Quality Control Assays

Absolute titer: Infection of a mid-log culture of *E. coli* ER2738 followed by plating yielded 1.0 x 10^13 pfu/ml.

Sequence verification: Sequencing of M13KE was carried out with ~96 glll Sequencing Primer (20-mer) (NEB #S1259S). Single digests of M13KE vector by KpnI and EagI were carried out to confirm presence of cloning sites.

Wild-type M13 contamination: A plate with >10^9 blue plaques showed no white plaques.

Protocol:

M13 Amplification
1. Grow overnight culture of *F*+ *E. coli* (e.g. ER2738).
2. Inoculate a 20 ml culture in a 250 ml Erlenmyer flask with 200 µL overnight *E. coli* culture. Add 1 µL phage suspension. Shake flask at 37°C, 250 rpm for 4–5 hrs.
3. Remove cells by centrifugation at 4500 g for 10 min. Transfer supernatant to a fresh tube. Repeat centrifugation.

M13E Amplification
1. Grow overnight culture of *F*+ *E. coli* (e.g. ER2738).
2. Inoculate a 20 ml culture in a 250 ml Erlenmyer flask with 200 µL overnight *E. coli* culture. Add 1 µL phage suspension. Shake flask at 37°C, 250 rpm for 4–5 hrs.
3. Remove cells by centrifugation at 4500 g for 10 min. Transfer supernatant to a fresh tube. Repeat centrifugation.

4. Transfer top 16 ml of supernatant to a new tube and add 4 ml of 2.5 M NaCl/20 % PEG-8000 (w/v). Briefly mix. Precipitate phage for 1 hr or overnight at 4°C.
5. Pellet phage by centrifugation at 12000 g for 15 min. Decant supernatant. Resuspend pellet in 1 ml TBS. Transfer to an eppendorf tube. Spin briefly to remove any cell debris.
6. Transfer supernatant to a fresh tube. Add 200 µl of 2.5 M NaCl/20 % PEG-8000. Incubate on ice for 15-60 min. Spin 12000 –14000 rpm in a benchtop centrifuge for 10 min. Discard supernatant. Spin again briefly and remove remaining supernatant with pipette. Resuspend pellet in 200 µL TBS. For long-term storage at ~20 °C, add 200 µL sterile glycerol.

To scale up the above protocol, use multiple culture flasks. Alternatively, after incubating 20 ml culture for 2 hrs, add the entire culture to 1L LB. Incubate the large culture for 4 hrs, then modify the protocol to remove cells and purify phage.

Supplied in: 1X TBS and 50% glycerol.

References: