Fusion BioLabs Short Protocol for Transformation of Phage Display Cells

1. Product description

Catalogue #	Strain	Amber Suppressor (Y/N)	Main application	Genotype
PDCE	ER2738	N	Phage display library construction	*F'proA+B+ lacIq Δ(lacZ)M15 zzf::Tn10 (tetr)] fhuA2 glnVΔ(lac-proAB) thi-1Δ(hsdS-mcrB)5*
PDCS	SS320	Y	Antibody fragment expression	*[F'proA+B+ lacIqlacZΔM15 Tn10 (tetr)] hsdR mcrB araD139 Δ(araABC-leu)7679 ΔlacX74 galUgalK rpsL thi*
PDCT	TG1	N	Phage display library construction	*[F´ traD36 proA+B+ lacIqZ ΔM15] supE thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5(rK- mK-)*

2. Panning Protocol using biotin labelled antigen target

1) Take Recovery Medium and 1.5 ml or 2.0 ml sterilized microcentrifuge tube readily available at room temperature (one tube for each transformation reaction).

2) Place the microcentrifuge tubes on ice (one microfuge tube for each transformation reaction).

3) Remove Phage Display Cells from the -80 °C freezer and place on wet ice until they thaw completely (10-15 minutes).

4) When the cells are thawed, mix them by tapping gently. Aliquot 50 µL of cells into the chilled microcentrifuge tubes on ice.

5) Add 1 µL monoclonal phage ELISA validated and/or Sequencing confirmed candidate (from panning of scFv, Fab, VHH, affibody or peptide phage library) to the 50 µL of cells on ice for 10 min. Stir briefly with pipette tip; do not pipet up and down to mix, which can introduce air bubbles and warm the cells.

6) Heat shock at 42°C water bath for 30 seconds

7) Put the heat shocked microfuge tube on ice immediately; add 450 µL of Recovery Medium to the microfuge tube.

8) Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.

9) Spread up to 100 µL of transformed cells on 2xYT agar plate containing the appropriate antibiotic.

10) Incubate the plates overnight at 37 °C.

11) Transformed single clone can be further grown in 2xYT medium to isolate for down-stream application, such as DNA plasmid preparation, or in TB medium for antibody fragment expression.

3. Media recipes

3.1 2xYT Agar plates for plate transformed cells (per liter)

16 g tryptone
10 g yeast extract
5 g NaCl
15 g agar

Add all components to distilled water. Adjust pH to 7.0 with 5N NaOH. Autoclave and cool to 55 °C.

3.2 2xYT Medium for growth of transformants (per liter)

16 g tryptone
10 g yeast extract
5 g NaCl
15 g agar

Add all components to distilled water. Adjust pH to 7.0 with 5N NaOH. Autoclave and cool to 55 °C.

3.3 TB media for antibody fragment expression (per liter)

12 g tryptone
24 g yeast extract
12.5 g K_2HPO_4
2.3 g KH_2PO_4
4 ml 100% glycerol

Add all components to deionised water. Autoclave and cool to 55 °C.