Ligation (sticky-ends)

Special Reagents:

Crystal violet (10 mg/ml solution in water)
Agarose gel reagents (agarose, TBE)
T4 ligase (and included buffers)
Restriction enzymes and buffers

1. Prepare a standard miniprep of plasmid DNA (including target vector and plasmid containing fragment to be subcloned in to target vector).

2. **Digest 5 µL of each miniprep in a 20 µL reaction**. This usually means 1 µL of each of the appropriate restriction enzyme(s), 2 µL of 10X buffer, and 11 µL of ddH_2O.

3. Run products of this reaction out on an **agarose gel stained with crystal violet.** To prepare gel, mix and boil gel as usual, but before pouring add 10 ug/ml crystal violet (ie, 50 µL of 10 mg/ml crystal violet solution in 50 mL gel solution). **Gel must be run in TBE with crystal violet as well!!!**

4. Visualize gel on a standard light box (no UV) and (with a razor blade) cut out bands corresponding to target vector and fragment, and transfer each to microfuge tubes.

5. Gel-purify DNA from these bands using the Qiaquick gel extraction kit (follow kit instructions exactly as written).

6. Set up ligations as follows (use 0.2 µL PCR tubes):
   - 5 µL vector DNA (from step 5)
   - 9 µL insert fragment DNA (from step 5)
   - 3 µL ddH_2O (sterile)
   - 2 µL 10X T4 ligase buffer (well-dissolved—this stuff precipitates during storage and must be vigourously mixed to re-dissolve the ATP, DTT, etc.)
   - 1 µL T4 ligase (roughly 2.5 Weiss units)

7. Incubate at 16^0C overnight (in PCR machine), or 2 hours at room temperature.

8. Heat-inactivate digestion mix (65^0C for 15 minutes).

9. Transform entire digestion mix in to MC1061 heat-shock competent E. coli. Plate on appropriate selection media.