

## How to isolate DNA from LISA glycerol stocks

The yield of DNA from pGIPZ-shRNAmir cultures is generally low, e.g. we usually need two miniprep to obtain the 15 µg of DNA we require for bulk virus preparation. If your yield is even lower, consider the following improvements:

- avoid glycerol, i.e. do not grow them in storage medium
- do not use Zeocin, it will reduce your yield
- culture at 37°C, not 30°C; the lower temperature is meant to reduce recombination for long-term storage cultures only

### Plasmid Preparation Protocol:

- First thaw your glycerol stock culture and mix to resuspend any *E. coli* that may have settled to the bottom of the well or tube.
- Inoculate 10 µl from the glycerol stock into 5 ml of 2X-LB (low salt) with 100 µg/ml carbenicillin and return the glycerol stock(s) to -80°C.
- Grow all pGIPZ clones at 37°C in 2X-LB broth (low salt) media plus 100 µg/ml carbenicillin only.
- With pGIPZ-shRNAmir constructs, 5 ml of culture can be used for one plasmid mini-prep generally producing 5-10 µg of plasmid DNA.

### 2X-LB broth (low salt) media preparation:

Put some 100 ml of water and a stirring rod into a measuring cylinder, and then weigh in:

20.0 g Tryptone

10.0 g Yeast Extract

5.0 g Sodium Chloride

(OR 20.0 g premixed LB Lennox plus an extra 10.0 g Tryptone and an extra 5.0 g Yeast Extract)

Fill up to 900 ml with water, adjust pH to 7.0 (RT, NaOH), adjust volume to 1000 ml, and autoclave in a Schott bottle. Once cooled, add 2.0 ml of a sterile 50 g · l<sup>-1</sup> 1 carbenicillin stock only, i.e. no Zeocin.

### Notes:

- If a larger culture volume is desired, incubate the 3-5 ml culture for 8 hours at 37°C with shaking and use as a starter inoculum. Dilute the starter culture 1:500-1:1000 into the larger volume. Incubate at 37°C for 18-19 hours with vigorous shaking. Sediment the culture and begin preparation of plasmid DNA.
- Due to the tendency of all viral vectors to recombine, keep the incubation times as short as possible and avoid subculturing. Return to your original glycerol stock for each plasmid preparation.