

**Cloning Procedure-** For use with Invitrogen TA cloning kits using vector pCR2.1 and chemically competent cells INVaF'

- > See Ligation Protocol
  - > See Making agar plates protocol
1. Be careful for sample contamination- both from other samples but also from “environmental” sources.
  2. Wear gloves and wash bench top before working.
  3. Remove ligations from -20 freezer in Cloning room and thaw on ice
  4. Get super-competent cells and place immediately on ice (they are very fragile so handle carefully). For msat isolation, you will need 2 tubes (100uL total)
  5. Thaw the SOC. This must be at room temp. before use. Don't use it if there is sediment or it is cloudy.
  6. Turn on water bath and set to 42 degrees.
  7. Turn on incubator and set temperature to 37 degrees
  8. Divide cells... 1/4<sup>th</sup> reactions normal. (12.5uL cells per tube)
  9. Once everything is thawed, In each tube of cells place 0.5 uL ligation. Keep on ice. Mix gently with pipette tip.
  10. Cool on ice for 30-40 minutes.
  11. Heat shock in water bath at 42 degrees for **EXACTLY** 30 seconds. It is extremely critical that samples are taken directly from ice to warm water.
  12. **IMMEDIATELY** place back on ice for 5 minutes.
  13. After 5 minutes, add 100 uL room temperature (or 37 degrees) SOC to each sample.
  14. Place securely in orbital shaker at 37 degrees for one hour. Shake at approximately 200rpm. You may need to tape the samples down!
  15. While bacterial transformations are shaking, place x-gal, dry, and warm agar plates in incubator.
  16. After one hour, remove samples. Plate SOC/Bacteria mix on each plate. sterile glass beads to spread. Shake gently. Do this quickly to ensure that samples are not cooled below 37 degrees for more then a few minutes.
  17. Place upside down in incubator immediately after plating.
  18. Once all samples are plated, cover them all in tin foil and let grow for >15 hours.

- See protocol for picking colonies in the Glenn manual (step VIII)
- See clonecheck protocol in the Glenn manual (step VIII)
- See Exosap and Cycle sequencing protocols
- See ethanol precipitation protocol
- Enjoy sequences!