Aspergillus fumigatus].	3005839	286
Aspergillus fumigatus	rAsp f 13 [Aspergillus fumigatus].	3005841	152
Aspergillus fumigatus	Aspf1 allergen [Aspergillus fumigatus].	3021324	125
Aspergillus fumigatus	IgE-binding protein [Aspergillus fumigatus].	3219530	185
Aspergillus fumigatus	allergen [Aspergillus fumigatus].	3643813	427
Aspergillus fumigatus	metalloprotease (MEP) [Aspergillus fumigatus].	3776613	634

Aspergillus niger	beta-xylosidase [Aspergillus niger].	4235093	804
Aspergillus fumigatus	suppressor protein spt23-related, with ankyrin repeats [Aspergillus	42820661	1407
Aspergillus flavus	Oryzin precursor (Alkaline proteinase) (ALP) (Elastase)	464318	403
Aspergillus fumigatus	PPase [Aspergillus fumigatus].	5019414	178
Aspergillus fumigatus	Asp FII [Aspergillus fumigatus].	664852	250
Aspergillus fumigatus	rAsp f 8 [Aspergillus fumigatus].	6686624	111
Aspergillus flavus	Allergen Asp f1.	74665726	403
Aspergillus fumigatus	Major allergen Asp f2 precursor (Asp fII).	83300352	310
Aspergillus fumigatus	Heat shock protein 90 (Heat shock protein hsp1) (65 kDa IgE-binding	83303658	706
Aspergillus fumigatus	major allergen 1 18kDa antigen [Aspergillus fumigatus].	9280360	150
Aspergillus fumigatus	asperillopepsin i [Aspergillus fumigatus].	963013	395
Aspergillus fumigatus	Ribonuclease mitogillin precursor (Major allergen Asp f1) (Asp f	54039254	176
Candida albicans	Alcohol dehydrogenase 1 (40 kDa allergen) (Allergen (Aand a 1) (Can	1168348	350
Candida boidinii	Putative peroxiredoxin-A (Thioredoxin reductase) (Peroxisomal	130360	167
Candida boidinii	Putative peroxiredoxin-B (Thioredoxin reductase) (Peroxisomal	130361	167
Candida albicans	Fructose-bisphosphate aldolase (37 kDa major allergen) (IgE-binding	18203509	40
Candida albicans	Enolase 1 (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate	232054	440
Candida albicans	putative aminoacid permease protein [Candida albicans].	24461764	648
Candida albicans	29 kDa IgE-binding protein [Candida albicans].	37548637	236
Candida albicans	hypothetical protein CaO19.557 [Candida albicans SC5314].	46431500	341
Candida albicans	hypothetical protein CaO19.8192 [Candida albicans SC5314].	46431509	341
Coprinus comatus	Cop c1 allergen [Coprinus comatus].	4538529	81
Coprinus comatus	thioredoxin [Coprinus comatus].	5689669	106
Coprinus comatus	rCop c3 [Coprinus comatus].	5689671	328
Coprinus comatus	rCop c5 [Coprinus comatus].	5689673	141
Coprinus comatus	rCop c7 [Coprinus comatus].	5689675	152
Cryptococcus neoformans	allergen, putative [Cryptococcus neoformans var. neoformans JEC21].	57227639	234
Cryptococcus neoformans	thioredoxin (allergen cop c 2) [Cryptococcus neoformans var.	58265022	104
Davidiella tassiana	ribosomal protein P1 [Davidiella tassiana].	1143425	110
Davidiella tassiana	Minor allergen Cla h 7 (Cla h 5) (Cla h V).	1168970	204
Davidiella tassiana	60S acidic ribosomal protein P2 (Allergen Cla h 3) (Cla h III).	1173074	111
Davidiella tassiana	60S acidic ribosomal protein P2 (Minor allergen Cla h 4) (Cla h	21542440	111
Davidiella tassiana	putative nuclear transport factor 2 [Davidiella tassiana].	21748151	125
Davidiella tassiana	hydrophobin [Davidiella tassiana].	22796153	105
Davidiella tassiana	enolase; phosphopyruvate hydratase [Davidiella tassiana].	467660	440
Davidiella tassiana	Enolase (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate	6015094	440
Davidiella tassiana	Heat shock 70 kDa protein (Allergen Cla h 4) (Cla h IV).	729764	643
Davidiella tassiana	aldehyde dehydrogenase (NAD+) [Davidiella tassiana].	76666769	496
Embellisia allii	major allergen alt a1 [Embellisia allii].	49476559	138
Embellisia indefessa	major allergen alt a1 [Embellisia indefessa].	49476561	137
Embellisia novae-zelandiae	major allergen alt a1 [Embellisia novae-zelandiae].	49476563	136
Embellisia telluster	major allergen alt a1 [Embellisia telluster].	49476565	138
Epicoccum nigrum	[Segment 1 of 2] Major allergen Epi p 1 (Epi n 14625*).	24636821	12
Fusarium culmorum	60S acidic ribosomal protein P2 [Fusarium culmorum].	19879657	109
Fusarium culmorum	thioredoxin-like protein [Fusarium culmorum].	19879659	121
Fusarium culmorum	helix-loop-helix protein [Fusarium culmorum].	25361513	450
Fusarium culmorum	hypothetical protein [Fusarium culmorum].	27965571	342
Gibberella zeae	ENO_ALTAL Enolase (2-phosphoglycerate dehydratase)	46108928	438
Gibberella zeae	RLA2_ALTAL 60S acidic ribosomal protein P2 (Minor allergen Alt a 6)	46122455	109
Gibberella zeae	RLA1_CLAHE 60S acidic ribosomal protein p1 (allergen cla h 12) (CLA	46137705	108
Haematococcus haematococcus	Allergen Fus s 13596*.	3122132	8
Lewia ethzedia	major allergen alt a1 [Lewia ethzedia].	49476483	138
Macrospora scirpicola	major allergen alt a1 [Macrospora scirpicola].	49476555	138
Malassezia sympodialis	allergen [Malassezia sympodialis].	1261972	350
Malassezia furfur	Major allergen Mal f 1 precursor (Pit o 1).	13959403	350
Malassezia sympodialis	allergen [Malassezia sympodialis].	19069920	342
Malassezia sympodialis	manganese superoxide dismutase [Malassezia sympodialis].	28569698	237
Malassezia furfur	MF1 [Malassezia furfur].	3445490	177
Malassezia furfur	MF2 [Malassezia furfur].	3445492	166
Malassezia sympodialis	allergen [Malassezia sympodialis].	4138171	172
Malassezia sympodialis	allergen [Malassezia sympodialis].	4138173	162

Malassezia sympodialis	allergen [Malassezia sympodialis].	4138175	187
Malassezia furfur	major allergenic protein Mal f4 [Malassezia furfur].	4587985	342
Malassezia sympodialis	allergen [Malassezia sympodialis].	7271239	179
Malassezia furfur	allergen Mal f3 - Malassezia furfur.	7514251	166
Malassezia sympodialis	mala s 12 allergen precursor [Malassezia sympodialis].	78038796	618
Neurospora crassa	probable rAsp f 9 allergen [Neurospora crassa].	28949979	301
Neurospora crassa	related to rasp f 7 allergen [Neurospora crassa].	28950043	265
Nimbya caricis	major allergen alt a1 [Nimbya caricis].	49476557	138
Penicillium oxalicum	vacuolar serine protease [Penicillium oxalicum].	12005497	503
Penicillium citrinum	vacuolar serine protease [Penicillium citrinum].	12005501	358
Penicillium citrinum	enolase [Penicillium citrinum].	13991101	438
Penicillium chrysogenum	vacuolar serine protease [Penicillium chrysogenum].	14215732	494
Penicillium citrinum	Heat shock 70 kDa protein (Allergen Pen c 19).	14423733	503
Penicillium chrysogenum	alkaline serine protease [Penicillium chrysogenum].	21069093	398
Penicillium citrinum	unknown [Penicillium citrinum].	38326693	228
Penicillium citrinum	Pen c 1; alkaline serine protease [Penicillium citrinum].	4587983	397
Penicillium citrinum	alkaline serine protease Pen c2 [Penicillium citrinum].	4588118	457
Penicillium citrinum	peroxisomal membrane protein [Penicillium citrinum].	5326864	167
Penicillium chrysogenum	allergen Pen n 13 [Penicillium chrysogenum].	6684758	397
Penicillium chrysogenum	allergen Pen n 18 [Penicillium chrysogenum].	7963902	494
Penicillium chrysogenum	68 kDa allergen [Penicillium chrysogenum].	999009	117
Pleospora tarda	major allergen alt a1 [Pleospora tarda].	49476463	137
Pleospora herbarum	major allergen alt a1 [Pleospora herbarum].	49476469	137
Rhodotorula mucilaginosa	Enolase (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate	37078092	439
Saccharomyces cerevisiae	Chain A, Yeast Profilin, Cubic Crystal Form.	15988101	125
Schizophyllum commune	pSc7 protien [Schizophyllum commune].	169865	204
Schizophyllum commune	pSc14 protein [Schizophyllum commune].	386678	214
Scomber japonicus	parvalbumin [Scomber japonicus].	29420793	109
Stemphylium vesicarium	major allergen alt a1 [Stemphylium vesicarium].	49476465	137
Stemphylium callistephi	major allergen alt a1 [Stemphylium callistephi].	49476467	137
Trichophyton tonsurans	83 kDa hypersensitivity protein (Protein IV).	1708296	26
Trichophyton schoenleinii	tri s 4 allergen [Trichophyton schoenleinii].	23894227	726
Trichophyton schoenleinii	tri m 2 allergen [Trichophyton schoenleinii].	23894260	405
Trichophyton rubrum	Tri r 4 allergen [Trichophyton rubrum].	5813788	726
Trichophyton rubrum	Tri r 2 allergen [Trichophyton rubrum].	5813790	412
Ulocladium cucurbitae	major allergen alt a1 [Ulocladium cucurbitae].	49476545	137
Ulocladium alternariae	major allergen alt a1 [Ulocladium alternariae].	49476547	138
Ulocladium botrytis	major allergen alt a1 [Ulocladium botrytis].	49476549	137
Ulocladium atrum	major allergen alt a1 [Ulocladium atrum].	49476551	137
Ulocladium chartarum	major allergen alt a1 [Ulocladium chartarum].	49476553	137
Verticillium dahliae	allergen rAsp f9-like protein [Verticillium dahliae].	42742375	403
Verticillium dahliae	allergen Asp f2-like protein [Verticillium dahliae].	42742377	297

Aeroallergens mites

Species	Comments	GI #	AA
Acarus siro	lipid binding protein [Acarus siro].	4049356	64
Blomia tropicalis	allergen [Blomia tropicalis].	1377859	130
Blomia tropicalis	cysteine protease precursor [Blomia tropicalis].	14276828	221
Blomia tropicalis	parmyosin allergen [Blomia tropicalis].	21954740	875
Blomia tropicalis	trypsin [Blomia tropicalis].	25989482	266
Blomia tropicalis	allergen Blo t Mag 1 [Blomia tropicalis].	25989484	338
Blomia tropicalis	major IgE-binding protein Blo t 5 [Blomia tropicalis].	4204917	134
Blomia tropicalis	allergen precursor [Blomia tropicalis].	902012	144
Blomia tropicalis	allergen [Blomia tropicalis].	915347	73
Chironomus thummi	Globin CTT-III precursor (Erythrocrutorin III).	121219	151
Chironomus thummi	Globin CTT-IV precursor.	121227	151
Chironomus thummi	Globin CTT-VIII.	121237	151
Chironomus thummi	Globin CTT-VIIB-3 precursor.	121244	161
Chironomus thummi	Globin CTT-VIIB-6 precursor.	121248	161
Chironomus thummi	Globin CTT-VIIB-7 precursor.	121249	162

Chironomus thummi	Globin CTT-III.A.	121256	151
Chironomus thummi	Globin CTT-X.	121259	151
Chironomus thummi	Globin CTT-II beta precursor.	1707908	160
Chironomus thummi	Globin CTT-IX precursor.	1707911	161
Chironomus thummi	Globin CTT-I/CTT-IA precursor (Erythrocrucorin).	2506460	158
Chironomus thummi	Globin CTT-VI precursor.	2506461	162
Chironomus thummi	Globin CTT-VIIB-4 precursor (Erythrocrucorin).	56405052	161
Chironomus thummi	Globin CTT-VIIB-5/CTT-VIIB-9 precursor.	56405054	161
Chironomus thummi	Globin CTT-VIIA precursor.	56405306	161
Chironomus kiiensis	tropomyosin [Chironomus kiiensis].	7321108	285
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	10189811	215
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	10189816	213
Dermatophagoides pteronyssinus	group III allergen - house-dust mite (Dermatophagoides	102832	18
Dermatophagoides pteronyssinus	Der p 7 allergen polypeptide.	1045602	215
Dermatophagoides microceras	Major mite fecal allergen Der m 1 (Der m 1).	127205	30
Dermatophagoides farinae	Der f 3 mite allergen.	1314736	232
Dermatophagoides pteronyssinus	Alpha-amylase (Allergen Der p 4) (Der p IV).	1351935	19
Dermatophagoides pteronyssinus	Mite group 2 allergen Der p 2 precursor (Der p II) (DPX).	1352237	146
Dermatophagoides pteronyssinus	Mite allergen Der p 5 (Der P V) (IgE-binding allergen).	1352238	132
Dermatophagoides pteronyssinus	Mite allergen Der p 6 (Der p VI) (DP5).	1352239	20
Dermatophagoides farinae	allergen Der f II precursor [Dermatophagoides farinae].	13560629	170
Dermatophagoides farinae	Mag44 [Dermatophagoides farinae].	1359436	299
Dermatophagoides farinae	paramyosin-like allergen [Dermatophagoides farinae].	13785807	692
Dermatophagoides farinae	Mite allergen Der f 6 precursor (Der f VI) (DF5).	14424450	279
Dermatophagoides pteronyssinus	ferritin heavy chain-like protein [Dermatophagoides pteronyssinus].	15072346	180
Dermatophagoides farinae	Mag3 [Dermatophagoides farinae].	1545803	349
Dermatophagoides farinae	major Der f 2 isoform [Dermatophagoides farinae].	17978844	129
Dermatophagoides pteronyssinus	group 14 allergen protein [Dermatophagoides pteronyssinus].	20385544	1662
Dermatophagoides pteronyssinus	Chain A, X-Ray Structure of Der P 2, The Major House Dust Mite	21465915	129
Dermatophagoides farinae	gelsolin-like allergen Der f 16 [Dermatophagoides farinae].	21591547	480
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725560	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725562	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725564	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725566	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725568	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725570	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725572	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725574	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725576	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725578	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725580	222
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725582	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725584	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725586	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725588	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725590	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725592	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725594	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725596	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725600	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725602	129
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	21725604	129
Dermatophagoides farinae	mite allergen Der f II precursor [Dermatophagoides farinae].	217306	146
Dermatophagoides farinae	mite allergen Der f II precursor [Dermatophagoides farinae].	217308	138
Dermatophagoides pteronyssinus	serine protease [Dermatophagoides pteronyssinus].	22595342	244
Dermatophagoides pteronyssinus	tropomyosin [Dermatophagoides pteronyssinus].	2353266	284
Dermatophagoides pteronyssinus	tropomyosin [Dermatophagoides pteronyssinus].	2440053	284
Dermatophagoides farinae	Mite allergen Der f 7 precursor (Der f VII).	2498299	213
Dermatophagoides farinae	Mite allergen Der f 3 precursor (Der f III).	2507248	259
Dermatophagoides farinae	trypsin-like protease=Der f III allergen homolog [N-terminal]	259012	20
Dermatophagoides farinae	Der f 1 allergen preproenzyme [Dermatophagoides farinae].	27530349	321

Dermatophagoides farinae	60 kDa allergen Der f 18p [Dermatophagoides farinae].	27550039	462
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	28798085	132
Dermatophagoides pteronyssinus	unnamed protein product [Dermatophagoides pteronyssinus].	29786835	302
Dermatophagoides pteronyssinus	trypsin-like serine protease [Dermatophagoides pteronyssinus].	31745576	273
Dermatophagoides pteronyssinus	serine protease LM-1 [Dermatophagoides pteronyssinus].	37654735	261
Dermatophagoides pteronyssinus	HDM allergen [Dermatophagoides pteronyssinus].	37778944	875
Dermatophagoides pteronyssinus	Tertiary Structure Of The Major House Dust Mite Allergen Der P 2,	3891991	129
Dermatophagoides farinae	DF5=allergen {N-terminal} [Dermatophagoides farinae=mites, Peptide	404371	20
Dermatophagoides pteronyssinus	alpha-amylase [Dermatophagoides pteronyssinus].	5059162	496
Dermatophagoides pteronyssinus	Der p 3 allergen.	511476	261
Dermatophagoides pteronyssinus	Der p 1 allergen preproenzyme [Dermatophagoides pteronyssinus].	511953	320
Dermatophagoides farinae	Der f II [Dermatophagoides farinae].	546852	142
Dermatophagoides farinae	mite allergen Der f 2 [Dermatophagoides farinae].	55859466	146
Dermatophagoides farinae	Der f 2 [Dermatophagoides farinae].	55859468	146
Dermatophagoides farinae	mite allergen Der f 2 [Dermatophagoides farinae].	55859470	146
Dermatophagoides farinae	group 2 allergen [Dermatophagoides farinae].	56378069	146
Dermatophagoides farinae	98kDa HDM allergen [Dermatophagoides farinae].	5815436	555
Dermatophagoides pteronyssinus	Der p 1 allergen [Dermatophagoides pteronyssinus].	61608445	216
Dermatophagoides farinae	allergen Der f I precursor - house-dust mite (Dermatophagoides	627141	319
Dermatophagoides farinae	Allergen MAG29.	729970	145
Dermatophagoides farinae	Allergen Mag.	729979	341
Dermatophagoides farinae	Major mite fecal allergen Der f 1 precursor (Der f I).	730035	321
Dermatophagoides pteronyssinus	Major mite fecal allergen Der p 1 precursor (Der p I).	730036	320
Dermatophagoides pteronyssinus	Major fecal allergen Der p 1-associated protein.	74798156	23
Dermatophagoides farinae	Major Der f 2 isoform.	74820084	129
Dermatophagoides pteronyssinus	major allergen p Dp 15 [Dermatophagoides pteronyssinus].	807138	219
Dermatophagoides pteronyssinus	IgE-binding protein C-terminal fragment (148 AA) [Dermatophagoides	9072	148
Dermatophagoides pteronyssinus	Der p V allergen [Dermatophagoides pteronyssinus].	913285	132
Euroglyphus maynei	Mite group 2 allergen Eur m 2 precursor.	14423649	145
Euroglyphus maynei	Mite allergen Eur m 3 precursor.	14423685	261
Euroglyphus maynei	group 2 allergen Eur m 2 0102 [Euroglyphus maynei].	3941386	135
Euroglyphus maynei	group 1 allergen Eur m 1 0101 precursor [Euroglyphus maynei].	3941388	321
Euroglyphus maynei	group 1 allergen Eur m 1 0102 [Euroglyphus maynei].	3941390	327
Euroglyphus maynei	alpha-amylase precursor [Euroglyphus maynei].	5059164	521
Euroglyphus maynei	high molecular weight allergen M-177 precursor [Euroglyphus	6492307	1668
Glycyphagus domesticus	Gly d 2 [Glycyphagus domesticus].	6179520	128
Glycyphagus domesticus	gly d 2.02 isoform [Glycyphagus domesticus].	7160811	125
Lepidoglyphus destructor	Mite allergen Lep d 7 precursor.	14423650	216
Lepidoglyphus destructor	Mite allergen Lep d 5.	14423651	110
Lepidoglyphus destructor	Fatty acid-binding protein (Allergen Lep d 13).	14423714	131
Lepidoglyphus destructor	Tropomyosin (Allergen Lep d 10).	14423956	284
Lepidoglyphus destructor	allergen Lep d 1.01.	1582222	141
Lepidoglyphus destructor	allergen Lep d 1.02.	1582223	141
Lepidoglyphus destructor	alpha tubulin [Lepidoglyphus destructor].	19702131	450
Lepidoglyphus destructor	Lep D 2 precursor [Lepidoglyphus destructor].	21213898	141
Lepidoglyphus destructor	Lep D 2 precursor [Lepidoglyphus destructor].	21213900	141
Lepidoglyphus destructor	allergen Lep d 1.02 precursor - Lepidoglyphus destructor.	2147108	141
Lepidoglyphus destructor	Lep D 2 allergen [Lepidoglyphus destructor].	999462	141
Tyrophagus putrescentiae	group 2 allergen [Tyrophagus putrescentiae].	2182106	141
Tyrophagus putrescentiae	fatty acid-biding protein [Tyrophagus putrescentiae].	51860756	131

Aeroallergens insects

Species	Comments	GI #	AA
Argas reflexus	Arg r 1 precursor [Argas reflexus].	58371884	159
Blattella germanica	allergen Bla g 4.	1166573	182
Blattella germanica	Aspartic protease Bla g 2 precursor (Allergen Bla g II).	1703445	352
Blattella germanica	major allergen Bla g 1.02 [Blattella germanica].	4240395	492
Blattella germanica	major allergen Bla g 1.0101 [Blattella germanica].	4572592	412
Blattella germanica	36 kda allergen {peptide 143-111} [Blattella germanica=German	544618	20
Blattella germanica	36 kda allergen {peptide 143-115} [Blattella germanica=German	544619	25

Blattella germanica	Glutathione S-transferase (GST class-sigma) (Major allergen Bla g	8225491	204
Blattella germanica	Chain A, The Structure Of Mutant (N93q) Of Bla G 2.	8277863	330
Blattella germanica	tropomyosin [Blattella germanica].	8101069	284
Ixodes ricinus	allergen like protein [Ixodes ricinus].	2856446	116
Lepisma saccharina	tropomyosin [Lepisma saccharina].	20387027	284
Lepisma saccharina	tropomyosin [Lepisma saccharina].	20387029	243
Periplaneta americana	allergen [Periplaneta americana].	1581589	631
Periplaneta americana	allergen [Periplaneta americana].	1580792	685
Periplaneta americana	allergen [Periplaneta americana].	1580794	470
Periplaneta americana	allergen [Periplaneta americana].	1580797	393
Periplaneta fuliginosa	tropomyosin [Periplaneta fuliginosa].	19310971	284
Periplaneta americana	ribosomal protein S12 [Periplaneta americana].	21217441	139
Periplaneta americana	Rab11 [Periplaneta americana].	21217443	204
Periplaneta americana	Cr-P11 allergen [Periplaneta americana].	2231297	446
Periplaneta americana	Cr-P11 allergen [Periplaneta americana].	2253610	274
Periplaneta americana	Cr-P11 [Periplaneta americana].	2580504	395
Periplaneta americana	allergen [Periplaneta americana].	2897849	228
Periplaneta americana	major allergen Per a 1.0105 [Periplaneta americana].	30144660	124
Periplaneta americana	major allergen Per a 1.0101 [Periplaneta americana].	4240399	231
Periplaneta americana	tropomyosin [Periplaneta americana].	4378573	284
Periplaneta americana	tropomyosin [Periplaneta americana].	4468639	284
Pieris rapae	nitrile-specifier protein [Pieris rapae].	40288346	632
Plodia interpunctella	arginine kinase [Plodia interpunctella].	15886861	355
Plodia interpunctella	unnamed protein product [Plodia interpunctella].	21335404	285
Plodia interpunctella	unnamed protein product [Plodia interpunctella].	21335406	705
Plodia interpunctella	unnamed protein product [Plodia interpunctella].	21335408	254
Polypodium nubilifer	Pol n I component MV =allergic monomeric hemoglobin {N-terminal}	1703681	37
Tenebrio molitor	cockroach allergen-like protein [Tenebrio molitor].	32967475	595

Aeroallergens pollen

Species	Comments	GI #	AA
Agrostis alba	pollen allergen Agr a 1 - bent grass (fragment).	320606	26
Agrostis alba	Group 1 allergen Agr a 1 (Form 2), pollen.	75139987	35
Agrostis alba	Group 1 allergen Agr a 1 (Form 1), pollen.	75139989	35
Alnus glutinosa	Aln g 1 [Alnus glutinosa].	261407	160
Alnus glutinosa	pollen allergen Aln g 4 [Alnus glutinosa].	3319651	85
Ambrosia artemisiifolia	Pollen allergen Amb a 1.1 precursor (Antigen E) (AgE) (Antigen Amb	113475	396
Ambrosia artemisiifolia	Pollen allergen Amb a 1.2 precursor (Antigen E) (Antigen Amb a I)	113476	398
Ambrosia artemisiifolia	Pollen allergen Amb a 1.3 precursor (Antigen E) (Antigen Amb a I).	113477	397
Ambrosia artemisiifolia	Pollen allergen Amb a 1.4 precursor (Antigen E) (Antigen Amb a I).	113478	392
Ambrosia elatior	Pollen allergen Amb a 5 (Amb a V) (Allergen Ra5).	114090	45
Ambrosia trifida	Pollen allergen Amb t 5 precursor (Amb t V) (Allergen Ra5G).	114091	73
Ambrosia artemisiifolia	Nonspecific lipid-transfer protein precursor (LTP) (Pollen allergen	14285595	118
Ambrosia artemisiifolia	antigen E.	166443	397
Ambrosia artemisiifolia	profilin-like protein [Ambrosia artemisiifolia].	34851178	131
Ambrosia artemisiifolia	profilin-like protein [Ambrosia artemisiifolia].	34851180	131
Ambrosia artemisiifolia	profilin-like protein [Ambrosia artemisiifolia].	34851182	133
Ambrosia elatior	Pollen allergen Amb a 3 (Amb a III) (Allergen Ra3).	416636	101
Ambrosia psilostachya	Amb p V allergen.	515953	77
Ambrosia psilostachya	Amb p V allergen.	515954	77
Ambrosia psilostachya	Amb p V allergen.	515955	77
Ambrosia psilostachya	Amb p V allergen.	515956	77
Ambrosia psilostachya	Amb p V allergen.	515957	77
Ambrosia artemisiifolia	allergen Amb a 1.2 precursor - common ragweed.	539048	398
Ambrosia artemisiifolia	allergen Amb a 1.3 precursor - common ragweed.	539049	397
Ambrosia artemisiifolia	Pollen allergen Amb a 2 precursor (Antigen K) (Antigen Amb a II).	113479	397
Anthoxanthum odoratum	pollen allergen Ant o 1 - sweet vernal grass (fragment).	320607	26
Anthoxanthum odoratum	Group 1 allergen Ant o I (Form 1), pollen.	75139986	32
Anthoxanthum odoratum	Group 1 allergen Ant o I (Form 2), pollen.	75139990	32
Artemisia vulgaris	major pollen allergen Art v 1 precursor [Artemisia vulgaris].	27818335	132
Betula pendula	allergenic isoflavone reductase-like protein Bet v 6.0102 [Betula	10764491	308

Betula pendula	Chain A, Birch Pollen Allergen Bet V 1 Mutant N28t, K32q, E45s,	11514622	159
Betula pendula	Calcium-binding allergen Bet v 3 (Bet v III).	1168696	205
Betula pendula	Major pollen allergen Bet v 1-B (Bet v I-B).	1168701	160
Betula pendula	Major pollen allergen Bet v 1-C (Bet v I-C).	1168702	160
Betula pendula	Major pollen allergen Bet v 1-D/H (Bet v I-D/H).	1168703	160
Betula pendula	Major pollen allergen Bet v 1-E (Bet v I-E).	1168704	160
Betula pendula	Major pollen allergen Bet v 1-F/I (Bet v I-F/I).	1168705	160
Betula pendula	Major pollen allergen Bet v 1-G (Bet v I-G).	1168706	160
Betula pendula	Major pollen allergen Bet v 1-J (Bet v I-J).	1168707	160
Betula pendula	Major pollen allergen Bet v 1-K (Bet v I-K).	1168708	160
Betula pendula	Major pollen allergen Bet v 1-L (Bet v I-L).	1168709	160
Betula pendula	Major pollen allergen Bet v 1-M/N (Bet v I-M/N).	1168710	160
Betula platyphylla	Bet vl jap1 [Betula platyphylla var. japonica].	12583681	160
Betula platyphylla	Bet vl jap2 [Betula platyphylla var. japonica].	12583683	160
Betula platyphylla	Bet vl jap3 [Betula platyphylla var. japonica].	12583685	160
Betula pendula	Profilin (Pollen allergen Bet v 2) (Bet v II).	130975	133
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321714	160
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321716	160
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321718	160
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321720	160
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321722	160
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321724	160
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321726	160
Betula pendula	major allergen Bet v 1 [Betula pendula].	1321728	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542857	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542861	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542863	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542865	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542867	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542869	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542871	160
Betula pendula	pollen allergen Bet v 1 [Betula pendula].	1542873	160
Betula pendula	Birch Pollen Profilin.	1942360	133
Betula pendula	peptidylprolyl isomerase (cyclophilin) [Betula pendula].	21886603	173
Betula pendula	Bet v I=major allergen [Betula verrucosa=birch trees, pollen,	239734	43
Betula pendula	major allergen Bet v 1 [Betula pendula].	2414158	160
Betula pendula	pollen allergen Betv1 [Betula pendula].	2564220	160
Betula pendula	pollen allergen Betv1 [Betula pendula].	2564222	160
Betula pendula	pollen allergen Betv1 [Betula pendula].	2564224	160
Betula pendula	pollen allergen Betv1 [Betula pendula].	2564228	160
Betula	isoallergen {N-terminal} [birch, pollen, Peptide Partial, 51 aa].	298736	51
Betula	isoallergen {N-terminal} [birch, pollen, Peptide Partial, 51 aa].	298737	51
Betula pendula	allergen Bet v 1x [Betula pendula].	30908931	21
Betula pendula	major pollen allergen Bet v I - European white birch (fragment).	320545	51
Betula pendula	major pollen allergen Bet v II - European white birch (fragment).	320546	51
Betula pendula	Chain A, Birch Pollen Allergen Bet V 1 Mutant E45s.	38492423	159
Betula pendula	pollen allergen Betv1, isoform at8 [Betula pendula].	4006928	160
Betula pendula	pollen allergen Betv1, isoform at10 [Betula pendula].	4006945	160
Betula pendula	pollen allergen Betv1, isoform at14 [Betula pendula].	4006947	120
Betula pendula	pollen allergen Betv1, isoform at37 [Betula pendula].	4006953	160
Betula pendula	pollen allergen Betv1, isoform at42 [Betula pendula].	4006955	160
Betula pendula	pollen allergen Betv1, isoform at45 [Betula pendula].	4006957	160
Betula pendula	pollen allergen Betv1, isoform at50 [Betula pendula].	4006959	160
Betula pendula	pollen allergen Betv1, isoform at59 [Betula pendula].	4006961	160
Betula pendula	pollen allergen Betv1, isoform at87 [Betula pendula].	4006963	120
Betula pendula	pollen allergen Betv1, isoform at5 [Betula pendula].	4006965	160
Betula pendula	pollen allergen Betv1, isoform at7 [Betula pendula].	4006967	160
Betula pendula	pollen allergen, Betv1 [Betula pendula].	4376216	159
Betula pendula	pollen allergen, Betv1 [Betula pendula].	4376219	159
Betula pendula	pollen allergen, Betv1 [Betula pendula].	4376220	159
Betula pendula	pollen allergen, Betv1 [Betula pendula].	4376221	159

Betula pendula	pollen allergen, Betv1 [Betula pendula].	4376222	159
Betula pendula	isoallergen bet v 1 b1 [Betula pendula].	4590392	160
Betula pendula	isoallergen Bet v 1 b2 [Betula pendula].	4590394	160
Betula pendula	isoallergen bet v 1 b3 [Betula pendula].	4590396	160
Betula pendula	Chain A, Solution Structure Of The Birch Pollen Allergen Bet V 4.	46014884	84
Betula pendula	1 Sc-3 [Betula pendula].	534898	160
Betula pendula	1 Sc2 [Betula pendula].	534900	159
Betula pendula	1-Sc1 [Betula pendula].	534910	160
Betula pendula	Chain A, Birch Pollen Allergen Bet V 1.	6980540	159
Betula pendula	Polcalcin Bet v 4 (Calcium-binding pollen allergen Bet v 4).	14423850	85
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545875	160
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545877	160
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545879	160
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545887	160
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545891	160
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545893	160
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545895	161
Carpinus betulus	pollen allergen Car b 1 [Carpinus betulus].	1545897	161
Carpinus betulus	Car b 1=major allergen [Carpinus betulus=hornbeam trees, pollen,	239735	40
Carpinus betulus	Car b 1 [Carpinus betulus].	402745	159
Carpinus betulus	Major pollen allergen Car b 1 isoforms 1A and 1B (Car B 1).	730048	160
Carpinus betulus	Major pollen allergen Car b 1 isoform 2 (Car b 1).	730049	160
Catharanthus roseus	Intracellular pathogenesis related protein.	75279798	140
Chamaecyparis obtusa	Chao1 [Chamaecyparis obtusa].	1514943	375
Chamaecyparis obtusa	Polygalacturonase precursor (PG) (Pectinase) (Major pollen allergen	47606004	514
Chenopodium album	Che a 1 allergen precursor [Chenopodium album].	22074346	168
Chenopodium album	pollen allergen Che a 2 [Chenopodium album].	29465666	131
Chenopodium album	pollen allergen Che a 3 [Chenopodium album].	29465668	86
Crocus sativus	profilin [Crocus sativus].	58700651	131
Cryptomeria japonica	Cry j 1 precursor [Cryptomeria japonica].	19570315	374
Cryptomeria japonica	isoflavone reductase-like protein CJP-6 [Cryptomeria japonica].	19847822	306
Cryptomeria japonica	thaumatin-like protein [Cryptomeria japonica].	22830593	232
Cryptomeria japonica	thaumatin-like protein [Cryptomeria japonica].	22830595	233
Cryptomeria japonica	thaumatin-like protein [Cryptomeria japonica].	22830597	230
Cryptomeria japonica	allergen Cry j 2 [Cryptomeria japonica].	24898904	514
Cryptomeria japonica	allergen Cry j 2 [Cryptomeria japonica].	24898906	514
Cryptomeria japonica	allergen Cry j 2 [Cryptomeria japonica].	24898908	514
Cryptomeria japonica	class IV chitinase [Cryptomeria japonica].	56550550	281
Cryptomeria japonica	pollen major allergen No.121 isoform 1 [Cryptomeria japonica].	62149368	278
Cryptomeria japonica	pollen major allergen No.121 isoform 2 [Cryptomeria japonica].	62149370	290
Cryptomeria japonica	pollen allergen CJP38 [Cryptomeria japonica].	62149372	348
Cryptomeria japonica	Polygalacturonase precursor (PG) (Pectinase) (Major pollen allergen	1171004	514
Cryptomeria japonica	Luji basic protein precursor (SBP) (Major allergen Cry j 1) (Cry j	1173367	374
Cupressus arizonica	putative allergen Cup a 1 [Cupressus arizonica].	19069497	367
Cupressus sempervirens	PR5 allergen Cup s 3.2 precursor [Cupressus sempervirens].	38456228	225
Cupressus sempervirens	PR5 allergen Cup s 3.3 precursor [Cupressus sempervirens].	38456230	225
Cupressus arizonica	cup a 1 protein [Cupressus arizonica].	6562326	346
Cupressus sempervirens	Cup s 1 pollen allergen precursor [Cupressus sempervirens].	8101711	367
Cupressus sempervirens	Cup s 1 pollen allergen precursor [Cupressus sempervirens].	8101713	367
Cupressus sempervirens	Cup s 1 pollen allergen precursor [Cupressus sempervirens].	8101715	367
Cupressus sempervirens	Cup s 1 pollen allergen precursor [Cupressus sempervirens].	8101717	367
Cupressus sempervirens	Cup s 1 pollen allergen precursor [Cupressus sempervirens].	8101719	367
Cupressus arizonica	Cup a 3 protein [Cupressus arizonica].	9929163	199
Cynodon dactylon	acidic Cyn d 1 isoallergen isoform 1 precursor [Cynodon dactylon].	10314021	244
Cynodon dactylon	B1 protein allergen [Cynodon dactylon].	1247373	71
Cynodon dactylon	B4 protein allergen [Cynodon dactylon].	1247375	73
Cynodon dactylon	B2 protein allergen [Cynodon dactylon].	1247377	247
Cynodon dactylon	Major pollen allergen Cyn d 1.	14423757	246
Cynodon dactylon	acidic allergen Cyn d 1 precursor [Cynodon dactylon].	15384338	244
Cynodon dactylon	acidic Cyn d 1 isoallergen isoform 2 precursor [Cynodon dactylon].	16076693	262
Cynodon dactylon	acidic Cyn d 1 isoallergen isoform 3 precursor [Cynodon dactylon].	16076695	262

Cynodon dactylon	acidic Cyn d 1 isoallergen isoform 4 precursor [Cynodon dactylon].	16076697	262
Cynodon dactylon	calcium-binding pollen allergen [Cynodon dactylon].	1871507	82
Cynodon dactylon	profilin 1 [Cynodon dactylon].	2154730	131
Cynodon dactylon	pollen allergen Cyn d 23 [Cynodon dactylon].	32344779	118
Cynodon dactylon	pollen allergen Cyn d 15 [Cynodon dactylon].	32344781	112
Cynodon dactylon	pollen 2-phosphoglycerate dehydrogenase 2 precursor [Cynodon	37222051	446
Cynodon dactylon	pollen allergen (group II) [Cynodon dactylon].	4006978	122
Cynodon dactylon	FAD-linked oxidoreductase BG60 [Cynodon dactylon].	41393750	522
Cynodon dactylon	major allergen Cyn d I=34 kda polypeptide {N-terminal} [Cynodon	451274	25
Cynodon dactylon	major allergen Cyn d I=29 kda polypeptide {N-terminal} [Cynodon	451275	38
Cynodon dactylon	Cyn d 24 [Cynodon dactylon].	51950706	153
Cynodon dactylon	Cyn d Ib isoallergen {N-terminal} [Cynodon dactylon=Bermuda grass,	691726	34
Cynodon dactylon	Acidic Cyn d 1 isoallergen isoform 4 precursor.	75249708	262
Dactylis glomerata	allergen Dac g II.	1093120	196
Dactylis glomerata	group 5 allergen precursor [Dactylis glomerata].	14423124	290
Dactylis glomerata	unnamed protein product [Dactylis glomerata].	18093971	265
Dactylis glomerata	unnamed protein product [Dactylis glomerata].	18093991	264
Dactylis glomerata	Dac gIII allergen.	1825459	96
Dactylis glomerata	pollen allergen DG3 - orchard grass (fragment).	280409	28
Dactylis glomerata	{Segment 1 of 4} Major pollen allergen Dac g 4.	32363464	12
Dactylis glomerata	{Segment 2 of 4} Major pollen allergen Dac g 4.	32363465	11
Dactylis glomerata	{Segment 3 of 4} Major pollen allergen Dac g 4.	32363466	17
Dactylis glomerata	{Segment 4 of 4} Major pollen allergen Dac g 4.	32363467	15
Dactylis glomerata	group 1 allergen Dac g 1.01 precursor [Dactylis glomerata].	33149333	240
Dactylis glomerata	pollen allergen (group II) [Dactylis glomerata].	4007040	122
Dicranopteris linearis	2S storage protein-like albumin precursor [Dicranopteris linearis].	6573243	125
Dicranopteris curranii	2S storage protein-like albumin precursor [Dicranopteris curranii].	6573245	125
Dicranopteris linearis	2S storage protein-like albumin precursor [Dicranopteris linearis].	6573247	125
Festuca arundinacea	pollen allergen Fes e 1 type A - reed fescue (fragment).	320610	17
Festuca arundinacea	pollen allergen Fes e 1 type B - reed fescue (fragment).	320611	20
Festuca arundinacea	Group I allergen FeS e 1, pollen.	75139991	35
Fraxinus excelsior	allergen Fra e 1.0101 [Fraxinus excelsior].	33327133	145
Fraxinus excelsior	allergen Fra e 1 [Fraxinus excelsior].	34978692	146
Fraxinus excelsior	Fra e 1.0102 major allergen [Fraxinus excelsior].	56122438	145
Holcus lanatus	protein with incomplete signal sequence [Holcus lanatus].	1167836	248
Holcus lanatus	pollen allergen Hol 1 5b [Holcus lanatus].	11991229	296
Holcus lanatus	group V grass pollen allergen [Holcus lanatus].	2266623	240
Holcus lanatus	group V allergen [Holcus lanatus].	2266625	264
Holcus lanatus	major group I allergen Hol 1 1 [Holcus lanatus].	3860384	263
Holcus lanatus	pollen allergen (group II) [Holcus lanatus].	4007084	122
Holcus lanatus	allergen Hol-II [Holcus lanatus].	414703	265
Holcus lanatus	30K allergen.	75140046	20
Humulus japonicus	Humj 1 [Humulus japonicus].	33113263	155
Humulus scandens	profilin-like protein [Humulus scandens].	34851174	131
Humulus scandens	profilin-like protein [Humulus scandens].	34851176	131
Juniperus oxycedrus	putative allergen jun o 1 [Juniperus oxycedrus].	15139849	367
Juniperus ashei	elongation factor [Juniperus ashei].	21632054	172
Juniperus rigida	PR5 allergen Jun r 3.1 precursor [Juniperus rigida].	38456222	225
Juniperus rigida	PR5 allergen Jun r 3.2 precursor [Juniperus rigida].	38456224	225
Juniperus ashei	pollen major allergen 1-1 [Juniperus ashei].	4138877	367
Juniperus virginiana	Pathogenesis-related protein precursor (Putative major pollen	51316532	110
Juniperus oxycedrus	pollen allergen Jun o 4 [Juniperus oxycedrus].	5391446	165
Juniperus ashei	allergen Jun a 3 [Juniperus ashei].	6940772	225
Juniperus virginiana	pollen major allergen 1-2 [Juniperus virginiana].	8843917	367
Juniperus virginiana	pollen major allergen 1-1 [Juniperus virginiana].	8843921	367
Juniperus ashei	pollen major allergen 2 protein [Juniperus ashei].	9955725	507
Ligustrum vulgare	major allergen [Ligustrum vulgare].	3256210	145
Ligustrum vulgare	major allergen [Ligustrum vulgare].	3256212	145
Lilium longiflorum	profilin 1 [Lilium longiflorum].	6425105	131
Lilium longiflorum	profilin 2 [Lilium longiflorum].	6425107	131
Lilium longiflorum	profilin 3 [Lilium longiflorum].	6425109	131

Lolium perenne	pollen allergen p1b precursor - perennial ryegrass.	100639	308
Lolium perenne	Pollen allergen Lol p 2-A (Lol p II-A).	126286	97
Lolium perenne	Pollen allergen Lol p 3 (Lol p III).	126387	97
Lolium perenne	pollen allergen.	168374	252
Lolium perenne	Major pollen allergen Lol p 5a precursor (Lol p Va) (Lol p Ib).	2498581	308
Lolium perenne	Major pollen allergen Lol p 5b precursor (Lol p Vb).	2498582	339
Lolium italicum	pollen allergen (group II) [Lolium italicum].	4067636	122
Lolium perenne	pollen allergen Lol p VA precursor: major allergen [Lolium	4416516	301
Lolium perenne	Major pollen allergen Lol p 11 (Lol p XI).	47605808	134
Lolium perenne	pollen allergen Lol p 4 [Lolium perenne].	55859464	423
Lolium perenne	pollen allergen [Lolium perenne].	6634467	301
Lolium perenne	50K allergen.	75140048	20
Lolium perenne	Allergen Lol p II.	75220940	88
Lolium perenne	Pollen allergen.	75274600	263
Lolium perenne	allergen Lol p II [Lolium perenne].	939932	88
Lolium perenne	Pollen allergen Lol p 1 precursor (Lol p I) (Allergen R7).	126385	263
Lupinus luteus	Chain A, Crystal Structure Of Pathogenesis-Related Protein	22218768	155
Lupinus luteus	Chain B, Crystal Structure Of Pathogenesis-Related Protein	22218772	155
Lupinus luteus	Chain B, Crystal Structure Of Pathogenesis-Related Protein L1pr-	61680377	157
Mercurialis annua	Profilin [Mercurialis annua].	2959898	133
Olea europaea	main olive allergen [Olea europaea].	13195753	130
Olea europaea	major allergen OLE16 - common olive (fragment).	1362128	137
Olea europaea	major allergen OLE17 - common olive (fragment).	1362129	136
Olea europaea	major allergen OLE19 - common olive (fragment).	1362130	136
Olea europaea	major allergen OLE1c - common olive (fragment).	1362131	145
Olea europaea	major allergen OLE20 - common olive (fragment).	1362132	137
Olea europaea	major allergen OLE26 - common olive (fragment).	1362133	136
Olea europaea	major allergen OLE33/OLE37 - common olive (fragment).	1362134	136
Olea europaea	major allergen OLE3c - common olive.	1362135	145
Olea europaea	major allergen OLE5c - common olive.	1362136	145
Olea europaea	major allergen OLE6c - common olive (fragment).	1362137	136
Olea europaea	beta-1,3-glucanase like protein [Olea europaea].	14279169	460
Olea europaea	Pollen allergen Ole e6.	14423643	50
Olea europaea	Calcium-binding allergen Ole e 8 (PCA18/PCA23).	14423648	171
Olea europaea	Pollen allergen Ole e 7 (Ole e VII).	22002032	21
Olea europaea	Ole e 1.0042 protein [Olea europaea].	2465127	146
Olea europaea	Ole e 1.0103 protein [Olea europaea].	2465129	146
Olea europaea	Ole e 1 protein [Olea europaea].	2465131	146
Olea europaea	Superoxide dismutase [Cu-Zn] (Allergen Ole e 5) (Ole e V).	2500822	29
Olea europaea	allergen Ole e 10 [Olea europaea].	29465664	123
Olea europaea	[Segment 1 of 2] Major pollen allergen Ole e 4 (Ole e IV).	32363448	10
Olea europaea	[Segment 2 of 2] Major pollen allergen Ole e 4 (Ole e IV).	32363449	14
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33325111	132
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33325115	132
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329732	132
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329738	132
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329744	131
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329748	129
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329750	131
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329752	131
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329754	132
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329756	132
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	33329758	131
Olea europaea	calcium-binding pollen allergen [Olea europaea].	3337403	84
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	37548753	132
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	37724593	135
Olea europaea	major pollen allergen Ole e 1 [Olea europaea].	37724597	134
Olea europaea	Ole e 6 allergen [Olea europaea var. sylvestris].	37725369	38
Olea europaea	Ole e 3 allergen [Olea europaea].	37725377	52
Olea europaea	Profilin-1 (Pollen allergen Ole e 2).	3914426	134
Olea europaea	Profilin-2 (Pollen allergen Ole e 2).	3914427	134

Olea europaea	Profilin-3 (Pollen allergen Ole e 2).	3914428	134
Olea europaea	Cu /Zn super-oxide dismutase [Olea europaea].	39840779	152
Olea europaea	calcium-binding protein [Olea europaea].	6901654	171
Olea europaea	Major pollen allergen (Allergen Ole e 1) (Ole e 1).	14424429	145
Parietaria officinalis	mAb 2F9-reactive major allergen {N-terminal} [Parietaria	1311509	17
Parietaria officinalis	mAb 8C7-reactive major allergen {N-terminal, band 1} [Parietaria	1311510	15
Parietaria officinalis	mAb 8C7-reactive major allergen {N-terminal, band 2} [Parietaria	1311511	15
Parietaria officinalis	mAb 3F8-reactive major allergen {N-terminal} [Parietaria	1311512	15
Parietaria officinalis	mAb 8B6-reactive major allergen {N-terminal} [Parietaria	1311513	30
Parietaria judaica	Profilin-2 (Pollen allergen Par j 3.0102).	14423869	131
Parietaria judaica	Profilin-1 (Pollen allergen Par j 3.0101).	14423876	132
Parietaria judaica	P8 protein [Parietaria judaica].	1532056	133
Parietaria judaica	P9 protein [Parietaria judaica].	1532058	176
Parietaria officinalis	Par o 1a=acidic allergen isoform {N-terminal} [Parietaria	1836010	25
Parietaria officinalis	Par o 1b=basic allergen isoform {N-terminal} [Parietaria	1836011	24
Parietaria judaica	Probable nonspecific lipid-transfer protein 1 precursor (LTP)	2497749	138
Parietaria judaica	Probable nonspecific lipid-transfer protein 2 precursor (LTP 2)	2497750	133
Parietaria judaica	Probable nonspecific lipid-transfer protein (LTP) (Major pollen	3915783	139
Parietaria judaica	major allergen Par j 1.	741844	143
Parietaria officinalis	Pollen major allergen Par o 1.	75139847	12
Phalaris aquatica	Major pollen allergen Pha a 1 precursor (Pha a 1).	2498576	269
Phalaris aquatica	Major pollen allergen Pha a 5.1 precursor (Pha A 5) (Clone 28).	2498577	320
Phalaris aquatica	Major pollen allergen Pha a 5.2 precursor (Pha a 5) (Clone 14).	2498578	305
Phalaris aquatica	Major pollen allergen Pha a 5.3 precursor (Pha a 5) (Clone 29).	2498579	294
Phalaris aquatica	Major pollen allergen Pha a 5.4 (Pha a 5) (Clone 5).	2498580	175
Phalaris aquatica	Pha a I=34 kda pollen allergen {N-terminal} [Phalaris	409328	20
Phleum pratense	major allergen Phl p Va.	1092249	285
Phleum pratense	group V allergen Phl p 5 precursor [Phleum pratense].	13430402	275
Phleum pratense	Polcalcine Phl p 7 (Calcium-binding pollen allergen Phl p 7) (P7).	14423846	78
Phleum pratense	major allergen Phl p 5 [Phleum pratense].	1684718	281
Phleum pratense	major allergen Phl p 5 [Phleum pratense].	1684720	276
Phleum pratense	unnamed protein product [Phleum pratense].	21725606	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725608	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725610	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725612	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725614	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725616	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725618	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725620	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725622	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725624	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725626	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725628	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725630	287
Phleum pratense	unnamed protein product [Phleum pratense].	21725632	287
Phleum pratense	pollen allergen Phl p 11 [Phleum pratense].	23452313	143
Phleum pratense	Major Pollen Allergen Phl p Va [Phleum pratense].	2398757	286
Phleum pratense	profilin 3 [Phleum pratense].	2415700	131
Phleum pratense	profilin 4 [Phleum pratense].	2415702	131
Phleum pratense	Chain A, Crystal Structure Of Phl P 1, A Major Timothy Grass Pollen	28373838	241
Phleum pratense	Chain N, Crystal Structure Of Phl P 6, A Major Timothy Grass Pollen	28374072	111
Phleum pratense	Pollen allergen Phl p 5b precursor (Phl p Vb).	2851457	284
Phleum pratense	Chain A, Crystal Structure Of The Functional Domain Of The Major	28948464	102
Phleum pratense	phl p5a allergen precursor [Phleum pratense].	29500897	284
Phleum pratense	Phl p6 allergen [Phleum pratense].	3004465	138
Phleum pratense	Phl p6 allergen [Phleum pratense].	3004467	138
Phleum pratense	Phl p6 IgE binding fragment [Phleum pratense].	3004469	106
Phleum pratense	major allergen Phl p 5 [Phleum pratense].	3135497	276
Phleum pratense	major allergen Phl p 5 [Phleum pratense].	3135499	276
Phleum pratense	major allergen Phl p 5 [Phleum pratense].	3135501	276
Phleum pratense	major allergen Phl p 5 [Phleum pratense].	3135503	276

Phleum pratense	group V allergen Phl p 5.0103 precursor [Phleum pratense].	3309039	312
Phleum pratense	group V allergen Phl p 5.0203 precursor [Phleum pratense].	3309041	295
Phleum pratense	group V allergen Phl p 5.0206 precursor [Phleum pratense].	3309045	290
Phleum pratense	group V allergen Phl p 5.0207 precursor [Phleum pratense].	3309047	287
Phleum pratense	pollen allergen Phl p1 [Phleum pratense].	3901094	263
Phleum pratense	Phl p 3 allergen [Phleum pratense].	39841264	97
Phleum pratense	Phlp5 [Phleum pratense].	398830	312
Phleum pratense	PHL P11, pollen allergen [Phleum pratense].	415896	122
Phleum pratense	PHLP5A protein - common timothy (fragment).	422005	257
Phleum pratense	unnamed protein product [Phleum pratense].	45108967	500
Phleum pratense	unnamed protein product [Phleum pratense].	45108973	500
Phleum pratense	unnamed protein product [Phleum pratense].	45823012	240
Phleum pratense	Profilin-1 (Pollen allergen Phl p 12) (Phl p 11).	464471	131
Phleum pratense	Phl p 1 allergen [Phleum pratense].	473360	263
Phleum pratense	allergen Phl p Vb - common timothy.	481397	280
Phleum pratense	polygalacturonase [Phleum pratense].	4826572	394
Phleum pratense	pollen allergen Phl p 4 [Phleum pratense].	54144332	508
Phleum pratense	pollen allergen Phl p 4 [Phleum pratense].	54144334	508
Phleum pratense	Pollen allergen Phl p V.	75139900	24
Phleum pratense	Pollen allergen Phl p1 precursor.	75221090	263
Phoenix dactylifera	profilin [Phoenix dactylifera].	21322677	131
Pinus radiata	Pollen allergen.	75099285	293
Platanus x	putative invertase inhibitor precursor [Platanus x acerifolia].	26190140	179
Platanus x	polygalacturonase [Platanus x acerifolia].	49523394	377
Poa pratensis	Pollen allergen KBG 31 precursor (Pollen allergen Poa p 9) (Poa p	113560	373
Poa pratensis	Pollen allergen KBG 41 precursor (Pollen allergen Poa p 9) (Poa p	113561	333
Poa pratensis	pollen allergen Poa p 7 [Poa pratensis].	11991227	303
Poa pratensis	pollen allergen Poa-p1 - Kentucky bluegrass (fragment).	280414	20
Poa pratensis	pollen allergen Poa p 1 - Kentucky bluegrass (fragment).	320620	26
Poa pratensis	pollen allergen (group 1) [Poa pratensis].	4007655	122
Poa pratensis	group 1 pollen allergen [Poa pratensis].	4090265	263
Poa pratensis	pollen allergen (clone 7.2) - Kentucky bluegrass (fragment).	539056	131
Poa pratensis	Pollen allergen KBG 60 precursor (Pollen allergen Poa p 9) (Poa p	113562	307
Quercus alba	major pollen allergen Que a 1 - white oak (fragment).	543675	24
Salsola kali	major antigen-like protein [Salsola kali].	22726221	320
Salsola kali	[Segment 1 of 4] Pollen allergen Sal k 1.	25090948	11
Salsola kali	[Segment 2 of 4] Pollen allergen Sal k 1.	25090949	8
Salsola kali	[Segment 3 of 4] Pollen allergen Sal k 1.	25090950	9
Salsola kali	[Segment 4 of 4] Pollen allergen Sal k 1.	25090951	14
Sambucus nigra	allergen-like protein BRSn20 [Sambucus nigra].	6561156	159
Syringa vulgaris	Polcalcine Syr v 3 (Calcium-binding pollen allergen Syr v 3).	14423847	81
Syringa vulgaris	allergen-like protein Syr v 1 isoform 1 - Syringa vulgaris.	631911	145
Syringa vulgaris	allergen-like protein Syr v 1 isoform 2 - Syringa vulgaris.	631912	145
Syringa vulgaris	allergen-like protein Syr v 1 isoform 3 - Syringa vulgaris.	631913	145
Taraxacum officinale	root allergen protein [Taraxacum officinale].	2707295	157

Food allergens animals

Species	Comments	GI #	AA
Batillus cornutus	major allergen Tur c1 - Turbo cornutus.	7441399	146
Bos taurus	allergen Bos d 2.0103 [imported] - bovine.	11277082	156
Bos taurus	allergen Bos d 2.0102 [imported] - bovine.	11277083	156
Bos taurus	kappa-casein [Bos taurus].	1228078	190
Bos taurus	Myelin basic protein (MBP) (Myelin A1 protein) (20 kDa	126796	169
Bos taurus	alpha-lactalbumin precursor (EC 2.4.1.22).	162644	142
Bos taurus	albumin [Bos taurus].	162648	607
Bos taurus	alpha-s1-casein.	162650	93
Bos taurus	beta-lactoglobulin.	162748	151
Bos taurus	beta-lactoglobulin.	162750	14
Bos taurus	alpha-s1-casein precursor.	162792	214
Bos taurus	alpha-S1-casein.	162794	214
Bos taurus	beta-casein precursor.	162797	224

Bos taurus	beta-casein.	162805	224
Bos taurus	kappa-casein precursor.	162811	190
Bos taurus	alpha-s1-casein.	162927	76
Bos taurus	alpha-s2-like casein precursor.	162929	222
Bos taurus	beta-casein precursor.	162931	224
Bos taurus	S100 calcium-binding protein A7 (Allergen Bos d 3) (Dander minor	2493414	101
Bos taurus	MD-2 protein [Bos taurus].	27806539	160
Bos taurus	Niemann-Pick disease, type C2 [Bos taurus].	27806881	149
Bos taurus	alpha-lactalbumin [Bos taurus].	295774	142
Bos taurus	bovine serum albumin [Bos taurus].	3336842	607
Bos taurus	beta-casein A3 [Bos taurus].	459292	224
Bos taurus	beta-lactoglobulin [Bos taurus].	520	178
Bos taurus	PREDICTED: similar to Odorant-binding protein precursor (OBP) [Bos	61819907	171
Bos taurus	beta-lactoglobulin variant B precursor [Bos taurus].	669061	178
Bos taurus	PREDICTED: similar to Odorant-binding protein precursor (OBP) [Bos	76677407	152
Bos taurus	PREDICTED: similar to Odorant-binding protein precursor (OBP) [Bos	76686423	215
Bos taurus	major allergen BDA20 [Bos taurus].	886215	172
Charybdis feriatus	heat stable allergen tropomyosin [Charybdis feriatus].	7024506	264
Crassostrea gigas	tropomyosin [Crassostrea gigas].	15419048	233
Crassostrea virginica	tropomyosin [Crassostrea virginica].	3668408	160
Cyprinus carpio	parvalbumin [Cyprinus carpio].	17977825	109
Cyprinus carpio	parvalbumin [Cyprinus carpio].	17977827	109
Farfantepenaeus aztecus	muscle tropomyosin, Pen a I=36 kda major allergen	632782	21
Gadus callarias	Parvalbumin beta (Allergen Gad c 1) (Gad c I) (Allergen M).	131112	113
Gadus morhua	parvalbumin beta [Gadus morhua].	14531014	109
Gadus morhua	parvalbumin beta [Gadus morhua].	14531016	109
Gallus gallus	Ovomucoid precursor (Allergen Gal d 1) (Gal d I).	124757	210
Gallus gallus	Lysozyme C precursor (1,4-beta-N-acetylmuramidase C) (Allergen Gal	126608	147
Gallus gallus	Ovalbumin (Plakalbumin) (Allergen Gal d 2) (Gal d II).	129293	386
Gallus gallus	Ovotransferrin precursor (Conalbumin) (Allergen Gal d 3) (Gal d	1351295	705
Gallus gallus	Chain A, Loop-Inserted Structure Of P1-P1' Cleaved Ovalbumin Mutant	15826578	385
Gallus gallus	very low density lipoprotein II precursor.	211156	106
Gallus gallus	lysozyme protein.	212279	24
Gallus gallus	vitellogenin.	212881	1852
Gallus gallus	ovalbumin [Gallus gallus].	212900	388
Gallus gallus	Chain D, Crystal Structure Of S-Ovalbumin At 1.9 Angstrom	34811333	385
Gallus gallus	PREDICTED: similar to MD-2 [Gallus gallus].	50731634	155
Gallus gallus	unnamed protein product [Gallus gallus].	63052	155
Gallus gallus	preproalbumin (serum albumin) [Gallus gallus].	63748	615
Gallus gallus	vitellogenin [Gallus gallus].	63885	81
Gallus gallus	vitellogenin [Gallus gallus].	63887	1850
Gallus gallus	ovotransferrin [Gallus gallus].	757851	705
Gallus gallus	unnamed protein product [Gallus gallus].	808969	386
Haliothis diversicolor	tropomyosin [Haliothis diversicolor].	9954249	284
Helix aspersa	tropomyosin [Helix aspersa].	4468224	284
Homarus americanus	Tropomyosin (Allergen Hom a 1).	14285796	284
Homarus americanus	fast tropomyosin isoform [Homarus americanus].	2660868	284
Kryptolebias marmoratus	parvalbumin 1 [Rivulus marmoratus].	50953781	109
Kryptolebias marmoratus	parvalbumin 2 [Rivulus marmoratus].	50953783	109
Metapenaeus ensis	tropomyosin.	607633	274
Mimachlamys nobilis	tropomyosin [Chlamys nobilis].	9954253	284
Panulirus stimpsoni	Tropomyosin (Allergen Pan s 1) (Pan s I).	14285797	274
Penaeus monodon	allergen Pen m 2 [Penaeus monodon].	27463265	356
Perna viridis	tropomyosin [Perna viridis].	9954251	284
Rana esculenta	parvalbumin alpha [Rana esculenta].	20796729	110
Rana sp.	parvalbumin alpha [Rana sp. CH-2001].	20796733	110
Rana esculenta	parvalbumin beta protein [Rana esculenta].	20797081	109
Rana sp.	parvalbumin beta protein [Rana sp. CH-2001].	20797085	109
Salmo salar	Parvalbumin beta 2 (Major allergen Sal s 1).	18281421	108
Salmo salar	Parvalbumin beta 1 (Major allergen Sal s 1).	2493445	109
Theragra chalcogramma	parvalbumin [Theragra chalcogramma].	14531018	109

Theragra chalcogramma

parvalbumin [Theragra chalcogramma].

14531020 109

Food allergens plants

Species

Actinidia chinensis

Actinidia deliciosa

Actinidia deliciosa

Actinidia deliciosa

Actinidia deliciosa

Actinidia deliciosa

Anacardium occidentale

Anacardium occidentale

Anacardium occidentale

Anacardium occidentale

Ananas comosus

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Apium graveolens

Arachis hypogaea

Arachis hypogaea

Arachis hypogaea

Arachis hypogaea

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Arachis hypogaea

Arachis hypogaea

Arachis hypogaea

Arachis hypogaea

Arachis hypogaea

Arachis hypogaea

Arachis hypogaea

Bertholletia excelsa

Bertholletia excelsa

Bertholletia excelsa

Beta vulgaris

Brassica rapa

Brassica napus

Brassica napus

Brassica napus

Brassica napus

Brassica napus

Brassica napus

Brassica napus

Comments

Actinidain precursor (Actinidin) (Allergen Act c 1).

unnamed protein product [Actinidia deliciosa].

actinidin.

phytoecystatin [Actinidia deliciosa].

pKIWI501.

thaumatin-like protein [Actinidia deliciosa].

vicilin-like protein [Anacardium occidentale].

vicilin-like protein [Anacardium occidentale].

2s albumin [Anacardium occidentale].

allergen Ana o 2 [Anacardium occidentale].

profilin [Ananas comosus].

Major allergen Api g 1 (Api g 1.0101) (Api g 1).

Chlorophyll a-b binding protein, chloroplast precursor (Allergen

Api g 1.0201 allergen [Apium graveolens].

[Segment 2 of 4] Allergen Api g 3.

[Segment 3 of 4] Allergen Api g 5.

[Segment 4 of 4] Allergen Api g 5.

[Segment 1 of 4] Allergen Api g 5.

profilin [Apium graveolens].

cofactor-independent phosphoglyceromutase [Apium graveolens].

NADP-dependent malate dehydrogenase (decarboxylating) [Apium

Allergen Ara h 1, clone P15 precursor (Ara h 1).

Allergen Ara h 1, clone P41B precursor (Ara h 1).

oleosin variant A [Arachis hypogaea].

oleosin variant B [Arachis hypogaea].

unnamed protein product [Arachis hypogaea].

conglutin [Arachis hypogaea].

allergen Ara h 3/Ara h 4 [Arachis hypogaea].

trypsin inhibitor [Arachis hypogaea].

peanut agglutinin precursor; prePNA [Arachis hypogaea].

allergen Ara h 2.02 [Arachis hypogaea].

allergen Ara h 2 isoform [Arachis hypogaea].

glycinin [Arachis hypogaea].

Ara h 6 allergen [Arachis hypogaea].

conarachin [Arachis hypogaea].

conarachin [Arachis hypogaea].

conarachin [Arachis hypogaea].

oleosin isoform [Arachis hypogaea].

glycinin [Arachis hypogaea].

Ara h 6 allergen [Arachis hypogaea].

glycinin [Arachis hypogaea].

profilin [Arachis hypogaea].

allergen Ara h 6 [Arachis hypogaea].

allergen [Arachis hypogaea].

mannose/glucose-binding lectin precursor.

2S sulfur-rich seed storage protein precursor (Allergen Ber e 1)

2S albumin [Bertholletia excelsa].

11S globulin [Bertholletia excelsa].

RS2 protein [Beta vulgaris subsp. vulgaris].

Thioredoxin H-type (TRX-H).

pollen allergen group II (clone 42) - rape.

pollen allergen group II (clone 44) - rape.

pollen allergen group II (clone 4) - turnip (fragment).

recombinant lb pronapin precursor [Brassica napus].

Lipid transfer protein [Brassica rapa].

[Segment 1 of 5] Chitin-binding allergen Bra r 2.

[Segment 2 of 5] Chitin-binding allergen Bra r 2.

GI# AA

113285 380

15984 380

166317 380

40807635 116

450239 184

71057064 225

21666498 536

21914823 538

24473800 138

25991543 457

14161637 131

1346568 154

14423661 264

1769847 159

32363124 30

32363125 24

32363126 10

33300921 22

4761578 134

6706331 559

6706333 570

1168390 614

1168391 626

13161005 176

13161008 176

14347293 207

17225991 144

21314465 538

22135348 219

253289 273

26245447 172

31322017 169

3703107 507

37499626 157

46560472 303

46560474 299

46560476 428

47156059 176

47933675 510

57118278 124

5712199 530

5902968 131

5923742 129

5931948 160

951118 254

112754 146

17713 154

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11691639 158

11135129 123

2129801 83

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Secale cereale	pollen allergen Sec c 4 [Secale cereale].	55859454	518
Secale cereale	pollen allergen Sec c 4 [Secale cereale].	55859456	520
Secale cereale	30K allergen.	75140047	16
Secale cereale	Major BAKER'S ASTHMA allergen SEC C 1.	75198875	26
Sesamum indicum	2S albumin [Sesamum indicum].	13183175	153
Sesamum indicum	7S globulin [Sesamum indicum].	13183177	585
Sesamum indicum	2S albumin precursor [Sesamum indicum].	5381323	148
Sinapis alba	allergen sin a 1.0104 [Sinapis alba].	1009434	145
Sinapis alba	allergen sin a 1.0105 [Sinapis alba].	1009436	145
Sinapis alba	allergen sin a 1.0106 [Sinapis alba].	1009438	145
Sinapis alba	allergen sin a 1.0107 [Sinapis alba].	1009440	145
Sinapis alba	allergen sin a 1.0108 [Sinapis alba].	1009442	145
Sinapis alba	Allergen Sin a 1 precursor (Sin a 1) [Contains: Allergen Sin a 1	51338758	145
Sinapis alba	major allergen Sin a 1 [Sinapis alba].	7545129	145
Solanum tuberosum	Aspartic protease inhibitor 11 (Cathepsin D inhibitor PDI)	124148	188
Solanum tuberosum	Patatin B1 precursor (Potato tuber protein).	129641	377
Solanum tuberosum	patatin.	169500	386
Solanum tuberosum	Cysteine protease inhibitor 1 precursor (PCPI 8.3) (P340) (P34021).	20141344	222
Solanum tuberosum	Serine protease inhibitor 7 precursor (PIG) (PIGEN1) (Allergen Sola	20141714	221
Solanum tuberosum	aspartic proteinase inhibitor [Solanum tuberosum].	21413	217
Solanum tuberosum	unnamed protein product [Solanum tuberosum].	21510	386
Solanum tuberosum	patatin [Solanum tuberosum].	21512	386
Solanum tuberosum	patatin [Solanum tuberosum].	21514	386
Triticum aestivum	hypothetical 11.8K protein - wheat.	100794	106
Triticum turgidum	alpha-amylase inhibitor, tetrameric, chain CM3 precursor - durum	100834	168

Triticum aestivum	profilin [Triticum aestivum].	1008443	141
Triticum aestivum	profilin [Triticum aestivum].	1008445	140
Triticum aestivum	profilin [Triticum aestivum].	1052817	138
Triticum aestivum	gamma-gliadin precursor [Triticum aestivum].	1063270	279
Triticum aestivum	triosephosphat-isomerase [Triticum aestivum].	11124572	253
Triticum aestivum	allergenic peptide=low-molecular-weight glutenin chain [wheat,	1168771	30
Triticum aestivum	Endogenous alpha-amylase/subtilisin inhibitor (WASI).	123975	180
Triticum aestivum	alpha-gliadin [Triticum aestivum].	1204264	259
Triticum aestivum	IgE-binding polypeptide 4[major allergen {N-terminal} (wheat,	1311642	16
Triticum aestivum	agglutinin isolectin A precursor.	170666	212
Triticum turgidum	wheat germ agglutinin.	170668	186
Triticum aestivum	agglutinin isolectin D precursor.	170670	213
Triticum aestivum	gamma gliadin precursor.	170702	302
Triticum aestivum	gamma-gliadin B precursor.	170708	291
Triticum aestivum	alpha-type gliadin precursor protein.	170710	318
Triticum aestivum	pre-alpha/beta-gliadin A-II.	170712	291
Triticum aestivum	pre-alpha/beta-gliadin A-V.	170716	319
Triticum aestivum	alpha/beta-gliadin precursor.	170718	313
Triticum aestivum	alpha/beta-gliadin precursor [Triticum aestivum].	170720	286
Triticum aestivum	pre-alpha/beta-gliadin A-I.	170722	262
Triticum aestivum	pre-alpha/beta-gliadin A-IV.	170724	297
Triticum aestivum	pre-alpha/beta-gliadin A-III.	170726	282
Triticum aestivum	alpha-type gliadin.	170728	186
Triticum aestivum	gamma-gliadin B-I precursor [Triticum aestivum].	170730	304
Triticum aestivum	gamma-gliadin.	170732	323
Triticum aestivum	gamma gliadin B-III.	170734	244
Triticum aestivum	gamma-gliadin.	170736	251
Triticum aestivum	gamma-gliadin.	170738	327
Triticum urartu	gliadin.	170740	296
Triticum aestivum	HMW glutenin subunit 1x2* [Triticum aestivum].	170743	815
Triticum aestivum	serpin [Triticum aestivum].	1885350	399
Triticum aestivum	unnamed protein product [Triticum aestivum].	21673	307
Triticum aestivum	unnamed protein product [Triticum aestivum].	21701	145
Triticum aestivum	CM 17 protein precursor [Triticum aestivum].	21711	143
Triticum aestivum	unnamed protein product [Triticum aestivum].	21713	168
Triticum aestivum	high molecular weight glutenin subunit 1Ax1 [Triticum aestivum].	21743	830
Triticum aestivum	high molecular weight glutenin subunit 10 [Triticum aestivum].	21751	648
Triticum aestivum	unnamed protein product [Triticum aestivum].	21755	286
Triticum aestivum	unnamed protein product [Triticum aestivum].	21757	296
Triticum aestivum	unnamed protein product [Triticum aestivum].	21761	286
Triticum aestivum	unnamed protein product [Triticum aestivum].	21765	313
Triticum aestivum	seed storage protein (154AA) [Triticum aestivum].	21769	154
Triticum aestivum	unnamed protein product [Triticum aestivum].	21773	307
Triticum aestivum	unnamed protein product [Triticum aestivum].	21779	660
Triticum aestivum	unnamed protein product [Triticum aestivum].	21783	356
Triticum aestivum	unnamed protein product [Triticum aestivum].	21793	39
Triticum turgidum	precursor (AA -24 to 119) [Triticum turgidum subsp. durum].	21916	143
Triticum turgidum	CM2 protein [Triticum turgidum subsp. durum].	21920	145
Triticum turgidum	unnamed protein product [Triticum turgidum subsp. durum].	21926	295
Triticum turgidum	LMW glutenin [Triticum turgidum subsp. durum].	21930	285
Triticum aestivum	HMW glutenin subunit 1By9 [Triticum aestivum].	22090	705
Triticum turgidum	tetrameric alpha-amylase inhibitor 16 kDa subunit, CM16* [Triticum	244610	18
Triticum aestivum	27K protein [Triticum aestivum].	30793446	203
Triticum aestivum	Allergen C-C.	3913017	27
Triticum aestivum	pollen allergen (group II) [Triticum aestivum].	4007852	122
Triticum aestivum	putative pollen allergen precursor [Triticum aestivum].	40644792	178
Triticum aestivum	putative group V allergen [Triticum aestivum].	40644794	119
Triticum aestivum	putative major allergen Phl p 5 [Triticum aestivum].	40644796	231
Triticum aestivum	pollen allergen homolog [Triticum aestivum].	4099919	271
Triticum aestivum	alpha-gliadin.	473876	287
Triticum aestivum	pollen allergen Tri a 4 [Triticum aestivum].	55859458	518

Triticum aestivum	pollen allergen Tri a 4 [Triticum aestivum].	55859460	518
Triticum aestivum	putative gamma-gliadin [Triticum aestivum].	62484809	285
Triticum aestivum	putative LMW-glutenin subunit [Triticum aestivum].	62550933	326
Triticum aestivum	putative omega-gliadin [Triticum aestivum].	63252971	375
Triticum aestivum	glutenin [Triticum aestivum].	736319	838
Triticum aestivum	Gliadin omega-5.	75139902	32
Triticum turgidum	16K protein.	75140060	10
Triticum aestivum	LMM glutenin 1.	75219081	285
Triticum aestivum	LMM glutenin 3.	75317968	373
Triticum urartu	unknown protein.	806315	106
Triticum aestivum	low molecular weight glutenin [Triticum aestivum].	886963	229
Triticum aestivum	low molecular weight glutenin [Triticum aestivum].	886965	261
Triticum aestivum	low molecular weight glutenin [Triticum aestivum].	886967	276
Triticum aestivum	unnamed protein product [Triticum aestivum].	897811	101
Triticum aestivum	pollen allergen-like protein [Triticum aestivum].	972513	118
Vitis sp.	Nonspecific lipid-transfer protein P2 (LTP P2).	462717	38
Vitis sp.	Nonspecific lipid-transfer protein P3 (LTP P3).	462718	37
Vitis sp.	Nonspecific lipid-transfer protein P4 (LTP P4).	462719	37
Zea mays	Nonspecific lipid-transfer protein precursor (LTP) (Phospholipid	128388	120
Zea mays	Zm13.	1588669	170
Zea mays	beta-expansin 1a precursor (Pollen allergen Zea m 1) (Zea m 1).	20138191	269
Zea mays	profilin [Zea mays].	2642324	131
Zea mays	beta-expansin 9 protein [Zea mays].	28630919	269
Zea mays	beta-expansin 1 protein [Zea mays].	28630923	269
Zea mays	group 3 pollen allergen [Zea mays].	33188338	120
Zea mays	pectate lyase homolog [Zea mays].	405535	438
Zea mays	Bet v 1 allergen [Zea mays].	54111527	154
Zea mays	Pectate lyase homolog.	75221515	104

Other contact allergens

Species	Comments	GI #	AA
Acanthamoeba castellanii	Acanthamoeba Castellanii Profilin Ia.	2781014	125
Acanthamoeba castellanii	Chain B, Crystal Structure Of Acanthamoeba Castellanii Profilin Ii,	9257090	125
Equus caballus	latherin [Equus caballus].	20143977	228
Equus caballus	Dander allergen Equ c 2.0101.	3121755	29
Equus caballus	Dander allergen Equ c 2.0102.	3121756	19
Equus caballus	Major allergen Equ c 1 precursor.	3121758	187
Equus caballus	preproalbumin [Equus caballus].	399672	607
Hevea brasiliensis	IgE-binding protein MnSOD [Hevea brasiliensis].	10862818	205
Hevea brasiliensis	Chain A, Latex Profilin Hevb8.	11513601	131
Hevea brasiliensis	beta-1,3-glucanase.	1184668	374
Hevea brasiliensis	Pro-hevein precursor (Major hevein) [Contains: Hevein (Allergen Hev	123062	204
Hevea brasiliensis	Rubber elongation factor protein (REF) (Allergen Hev b 1).	132270	138
Hevea brasiliensis	Profilin-6 (Pollen allergen Hev b 8.0204).	14423856	131
Hevea brasiliensis	Profilin-5 (Pollen allergen Hev b 8.0203).	14423858	131
Hevea brasiliensis	Profilin-4 (Pollen allergen Hev b 8.0202).	14423859	131
Hevea brasiliensis	Profilin-3 (Pollen allergen Hev b 8.0201).	14423860	131
Hevea brasiliensis	Profilin-2 (Pollen allergen Hev b 8.0102).	14423868	131
Hevea brasiliensis	Small rubber particle protein (SRPP) (22 kDa rubber particle	14423933	204
Hevea brasiliensis	putative class I chitinase [Hevea brasiliensis].	14575525	295
Hevea brasiliensis	latex allergen.	1480457	151
Hevea brasiliensis	latex patatin homolog [Hevea brasiliensis].	1916805	388
Hevea brasiliensis	lipid transfer precursor protein [Hevea brasiliensis].	20135538	116
Hevea brasiliensis	class I chitinase [Hevea brasiliensis subsp. brasiliensis].	27526732	295
Hevea brasiliensis	prohevein [Hevea brasiliensis].	2832430	187
Hevea brasiliensis	latex allergen [Hevea brasiliensis].	3087805	388
Hevea brasiliensis	ENSP-like protein [Hevea brasiliensis].	30909057	391
Hevea brasiliensis	profilin [Hevea brasiliensis].	3183706	131
Hevea brasiliensis	beta-1,3-glucanase [Hevea brasiliensis].	32765543	374
Hevea brasiliensis	latex allergen [Hevea brasiliensis].	3288200	388
Hevea brasiliensis	superoxide dismutase (manganese).	348137	233

Hevea brasiliensis	putative latex allergen hev b 7.02 [Hevea brasiliensis].	44581137	387
Hevea brasiliensis	major latex allergen Hev b 4 [Hevea brasiliensis].	46410859	366
Hevea brasiliensis	MnSOD [Hevea brasiliensis].	5777414	205
Hevea brasiliensis	latex protein allergen Hev b 7 [Hevea brasiliensis].	6707078	388
Hevea brasiliensis	enolase, isoform 1 [Hevea brasiliensis].	9581744	445
Hevea brasiliensis	enolase, isoform 2 [Hevea brasiliensis].	9581746	445
Methanococcus maripaludis	Alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal	45047603	217
Nicotiana tabacum	beta-expansin-like protein [Nicotiana tabacum].	12330698	273
Nicotiana tabacum	villin 1 [Nicotiana tabacum].	57283137	559
Nicotiana tabacum	villin 2 [Nicotiana tabacum].	57283139	520
Nicotiana tabacum	Polcalcin Nic t 1 (Calcium-binding pollen allergen Nic t 1).	59798467	84
Nicotiana tabacum	Polcalcin Nic t 2 (Calcium-binding pollen allergen Nic t 2).	59798468	86
Plasmodium falciparum	conserved protein [Plasmodium falciparum 3D7].	23505219	171
Styela plicata	major allergen [Styela plicata].	58257626	126

Nematodes and worms

Species	Comments	GI #	AA
Acanthocheilonema viteae	ladder protein [Acanthocheilonema viteae].	4102959	131
Ancylostoma caninum	secreted protein ASP-2 precursor [Ancylostoma caninum].	3608493	218
Ancylostoma duodenale	ancylostoma-secreted protein 1 precursor [Ancylostoma duodenale].	3719257	425
Ancylostoma caninum	ancylostoma-secreted protein 1 precursor ASP-1 [Ancylostoma	4884851	424
Ancylostoma caninum	Aspartic protease.	74936004	442
Anisakis simplex	Tropomyosin (Allergen Ani s 3).	14423976	284
Anisakis simplex	21k allergen [Anisakis simplex].	31339067	194
Anisakis simplex	Allergen Ani s 4.	47605398	14
Anisakis simplex	Major allergen Ani s 1 precursor (Excretory gland allergen Ani s 1)	47605452	194
Anisakis simplex	troponin-like protein [Anisakis simplex].	6065738	161
Anisakis simplex	putative nucleosome binding protein [Anisakis simplex].	6065744	321
Anisakis simplex	paramyosin [Anisakis simplex].	8117843	869
Anisakis simplex	paramyosin isoform [Anisakis simplex].	8453086	473
Ascaridia galli	fatty acid binding protein [Ascaridia galli].	3152922	135
Ascaris suum	allergen [Ascaris suum].	159653	395
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735096	134
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735098	134
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735100	134
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735102	133
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735106	133
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735108	267
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735110	267
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735112	267
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735114	134
Ascaris lumbricoides	ABA-1 allergen [Ascaris lumbricoides].	2735118	134
Ascaris suum	polyprotein allergen/antigen [Ascaris suum].	2970629	1095
Ascaris suum	major allergen ABA-1=TBA-1 allergen homolog {N-terminal} [Ascaris	299550	68
Brugia malayi	venom allergen antigen-like protein 1 [Brugia malayi].	13095442	220
Brugia malayi	major allergen [Brugia malayi].	13310414	227
Brugia malayi	larval allergen [Brugia malayi].	24711753	248
Dictyocaulus viviparus	DVA-1 polyprotein precursor (Antigen 1) (Allergen 1) (Nematode	2498317	1557
Dirofilaria immitis	venom allergen antigen 5-like protein [Dirofilaria immitis].	2245508	221
Heterodera glycines	secreted venom allergen-like protein vap2 [Heterodera glycines].	13447461	212
Heterodera glycines	venom allergen-like protein [Heterodera glycines].	14211968	212
Heterodera glycines	vap-1 [Heterodera glycines].	14326230	215
Heterodera glycines	probable polyprotein allergen Hgg-14 [Heterodera glycines].	18677166	288
Litomosoides carinii	ladder protein [Litomosoides carinii].	4102911	132
Loa loa	ladder protein [Loa loa].	4102951	132
Loa loa	LL20 15kDa ladder antigen.	414553	588
Meloidogyne incognita	secreted protein MSP-1 [Meloidogyne incognita].	4102596	231
Necator americanus	ancylostoma secreted protein 1 precursor [Necator americanus].	3396070	424
Necator americanus	calreticulin [Necator americanus].	3687326	403
Onchocerca volvulus	activation-associated secreted protein-2 [Onchocerca volvulus].	11762066	224
Onchocerca volvulus	myosin-like antigen.	159881	343

Onchocerca volvulus	vespid allergen antigen homolog [Onchocerca volvulus].	2796175	220
Onchocerca cervicalis	ladder protein [Onchocerca cervicalis].	4102953	133
Onchocerca volvulus	beta-galactoside-binding lectin.	433317	280
Onchocerca volvulus	activation-associated secreted protein-1 [Onchocerca volvulus].	5868902	220
Schistosoma japonicum	22.6 kDa tegumental antigen [Schistosoma japonicum].	2739154	191
Schistosoma japonicum	hypothetical protein, putative Profilin/allergen [Schistosoma	29841461	129
Setaria cervi	ladder protein [Setaria cervi].	4102957	133
Strongyloides stercoralis	IgG and IgE immunoreactive antigen recognized by sera from patients	2290388	152
Strongyloides stercoralis	IgG and IgE immunoreactive antigen recognized by sera from patients	2290390	90
Strongyloides stercoralis	IgG and IgE immunoreactive antigen recognized by sera from patients	2290392	128
Strongyloides stercoralis	IgG and IgE immunoreactive antigen recognized by sera from patients	2290394	144
Strongyloides stercoralis	IgG immunoreactive antigen [Strongyloides stercoralis].	2801529	156
Strongyloides stercoralis	allergen polyprotein homolog [Strongyloides stercoralis].	2801531	95
Stylonychia lemnae	unnamed protein product [Stylonychia lemnae].	10142	104
Toxocara canis	TBA-1 precursor [Toxocara canis].	1731859	140
Toxocara canis	ladder protein [Toxocara canis].	4102955	133
Toxocara canis	Allergen TBA-1.	74827032	36
Trichostrongylus colubriformis	aspartyl protease inhibitor precursor [Trichostrongylus	28274792	228
Wuchereria bancrofti	translationally controlled tumor protein-like protein [Wuchereria	14700054	181
Wuchereria bancrofti	vespid allergen antigen homolog [Wuchereria bancrofti].	4324680	220
Wuchereria bancrofti	vespid allergen antigen homolog [Wuchereria bancrofti].	4704758	220
Wuchereria bancrofti	cuticular endochitinase [Wuchereria bancrofti].	7673688504	

Cross Reference Number 5

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 55-60 of 182
Page Title	Appendix 2. Bioinformatic analysis of putative predicted polypeptide 5_1.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 55-60 of 182 are found in the **Confidential Attachment**, Pages 9-14 of 136.

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Cross Reference Number 6

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 61-72 of 182
Page Title	Appendix 3. Bioinformatic analysis of putative predicted polypeptide 5_3.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 61-72 of 182 are found in the **Confidential Attachment**, Pages 15-26 of 136.

Cross Reference Number 7

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 73-85 of 182
Page Title	Appendix 4. Bioinformatic analysis of putative predicted polypeptide 4.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 73-85 of 182 are found in the **Confidential Attachment**, Pages 27-39 of 136.

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This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 86-97 of 182
Page Title	Appendix 5. Bioinformatic analysis of putative predicted polypeptide 5_5.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 86-97 of 182 are found in the **Confidential Attachment**, Pages 40-51 of 136.

Cross Reference Number 9

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 98-103 of 182
Page Title	Appendix 6. Bioinformatic analysis of putative predicted polypeptides_6
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 98-103 of 182 are found in the **Confidential Attachment**, Pages 52-57 of 136.

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Cross Reference Number 10

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 104-122 of 182
Page Title	Appendix 7. Bioinformatic analysis of putative predicted polypeptide 3_1.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 104-122 of 182 are found in the **Confidential Attachment**, Pages 58-76 of 136.

Cross Reference Number 11

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 123-141 of 182
Page Title	Appendix 8. Bioinformatic analysis of putative predicted polypeptide 2.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 123-141 of 182 are found in the **Confidential Attachment**, Pages 77-95 of 136.

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Cross Reference Number 12

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 142-150 of 182
Page Title	Appendix 9. Bioinformatic analysis of putative predicted polypeptide 3_3.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 142-150 of 182 are found in the **Confidential Attachment**, Pages 96-104 of 136.

Cross Reference Number 13

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 151-173 of 182
Page Title	Appendix 10. Bioinformatic analysis of putative predicted polypeptide 5_5
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 151-173 of 182 are found in the **Confidential Attachment**, Pages 105-127 of 136.

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Cross Reference Number 14

This cross reference number is used in the report in place of the following page(s) that are found in of the **Confidential Attachment**.

Page	MSL-20306 Pages 174-182 of 182
Page Title	Appendix 11. Bioinformatic analysis of putative predicted polypeptide 3_6.
Reason for Deletion	Discloses commercial information.
FIFRA Reference	10(d)(1)(A)

MSL-20306 Pages 174-182 of 182 are found in the **Confidential Attachment**, Pages 128-136 of 136.

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Product Characterization Center
Analytical Protein Standards Program

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Characterization Plan Title

Characterization of the Cry1A.105 Protein Purified from the Corn Grain of MON 89034 and Comparison of the Physicochemical and Functional Properties of the Plant-Produced and *E. coli*-Produced Cry1A.105 Proteins

Authors

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Statement of Compliance

This protein characterization was conducted in compliance with the Good Laboratory Practice Standards of 40 CFR Part 160. While not a study as defined by 40 CFR Part 160, it was intended to evaluate the functional and physicochemical properties of the plant-produced Cry1A.105 protein from grain of MON 89034 to fulfill the requirements of 40 CFR Part 160.105(a). The experimental work was conducted in adherence with the characterization plan and facility Standard Operating Procedures.

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Quality Assurance Unit Statement

Characterization Plan Title: Characterization of the Cry1A.105 Protein Purified from the Corn Grain of MON 89034 and Comparison of the Physicochemical and Functional Properties of the Plant-Produced and *E. coli*-Produced Cry1A.105 Proteins

Characterization Plan Number: 60-100076

Reviews conducted by the Quality Assurance Unit confirm that the final report accurately describes the methods and standard operating procedures followed, and accurately reflects the raw data of the characterization plan.

Following is a list of reviews conducted by the Monsanto Regulatory Quality Assurance Unit on the characterization plan reported herein.

Date of Inspection or Audit	Phase	Date Reported to APS Officer	Date Reported to Management
07/26/2005	Western Blot	07/29/2005	07/29/2005
07/26/2005	Glycosylation	07/29/2005	07/29/2005
08/08/2005	Purity and Molecular Weight Determination	08/16/2005	08/16/2005
09/30/2005	Raw Data Audit	10/14/2005	10/14/2005
10/24/2005	Draft Report Audit	11/01/2005	11/01/2005

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November 2, 2005
Date

Characterization Plan Information

Characterization Plan

Number: 60-100076

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Title: Characterization of the Cry1A.105 Protein Purified from the Corn Grain of MON 89034 and Comparison of the Physicochemical and Functional Properties of the Plant-Produced and *E. coli*-Produced Cry1A.105 Proteins

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Records Retention: All characterization plan specific raw data, electronically stored Atlas files, the characterization plan, amendment, and final report will be retained at Monsanto Company, St. Louis.

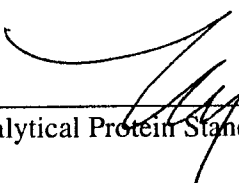
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Characterization Plan Certification

The results reported in this report accurately reflect the data generated under Characterization Plan Number 60-100076.

Signatures of Final Report Approval:



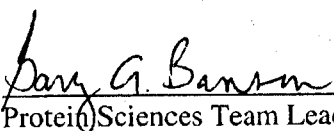
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11/02/2005
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Abbreviations and Definitions¹

AA	Amino acid
AEX	Anion Exchange Chromatography
APS	Analytical Protein Standard
<i>B.t.</i>	<i>Bacillus thuringiensis</i>
CEW	Corn Earworm
COA	Certificate of Analysis
EC ₅₀	Effective protein concentration to inhibit the growth of the target insect by 50%
ECL	Enhanced chemiluminescence
<i>E. coli</i>	<i>Escherichia coli</i>
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GLP	Good Laboratory Practice
HRP	Horseradish peroxidase
LB	Laemmli buffer [62.5mM Tris-HCl, 5% (v/v) 2-mercaptoethanol, 2% (w/v) sodium dodecyl sulfate, 0.005% (w/v) bromophenol blue, 10% (v/v) glycerol, pH 6.8.
5× LB	Five times concentrated LB
MALDI-TOF MS	Matrix assisted laser desorption ionization - time of flight mass spectrometry
MH+	Protonated mass ion
MSL	Monsanto Technical Report
NIST	National Institute of Standards and Technology
NFDM	Non-fat dried milk
N-terminal peptide antibody	Antibody raised against a synthetic peptide consisting of the first 14 amino acids of the N-terminus of Cry1A.105
PBST	Phosphate buffered saline - Tween® 20
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpyrrolidone
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOP	Standard operating procedure
TC	Trypsin-resistant core
US	United States

¹ Standard abbreviations, e.g. units of measure, concentration, mass, time etc., are used without definition according to the format described in "Instructions to Authors" in The Journal of Biological Chemistry.

1.0 Summary

Monsanto has developed corn, MON 89034, which produces the Cry1A.105 and Cry2Ab2 insecticidal proteins and is protected from feeding damage caused by European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. Cry1A.105 is a modified *Bacillus thuringiensis* (*B.t.*) Cry1A protein with 93.6 % overall amino acid sequence identity to the Cry1Ac protein. Cry2Ab2 is a *B.t.* (subsp. *kurstaki*) protein. The combination of the Cry1A.105 and Cry2Ab2 insecticidal proteins in a single plant allows better insect control and offers an additional insect-resistance management tool.

This report describes the physicochemical and functional properties of the Cry1A.105 protein isolated from MON 89034 and demonstrates the equivalence of this plant-produced Cry1A.105 protein to the previously characterized *E. coli*-produced Cry1A.105 protein.

A panel of analytical techniques was used to characterize the plant-produced Cry1A.105 protein. These analytical techniques included sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, densitometry, matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry, glycosylation analysis, and Cry1A.105 activity assay. The short-term stability of the plant-produced Cry1A.105 protein was also assessed using SDS-PAGE by estimating the purity and molecular weight of the protein present after storage at -4°C.

Full length Cry1A.105 protein (~130 kDa) and its fragments were identified using western blot analysis with previously characterized anti-Cry1A.105 antibody. MALDI-TOF mass spectrometry analysis of the ~130 kDa band, after trypsin digestion, yielded peptide masses consistent with the peptide masses expected for the Cry1A.105 protein. Together, the identified peptide masses yielded 43.8% overall coverage of the expected peptide sequence (516 out of 1177 amino acids) of the plant-produced Cry1A.105 protein. The immunoreactivity with the N-terminal peptide antibodies inferred the intactness of the N-terminus in the plant-produced full-length Cry1A.105 protein. The concentration of the full-length plant-produced Cry1A.105 protein determined by quantitative western blot analysis was 3.0 ng/μl. Purity and apparent molecular weight of the plant-produced Cry1A.105 protein was determined using densitometric analysis of a silver stained SDS-PAGE gel. Purity, calculated as the sum of the percent optical density of the stained bands (corresponding to immunoreactive bands identified using anti-Cry1A.105 antibodies) was 100%. The apparent molecular weight of the full-length plant-produced Cry1A.105 protein was estimated to be 130.0 kDa. The plant-produced Cry1A.105 protein was not glycosylated. The functional activity of the plant-produced Cry1A.105 protein was determined using an insect bioassay with the larvae of a sensitive insect, corn earworm (CEW). The Cry1A.105 protein was shown to be active with a

mean EC₅₀ value of 0.0074 µg/ml of diet. The plant-produced Cry1A.105 protein was stable for at least 18 days at a storage temperature of ~4°C.

The equivalence of the plant- and *E. coli*-produced Cry1A.105 proteins was evaluated by comparing their full-length molecular weights, immunoreactivity with anti-Cry1A.105 antibodies, glycosylation status, and functional activity. Based on preset acceptance criteria and the results obtained, the plant-produced Cry1A.105 protein is equivalent to the *E. coli*-produced Cry1A.105 protein.

These data provide a detailed characterization of the Cry1A.105 protein isolated from MON 89034 and establish the equivalence of the plant-produced Cry1A.105 protein to the *E. coli*-produced Cry1A.105 protein.

2.0 Introduction

Monsanto has developed corn, MON 89034, which produces the Cry1A.105 and Cry2Ab2 insecticidal proteins and is protected from feeding damage caused by European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. Cry1A.105 is a modified *Bacillus thuringiensis* (*B.t.*) Cry1A protein with 93.6 % overall amino acid sequence identity to the Cry1Ac protein. Cry2Ab2 is a *B.t.* (subsp. *kurstaki*) protein. The combination of the Cry1A.105 and Cry2Ab2 insecticidal proteins in a single plant allows better insect control and offers an additional insect-resistance management tool. The nucleotide sequence of the *cry1A.105* gene in MON 89034 was confirmed by molecular characterization (Rice et al., Draft).

Because the expression level of Cry1A.105 protein in the grain of MON 89034 is at a low level, it was necessary to express the protein in bacteria in order to produce sufficient quantities for safety testing. The *E. coli*-produced Cry1A.105 reference standard was engineered to match the Cry1A.105 protein produced in MON 89034. Consequently, it was necessary to demonstrate equivalence between the plant-produced and *E. coli*-produced proteins.

3.0 Purpose

The purpose of this plan was to characterize the physicochemical and functional properties of the Cry1A.105 protein produced in corn grain of MON 89034, and to compare the physicochemical and functional properties of the plant-produced protein with the previously characterized *E. coli*-produced Cry1A.105 protein (APS lot 20-100073).

4.0 Materials

4.1 Plant-Produced Cry1A.105 Protein

The plant-produced Cry1A.105 protein was purified from grain of MON 89034. The grain used for the isolation of Cry1A.105 protein, LIMS number 04ZMGRO00393, was produced under production plan 04-01-39-22 from seed lot number GLP-0404-14916-S. The identity of the grain containing MON 89034 was confirmed by event-specific PCR. A copy of the verification of identity (VOI) is archived under APS lot 60-100076. The purified plant-produced Cry1A.105 protein was stored in a 4 °C refrigerator in a buffer solution containing 50 mM CAPS, 1 mM PMSF, 2 mM benzamidine-HCl, 1 mM EDTA, 0.8 M NaCl, and 30% (v/v) ethylene glycol, pH 10.0. The data for purification of the Cry1A.105 protein from MON 89034, conducted prior to the initiation of this plan, are archived under APS lot 60-100076.

4.2 *E. coli*-produced Cry1A.105 Reference Standard

E. coli-produced Cry1A.105 protein (lot 20-100073) was used as a reference standard for determination of protein concentration and immunoblot analysis using N-terminal peptide antibody. This protein was also used as a reference standard to evaluate equivalence between plant- and *E. coli*-produced Cry1A.105 proteins for the molecular weight and functional activity assay, as a reference and a negative control in glycosylation analysis, and as a reference and a positive control in immunoblot analysis using anti-Cry1A.105 antibody. The *E. coli*-produced Cry1A.105 reference standard was previously characterized (Characterization Plan 20-100073) and a copy of the certificate of analysis (COA) is included as Appendix 2 of this report. The Cry1A.105 protein was stored in a -80 °C freezer in a buffer solution (25 mM CAPS, 1 mM benzamidine-HCl, 0.1 mM EDTA, and 0.2 mM DTT, pH ~10.3) at a total protein concentration of 1.2 mg/ml by amino acid analysis.

4.3 Assay Controls

Protein molecular weight standards (Bio-Rad, Hercules, CA) were used to calibrate SDS-PAGE gels and verify protein transfer to PVDF membranes. The *E. coli*-produced Cry1A.105 reference standard (lot 20-100073) was used to create a standard curve for the quantitative western blot. The Cry1A.105 trypsin-resistant core (lot 7518937) was used as a negative control for the western blot analysis using N-terminal peptide antibody. A peptide mixture (Sequazyme Peptide Mass Standards kit, Applied Biosystems) was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. Transferrin (Amersham Biosciences, Piscataway, NJ) and horseradish peroxidase (Sigma, St.

Louis, MO) were used as positive controls for glycosylation analysis. CandyCane™ glycoprotein molecular weight standards were used as molecular weight markers and positive and negative controls for glycosylation analysis.

5.0 Methods

5.1 Protein Purification

The plant-produced Cry1A.105 protein was purified from the corn grain of MON 89034 prior to initiation of this characterization plan. The purification procedure was not performed under a GLP study or plan; however, all procedures were documented on worksheets and, where applicable, SOPs were followed. The Cry1A.105 protein was purified at 4°C from an extract of ground grain using a combination of ammonium sulfate fractionation, anion exchange chromatography, and immunoaffinity chromatography.

The ground grain (10 kg) was mixed in PBS extraction buffer [$1 \times$ PBS: 1 mM KH_2PO_4 , 10 mM Na_2HPO_4 , 137 mM NaCl, and 2.7 mM KCl, pH 7.4] for 2.5 hours at approximately a 1:10 sample weight to buffer volume ratio. The slurry was filtered using an Ertel Alsop filter press (Kingston, NY) and the PBS washed filter cakes were retained. The filter cakes were resuspended in ~100 L of CAPS extraction buffer [50 mM CAPS, 1 mM EDTA, 10 mM DTT, 1 mM PMSF, 2 mM benzamidinium-HCl, and 1% PVPP (w/v), pH 10.8] and stirred for 2 hours. To remove lipids from the extract, CelPure P65 Diatomaceous Earth (Advanced Minerals Corp, Goleta, CA) was added to the slurry at 7.5% (w/v) and allowed to mix for ~10 minutes. The extract was clarified by filtration using the Ertel Alsop filter press and concentrated using a Hollow Fiber Cartridge (Amersham Biosciences, Piscataway, NJ). The contaminant DNA was removed by a combination of benzonase treatment and precipitation with polyethyleneimine. Benzonase was added to a final concentration of ~7 U/ml in the presence of 5 mM MgCl_2 and allowed to mix overnight. A polyethyleneimine solution [10% (w/v)] was added to the concentrated extract to a final concentration of 0.05% (w/v) and the extract was clarified by centrifugation to remove the remaining contaminant DNA. An ammonium sulfate pellet (0% – 35% saturation) was prepared by the addition of ammonium sulfate salt to the clarified extract and was allowed to dissolve overnight. After centrifugation, the ammonium sulfate pellet was dissolved over the weekend in ~15 L of resuspension buffer [50 mM CAPS, 1 mM EDTA, 10 mM DTT, 1 mM PMSF, and 2 mM benzamidinium-HCl, pH 10.8]. The resuspended sample was clarified by centrifugation, diafiltrated against fresh buffer (same as the resuspension buffer) to remove any residual ammonium sulfate and concentrated to final volume of ~8 L and CHAPS was added to a final concentration of 0.5 mM.

The concentrated sample was loaded onto a 2.1 L (6.7 cm x 20 cm) Q Sepharose Fast Flow anion exchange resin column, which was equilibrated with AEX buffer A [50 mM CAPS, 1 mM EDTA, 10 mM DTT, 1 mM PMSF, 2 mM benzamidinium-HCl, and 0.5 mM CHAPS, pH 10.8]. The bound Cry1A.105 protein was eluted with a linear salt gradient that increased from 0 M to 0.20 M sodium chloride over ~12 L and then maintained a 0.20 M sodium chloride concentration for ~4 L. Next, the salt gradient increased to 0.65 M sodium chloride over ~21 L, and finally stepped up to 1.0 M sodium chloride instantly. Fractions containing the Cry1A.105 protein (based on stained SDS-PAGE gel and western blot analysis of all fractions) were pooled to a final volume of ~10 L. Salt was removed from these pooled fractions by diafiltration with fresh buffer [50 mM EPPS, 1 mM EDTA, 10 mM DTT, 1 mM PMSF, and 2 mM benzamidinium-HCl, pH 7.5].

The buffer exchanged sample was loaded onto a 2.1 L (13.7 cm x 14.0 cm) Q Sepharose XL anion exchange resin column, which was equilibrated with AEX buffer A [50 mM EPPS, 1 mM EDTA, 10 mM DTT, 1 mM PMSF, 2 mM benzamidinium-HCl, and 0.5 mM CHAPS, pH 7.5]. The bound Cry1A.105 protein was eluted with a linear salt gradient that increased from 0 M to 0.25 M sodium chloride over ~4 L and then held at 0.25 M sodium chloride for ~10 L. Next, the salt gradient increased to 0.65 M sodium chloride over ~21 L and finally increased to 1.0 M sodium chloride over ~4 L and held constant for ~8 L. The fractions containing Cry1A.105 protein (based on stained SDS-PAGE gel and western blot analysis of all fractions) were pooled to a final volume of ~10 L. These fractions were diafiltrated into fresh buffer [50 mM EPPS, 1 mM EDTA, 10 mM DTT, 1 mM PMSF, and 2 mM benzamidinium-HCl, pH 7.5] to remove salt and concentrated to ~2.0 L using a hollow fiber cartridge.

Prior to the affinity purification step, the sample was diafiltrated into fresh buffer [50 mM EPPS, 1 mM EDTA, 1 mM PMSF, 2 mM benzamidinium-HCl, and 150 mM NaCl, pH 7.6] and concentrated down to ~1 L. The solution containing Cry1A.105 protein was re-circulated over the 9.3 ml (1.75 cm x 2.6 cm) protein A agarose column (Sigma) conjugated with monoclonal anti-Cry1Ac antibody (Strategic Biosolutions, Newark DE). Bound Cry1A.105 protein was eluted using 50 mM CAPS, 1 mM EDTA, 1 mM PMSF, 2 mM benzamidinium-HCl, 0.8 M NaCl, and 30% (v/v) ethylene glycol, pH 10.0. After analysis of fractions by lateral flow strips, stained SDS-PAGE gel and western blot, fraction 25 was determined to contain the majority of the full-length Cry1A.105. This fraction was submitted to the APS program as two aliquots that were assigned APS lot number 60-100076.

5.2 Protein Concentration

The concentration of the full-length (~130kDa) plant-produced Cry1A.105 protein was estimated using quantitative immunoblot analysis. The *E. coli*-produced Cry1A.105 protein (amounts ranging from 1 to 6 ng purity corrected for the full-length protein, which was 82%) was used to create a standard curve. Aliquots of the plant-produced Cry1A.105 protein and reference standard were diluted in deionized water and 5x Laemmli buffer (5x LB), heated at 95.5 °C for 5 min, and applied to a pre-cast tris-glycine 4→20% polyacrylamide gradient 12-well gel. Three different amounts of the plant-produced protein were loaded in duplicate. Electrophoresis was performed at a constant voltage of 125 V for 15 min followed by a constant voltage of 170 V for 75 min. Pre-stained molecular weight markers (Bio-Rad Precision Plus Dual Color, Hercules, CA) were used to verify electrotransfer of protein to the membrane and estimate the size of the immunoreactive bands observed. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 25 V.

The membrane was blocked for 1 hour with 5% (w/v) NFDM in 1× PBST. The membrane was probed with a 1:1000 dilution of rabbit anti-Cry1A.105 antibody (lot 070705JL) in 1% (w/v) NFDM in PBST for 60 min. Excess antibody was removed using four 5 min washes with PBST. Finally, the membrane was probed with horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma, St. Louis, MO) at a dilution of 1:7500 in 1% (w/v) NFDM in PBST for 60 minutes. Excess HRP-conjugate was removed using three 5 min washes with PBST. All incubations were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (Amersham Biosciences, Piscataway, NJ) and exposed (5 min, 10 min, 20 min) to Hyperfilm ECL high performance chemiluminescence film (Amersham Biosciences). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

The immunoreactive band of the plant-produced Cry1A.105 protein in each lane migrating at the same level as the full-length reference standard protein was quantitated relative to the standard curve. Quantitation was performed using the volume tool and the linear regression method in the Quantity One software (version 4.4.0) after scanning on the Bio-Rad GS-800 densitometer. The concentration of the plant-produced Cry1A.105 was determined by dividing the amount of protein in each lane by the volume of protein loaded in that lane [Microsoft Excel 2002 (10.6730.6718)SP3].

Throughout the report, all load quantities of plant-produced Cry1A.105 refer to the amount of the full-length protein.

5.3 Immunoblot Analysis

Immunoblot analysis was performed to confirm the identity of the Cry1A.105 protein, the intactness of its N-terminus, and compare immunoreactivity of the plant- and *E. coli*-produced proteins.

5.3.1 Immunoblot Analysis Using anti-Cry1A.105 Antibody

The western blot described in section 5.2 was also used to establish the identity of the plant-produced protein and to compare the immunoreactivity of the plant-produced and *E. coli*-produced Cry1A.105 proteins. Equivalence was demonstrated for bands representing full-length (~130 kDa) proteins that were identified by the anti-Cry1A.105 antibody and showed similar electrophoretic mobility.

5.3.2 Immunoblot Analysis Using the N-terminal Peptide Antibody

A western blot analysis using the N-terminal peptide antibody (lot 42005MG) was performed to confirm the intactness of the N-terminus of the plant-produced Cry1A.105 protein. The N-terminal peptide antibodies were produced against a synthetic peptide consisting of the first 14 amino acids of the Cry1A.105 protein N-terminus. The trypsin resistant core (lot 7518937) was used as a negative control because its N-terminus was removed by trypsin. The plant-produced Cry1A.105 protein, the *E. coli*-produced Cry1A.105 reference standard (corrected for the purity of the full-length protein), and the Cry1A.105 trypsin-resistant core (~56 kDa, lot 7518937) were each loaded on gels at 20 ng and 40 ng per lane. Each protein was mixed with 5× LB, heated at 96.0 °C for 5 min, and applied to a pre-cast tris-glycine 4→20% polyacrylamide gradient 10-well gel. Electrophoresis was performed at a constant voltage of 150 V for 5 min followed by a constant voltage of 200 V for 60 min. Pre-stained molecular weight markers (Bio-Rad Precision Plus Dual Color,) were used to verify electrotransfer of protein to the membrane and estimate the size of the immunoreactive bands. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen) was performed for 90 min at a constant voltage of 25 V.

The membrane was blocked overnight with 5% (w/v) NFDM in 1× PBST. The membrane was probed with a 1:33.33 dilution of rabbit N-terminal peptide antibody (lot 42005MG) in 1% (w/v) NFDM in PBST for 60 min. Excess antibody was removed using three 5 min washes with PBST. Finally, the membrane was probed with HRP-conjugated anti-rabbit IgG (Sigma) at a dilution of 1:7500 in 1% (w/v) NFDM in PBST for 60 minutes. Excess HRP-conjugate was removed using three 5 min washes with PBST. The blocking step was performed at 4 °C. All other incubations were performed at room temperature. Immunoreactive bands

were visualized using the ECL detection system (Amersham Biosciences) and exposed (1 min, 2 min, 5 min, 10 min) to Hyperfilm ECL high performance chemiluminescence film (Amersham Biosciences). Films were developed using a Konica SRX-101A automated film processor.

5.4 MALDI-TOF Tryptic Mass Map Analysis

MALDI-TOF mass spectrometry was used to confirm the identity of the plant-produced Cry1A.105 protein.

5.4.1 Concentration of Protein

The plant-produced protein (4.5 ml) was diluted to 9.0 ml with Cry1A.105 concentration buffer [25 mM CAPS, 0.02% (w/v) sodium dodecyl sulfate] followed by concentration down to 64 μ l using Amicon Ultrafree CL concentrators (Millipore Corporation, Bedford, MA). Sixty microliters of 5 \times LB was used to wash the concentrator walls and membranes. The membranes were heated with 5 \times LB for 5 minutes at 95.1°C. Sixteen microliters of this 5 \times LB was then used as the loading buffer for the Cry1A.105 sample for SDS-PAGE separation.

5.4.2 SDS-PAGE Separation of Proteins

The concentrated plant-produced Cry1A.105 protein was subjected to electrophoresis on an SDS-polyacrylamide gel. The protein sample was loaded in two lanes. Broad Range molecular weight markers (Bio-Rad) were used to estimate molecular weights. Plant-produced Cry1A.105 protein and molecular weight markers were heated at 95.3 °C for 5 min and then applied to a pre-cast tris-glycine 4 \rightarrow 20% polyacrylamide gradient 10-well mini-gel. Electrophoresis was performed at constant voltage (125 V for 10 min followed by 170 V for 70 min). Proteins were stained with Bio-Rad Coomassie 1 \times stain for 2 hours, and destained by washing with Coomassie R-250 1 \times destain solution (Bio-Rad) for 2 hours with one change of the destain solution.

5.4.3 In-gel Protein Digestion

The bands representing full-length plant-produced Cry1A.105 protein (~130 kDa) were excised from two gel lanes, destained, reduced, alkylated, and subjected to an in-gel trypsin digestion (Williams *et al.*, 1997). Briefly, each gel band was individually destained for 30 min by incubation in 100 μ L of 40% (v/v) methanol and 10% (v/v) glacial acetic acid in a microfuge tube. This was repeated two additional times. Following destaining, the gel bands were incubated in 100 μ L of 100 mM ammonium bicarbonate buffer for 30 min at room temperature. The protein was reduced in 100 μ L of 10 mM dithiothreitol solution for

2 hours at 37 °C. The protein was alkylated by the addition of 100 μ L of 200 mM iodoacetic acid. The alkylation reaction was allowed to proceed at room temperature for 20 min in the dark. The gel bands were incubated in 100 μ L of 100 mM ammonium bicarbonate buffer for 30 min at room temperature at which time 100 μ L of acetonitrile was added and the incubation was continued for an additional 30 minutes. The ammonium bicarbonate/acetonitrile incubations were repeated two additional times to remove the reducing and alkylating reagents and other salts from the gel. The gel bands were dried in a SpeedVac concentrator, rehydrated with 40 μ L 25 mM ammonium bicarbonate containing 33 μ g/ml trypsin, and the protein contained in the gel band was digested for 16 hours at 37.1 °C. Digested peptides were extracted for 60-80 min at room temperature with 50 μ L 70% (v/v) acetonitrile containing 0.1% (v/v) TFA per gel band. Both extraction supernatants were combined into a single tube and dried in a SpeedVac concentrator. This process of extracting the peptides was repeated two more times. The final dried materials were reconstituted in 5 μ L of 0.1% (v/v) TFA.

5.4.4 Sample Preparation

An aliquot (4 μ L) of the digested sample was desalted (Bagshaw *et al.*, 2000) using Millipore (Bedford, MA) ZipTip C18 pipette tips. Prior to desalting, the tips were wetted with methanol and equilibrated with 0.1% (v/v) TFA. The sample was applied to a ZipTip and eluted with 4 μ L of Wash 1 [0.1% (v/v) TFA], followed by 4 μ L of Wash 2 [20% (v/v) acetonitrile containing 0.1% (v/v) TFA], followed by 4 μ L of Wash 3 [50% (v/v) acetonitrile containing 0.1% (v/v) TFA], and finally with 4 μ L of Wash 4 [90% (v/v) acetonitrile containing 0.1% (v/v) TFA].

5.4.5 MALDI-TOF Instrumentation and Mass Analysis

Mass spectral analyses were performed as follows. Mass calibration of the instrument was performed using an external peptide mixture from a Sequazyme Peptide Mass Standards kit (Applied Biosystems). Samples (0.5 μ L) from each of the desalting steps, as well as a sample of solution taken prior to desalting, were co-crystallized with 0.8 μ L α -cyano-4-hydroxy cinnamic acid on the analysis plate. The sample was analyzed in the 500 to 5000 Dalton range using 100 shots at a laser intensity setting of 2781 (a unit-less MALDI-TOF instrument specific value). Protonated (MH⁺) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical trypsin digest of the expected Cry1A.105 (plant) protein sequence based upon the nucleotide sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Experimental masses (MH⁺)

were assigned to peaks in the 500 to 1000 Da range if there were two or more isotopically resolved peaks, and in the 1000 to 5000 Da range if there were three or more isotopically resolved peaks in the spectra. Peaks were not assessed if the peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned due to overlap with a stronger signal ± 2 Daltons from the mass analyzed. Known autocatalytic fragments from trypsin digestion were identified in the raw data. The tryptic mass map coverage was considered acceptable if $\geq 40\%$ of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments.

5.5 Molecular Weight and Purity Estimation – SDS-PAGE

Aliquots of the *E. coli*-produced reference standard and plant-produced Cry1A.105 proteins were mixed with $5\times$ LB to a final protein concentration of 10 ng/ μ l and 2.4 ng/ μ l, respectively. The plant-produced protein was analyzed in duplicate at 48, 72, and 96 ng of total protein per lane. The *E. coli*-produced Cry1A.105 reference standard was analyzed at 96 ng of purity corrected full-length protein. All samples were heated in a thermo-block at 99 °C for 3 min and applied to a pre-cast tris-glycine 4 \rightarrow 20% polyacrylamide gradient 10-well mini-gel (Invitrogen). Electrophoresis was performed at a constant voltage of 125 V for 10 min followed by a constant voltage of 170 V for 70 min.

The gel was stained using the SilverXpress® Silver Staining Kit Protocol (Invitrogen, Carlsbad, CA). The gel was fixed for 10 minutes in 200 ml of fixing solution (90 ml ultra pure water, 100 ml methanol, and 20 ml acetic acid). This was followed by 10 minutes in 100 ml sensitizing solution (105 ml ultra pure water, 100 ml methanol, and 5 ml sensitizer) and repeated once. The excess of the sensitizing solution was removed using two 5 minutes washes in 200 ml ultra pure water. The gel was stained for 15 minutes in 100 ml staining solution (5 ml Stainer A, 5 ml Stainer B, and 90 ml ultra pure water). The stain was removed using two 5 minutes washes of 200 ml ultra pure water. Developing occurred in 100 ml of solution (5 ml developer and 95 ml ultra pure water) for 3 - 15 minutes, and was stopped by addition of 5 ml stopping solution for 10 minutes. The gel was washed three times for 10 minutes each with 200 ml of ultra pure water. Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). Molecular weight markers (Bio-Rad Broad-Range) were used to estimate the apparent molecular weight of the plant-produced Cry1A.105 protein. For the purity evaluation, all visible bands within each lane were quantified. Stained bands corresponding to immunoreactive bands identified by anti-Cry1A.105 antibody and migrating from ~56 to ~130 kDa were included in the purity calculation for the protein of interest.

as they represent various lengths of the insecticidal protein from the ~56 kDa tryptic core to the ~130 kDa full-length protein (Bietlot et al., 1989). The purity and estimated full-length molecular weight of the plant-produced Cry1A.105 protein were reported as the average of the six values obtained by densitometric analysis.

5.6 Glycosylation Analysis

Glycosylation analysis was used to determine whether the plant-produced Cry1A.105 protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the plant-produced Cry1A.105 protein, the *E. coli*-produced Cry1A.105 reference standard, and the positive controls, transferrin (Amersham Biosciences) and horseradish peroxidase (Sigma), were each mixed with 5× LB. These samples were heated at 95 °C for 4 min, cooled, and loaded on a tris-glycine 4→20% polyacrylamide gradient 10-well mini-gel. Each sample was loaded at 48 and 96 ng (purity corrected for the full length protein) per lane. Precision Plus Dual Color pre-stained protein molecular weight markers (Bio-Rad) were loaded to verify electrotransfer of the proteins to the membrane, and the CandyCane™ Glycoprotein Molecular Weight Standards (Molecular Probes, Eugene, OR) were loaded as positive/negative controls and markers for molecular weight. Electrophoresis was performed at a constant voltage of 125 V for 10 min followed by a constant voltage of 170 V for 70 min. Electrotransfer to a 0.2 µm PVDF membrane was performed for 90 min at a constant voltage of 25 V.

Carbohydrate detection was performed directly on the PVDF membrane using the Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit (Molecular Probes). The manufacturer's protocol was followed. All steps were performed at room temperature. The PVDF membrane was fixed in 25 ml of a solution containing 50% methanol and 5% glacial acetic acid for 1 hour, and then the solution was changed and the membrane was incubated overnight. Two 15 minute washes (50 ml each) of 3% (v/v) glacial acetic acid (wash solution), were followed by a 20 minute oxidation in 25 ml of the kit supplied oxidizing solution. After oxidation, three 15 minute washes (50 ml each) prepared the membrane for staining. The blot was incubated in 25 ml of Pro-Q Emerald Staining Solution that was prepared using the kit reagents. After 1 hour of staining in the dark, two 30 minute, 50 ml wash cycles were followed by two 45 minute, 50 ml wash cycles. The final wash cycles included two 25 ml, 1 minute deionized water washes followed by three 25 ml, 5 minute methanol washes (B&J Brand, Muskegon, MI). The blot was then scanned using the BioRad Molecular Imager FX using the Alexa 488 illumination setting (Quantity One software; version 4.6, build 036) in order to visualize the fluorescing glycosylated proteins.

5.7 Functional Activity Assay

In order to assess the functional activity of the plant-produced Cry1A.105 protein and to compare its activity to the *E. coli*-produced Cry1A.105 reference standard, aliquots of the plant-produced Cry1A.105 protein and *E. coli*-produced Cry1A.105 reference standard protein were transferred to the Monsanto Ecological Technology Center. These aliquots were used to estimate the effective protein concentration necessary to inhibit the growth of the target insect by 50% (EC₅₀ value) and the rate of weight loss as test protein concentration in the diet increased (slope parameter) as described in Appendix 4. The functional activity of the plant-produced Cry1A.105 was considered equivalent to that of the *E. coli*-produced protein activity if there was less than a 4-fold difference in mean EC₅₀ values. Logistic regression was used to model concentration-response curves for growth inhibition and for EC₅₀ determinations.

5.8 Storage Stability

The short-term stability of the plant-produced Cry1A.105 protein in a 4 °C refrigerator was evaluated by comparing the purity and molecular weight values obtained on day 0 to the purity and molecular weight values obtained on day 18 of storage. An aliquot of the plant-produced Cry1A.105 protein was stored in a 4 °C refrigerator. Day 0 stability analysis corresponds to the purity and molecular weight determination, described in Section 5.5. On day 18, the sample was removed from storage, diluted with loading buffer to a final concentration of 2.4 ng/μl, heated at 95.3 °C for 5 min, and loaded in duplicate (48, 72, and 96 ng per lane) onto a tris-glycine 4-20% gradient polyacrylamide gel. Staining and densitometric analysis were performed as described in section 5.5 for Molecular Weight and Purity Estimation-SDS-PAGE. The protein samples were considered to have undergone degradation if a >10% decrease in purity and/or molecular weight was observed relative to the value determined on Day 0.

6.0 Data Rejected or Not Reported

One quantitative western blot was rejected because of the unusual appearance of protein bands in two of the twelve lanes. The protein bands had migrated outside the edge of the lane or were not parallel, relative to other bands in the lane. Since these two lanes significantly affected the standard curve, these data were rejected.

7.0 Characterization Plan Amendment

The characterization plan was amended to transfer project lead responsibilities from Tuhin Ganguly to Brian Goertz. There was no impact on the characterization plan as a result of this change.

8.0 Control of Bias and Quality Measures

Controls and standards were included with each analysis. A four-peptide mixture from the Sequazyme Peptide Mass Standards kit (Applied Biosystems) was used to calibrate the MALDI-TOF mass spectrometer for masses observed between 500-5000 Daltons. Replicate analyses were used for the insect bioassay, quantitative immunoblot, N-terminal peptide antibody immunoblot, purity and molecular weight determination, stability, and glycosylation analyses.

9.0 Results and Discussion

9.1 Protein Concentration

The concentration of the full-length Cry1A.105 (~130 kDa) protein was estimated to be 3.0 ng/μl based on a quantitative immunoblot (Figure 1, Table 1).

9.2 Protein Identity

The identity of the plant-produced Cry1A.105 protein was confirmed using three analytical methods: immunoblot analysis using anti-Cry1A.105 antibody, immunoblot analysis using the N-terminal peptide antibody, and MALDI-TOF MS tryptic mass map analysis. In addition, immunoblot analysis using anti-Cry1A.105 antibody confirmed equivalent immunoreactivity of the plant- and *E. coli*-produced proteins.

9.2.1 Immunoblot Analysis Using Anti-Cry1A.105 Antibody

The quantitative immunoblot used for determination of the protein concentration (section 9.1) was also utilized for confirmation of the Cry1A.105 protein identity. The plant-produced Cry1A.105 protein and *E. coli*-produced reference standard were loaded on the same gel. Similar immunoreactive bands migrating between approximately 85 and 130 kDa were observed in the plant-produced Cry1A.105 and *E. coli*-produced reference samples (Figure 1, lanes 2-11). As expected, the immunoreactive signal increased with increased loading levels of both the plant- and *E. coli*-produced proteins. The major protein band, migrating with an apparent molecular weight of ~130 kDa, was present in both the plant-produced and *E. coli*-produced Cry1A.105 samples. This band represents the full-length Cry1A.105 protein. The immunoreactive bands with lower molecular weight most likely represent degradation products of the Cry1A.105 protein. An additional immunoreactive band with molecular weight above 250 kDa was observed in the samples of the plant-produced and *E. coli*-produced Cry1A.105 protein. This band most likely represents the aggregation of the Cry1A.105 protein. The western

blot analysis confirmed the identity of the plant-produced Cry1A.105 protein and demonstrated that both the plant- and *E. coli*-produced protein had equivalent immunoreactivity.

9.2.2 *Immunoblot Analysis Using the N-terminal Peptide Antibody*

Western blot analysis was performed using previously characterized N-terminal peptide antibody (lot 42005MG). The antibodies were raised against a synthetic peptide consisting of the first 14 amino acids of the Cry1A.105 N-terminus (MDNNPNINECIPYN). The *E. coli*-produced Cry1A.105 standard containing the N-terminal sequence, the Cry1A.105 trypsin-resistant core standard lacking the N-terminus, and the plant-produced Cry1A.105 protein were loaded on the gel at 20 and 40 ng protein per lane. *E. coli*-produced Cry1A.105 protein and Cry1A.105 trypsin-resistant core served as positive and negative controls, respectively. As expected, no immunoreactive bands were observed in the Cry1A.105 trypsin-resistant core lanes (Figure 3, lanes 6 and 7). The band corresponding to the full-length Cry1A.105 protein (~130 kDa) was observed in both the plant- and *E. coli*-produced Cry1A.105 samples. Additionally, a fragment of lower molecular weight (~85 kDa) was observed in both proteins (Figure 3, lanes 2-5). This fragment most likely represents a product of C-terminal degradation of Cry1A.105 protein. As expected, the intensity of the bands increased in a load dependent manner. The data indicate that the Cry1A.105 protein contains an intact N-terminus.

9.2.3 *MALDI-TOF Tryptic Mass Map Analysis*

The plant-produced, full-length Cry1A.105 protein was further assessed by MALDI-TOF mass spectrometry. Prior to analysis, the protein sample was chemically reduced, alkylated and digested with trypsin. The ability to identify a protein using this method is dependent on matching a sufficient number of observed mass fragments to expected (theoretical) mass fragments. A protein can typically be identified when 40% of the mass fragments are identified from the analyzed protein (Jiménez et al., 1998).

There were 52 protein fragments (out of 79 masses) identified that matched the expected masses of the Cry1A.105 trypsin-digested peptides (Table 2). The identified masses were used to assemble a coverage map that indicates those matched peptide sequences for the protein (Figure 2). The protein was confirmed as Cry1A.105 because 516 of 1177 amino acids (43.8%) were identified.

9.3 Molecular Weight and Purity Determination

The plant-produced Cry1A.105 protein was separated using SDS PAGE and stained using the SilverXpress® Silver Staining Kit Protocol (Invitrogen). Purity and apparent molecular weight of the plant-produced Cry1A.105 protein were estimated using densitometric analysis (Figure 4) and the data are summarized in Table 3. The full-length Cry1A.105 protein in the plant-produced sample had an estimated molecular weight of 130.0 kDa (calculated as the average of the six loads) and migrated to the identical position on the gel as *E. coli*-produced Cry1A.105 reference standard (Figure 4, lane 2). The apparent molecular weight of the full-length *E. coli* produced Cry1A.105 reference standard on the COA is 131.5 kDa (Appendix 2). The difference in molecular weight between the plant- and *E. coli*-produced Cry1A.105 full-length proteins was 1% (Table 4). Because the difference in the molecular weights met the pre-set acceptance criteria ($\leq 10\%$), the plant- and *E. coli*-produced Cry1A.105 proteins are considered equivalent based on their molecular weight.

The purity value for the plant-produced Cry1A.105 protein (Figure 4, Lanes 3-8) was calculated as the sum of the stained protein bands corresponding to the immunoreactive bands in Figure 1 (Lanes 6-11). The average purity of the plant-produced Cry1A.105 protein was 100% (Table 3).

9.4 Glycosylation Analysis

Many eukaryotic proteins are post-translationally modified with carbohydrate moieties (Rademacher *et al.*, 1988). These carbohydrate moieties may be complex, branched polysaccharide structures or simple monosaccharides. In contrast, prokaryotic organisms such as *E. coli* lack the necessary biochemical "machinery" required for protein glycosylation. To test whether potential post-translational glycosylation of the plant-produced Cry1A.105 protein occurred, it was analyzed for the presence of covalently bound carbohydrate moieties. The *E. coli*-produced Cry1A.105 reference standard (negative control), horseradish peroxidase (positive control), and transferrin (positive control) were analyzed concurrently with the plant-produced Cry1A.105 protein. The results of this analysis are presented in Figure 5. The positive controls were detected at the expected molecular weights, in a concentration-dependent manner (Figure 5, lanes 2-5). No detectable signal was observed for the test or reference standard Cry1A.105 proteins (Figure 5, lanes 6-9) on the blot. Thus, the plant-produced protein is not glycosylated and, thus, is equivalent to the *E. coli*-produced Cry1A.105 reference standard with respect to glycosylation.

9.5 Functional Activity

The results of the functional activity assays are summarized on Figure 6 and in Appendix 4. The mean EC_{50} values for the plant-produced Cry1A.105 protein and *E. coli*-produced reference standard were determined to be 0.0074 and 0.012 μ g Cry1A.105 per ml diet, respectively. The mean EC_{50} values met the pre-set acceptance criteria (<4 fold difference), therefore the plant-produced Cry1A.105 protein is determined to have equivalent functional activity to the *E. coli*-produced protein.

9.6 Storage Stability

Storage stability of the plant-produced Cry1A.105 protein stored at $\sim 4^{\circ}\text{C}$ for 18 day period was assessed (Tables 5 and 6, Figure 7). Stability was evaluated by comparison of the molecular weight and purity of the protein after storage to the initial molecular weight and purity values determined on day 0. The molecular weight of the full-length Cry1A.105 protein was estimated to be 135.2 kDa on day 18. This value differed from the molecular weights obtained on day 0 (130.0 kDa) by 4% (Table 6). Therefore, based on molecular weight, the plant-produced Cry1A.105 protein was stable when stored in a 4°C refrigerator for 18 days. The purity value for the plant-produced Cry1A.105 protein (Figure 7, Lanes 2-7) was calculated as the sum of the stained protein bands corresponding to the immunoreactive bands in Figure 1 (Lanes 6-11). The average purity on day 18 was 100% by densitometric analysis of a silver stained SDS-polyacrylamide gel. The purity on day 18 was the same as the purity on day 0 (100%) (Tables 5 and 6). Therefore, based on purity, the plant-produced Cry1A.105 protein was stable when stored in a 4°C refrigerator for 18 days.

10.0 Conclusions

A panel of analytical techniques was used to characterize the plant-produced Cry1A.105 protein. These analytical techniques were: sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, densitometry, matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry, glycosylation analysis, and Cry1A.105 activity assay. The short-term stability of the plant-produced Cry1A.105 protein was also assessed using SDS-PAGE by estimating the molecular weight and purity of the protein after storage in a 4°C refrigerator.

Full length Cry1A.105 protein (~ 130 kDa) and its fragments were identified using western blot analysis with previously characterized anti-Cry1A.105 antibody. MALDI-TOF mass spectrometry analysis of the ~ 130 kDa band, after trypsin digestion, yielded peptide masses consistent with the peptide masses expected for the Cry1A.105 protein. Together, the identified peptide masses yielded 43.8% overall coverage of the expected

peptide sequence (516 out of 1177 amino acids) of the plant-produced Cry1A.105 protein. The immunoreactivity with the N-terminal peptide antibodies indicates the intactness of the N-terminus in the plant-produced full-length Cry1A.105 protein. The concentration of the full-length plant-produced Cry1A.105 protein determined by quantitative western blot analysis was 3.0 ng/ μ l. Purity and apparent molecular weight of the plant-produced Cry1A.105 protein was determined using densitometric analysis of a silver stained SDS-PAGE gel. Purity, calculated as the sum of the percent optical density of the stained bands corresponding to the immunoreactive bands in Figure 1 (Lanes 6-11), was 100%. The apparent molecular weight of the full-length plant-produced Cry1A.105 protein was estimated to be 130.0 kDa. The plant-produced Cry1A.105 protein was not glycosylated. The functional activity of the plant-produced Cry1A.105 protein was determined using an insect bioassay with the larvae of a susceptible pest, corn earworm (CEW). The Cry1A.105 protein produced in MON 89034 and in *E. coli* were shown to be active with mean EC_{50} values of 0.0074 and 0.012 μ g/ml of diet, respectively. The plant-produced Cry1A.105 protein was stable for at least 18 days at a storage temperature of $\sim 4^{\circ}\text{C}$.

The equivalence of the plant- and *E. coli*-produced Cry1A.105 proteins was evaluated by comparing their full-length molecular weights, immunoreactivity with anti-Cry1A.105 antibodies, glycosylation status, and functional activity. Based on preset acceptance criteria and the results obtained, the plant-produced Cry1A.105 protein is equivalent to the *E. coli*-produced Cry1A.105 protein.

These data provide a detailed characterization of the Cry1A.105 protein isolated from MON 89034 and establish the equivalence of the plant-produced Cry1A.105 protein to the *E. coli*-produced Cry1A.105 protein.

11.0 References

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Table 1. Concentration of the Full-Length Plant-Produced Cry1A.105 Protein.

The concentration of the full-length plant-produced Cry1A.105 sample was determined by densitometric analysis of the quantitative western blot shown in Figure 1.

Sample	Gel Lane	Amount (ng)	Amount of Original Sample Loaded (μl)	Calculated Concentration (ng/μl)	Average Cry1A.105 Concentration (ng/μl)
Cry1A.105 plant-produced protein	6	1.20	0.38	3.16	3.0
	7	1.20	0.38	3.16	
	8	1.48	0.50	2.96	
	9	1.37	0.50	2.74	
	10	2.73	1.00	2.73	
	11	3.02	1.00	3.02	

Table 2. Summary of the Tryptic Masses Identified for the Full-Length Plant-Produced Cry1A.105 Protein (~130 kDa) Using MALDI-TOF Mass Spectrometry.

Only experimental masses that matched expected masses are listed in the table. The composition of each wash step used to desalt samples is described in Section 5.4.4.

No	Observed Mass (Da)				Expected Mass (Da)	Δ^c	AA ^d Position	Fragment Sequence(s)
	Desalting ^a	Wash 1 ^b	Wash 2 ^b	Wash 3 ^b	Wash 4 ^b			
515.15		515.22	515.25	515.27	515.27	0.19	525-528	ARIR
		529.16				0.11	695-698	QPER
579.13		579.21	579.25	579.28		0.20	749-752	YQLR
589.12		589.18	589.23	589.26	589.25	0.19	225-228	DWIR
					589.28 ^c	0.16	1023-1027	VCPGR
					606.01	0.70	666-670	ELSEK
		611.25	611.29	611.32	611.29	0.11	936-940	VHSIR
621.17			621.29	621.31	621.32	0.20	1028-1032	GYILR
649.16		649.94	649.29	649.32	649.99	0.21	254-258	TYPIR
		688.26	688.30			0.11	94-99	NQAISR
727.14		727.25	727.28	727.31		0.21	229-233	YNQFR
731.15		731.24	731.29	731.33		0.21	424-429	QGFSHR
764.18		764.27	764.33	764.35	764.35	0.21	88-93	IEEFAR
		781.28	781.32		781.34	0.10	193-198	YNDLTR
		784.26				0.11	853-859	TQDGHAR
804.22		804.33	804.39	804.43	804.41	0.24	259-265	TVSQLTR
816.17		816.29	816.33	816.36		0.23	218-224	VWGPDSR
		854.94			855.00	0.53	1114-1120	SYTDGRR
907.21			907.40	907.43	907.43	0.25	174-181	DVSVFGQR
925.24		925.37	925.42	925.46	925.45	0.23	529-536	YASTTNLR
940.26			940.45	940.47	940.48	0.25	361-368	TLSSSTLYR
976.26			976.44	976.48	976.47	0.24	430-437	LSHVS MFR

Table continued from page 30

No Desalting ^a	Observed Mass (Da)				Expected Mass (Da)	Δ^c	AA ^d Position	Fragment Sequence(s)
	Wash 1 ^b	Wash 2 ^b	Wash 3 ^b	Wash 4 ^b				
1007.31	1007.44	1007.49		1007.52	1007.55	0.24	537-545	IYTVVAGER
1066.17	1065.97	1066.38		1066.03	1066.43	0.26	1121-1128	ENPCEFN
1074.28		1074.50		1074.52	1074.55	0.27	282-292	GSAQGIIEGSIR
				1074.54	1074.55	0.04	682-690	NLLQDSNFK
1089.30		1089.52	1089.54	1089.55	1089.57	0.27	491-501	GPFGTGGDILR
1144.29		1144.53	1144.55	1144.54	1144.57	0.28	450-458	APMFSWIHR
1203.40		1203.64	1203.67	1203.66	1203.68	0.28	350-360	IVAQLGQGVYR
1237.30		1237.55	1237.58	1237.57	1237.60	0.30	182-192	WGFDAAATNSR
1253.36		1253.61	1253.64	1253.63	1253.65	0.29	438-449	SGFSNSSVSIIR
1258.36		1258.61	1258.63	1258.64	1258.65	0.29	199-209	LIGNYTDHAVR
1269.40		1269.65			1269.69	0.29	479-490	AHTLQSGTTVVR
1398.34		1398.63	1398.65	1398.65	1398.67	0.33	146-157	EWEADPTNPALR
1424.32	1424.53	1424.62			1424.65	0.33	904-1005	GHVDVVEEQNNQR
				1576.81	1576.81 ^e	0.00	682-694	NLLQDSNFKDINR
					1576.87 ^e	0.06	623-637	AVNANETSTNQLGLK
				1598.81	1598.71	0.10	1120-1131	RENPCETNRYR
1800.47		1800.84	1800.84	1800.84	1800.87	0.40	753-767	GSFSSODLENYSIR
1900.48		1900.88	1900.89	1900.89	1900.91	0.43	266-281	EVNTNPLENFDGSR
1902.52			1902.92	1902.90	1902.96	0.44	100-115	LEGLSNLYQVAESFR
1955.58			1955.98	1955.97	1956.01	0.43	1006-1022	SVLVVPEWFAEVSQEV
2097.69			2098.14	2098.12	2098.15	0.46	860-878	LGNLEFLEERPLNGEALR
2107.63			2108.07	2108.05	2108.09	0.46	602-619	FELIPVTATLEAEVNER
2133.62			2134.08		2133.11	0.51	503-522	TSGGPFAYTIIVNINGQER
2148.59		2149.03	2149.06	2149.02	2149.05	0.46	404-423	SGTVDSLDEIPPNVPPR

Table continued from page 31

No	Observed Mass (Da)				Expected Mass (Da)	Δ^c	AA ^d Position	Fragment Sequence(s)
	Desalting ^a	Wash 1 ^b	Wash 2 ^b	Desalting Wash 3 ^b	Wash 4 ^b			
2196.64				2197.11	2197.06	2197.11	0.47	SPHLM DILNSITYTDAHR
				2211.11	2211.05	2211.13	0.02	LSHVSMFRSGFSNSSVSIIR
		2277.12				2277.10 ^e	0.02	GYIEDSQDLEIYSIRYNAK
2615.78				2616.33	2616.37	2277.15 ^e	0.03	KSGTVDSLDEIPPQNNNVPPR
						2616.36	0.58	EAYLPELSVIPGVNA AIFEELEGR
3728.03				3729.00		3728.87	0.84	RPFNIGINNQQLSVLDGTEFAYGTSSNLP SAVYR

^a Sample, 0.5 μ L, was analyzed prior to Zip Tip desalting
^b The Zip Tip was washed with 0.1% (v/v) trifluoroacetic acid containing acetonitrile at varying concentrations of acetonitrile as described in the methods section (Section 5.4.4): Wash 1 – 0% (v/v), Wash 2 – 20% (v/v), Wash 3 – 50% (v/v), and Wash 4 – 90% (v/v) acetonitrile.
^c A difference of less than one Dalton between the observed (first column where this mass is documented) and expected masses was necessary for consideration as a match.
^d AA position refers to amino acid position within the predicted CryIA.105 sequence as depicted in Figure 2.
^e Two expected fragments having nearly identical masses were matched to one observed mass.

Table 3. Molecular Weight and Purity Estimation for the Plant-Produced Cry1A.105 Using SDS-PAGE Analysis.

The apparent molecular weights of the protein bands representing the full-length plant-produced Cry1A.105 protein and the purity of the plant-produced Cry1A.105 protein were determined by densitometric analysis of the silver stained SDS polyacrylamide gel (Day 0) shown in Figure 4.

Cry1A.105 Sample Loaded	Apparent Mol. Wt. (kDa) of Full-Length Cry1A.105	Purity ¹ (%)
48 ng Load (Figure 4, lane 3)	130.49	100.0
48 ng Load (Figure 4, lane 4)	129.36	100.0
72 ng Load (Figure 4, lane 5)	128.70	99.9
72 ng Load (Figure 4, lane 6)	128.85	100.0
96 ng Load (Figure 4, lane 7)	129.43	100.0
96 ng Load (Figure 4, lane 8)	132.93	100.0
Average Values (Total for each lane / 6)	130.0	100

Note: Molecular weights were rounded to one decimal place and purity was rounded to nearest whole number.

¹ Summation of stained protein bands corresponding to immunoreactive bands in Figure 1, Lanes 6-11.

Table 4. Molecular Weight Difference Between Full-Length Plant- and *E. coli*-Produced Cry1A.105 Proteins.

Molecular Weight of Full-Length Plant-Produced Cry1A.105 Protein¹	Molecular Weight Difference from <i>E. coli</i>-Produced Cry1A.105 Protein²	Percent Difference from <i>E. coli</i>-Produced Cry1A.105 Protein³
130.0 kDa	1.5 kDa	1 %

¹ See Table 3 for the molecular weight of the full-length plant-produced protein.

² See COA (Appendix 2) for the molecular weight of the full-length *E. coli*-produced reference standard.

³ Percent difference was calculated as follows: $\frac{1.5 \text{ kDa}}{131.5 \text{ kDa}} \times 100\% = 1\%$

Table 5. Molecular Weight and Purity Estimation for the Plant-Produced Cry1A.105 after 18 Days of Storage in a 4 °C Refrigerator.

The day 18 purity and molecular weight values for the plant-produced Cry1A.105 protein were determined by densitometric analysis of the silver stained SDS polyacrylamide gel shown in Figure 7.

Plant-Produced Cry1A.105 Sample Loaded	Apparent Mol. Wt. (kDa) of Full Length Cry1A.105	Purity ¹ (%)
48 ng (Figure 7, lane 2)	136.32	100.0
48 ng (Figure 7, lane 3)	135.42	100.0
72 ng (Figure 7, lane 4)	134.18	99.9
72 ng (Figure 7, lane 5)	134.46	100.0
96 ng (Figure 7, lane 6)	134.71	99.9
96 ng (Figure 7, lane 7)	135.90	100.0
Average Values (Sum of Lanes/ 6)	135.2	100

Note: Molecular weights were rounded to one decimal place, and purity was rounded to the nearest whole number.

¹ Summation of stained protein bands corresponding to immunoreactive bands in Figure 1, Lanes 6-11.

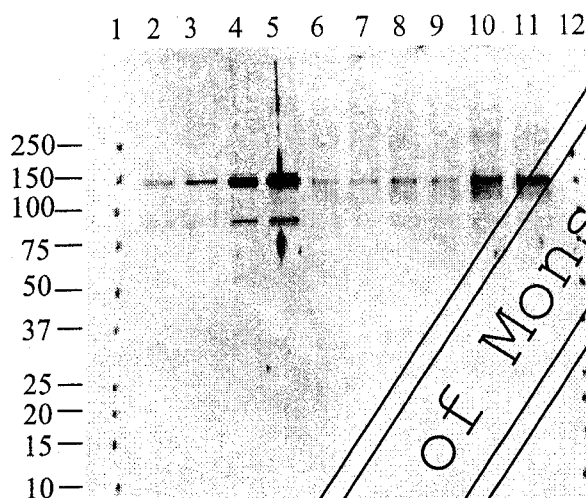
Table 6. Storage Stability (Day 18 vs. Day 0) of the Cry1A.105 Plant-Produced Protein at ~4 °C.

For the day 0 purity and molecular weight values for the plant-produced Cry1A.105 protein see Table 3. For the day 18 purity and molecular weight values see Table 5.

Apparent Mol. Wt. (kDa) of Full Length Cry1A.105 (kDa)			Purity ² (%)		
Day 0	Day 18	% Difference (Day 18 vs. Day 0) ¹	Day 0	Day 18	% Difference (Day 18 vs. Day 0) ¹
130.0	135.2	4	100	100	0

¹ Percent difference was calculated as follows: $\frac{|Day0 - Day18|}{Day0} \times 100 = \%Difference$

² Summation of stained protein bands corresponding to immunoreactive bands in Figure 1, Lanes 6-11.



Lane	Sample	Amount Loaded (ng)	Amount Loaded (μl)
1	Precision Plus Dual Color molecular weight markers	—	—
2	<i>E. coli</i> -produced Cry1A.105 standard	1	—
3	<i>E. coli</i> -produced Cry1A.105 standard	2	—
4	<i>E. coli</i> -produced Cry1A.105 standard	4	—
5	<i>E. coli</i> -produced Cry1A.105 standard	6	—
6	Plant-produced Cry1A.105 protein	—	7.5
7	Plant-produced Cry1A.105 protein	—	7.5
8	Plant-produced Cry1A.105 protein	—	10
9	Plant-produced Cry1A.105 protein	—	10
10	Plant-produced Cry1A.105 protein	—	20
11	Plant-produced Cry1A.105 protein	—	20
12	Precision Plus Dual Color molecular weight markers	—	—

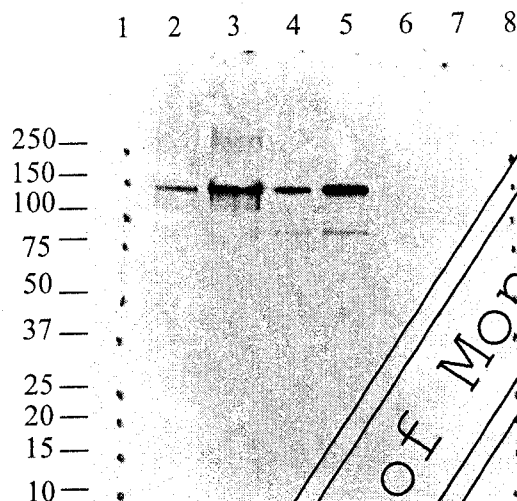
Figure 1. Quantitative Western Blot Analysis Using Anti-Cry1A.105 Antibody.

Aliquots of the plant-produced Cry1A.105 and *E. coli*-produced Cry1A.105 reference standard were separated by SDS-PAGE, and electrotransferred to a PVDF membrane. The membrane was incubated with rabbit anti-Cry1A.105 antibody (lot 070705JL) and developed using an ECL system. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in lane 1 and lane 12. The 10 minute exposure is shown.

0001	MDNNPNINEC	IPYNCLSNPE	VEVLGGERIE	TGYTPIDISL	SLTQFLLSEF
0051	VPGAGFVLGL	VDIIWGIFGP	SQWDAFLVQI	EQLINQRIEE	FARNOAISRL
0101	EGLSNLYQIY	AESFREWEAD	PTNPALREEM	RIQFNDMNSA	LTTAIPLFAV
0151	QNYQVPLLSV	YVQAANLHLS	VLRDVSFVGQ	RWGFDAATIN	SRYNDLTRLI
0201	GNYTDHAVRW	YNTGLERVWG	PDSRDWIRYN	QFRRELTLTV	LDIVSLFPNY
0251	DSRTYPIRTV	SQLTREIYTN	PVLENFDGSF	RGSAQGIEGS	IRSPHLMIDL
0301	NSITIIYTDH	RGEYYWSGHQ	IMASPVGFSG	PEFTFPLYGT	MGNAAPQORI
0351	VAQLGQGVYR	TLSSLYRRP	FNIGINNOQL	SVLDGTEFAY	GTSSNLPSAV
0401	YRKSGTVDSL	DEIPPQNNNV	PPRQGFHRL	SHVSMFRSGF	SNSSVSIIRA
0451	PMFSWIHRSA	EFNNIIASDS	ITQIPLVKAH	TLQSGTTVVR	GPGFTGGDIL
0501	RRTSGGPFAY	TIVNINGQLP	QRYRARIRYA	STTNLRIYVT	VAGERIFAGQ
0551	FNKTMDTGDP	LTFQSFSYAT	INTAFTFPMS	QSSFTVGADT	FSSGNEVYID
0601	RFELIPVTAT	LEAEYNLERA	QKAVNALFTS	TNQLGLKTNV	TDYHIDQVSN
0651	LVTYLSDEFC	LDEKRELSEK	VKHAKRLSDE	RNLLQDSNEK	DINROPERGW
0701	GGSTGITIQG	GDDVFKENYV	TLSGTFDECY	PTYLYQKIDE	SKLKAFTRYQ
0751	LRGYIEDSQD	LEIYSIRYNA	KHETVNVPGT	GSLWPLSAQS	PIGKCGEPNR
0801	CAPHLEWNP	LDCSCRDGEK	CAHSHHFSL	DIDVGCTDLN	EDLGWVVIK
0851	IKTQDGHARL	GNLEFLEEK	LVGEALARVK	RAEKKWRDKR	EKLEWETNIV
0901	YKEAKESVDA	LFVNSQYDQL	QADTNIAMIH	AADKRVHSIR	EAYLPELSVI
0951	PGVNAAIFEE	LEGRIFTAFS	LYDARNVIKN	GDFNGLSCW	NVKGHVDVEE
1001	ONNQRSVLV	PEWEAEVSQE	VRVCPGRGYI	LRVTAYKEGY	GEGCVTIHEI
1051	ENNTDELKFS	NCVEEEIYPN	NTVTCNDYTV	NQEEYGGAYT	SRNRGYNEAP
1101	SVPADYASVY	EEKSYTDGRR	ENPCEFNRGY	RDYTPLPVGY	VTKELEYFPE
1151	TDKVWIEIGE	TEGTFIVDSV	ELLMEE		

Figure 2. MALDI-TOF MS Coverage Map of the Plant-Produced Cry1A.105 Protein.

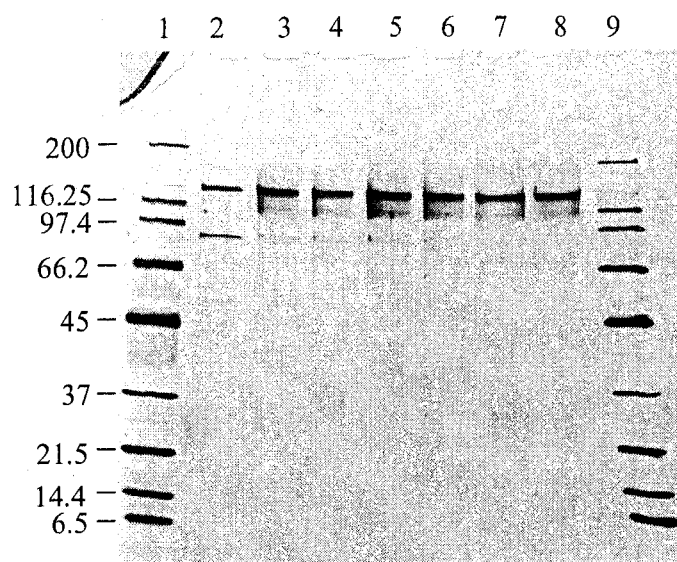
The amino acid sequence of the plant-produced Cry1A.105 protein was deduced from the coding region of the full-length *Cry1A.105* gene present in the grain of MON89034 (Rice et al., Draft). Shaded regions correspond to tryptic peptide masses that were identified from the 130 kDa protein band using MALDI-TOF MS. In total, 43.8% (516 of 1177 total amino acids) of the expected protein sequence was identified.



<u>Lane</u>	<u>Sample</u>	<u>Amount (ng)</u>
1	Precision Plus Dual Color molecular weight markers	—
2	Plant-produced Cry1A.105 protein	20
3	Plant-produced Cry1A.105 protein	40
4	<i>E. coli</i> -produced Cry1A.105 reference standard	20
5	<i>E. coli</i> -produced Cry1A.105 reference standard	40
6	Cry1A.105 trypsin-resistant core standard	20
7	Cry1A.105 trypsin-resistant core standard	40
8	Precision Plus Dual Color molecular weight markers	—

Figure 3. Western Blot Analysis Using N-terminal Peptide Antibody.

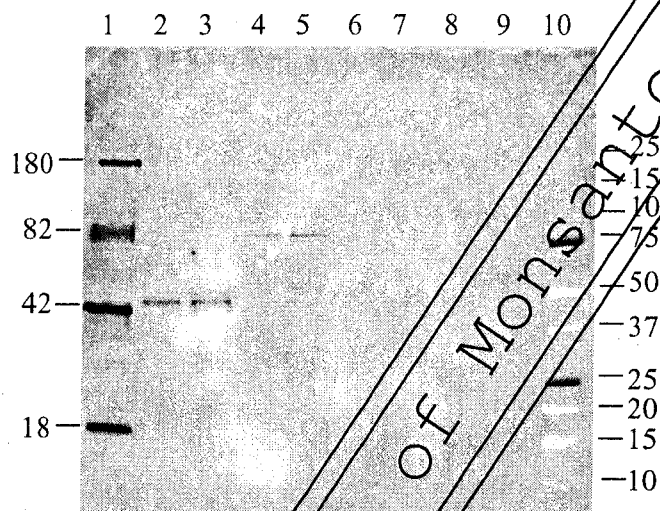
Aliquots of the plant-produced Cry1A.105 protein, *E. coli*-produced Cry1A.105 reference standard, and Cry1A.105 trypsin-resistant core standard were separated by SDS-PAGE and electrotransferred to a PVDF membrane. The membrane was incubated with the N-terminal peptide antibody (lot 42005MG) and immunoreactive bands were visualized using an ECL system. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in lane 1 and lane 8. The 5 min exposure is shown. Empty lanes were cropped.



<u>Lane</u>	<u>Sample</u>	<u>Amount (ng)</u>
1	Broad Range molecular weight markers	—
2	<i>E. coli</i> -produced Cry1A.105 reference standard	96
3	Plant-produced Cry1A.105 protein	48
4	Plant-produced Cry1A.105 protein	48
5	Plant-produced Cry1A.105 protein	72
6	Plant-produced Cry1A.105 protein	72
7	Plant-produced Cry1A.105 protein	96
8	Plant-produced Cry1A.105 protein	96
9	Broad Range molecular weight markers	—

Figure 4. Purity and Molecular Weight Analysis of the Plant-Produced Cry1A.105 Protein.

Aliquots of the plant-produced Cry1A.105 protein and the *E. coli*-produced Cry1A.105 reference standard were separated by a tris-glycine 4→20% polyacrylamide gradient gel and stained with an Invitrogen SilverXpress Silver Staining Kit. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in Lanes 1 and 9. Empty lanes were cropped.



<u>Lane</u>	<u>Sample</u>	<u>Amount (ng)</u>
1	CandyCane Glycoprotein molecular weight standards	—
2	Horseradish Peroxidase (positive control)	48
3	Horseradish Peroxidase (positive control)	96
4	Transferrin (positive control)	48
5	Transferrin (positive control)	96
6	Plant-produced Cry1A.105	48
7	Plant-produced Cry1A.105	96
8	<i>E. coli</i> -produced Cry1A.105 (negative control)	48
9	<i>E. coli</i> -produced Cry1A.105 (negative control)	96
10	Precision Plus Dual Color molecular weight markers	—

Figure 5. Glycosylation Analysis of the Plant-Produced Cry1A.105 Protein.

Aliquots of the plant-produced Cry1A.105 protein, *E. coli*-produced Cry1A.105 reference standard (negative control), horseradish peroxidase (positive control) and transferrin (positive control) were separated by SDS-PAGE (4→20%) and electrotransferred to a PVDF membrane. Where present, periodate-oxidized protein-bound carbohydrate moieties reacted with Pro-Q Emerald 488 glycoprotein stain and emitted a fluorescent signal at 488 nm. The signal was captured using a BioRad Molecular Imager FX. Approximate molecular weights (kDa) correspond to the glycosylated markers loaded in Lane 1 and the dual color markers (used to verify transfer) in Lane 10.

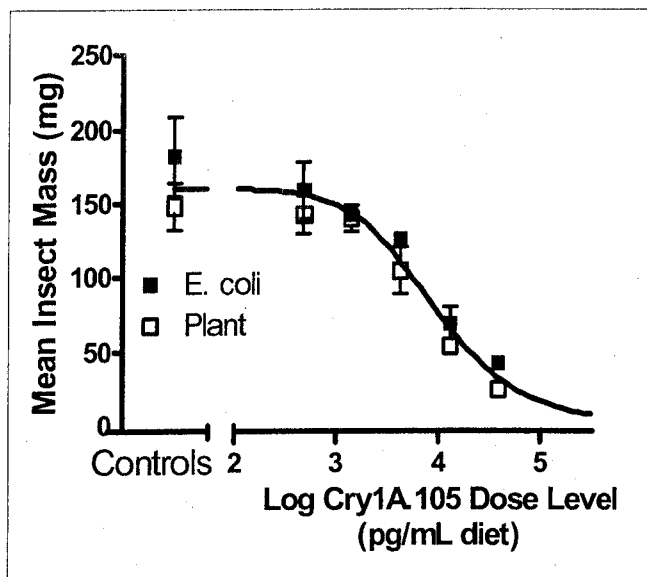
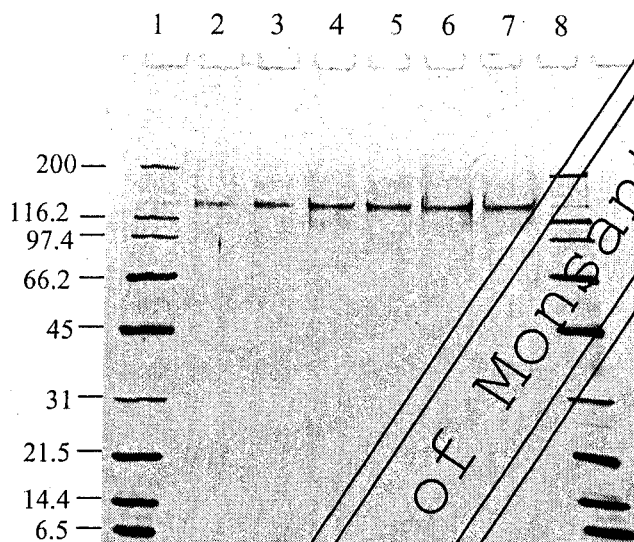


Figure 6. Functional Assay.

Equivalent slopes for dose-response relationships for the *E. coli*-produced Cry1A.105 reference standard and plant-produced Cry1A.105 protein in the corn earworm insect bioassay. Bioassay results from three replicates have been combined for illustration purposes only and are shown on a log concentration scale. Each data point represents the mean of the three bioassays along with the standard error of the mean. The units on the x-axis are expressed in picograms to avoid having negative values.



<u>Lane</u>	<u>Sample</u>	<u>Amount (ng)</u>
1	Broad Range molecular weight markers	—
2	Plant-produced Cry1A.105 protein	48
3	Plant-produced Cry1A.105 protein	48
4	Plant-produced Cry1A.105 protein	72
5	Plant-produced Cry1A.105 protein	72
6	Plant-produced Cry1A.105 protein	96
7	Plant-produced Cry1A.105 protein	96
8	Broad Range molecular weight markers	—

Figure 7. Storage Stability (Day 18) of the Plant-Produced Cry1A.105 Protein Stored in a 4°C Refrigerator.

SDS-PAGE analysis was performed on samples refrigerated for 18 days. Gels were stained with an Invitrogen SilverXpress® Silver Staining Kit. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in Lanes 1 and 8. Empty lanes were cropped.

Appendix 1. List of Applicable SOPs.

SOP Number	SOP Title
BR-ME-0388-02	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
BR-ME-0392-01	Western Blot Analysis (Immunoblotting)
BR-PO-0537-02	Characterization of Proteins for Use as TCR Substances
BR-EQ-0599-02	Bio-Rad GS-710 and GS-800 Densitometers
BR-PO-0655-02	Biotechnology Analytical Protein Standards Program
BR-PO-0722-01	Production and Isolation of Protein Standards
BR-EQ-0783-01	Applied Biosystems Voyager DE Pro Biospectrometry™ Workstation
BR-ME-0802-01	Protein Fragmentation via In-Gel Trypsin Digestion
BR-ME-0924-01	Electrotransfer of Proteins to Membranes
BR-ME-0932-02	Assessment of Immunoreactive Bands from Western Blots Exposed to X-Ray Films Using Bio-Rad GS-710 and GS-800 Densitometers
BR-EQ-0935-01	Konica SRX X-Ray Film Processors
BR-ME-0956-02	Protein Percent Purity and Apparent Molecular Weight Determination
BR-ME-0985-01	Zip-Tip Fractionation of Proteins and Peptides
BR-ME-0973-01	Drying of Polyacrylamide Mini-Gels Using Invitrogen Gel Drying System (Adaptation of Invitrogen Gel Drying Procedure)
BR-ME-0994-01	Coomassie Blue Staining of Polyacrylamide Gels
AG-EQ-0996-01	Adjustable and Fixed Volume Pipets
AG-EQ-0662-01	Analytical & Top-Loading Balances
BR-ME-0044-03	Diet Incorporation Insect Bioassay for Use in Determining Biological Activity

Monsanto Company
Product Characterization Center
Analytical Protein Standards Programs

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M8L 19960
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Appendix 2. Certificate of Analysis APS Lot number 20-100073

Analytical Protein Standard Certificate of Analysis

MONSANTO

ANALYTICAL PROTEIN STANDARDS

Sample Information:

Name of APS <i>E. coli</i> -produced Cry1A 105 protein	APS Lot Number 20-100073	Recertification Date August 31, 2005
Common or Alias Name(s)	Historical APS Lot Number(s)	Storage Requirements (until use) -80 °C
Source: Fermentation of <i>Escherichia coli</i> containing the pMON96861 expression plasmid		Comment(s) None
Additional Background Information:		

Characteristic	Method	Assay Date	Result
Concentration	Amino Acid analysis	10 February 2005	1.2 mg/mL (total protein)
Purity	SDS-PAGE/Densitometry	14 February 2005	92%
Molecular weight	SDS-PAGE/Densitometry	14 February 2005	131.5 - 58.1 kDa
Molecular weight	MALDI-TOF MS	18 February 2005	133604.32 Da
Identity	Immunoblot	18 February 2005	Confirmed
Identity	N-terminal sequence	18 February 2005	Confirmed 131.5 kDa - MDNPNPINE(C)IPY(N) 88.1 kDa - MDNPNPINE(XIP(Y)(N)
Identity	MALDI-TOF MS	17 February 2005	Confirmed sequence 42.6% coverage of expected sequence
Activity	Insect Bioassay	10 March 2005	EC ₅₀ = 5.8 ng Cry1A 105/mL diet

Buffer composition: 25 mM CAPS, pH 10.3, 1 mM benzamidine HCl, 0.1 mM EDTA, and 0.2 mM DTT

Physical description: Clear solution

Short-term storage stability (28 days) was evaluated during the certification process. Based upon the criteria provided in Characterization Plan 20-100073, no significant degradation was observed for samples stored at 4 °C, -20 °C and -80 °C.

Purity corrected concentration is 1.1 mg/mL (1.2 mg/mL \times 0.92 = 1.1 mg/mL)

John M. R. [Signature]
Quality Assurance Specialist

May 6, 2005
Date

[Signature]
Testing Facility Management

5/6/05
Date

[Signature]
Analytical Protein Standards Officer

5/6/2005
Date

Appendix 3. Certificate of Analysis APS Lot number 60-100076

Analytical Protein Standard Certificate of Analysis

MONSANTO

ANALYTICAL PROTEIN STANDARDS

Sample Information:

Name of APS Plant-produced Cry1A.105 Protein		APS Lot Number 60-100076	Recertification Date —
Common or Alias Name(s) YieldGard II Cry1A.105	Historical APS Lot Number —		Storage Requirements -80 °C
Source YieldGard II corn grain MON 89034			Comment(s) None
Additional Background Information Historic lot 7555526			

Characteristic	Method	Assay Date	Result
Concentration	Quantitative immunoblot	21 July 2005	3.0 ng/μL
Purity	SDS-PAGE/Densitometry	21 July 2005	100% ^a
Molecular weight	SDS-PAGE/Densitometry	21 July 2005	130.0 KDa
Identity	Immunoblot	21 July 2005	Confirmed ^b with anti-Cry1A.105 Ab
		22 July 2005	Confirmed ^b with N-terminal peptide Ab
Identity	MALDI-TOF MS	4 August 2005	Confirmed sequence 43.8% coverage of expected sequence
Composition	Glycosylation	26 July 2005	None detected
Activity	Insect Bioassay	5 August 2005	EC ₅₀ = 7.4 ng of Cry1A.105 protein/mL diet

^a Purity is the summation of multiple bands.

^b Immunoreactive band of expected size was observed.

Buffer composition: 50 mM CAPS, 1 mM PMSF, 2 mM benzamidine-HCl, 1 mM EDTA, 800 mM NaCl, 30% (v/v) ethylene glycol, pH 10.0

Physical description: Clear solution

Short-term storage stability (18 days) was evaluated during the certification process. Based upon the criteria provided in Characterization Plan 60-100076, no significant degradation was observed when the sample was stored at 4°C.

Purity corrected concentration is 3 ng/μL (3 ng/μL × 1 = 3 ng/μL)

Jean M. Reida-Heath
Quality Assurance Specialist

Oct. 28, 2005
Date

James M. Lee
Testing Facility Management

11-1-2005
Date

[Signature]
Analytical Protein Standards Officer

11/1/2005
Date

Appendix 4. Insect Bioassay Summary

Monsanto Company
Biotechnology Regulatory Sciences

Plan # 60-100076
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Insect Bioassay Summary for Characterization of the Cry1A.105 Protein Purified from the Corn Grain of MON 89034 and Comparison of the Physicochemical and Functional Comparability of the Plant- Produced and *E. Coli*-Produced Cry1A.105 Proteins

Purpose:

The purpose of this analysis was to compare the biological activity between plant-produced Cry1A.105 protein isolated from the corn grain of MON 89034 and a reference *E. coli*-produced Cry1A.105 protein by determining EC₅₀ values for each protein in a corn earworm (CEW) diet-incorporation insect bioassay. The EC₅₀ value is defined as the level of Cry1A.105 protein in the diet that results in 50% growth inhibition.

Materials:

Plant-Produced Cry1A.105 protein, *E. coli*-produced Cry1A.105 Reference Standard Protein and Control Substance:

The reference standard, an *E. coli*-produced Cry1A.105 protein (lot # 20-100073) and a plant-produced Cry1A.105 protein (lot # 00-100076) isolated from the corn grain of MON 89034, were received from the Monsanto Product Characterization Center (PCC). The total protein concentration of the *E. coli*-produced Cry1A.105 protein aliquots was 1.2 mg/mL, with a purity of 92%, and a purity corrected concentration of 1.1 mg Cry1A.105/mL. The Cry1A.105 protein concentration of the plant-produced Cry1A.105 protein aliquots was 3 µg/mL. The control substances used in the bioassays were buffers of the same composition used for storing the *E. coli*-produced and plant-produced proteins. The *E. coli*-produced reference standard was suspended in 25 mM CAPS, pH ~10.3, 1 mM benzamidine-HCl, 0.1 mM EDTA, 0.2 mM DTT buffer solution. The composition of the plant storage buffer was 50 mM CAPS, 1.0 mM PMSF, 2.0 mM benzamidine-HCl, 1 mM EDTA, 0.8 M NaCl, 30% (v/v) ethylene glycol, pH 10.0. The plant-produced and *E. coli*-produced Cry1A.105 proteins were stored at 4° C and -80° C, respectively, and the buffers for both proteins were stored at 4° C.

Methods:

Insects. CEW were obtained from Benzon Research Inc. Insect eggs were incubated at a temperatures ranging from 10° C to 27° C, to achieve the desired hatch time.

Bioassays. CEW were used to measure activity of the plant- and *E. coli*-produced Cry1A.105 protein samples in accordance with the Monsanto SOP BR-ME-0044-03. The bioassay was replicated three times on separate days with separate batches of insects. The plant- and *E. coli*-produced proteins were run in parallel during each bioassay. Each bioassay replicate for the *E.*

coli-produced and plant-produced Cry1A.105 proteins consisted of a series of five protein levels yielding a dose series ranging from 0.00048 – 0.039 µg Cry1A.105 protein/mL diet with a 3-fold separation factor between dose levels. This dose series was chosen to adequately characterize the dose-effect relationship for the proteins from both sources. Each dose level, including the control, had an equivalent volume of buffer added to the dosing solution. The Cry1A.105 protein dosing solutions were prepared by diluting the protein with purified water and incorporating the dilution into an agar-based insect diet (Southland). Diet mixture was then dispensed in 1 mL aliquots into a 128 well tray (#BIO-BA-128, CD International, Pitman, NJ). Insect larvae were placed on these diets using a fine paintbrush, with a target number of 16 insects per treatment. The infested wells were covered by a ventilated adhesive cover (#BIO-CV-16, CD International, Pitman, NJ) and the insects were allowed to feed for a period of 6-7 days in an environmental chamber programmed at 27° C, ambient relative humidity and a lighting regime of 14h:10h, light:dark. The combined weight of the surviving insects at each dose level for each source of protein was recorded at the end of the 6-7 day incubation period.

Dose-response modeling and Results:

The following three-parameter logistic model, with an extra parameter for the change in variation with the expected weight (equation below), was used to model the dose-response curves for each protein source and each replicate under the PROC NLMIXED procedure in SAS:

Equation:

$$W_t = \frac{W_0}{1 + \left(\frac{\text{DietDose}}{EC50} \right)^B} + e$$

$$\text{Var}(e) = \left\{ \frac{s \cdot W_0}{1 + \left(\frac{\text{DietDose}}{EC50} \right)^B} \right\}^2$$

where W_t is the average CEW larvae weight and DietDose is the Cry1A.105 protein diet dose level. The residual variation was assumed to be proportional to the expected mean weight. The parameters that are included in the model are W_0 which represents the expected weight at $\text{DietDose} = 0.0$, EC_{50} which represents the concentration needed to inhibit the growth of the target insect by 50%, B which reflects the rate of the weight loss as DietDose increases, s which represents the proportion of the standard deviation to the expected weight, and e which denotes the residual (error).

The EC₅₀ values for each replicate bioassay are summarized in Table 1, and the dose response relationships for the two protein sources are illustrated in Figure 1. The ranges of the estimated EC₅₀ values overlapped for the two sources of protein. The EC₅₀ values for the plant-produced protein ranged from 0.0055 to 0.0089 µg Cry1A.105/mL diet and the EC₅₀ values for the *E. coli*-produced protein ranged from 0.0053 to 0.0169 µg Cry1A.105/mL diet. The mortality rate of the control substance groups was less than 20% in each insect bioassay, which met the acceptability criteria for the assay.

Table 1. EC₅₀ values and standard errors for *E. coli*- and plant-produced Cry1A.105 proteins in a CEW diet-incorporation bioassay

Bioassay ID	EC ₅₀ Estimates (µg Cry1A.105/mL diet)	Standard Error (µg Cry1A.105/mL diet)
<i>E. coli</i> -produced replicate 1	0.013	0.0025
Plant-produced replicate 1	0.0055	0.0014
<i>E. coli</i> -produced replicate 2	0.0053	0.0022
Plant-produced replicate 2	0.0089	0.0018
<i>E. coli</i> -produced replicate 3	0.017	0.0021
Plant-produced replicate 3	0.0077	0.0012
Mean EC ₅₀ for <i>E. coli</i> -produced Cry1A.105 protein: 0.012 µg Cry1A.105/mL diet		
Mean EC ₅₀ for plant-produced Cry1A.105 protein: 0.0074 µg Cry1A.105/mL diet		
Standard Deviation for <i>E. coli</i> -produced Cry1A.105 protein: 0.0062 µg Cry1A.105/mL diet		
Standard Deviation for plant-produced Cry1A.105 protein: 0.0017 µg Cry1A.105/mL diet		

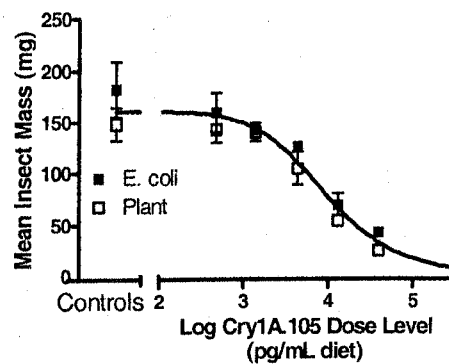


Figure 1. Corn earworm dose-response relationships for Cry1A.105 from both protein sources in a diet-incorporation bioassay. Each data point represents the mean of the insect weights for the bioassay replicates ($n = 3$) at each dose level along with the standard error of the mean. Dose units on the x -axis are expressed in log picograms Cry1A.105/mL diet. Dose-response curves were prepared using GraphPad Prism software (v. 4.02 for Windows).

Conclusions:

The mean EC_{50} values for the *E. coli*-produced protein and the plant-produced protein were comparable and were determined to be 0.012 and 0.0074 μg Cry1A.105/mL diet, respectively.

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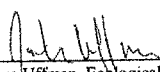
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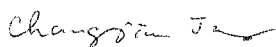
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Product Characterization Center
Analytical Protein Standards Program

Lot 60-100075
MSL 20071
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Characterization Plan Title

Characterization of the Cry2Ab2 Protein Purified from the Corn Grain of
MON 89034 and Comparison of the Physicochemical and Functional Properties of
the Plant-Produced and *E. coli*-Produced Cry2Ab2 Proteins

Authors

Kanthasamy Karunanandaa, Ph.D., Jamie J. Thorp, Michael E. Goley,
Steven L. Levine, Ph.D., and Andre Silvanovich, Ph.D.

Report Completed On

January 12, 2006

Performing Laboratory

Monsanto Company
Product Characterization Center
Biotechnology Regulatory Sciences
800 North Lindbergh Boulevard
St. Louis, Missouri 63167

Laboratory Project ID

MSL Number: 20071
Analytical Protein Standards Lot 60-100075

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Lot 60-100075
MSL-20071
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Statement of Compliance

This protein characterization was conducted in compliance with the Good Laboratory Practice Standards at 40 CFR Part 160. While not a study as defined by 40 CFR Part 160, it was intended to evaluate the functional and physicochemical properties of the plant-produced Cry2Ab2 protein isolated from the corn grain of MON 89034 to fulfill the requirements of 40 CFR Part 160.105(a). The experimental work was conducted in adherence with the characterization plan and facility Standard Operating Procedures.

Submitter

Date

Scott Miller
Sponsor Representative

1/12/06
Date

Analytical Protein Standards Officer

1/12/2006
Date

ILV
Project Lead, Author

1/12/2006
Date

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Quality Assurance Unit Statement

Characterization Plan Title: Characterization of the Cry2Ab2 Protein Purified from the Corn Grain of MON 89034 and Comparison of the Physicochemical and Functional Properties of the Plant-produced and *E. coli*-Produced Cry2Ab2 Proteins

Characterization Plan Number: 60-100075

Reviews conducted by the Quality Assurance Unit confirm that the final report accurately describes the methods and standard operating procedures followed, and accurately reflects the raw data of the characterization plan.

Following is a list of reviews conducted by the Monsanto Regulatory Quality Assurance Unit on the characterization plan reported herein.

Date of Inspection or Audit	Phase	Date Reported to APS Officer	Date Reported to Management
05/31/2005	Glycosylation	06/03/2005	06/03/2005
06/15/2005	Insect Bioassay	06/15/2005	06/15/2005
08/26/2005	Raw Data Audit	09/07/2005	09/07/2005
11/15/2005	Draft Report Audit	11/30/2005	11/30/2005

Joan Rejda-Heath
Joan Rejda-Heath, Ph.D.
Quality Assurance Specialist
Monsanto Regulatory
Monsanto Company

Jan. 12, 2006
Date

Characterization Plan Information

Characterization Plan Number: 60-100075

MSL Number: 20071

Title: Characterization of the Cry2Ab2 Protein Purified from the Corn Grain of MON 89034 and Comparison of the Physicochemical and Functional Properties of the Plant-produced and *E. coli*-Produced Cry2Ab2 Proteins

Testing Facility: Monsanto Company
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Contributors: Tandi Collisson, Tallis P. Brown, Chris Dalton, Richard S. Thoma, and Josh Uffman

Plan Initiation Date: May 18, 2005

COA Completion Date: September 8, 2005

Report Completion Date: January 12, 2006

Records Retention: All characterization plan specific raw data, electronically stored Atlas files, the characterization plan, and final report will be retained at Monsanto Company, St. Louis.


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Characterization Plan Certification

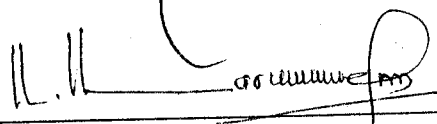
The results reported in this report accurately reflect the data generated under Characterization Plan Number 60-100075.

Signatures of Final Report Approval:



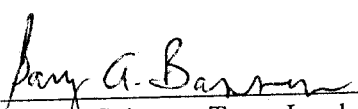
Analytical Protein Standards Officer

1/12/2006
Date



Project Lead, Author

1/12/2006
Date



Protein Sciences Team Lead

1/12/2006
Date

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