**Disclaimer:**

1. **All measurements are based on prepping 4 clones per group.**
2. **When you are labelling tubes, label the side with your full clone name (04XX#.15) and the cap with initials only, unless otherwise specified.**

**Overnight Cultures (ON) #.14**

___ 1. Obtain 4 culture tubes. Label the SIDE of each tube 04XX#.15
- ___ a. Make sure caps are loose

___ 2. Obtain a pipet bulb, a 10mL serological pipet, and LB+Amp (the yellow liquid)
***remember your A-S-E***
- ___ a. Use the pipet bulb to draw up 8 mL of LB+Amp into the serological pipet
- ___ b. +2mL to each culture tube. Do this quickly. Speed > accuracy in this step.

___ 3. Obtain a library plate (petri dish with bacteria on it), wooden splints, and a sharpie
- ___ a. Using a sharpie, circle 4 bacterial colonies on the bottom of the plate (do not touch the agar). Have each member write their initials next to one circle. Look for circular, isolated, and light blue or white colonies.
- ___ b. Using a different splint for each prep, gently tap the stick to the bacteria, quickly dip stick in the culture tube, and swish around (AKA inoculate media)

___ 4. Make sure all culture tubes are labelled and sealed. Bring culture tubes to front.

___ 5. Clean up and hand in lab preps

___ 6. Enter clone information on Clone Report Sheet on one of the side computers

**PCR & Solution I #.15 (remember these are two labs squished into one day)**

**PCR**

**___ 1. Receive ON culture tubes. Tap to resuspend.**

**___ 2. As an individual, make a dilute culture**

**___ a. Label a microfuge tube with clone name on side and initials + "DIL" on cap.**

**___ b.** +200ul $ddH_2O$

___ c. Tap ON culture tube and +5ul from culture tube to the "DIL" tube

___ d. Set aside culture tubes for later

___ 3. As a group, prepare 5X mix (5X is for 4 people)

___ a. Obtain a fresh microfuge tube and label the cap with "5X"

___ b. +90ul $ddH_2O$

___ c. +12.5ul FOR primer

___ d. +12.5ul REV primer

___ e. Tap tube to mix

___ 4. As an individual, set up the PCR rxn

___ a. Obtain and label a fresh PCR tube with INITIALS ONLY on the side and cap

___ b. +23ul of 5X mix to each PCR tube

___ c. Tap your "DIL" tube to resuspend cells. +2ul of your dilute culture to your PCR tube. Tap tube to mix.

___ d. Make sure everything is labelled and bring up PCR tubes to the front.

___ 5. Discard your "DIL" tube. DO NOT discard your original culture tube.

SOLUTION I

___ 1. Obtain a fresh microfuge tube and label the side w/ full clone name and the top w/ initials

___ 2. Obtain your original culture tube

___ a. Tap to resuspend cells

___ b. Pour all of the contents of your culture tube into your new microfuge tube (yes, physically pour it)

___ c. Discard your culture tube

___ 3. Centrifuge your microfuge tubes at full speed for 1 minute.

___ a. Make sure the centrifuge is balanced

___ b. After the minute is up, remove the microfuge tubes from the centrifuge. You should see white stuff at the bottom and clear yellow liquid above.

___ c. Pour out and discard the clear yellow liquid (the supernatant).

*PRO TIP* Try not to lose the white stuff at the bottom. It is better to have a little bit of yellow liquid left in the tube than to lose your white stuff.

___ 4. Using a P1000, +200ul of Solution I to the microfuge tubes

___ a. Gently pipet up and down several times to suspend cells in Solution I. There should be no clumps in the microfuge tube at the end

___ 5. Making sure they are properly labelled, bring your microfuge tubes (with your cells suspended in solution I) to the front.

___ 6. Clean up and hand in lab prep for PCR & Solution I

**PCR Gels (PCRG) #.15**

***DO NOT CONFUSE LOADING DYE WITH STANDARD/MARKER***

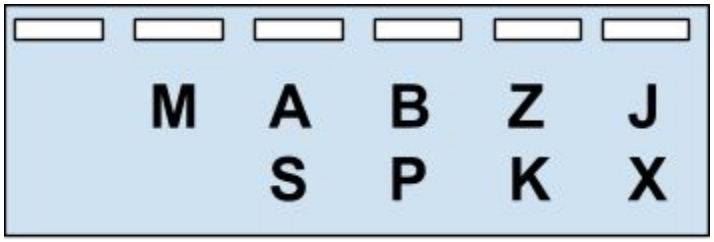
___ 1. Retrieve PCR tubes and +2.5ul of 10X loading dye to each tube

___ 2. Go to the front and exchange loading dye for standard/marker

___ 3. Look at front board for gel assignments. If the lab group you are sharing your gel with is already at the gel station, WAIT UNTIL THEY HAVE FINISHED. If they are not there, go to your gel.

___ 4. Load your gel

- ___ a. Record what part of the gel you are using (top/bottom)
- ___ b. Using a P20, pipet 20ul of standard into the SECOND WELL. (Standard = Marker = M)
  - ___ i. Return standard to front of room
- ___ c. Have each member of your group +20ul of your PCR tube contents into a separate well
- ___ d. Record the the contents of each well on your lab prep. Example:




Lab Group 0
Top of Gel

___ 5. Pull out your yellow sheet and leave it next to your gel, clearly indicating your lab group number. Clean up.

**Miniprep (MP) #.15**

***IMPORTANT: Before you start, review the information on the board about the difference between microfuge tubes, spin columns, collection tubes, supernatant, and flow-through***

___ 1. Receive your tube with your bacterial cells suspended in Solution I and thaw
- ___ a. +200ul solution II. Gently invert 10-15 times until solution is clear.
- ___ b. +400ul solution III. Gently invert 4-6 times. You should see a white precipitate
- ___ c. Centrifuge at full speed 5 min. There should be a pellet at the bottom and liquid supernatant above it

___ 2. Receive a brand new spin column and collection tube. Label only spin column with initials.
- ___ a. Place spin column in the collection tube. Pour supernatant from the tube you just centrifuged into the spin column
  - *Note* Do not get any of the white precipitate into the spin column. It is better to get less supernatant than getting all of the supernatant and some of the white precipitate
- ___ b. Keep tubes at room temperature for 1 minute
- ___ c. Centrifuge for 1 min. Discard the flow-through.

___ 3. Place spin column back in collection tube
- ___ a. +400ul wash buffer to spin column
- ___ b. Centrifuge 1 min
- ___ c. Discard flow-through
- ___ d. Centrifuge again at full speed for 1 minute
- ___ e. Leave cap open for 1 minute

___ 4. Receive a brand new YELLOW microfuge tube. Label cap and side with full clone name.
- ___ a. Place spin column in the yellow tube and +60 ul elution buffer
- ___ b. Leave at room temp for 1 minute
- ___ c. Centrifuge at full speed for 1 minute
- ___ d. Discard spin column and keep the yellow tube.

___ 5. Close cap of yellow tube. The yellow tube now contains your plasmid DNA! (if you have any)
- ___ a. Bring yellow tubes to front
- ___ b. Clean up and hand in lab preps to front

**Restriction Digest (RD) #.15**

PSA: Always keep enzyme on ice and never put anything into yellow tube (you can only take stuff out)

___ 1. Obtain yellow miniprep tubes, fresh microfuge tubes, and a pipet. Thaw out yellow tube.
- ___ a. Label the fresh microfuge tube with initials and the letter "D" on cap for digest. Also, label the side with full clone name.
- ___ b. +10ul of MP DNA from yellow tube to the newly labelled microfuge tube.
- ___ c. Return MP tubes to front of room.

___ 2. Make a 5X Mix (one per lab group)
- ___ a. Label a fresh microfuge tube with "5X" on cap
- ___ b. +35ul $ddH_2O$
- ___ c. +12ul 10X restriction buffer
- ___ d. +5ul PvuII-HF enzyme (ALWAYS ADD THIS LAST)
- ___ e. Mix by tapping tube (actually very important). Then, place on ice.

___ 3. +10ul of 5X mix to "D" tube. Gently tap tube to mix.

___ 4. Bring "D" tubes to front for incubation.
- ___ a. Clean up and hand in lab preps to front

**Restriction Digest Gels (RDG) #.15**

___ 1. Making your uncut sample

___ a. Receive and thaw yellow MP tubes. Obtain loading dye, distilled water, and fresh microfuge tubes.

___ b. Label top of fresh microfuge tube with initials and the letter "U" for uncut

___ c. +8ul $ddH_2O$

___ d. +2ul loading dye

___ e. +10ul MP DNA from yellow tube to "U" tube

___ f. Return yellow tubes to front

___ 2. Swap loading dye for standard/marker

___ 3. Receive "D" tubes from freezer and thaw.

___ 4. Look at front board for gel assignments. If the lab group you are sharing your gel with is already at the gel station, WAIT UNTIL THEY HAVE FINISHED. If they are not there, go to your gel.

___ 5. Load your gel

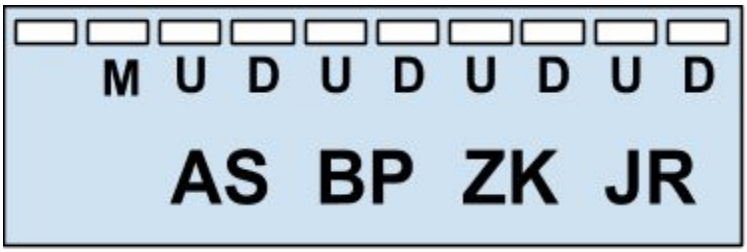
___ a. Record what part of the gel you are using (top/bottom)

___ b. Using a P20, pipet 20ul of standard into the SECOND WELL. (Standard = Marker = M)

___ i. Return standard to front of room

___ c. Have each member of your group +20ul of the uncut "U" DNA into one well and +20ul of the digest "D" DNA into the next well

___ d. Record the the contents of each well on your lab prep. Example:




Lab Group 0
Bottom of Gel

___ 6. Pull out your yellow sheet and leave it next to your gel, clearly indicating your lab group number. Clean up.