EBI FOOD SAFETY

PCR of phage DNA:

Phage DNA does not need to be extracted from bacteriophage, rather phages can be used directly in the PCR reaction.

Protocol:

1. Remove a plaque from the top-agar with a Pasteur pipette and transfer to an eppendorf cap with 200 µl of SM-phage buffer.
2. Incubate at 30˚C for 30 min while shaking.
3. Use 1µl of the supernatant as template for the PCR reaction.

PCR settings:

Cycle 30x
30 sec 94˚C denaturing
60 sec 50-53˚C annealing
60 sec 68-72˚C elongation

Using the following primers:

Forward: 5'-ccttcacgcatctttgttacag (binds P100 genome bp:108867-108888)

Reverse: 5'-cagggttgtatttaggtactc (binds P100 genome bp: 109957-109937)

Samples containing P100 will result in fragments of 1090bp length being generated.

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