JUNE 14, 2014

Liquid culture of JM109 cells started from glycerol stock

A sterile inoculating loop was used to transfer a small quantity of frozen JM109 cells into a culture tube with 5 mL LB broth. Culture left overnight in a 37°C shaking incubator.

JUNE 15, 2014

Liquid culture removed from incubator and moved to fridge

JUNE 16, 2014

JM109 cells rendered chemically competent and transformed with pET15b plasmid

The protocols **Making calcium-competent cells** and **Transformation of calcium-competent cells** (found in the Protocols [Antibody] folder) were followed to transform JM109 cells with pET15b plasmids with the following exceptions:

-OD_{600} was ~0.850.

-The antibiotic in the plates was ampicillin.

Both the transformed and control plates were left overnight in the 37°C incubator.

JUNE 17, 2014

Plates recovered from incubator and liquid cultures started

The plate containing transformed cells had a few dozen small colonies.

The control plate had no colonies.

Two 4 mL liquid cultures were started from two different colonies using a sterile inoculating loop. The cultures were left approximately 24 hours in a 37°C shaking incubator.

JUNE 17, 2014

JM109+pET15b liquid cultures moved to fridge

JUNE 21, 2014

pET15b purified from JM109 cells using Qiagen Miniprep and Plasmid Miniprep

Spin Miniprep:

The protocols provided with the kit were followed with the following exceptions:

- 1.5 mL of liquid culture was used (recommended was 1 mL to 5 mL)
- Centrifugation took place at ~ 11000 RPM in tabletop centrifuge (protocols called for higher RPM)
- DNA was incubated for ~ 30 min at 37°C after purification to help resuspension

The purified plasmid was placed in the freezer

Plasmid Miniprep:

The protocols provided with the kit were followed with the following exceptions:

- ~2.5 mL of liquid culture was used (recommended with 3 mL)
- **Cells were not resuspended in P1 buffer before adding P2. In an attempt to avoid premature lysis, the entire mixture was discarded. P1 was then added, followed by P2.**
- All centrifugation steps recommending 4°C were carried out a room temperature.

-Centrifugation was run at ~ 11000 RPM.
-Eluted DNA was resuspended in 50 uL ultrapure water.
The purified plasmid was placed in the freezer.

JUNE 27, 2014

Miniprep samples analyzed by nanodrop

pET15b purified from JM109 cells

	[Nucleic acid]	A260	A280	260/280
Spin Mini Kit	13.9 ng/uL	0.279	0.14	1.99
Plasmid-prep Kit	1.8 ng/uL	0.035	0.012	3

JUNE 29, 2014

Glycerol stock made

(500 uL 50% glycerol) + (500 uL JM109 cells containing pET15b).

Frozen at -80C.