Ligation-Mediated PCR (LM-PCR)

Repair of Overhangs

1. Isolate genomic DNA using Qiagen DNAeasy spin column.
2. Use 10μg DNA per reaction. Add 3.3μL T4 DNA polymerase, 5μL 10X T4 polymerase buffer, 0.5μL 10mM dNTP (100μM final), and 0.5μL BSA. Make reaction up to 50μL with dH_2O.
3. Incubate for 15 min at 12ºC.
4. Stop reaction with 1μL 0.5mM EDTA at 75ºC for 20 min.
5. Purify DNA over Qiagen gel purification column. Elute in 30μL.

Linker preparation

1. Dilute oligos (BW-1 and BW-2) to 100 μM in dH_2O.
2. Use 2.5μL of each oligo in total of 50μL TENS buffer (250mM NaCl, 10mM Tris pH 8.0, 0.2mM EDTA).
3. Heat to 94ºC for 2min, 70ºC for 5min, 55ºC for 5min, 50ºC 5min, 45ºC 5min, 40ºC 5min, 35ºC 5min, 30ºC 5min, 23ºC 5min, and finally on ice for 30 min. Always keep annealed oligos on wet ice, never greater temperature.
4. Dilute annealed linkers with 200μL TENS buffer for 1μM stock. Use 1μL of 1μM annealed linker per ligation reaction.

Ligation

1. Add: 10μL repaired genomic DNA, 1μL of 1μM annealed linker, 1μL T4 DNA ligase (4U/μL), 2μL 10X T4 ligase buffer, and 6μL dH2O.
2. Incubate 16hr at 16ºC in PCR machine.

Quantitative PCR (QPCR)

1. Perform QPCR. Use 2-5μL ligated, repaired genomic DNA with linker-2 and GFP#3 primers. Use GFP #7 primers as a normalization control for the amount of DNA in reactions.