Qiagen® Miniprep

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:

Primary Experimenter (contact):
Other Experimenters:

Source: Kit manual, Dujduan Waraho, Caleb Radens

Assembled: 6/17/2012

Reagent	Details	Quantity
Qiagen® Miniprep Kit		n/a
Overnight liquid culture		1.5-8 mL

Strain	Plasmid	Volume minipreped	Yield (ng / uL)

Procedure:

Critical Steps:

- Don't forget to label all tubes, including QIAprep® column and flow through tube!

NOTE:

- Qiagen describes how to make their buffers online, if a reagent runs out, sometimes you can make your own!

☐ **Grow cells overnight (at least 12 h) in appropriate media and appropriate antibiotics**

☐ **Pour culture into a labeled 1.5 mL Eppindorf tube**

☐ **Centrifuge at 13,000 rpm for 1 min, discard supernatant (dump out liquid)**

☐ **Repeat steps 2-3 until all of the culture has been spun down, or up to 8 mL**

- Choose mL of culture based on copy number of plasmid and/or end use of plasmid

☐ **Resuspend pellet in 250 µL of P1 Buffer (a.k.a. Resuspension buffer)**

- Stored at 4 °C, make sure that RNase has been added as indicated on bottle

☐ **Add 250 µL of P2 Buffer (a.k.a. Lysis buffer)**

- Your mixture will turn blue if pH indicator was added

☐ **Mix by inverting four to six times**

☐ **Add 350 µL of N3 Buffer (a.k.a. Neutralizing buffer) and <u>immediately!</u> mix by inverting four to six times**

- If indicator was added, mixture should turn clear again, if it doesn't pH needs correction before continuing

☐ **Centrifuge for 10 minutes at 13,000 rpm**

☐ **Transfer supernatant to a QIAprep® column**

☐ **Centrifuge at 13,000 rpm for 1 min**

☐ **Discard flow through (liquid in bottom chamber) and add 500 µL of PB buffer (a.k.a. Binding buffer)**

☐ **Centrifuge at 13,000 rpm for 1 min**

☐ **Discard flow through (liquid in bottom chamber) and add 750 µL of PE buffer (a.k.a. Wash buffer)**

- Make sure that ethanol has been added as indicated on bottle

☐ **Centrifuge at 13,000 rpm for 1 min**

☐ **Discard flow through and centrifuge the column at 13,000 rpm for 1 min again**

- This second centrifuge step is critical to remove excess PE buffer

☐ **Transfer column out of flow through tube to a labeled 1.5 mL Eppindorf tube**

☐ **Add 30-50 µL of EB Buffer (a.k.a. Elution buffer) directly to the center of the column**

- When plasmid >10 kb, improve yield by pre-warming EB Buffer to 70 °C

☐ **Let the column sit for at least a minute, up to 30 minutes**

- When adding 30 µL of EB, let it sit for at least 2 minutes

☐ **Centrifuge at 13,000 rpm for 1 min**

☐ **OPTIONAL: repeat the elution step into a fresh tube**

- A second elution will recover less DNA than the first, but may still be a usable amount

☐ **Discard column, plasmid is now in Eppindorf tube**

☐ **Quantify the amount of plasmid with a Nanodrop (See: How to use Nanodrop)**