**Bacterial Transformation Lab Protocol (Bio-Rad)**

(October **28**, 2013)

**CHECK OFF EACH STEP AS YOU READ IT THOROUGHLY AND FOLLOW THE DIRECTIONS**

 **1**. Label your three agar plates on the bottom (**NOT THE LID**). Label the plates

 as follows:

**LB -**

**LB/amp +**

**LB/amp -**

 **2**. On their lids, label the two micro testtubes as follows:

Lids

**+**

**-**

 The **+** indicates the micro testtube that

 will receive the plasmid with the two

 genetically engineered genes on them,

 while the **-** indicates the micro testtube that will not receive the plasmid.

 **3**. Place them in the foam “floatie”.

 **4**. Using a plastic disposable pipette, transfer .250 ml of CaCl**2**,to bothtubes, close and place them **on ice for at least 2 minutes**.



**.100 ml**

**.5 ml**

**1.0 ml**

**.250 ml**



 **5**. Using a sterile loop, **gently & lightly (don’t**

 **dig out** any agar) scrape off 4 colonies of

 bacteria off the surface of the “starter plate”

 **6**. Transfer the colonies of bacteria

 to one of the micro tubes. Swirl,

 spin and twist the loop to make

 sure all of the bacteria mixes with

 the CaCl**2** solution (with no floating



 chunks). Place the tube back in the

 ice. Discard this loop. **Using a new**

 **loop**, repeat this process in the

 **second micro tube**, and place that

 micro tube on ice as well.

 **7**. Have your teacher pipette 10ul of plasmid solution into your **+** micro tube.

 **8**. Incubate both tubes on ice for **10 minutes**. Make sure the micro testtubes are immersed in the ice (“floatie” in icewater).

 **9**. **Heat shock** time. Remove the micro test tubes from the ice bath and take them to a 42**o** water bath. Get a timer ready because you are going to place both test-tubes in the hot water bath for **EXACTLY** **50** **seconds**. After 50 seconds, **immediately** put micro testtubes back in the floatie and on ice for **2 minutes**.

**Immediately !!**



**420**

 **10**. Remove the floatie from the ice and place on the table top. Using a new sterile plastic pipette, add **.250 ml** of LB-broth to one of the tubes (and reclose it).Using a **new** sterile plastic pipette, add **.250 ml** of LB-broth to the other tube (and reclose it).Let sit on the table top for 10 minutes (room temperature).

 **11**. Gently flick both closed tubes with your finger to mix the solution. Using a new sterile pipette, pipette **.100 ml** from your **+** micro tube onto the

 LB/amp **+** plate.



**LB/amp +**

 **12**. Using a **sterile** loop, spread these suspensions evenly around the surface of the LB/AMP + plates by quickly “skating” (**lightly**) the flat surface of the loop back and forth across the surface of the plate.

**LB/amp +**

 **13**. Using **another new** sterile pipette, pipette **.100 ml** from your **--** micro tube onto the LB/amp **-** plate. Then pipette **.100 ml** from your **-** micro tube onto the LB **-** plate.



**LB/amp -**

**LB -**

 **14**. Using a **new** sterile loop, spread these suspensions evenly around the surface of these two agar plates by quickly “skating” (**lightly**) the flat surface of the loop back and forth across the surface of the plate.

 **15**. Stack up your plates and tape them together with blue tape. Using a black sharpie, put your group’s name on the blue tape. Give your stack to the teacher to put into the **37o** incubator.