Growing and Purifying shRNA Plasmids for Transfection

Materials:

1.) LB Broth with 100µg/µl Ampicillin – (Teknova #L8105)

2.) Chloramphenicol – (Fisher # ICN19032125)

3.) HiSpeed Plasmid Maxi Kit – (Qiagen # 12663)

4.) 15mL Centrifuge Tubes (Fisher # 05-538-53F)

5.) 50mL Centrifuge Tubes (Fisher # 07-203-510)

6.) 1.5mL RNase Free Microfuge Tubes (Fisher # NC9445663)

Before Starting:

1.) Prepare Chloramphenicol stocks

   a.) Fill a 50mL centrifuge tube with 100% ethanol
   b.) Weigh out 1.7g Chloramphenicol
   c.) Add Chloramphenicol to the ethanol and mix well
   d.) Aliquot 1250µl into eppendorf tubes (34mg/mL stock)
   e.) Store at -20ºC

2.) Thaw glycerol stocks for growth

Biohazard Concerns:

The shRNA plasmids are transformed into competent E. coli. Therefore, the bacteria are genetically altered and resistant to commonly used antibiotics. All bacterial cultures must be killed with bleach before being disposed of in the sink, or autoclaved prior to disposal.

Procedure:

1.) Add 3mL of LB broth with Ampicillin to a 15mL centrifuge tube.

2.) Inoculate the tube with 6µl of thawed glycerol stock.

   a.) This is a 1/500 ratio (good for low copy)
   b.) For a 1/1000 ratio, add 3µl (use for high copy)

3.) Shake overnight at 37ºC.

4.) The next morning, add 250mL of LB broth with Ampicillin to a 1mL flask.

5.) From the overnight starter culture, inoculate the flask broth with 500µl of the starter culture. Shake at 37C.

   a.) This is a 1/500 ratio (good for low copy plasmids)
   b.) For a 1/1000 ratio, add 250µl (use for high copy)

6.) Using remaining culture from the 15mL tube, aliquot 1mL of culture into 5-10 1.5mL microfuge tubes. Freeze the tubes at -80C for future use.

7.) After ~6 hours of growth, check the culture for turbidity. After the culture has reached turbidity, determine when the culture has reached log stage of growth by taking an OD600. The OD600 should be at least 0.5 (0.6-0.7 is better).

8.) Once the culture has reached log growth, add 1250µl of Chloramphenicol stock (34mg/mL).

9.) Shake overnight at 37ºC.

   a.) It is not necessary to add Chloramphenicol; however, it helps to prevent bacterial replication while allowing for plasmid replication thus increasing plasmid growth from low copy plasmids.

   b.) Some plasmids do better growing overnight only (no additional day growth). Inoculate the 250mL flask culture in the evening and allow to grow overnight. In this case, do not add Chloramphenicol.

10.) Extract and purify plasmids using Qiagen's Ultrafast Plasmid Maxi Kit according to the manufacturer's instructions.

11.) Quantitate plasmid yield on spectrophotometer.

a.) A constant of 50 should be used and the A260/280 ratio should be between 1.8 and 2.2.

b.) If the ratio is less than 1.8, there may be some protein contamination due from insufficient mixing of the buffers. It is advisable to use the blue dye supplied by Qiagen to ensure adequate mixing.

c.) If the ratios are greater than 2.2, then this suggests some RNA contamination. Once again, be sure to use the blue dye to ensure adequate mixing and be sure the proper amount of RNAse A is added to Buffer P1.