Cloning 101
Adapted from TOPO TA Cloning Kit for Sequencing by Invitrogen (Life Technologies)

IMPORTANT
Before cloning: Make sure LB/AMP plates are made
No more than 8 samples to clone at one time

LB/AMP Plates:

- See recipe card or follow guidelines for grams/liter on container
- Autoclave media and allow to cool before adding ampicillin
- Add 0.1g ampicillin/liter

1. Begin with mixed sample
2. Get genomic DNA (using zymo kit, MoBio kit, etc to get g DNA)
3. Nanodrop
4. Dilute to 50 ng/µl (with elution buffer or water)
5. PCR 20 µl reaction (using NEB 2X MM)
6. PCR clean-up kit
7. Nanodrop
8. Dilute to 10 ng/µl
9. Time for cloning
    a. Set LB/AMP plates out on bench to warm to room temperature
    b. Get SOC media and competent cells from -80°C and x amount of tubes for the remaining samples (Be sure to take ice bucket to -80°C when retrieving competent cells)
        i. Color lids purple and label with sample ID
        ii. Set on ice
    c. Set heat shock to 42°C and fill holes with water, according to number of samples or set water bath to 42°C
    d. Get enough PCR tubes for number of samples (found under the PCR hood)
    e. Get salt solution and water from -20°C
    f. In each LABELED PCR tube: Add in the order shown

Reagent	Volume	Location
Water	Add to a total volume of 6 µl	-20°C
Salt Solution	1 µl	-20°C
Fresh PCR Product	0.5-4 µl	
TOPO Vector	0.5-1 µl	-20°C
Final Volume	6 µl	

Important! When working with vector, work efficiently in order to put it back in -20°C as quickly as possible
Keep all regents on ice while working

    i. Before adding vector mix the water, salt solution, and DNA
    ii. When adding vector, tap PCR tube carefully, do NOT mix vector
    iii. Put samples at room temperature for 20 minutes for the ligation
g. Separate competent cells for the number of samples
    i. Example – the competent cells contain 50 µl, so for 5 samples, separate 10 µl in each purple colored tube. Don't split cells more than five ways
    ii. Cut the tip off of the pipette tip using a razor blade so as not to damage the competent cells
h. After incubation, place 2µl of ligation from PCR tube into LABELED purple colored tube, tap carefully (do NOT mix)
i. Place on ice for 5-30 minutes
j. Take ice bucket over to heat shock plate or water bath and heat shock for 30 seconds at 42°C
k. Place immediately back on ice for 2-3 minutes
l. Add 250 µl SOC media to each tube, using a flame for sterility
m. Tape tubes horizontally on a beaker and incubate while shaking for 1 hour at 37°C
n. While incubating samples
    i. Spread 50µl of x-gal onto LB/AMP plates then place in 37°C to warm up
o. Put SOC media back in -80°C
p. After incubation, get transformants from incubator, LB/AMP plates from 37°C, and hockey stick/ethanol/spinny device
    i. Using a flame, put 60 - 75 µl of transformants on plate and mix on LB/AMP plates with hockey stick device
q. Place LB/AMP plates with transformants in 37°C overnight
r. Put tranformants tubes in beaker and store in refrigerator in case they're needed later