Vector/Insert Digest
Last updated: December 10, 2010

Description: Digest circular plasmid with appropriate restriction enzymes. It is important to ensure that the digested piece does not contain restriction sites in the middle of the sequences of interest.

Total time: 4.5h Lab time: 45min Wait time: 3.5h

1. Thaw appropriate buffer, usually Roche buffer B. Look at charts on freezer door for the best buffers to use for certain enzymes.
2. Pre-heat 37C dry bath and 65C dry bath.
3. Prepare double digest reaction (keep all enzymes and buffers on ice!)

   4ul 10x buffer
   1-1.5ug plasmid DNA (~1.5ug for full digest, only 1ug for digest check)
   0.5ul enzyme 1
   0.5ul enzyme 2
   fill to volume in ultrapure H20
   = **40ul** digest reaction

   Restriction enzymes can be found in blue coolers in the -20C freezer. Buffers are either on the bottom shelf in the -80C freezer or in the -20C freezer.

4. Digest plasmid for at least 1.5hr at 37C (only 1h needed for digest check)
5. Prepare a gel.
6. **If insert digest, you can skip AP treatment, steps 6 and 7*
   Incubate with alkaline phosphatase (AP, in blue cooler in -20C freezer) for 1hr at 37C. This step will prevent self-ligation

   4ul AP buffer
   1ul AP enzyme

7. Heat shock at 65-70C for 5-10min (kills AP; use 70C tube dry bath)
   **If running gel next day, can store vector in freezer overnight*
8. Run the vector on a gel. If using large (~20ul) wells, split into two tubes.
9. Gel-purify relevant band(s).
10. Nano-spec DNA to get concentration.
11. Store purified vector DNA in -20C.