Genomikon protocol

Bead assembly

Buffers

W – Wash buffer. 20 mM Tris, 2 mM EDTA, 200 mM NaCl, pH 8.0
E – Elution buffer. 10 mM NaOH, unbuffered.
L – Ligation master mix. Per 7 ul mix, for ~10 ul total reaction volume: 1 ul Fermentas T4 DNA ligase 5 U/ul (EL0011), 1 ul 10x ligation buffer, 5 ul ddH2O.
N – Neutralization buffer (same as 50X TE). 0.5M Tris, 50 mM EDTA, pH 8.0.
T – Transformation buffer. 50 mM CaCl2.

DNA

1: Beads-AncXA 50 ul
2: OriblaAB 0.025 pm/ul 30 ul
3: LaczBA 0.025 pm/ul 30 ul
4: RfpAB 0.025 pm/ul 30 ul
5: LinkerBA 0.25 pm/ul 10 ul
6: CapAX 1 pm/ul 20 ul
7: CapBX 1 pm/ul 20 ul
8: pUC19 1 ng/ul 20 ul

Use 5 ul of Beads-AncXA per 0.05 pmol reaction, each 5 ul contains 0.20 pmol of AncXA annealed to 5 ul of NEB Oligo-dT(25) beads.

Design construct. The construct should begin with an Anchor, end with a Cap, contain a Rep (OriblaAB) and strictly alternate AB and BA parts. The Anchor and Cap have ends which allow spontaneous recircularization.

Wash beads. Resuspend 5 ul beads in 50 ul wash buffer. Pull beads to the side with a magnet, then remove all liquid, including any droplets on side of tube. This is a wash step. Do a second wash.

Anneal anchor. Add 2 ul of desired anchor (0.2 pm) to bead pellet, then add 10 ul of wash buffer. Mix and resuspend beads. Annealing should take place in less than 1 minute. Wash 2X.

Ligate first part. Add 2 ul of first part (0.05 pm) to bead pellet, then add 7 ul of ligation mix (1 ul ligase, 1 ul 10x ligation buffer, 5 ul H2O). Mix and thoroughly resuspend. Incubate for 5 minutes at room temperature. Wash 2X.

Repeat ligations for additional parts in design. Open reading frame parts (red, blue, ori) should be added at 3 ul (0.075 pm), the white is a short linker, and requires only 1 ul (0.25 pm). The last ligation can include both the cap (2 ul, 2 pm) and the last part. Wash 2X.

Elute. Add 20 ul of elution buffer (10 mM NaOH) to bead pellet. Resuspend, then pull beads and extract supernatant containing the construct. The supernatant can be added directly to competent cells for transformation. If some is retained to run on a gel, it should be neutralized with 2 ul of 50X TE before storage.

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