**Vector Preparation Protocols**

**Prep of Agar and LB broth:**

-Dissolve 8 capsules of Agar in 200 ml of water (in 500 ml bottles to give plenty of space when heating the solution later)

-Dissolve 10 g of LB broth in 400 ml water (in 500 ml bottles)

-Make up multiple bottles of each at the same time- good indefinitely at room temperature after autoclaving

-Autoclave solutions on liquid cycle

**Pouring plates:**

-Check antibiotic resistance of your vector (ampicillin, kanamycin, etc)

-Heat 1 bottle of agar in microwave until the entire volume is liquid. Let the agar cool (although not completely or it will solidify again).

-Add appropriate amount of antibiotic to agar solution and mix

-Pour plates (usually 200 ml agar ~ 14 plates

-Agar plates can be stored in the cold room for 1+ months

**Streaking out bacteria:**

-Vectors are often shipped in “stabs” or you may need to streak out from a glycerol stock

-Use a clean pipette tip to stab into the stock and spread onto plate as below- use a new pipette tip for each streak (3 in total)

-Incubate the plate overnight at 30C

**Picking colonies:**

-Make starter cultures using 3ml LB broth per culture + appropriate amount of antibiotic

-Use clean pipette tip to stab a single colony from the plate and place the entire tip in the tube containing broth and antibiotic

-Let the culture shake overnight at 30C

**Make glycerol stocks:**

-Add 500ul of 60% glycerol solution to 500ul of LB culture

-Mix thoroughly

-Put in -80 freezer- good indefinitely

Perform a miniprep and diagnostic digest for your vector to confirm sequence