**Protocol**

Use CULTURED CELLS instructions if looking at Qiagen Kit handout

updated 12 October 2016

This lab ran from 3:00 - 6:15 PM on Tuesday 10/11/2016

**Prep**

1. Assign test tube to students via number label.
2. Send notification to students informing them if their test tube numbers
3. Organize test tubes in numerical order for easy recovery
4. Remind students to label the LID of the eppendorf tubes and spin columns - not the side!

**Lysis**

1. Pipette 1ml **(1000ul)** vortexed/well shaken broth into 1.5ml tube, with the lid correctly labeled
2. Centrifuge for 2 min at max rpm, remove supernatant (the liquid on the top of the ‘pellet’)

*The first centrifuge is to condense the bacterial cells into a pellet. We pipet out the nutrient broth.*

1. Resuspend pellet in **200ul** PBS onto pellet.

*It is easiest to pipette the liquid up and down a couple of times over the pellet to resuspend. Stubborn pellets can be vortexed briefly.*

1. Pipette **20ul** Proteinase K into working tube.

*Proteinase K is added to lyse the cells, or to break them open and release the contents*

1. Pipette **200ul** of AL buffer into working tube

*The buffers added during this step are to provide conditions (pH, salts) to maximize enzyme activity.*

1. Vortex well.

*Double check that there is no trace of a pellet at the bottom of the tube.*

1. Put tube in the heat block (56C) for 10 minutes

*The incubation step is performed at the optimal temperature for comprehensive lysis of bacterial cells.*

**Precipitate**

1. Add **200ul** EtOH to the tube
2. Vortex

*Ethanol is added to precipitate the DNA molecules. The way this works is that normally, the negatively charged phosphate backbone of DNA is shielded by the water molecules. Note that the buffer solution also contains positive ions such as sodium. Adding ethanol disrupts the charge shielding, allowing the positive ions to bind both to the negative phosphate backbone and to the negatively charged silica column. Silica is what’s in the spin column. - Adam wrote this up from last quarter!*

\*Note: Precipitants may not be visible but doesn’t mean utter failure.

**Stick to the membrane**

1. Pipette the mixture into a spin column
2. **LABEL TOP OF SPIN COLUMN VIA RESPECTIVE NUMBER, NOT THE COLLECTION TUBE**
3. Centrifuge 1 min at 8000rpm

*The centrifugation step after adding ethanol passes the solution through the column and allows the DNA to stick to the column.*

1. Discard collection tube and liquid
2. Place spin column into **NEW** collection tube

**Wash part 1**

1. Pipette **500u**l Buffer AW1 into spin column
2. Centrifuge 1 min at 8000rpm
3. Discard collection tube and liquid
4. Place spin column into **NEW** collection tube

**Wash part 2**

1. Pipette **500u**l Buffer AW2 into spin column
2. Centrifuge 3 min at 14,000rpm
3. Discard collection tube and liquid

*The washes wash away any compounds that are not DNA while the DNA still sticks to the column.*

**Elute\***

1. Place spin column on a new, correctly labeled, 1.5 centrifuge tube
2. Pipette **200ul** Buffer AE into spin column
3. Wait 1 minute
4. Centrifuge 1 min at 8000rpm
5. Collect the liquid and throw out the spin colum

**DONE!**

*\*T AE solution is composed of 10 mM of Tris-HCl and 0.5 mM EDTA with an overall pH of 9.0. The high pH keeps the DNA from pretty much drying out. The actual process of how this all works is quite fascinating. The spin column is lined with positively-charged resin beads which interact with the negatively-charged backbones of the DNA. The AE buffer salt concentration and pH determine whether the DNA is bound to the wall or eluted. A higher concentrated salt buffer would compete for the positive and negative charges and cause the DNA to elute because the charge between the DNA and wall would be broken to to the salt’s interaction. pH can also affect the charge of the DNA.*