Quick Start Protocol PhD Phage Display Peptide Library Kit v2

Overview

1. Streak *E. coli* K12 ER2738 (NEB #E4104S) onto LB/Tet solid medium. Grow overnight at 37°C.
2. Select phage-target capture method: Prepare capture surface by coating with target and blocking, or select affinity (magnetic) beads method.
3. Incubate 10 µl phage library (or 10^{11} pfu) with target (on surface or free in solution, see Step 2), 10–60 min.
4. Wash unbound phage away with 10 x 1 ml washes of TBST.
5. Release bound phage with low pH buffer or with a known ligand to the target.
6. Amplify the selection's phage eluate in 20 ml *E. coli* culture for 4–5 hr.
7. Concentrate phage from culture supernatant by adding 1/5 volume of 20% PEG/2.5 M NaCl.
8. Titer a) unamplified eluate from Step 5, for future reference, and b) enriched phage pool from Step 7, to determine volume of input for round 2 selection.
9. Repeat steps 3–6, with 10^{11} pfu input of enriched phage pool each time, increasing selection or wash stringency if desired.
10. After three rounds of selection, do not amplify elution. Instead, titer the third-round elution to obtain individual plaques for phage sequencing reactions.
11. Post-panning analysis: Identify sequence motifs and/or carry out binding studies with selected clones.