

Transformation of XL-10 Gold Cells

- Determine antibiotic resistance of plasmid. Get LB/Antibiotic plate and dry upside-down in warm room till ready to use.
- Take an aliquot of plasmid and dilute to (.1-50ng) 50ng/ μ l*. Save stock in plasmid box in freezer.
- Thaw XL Gold cells on ice.
- Place 150 μ l into 14 μ l chilled Falcon tube.
- Add 2 μ l B-mercaptoethanol to cells. Incubate 10 min on ice. (Timing not extremely critical.)
- Add 10ng of plasmid (in 1 μ l) to mixture (or all of ligation mixture) to cells.
- Incubate on ice for 30 minutes.
- Heat shock at 42C for 30S. Be rather exact!
- Plunge tubes immediately into ice for 2 minutes.
- Add 300 μ l LB broth.
- Place tubes in shaker incubator for 45 minutes. Timing important!
- For supercoiled templates, plate 1/2 the culture on LB/antibiotic plate using half-moon pattern. (Plate entire culture for ligated templates.)
- Leave upside down in warm room for apprx. 14hr then check for growth.

After colonies grow on plate:

To prepare glycerol stock grow 5ml culture:

- Pick one colony from plate w tip and transfer into 5ml of LB broth containing proper antibiotic. Put into 100ml culture if plan on prepping plasmid for use.

Amp liquid cultures: final conc = 100 μ g/ml

In a 100ml culture: 100ml broth plus 50 μ l Amp stock.

In a 5ml culture: 5ml broth plus 2.5 μ l Amp stock.

Kan liquid cultures: final conc = 20 μ g/ml

In a 100ml culture: 100ml broth plus 200 μ l Kan stock.

In a 5ml culture: 5ml broth plus 10 μ l Kan stock.

- Grow in shaker incubator overnight. (If prepping plasmid, begin maxiprep here).
- Enter information in database, being as detailed as possible. (Assign it a name, location, vendor# or cat# if from kit, host (what it was grown up in), fill in description field telling about any special inserted sequences, what kind of vector i.e. expression vector,
- Label small round bottom tubes with generic name on lid and generic name again on the side with real name and resistance.
- In small round bottom tubes: add 750 μ l of 65% glycerol (kept at 4C) and 750 μ l of overnite culture.
- Put in proper place/box in -80 and information sheets into notebook.