Week 3

4/12/2016

**PCR, Clean-Up, and Quantification**

***Summary page***

*Stuff in italics is what we forgot*

Clean-Up Station

*Eppy tube racks - lots*

1 set of pipettes and tips

Eppendorf tubes (check classroom)

Centrifuge

8 racks for PCR tubes

PCR clean-up kit

Waste bag/basket

Discussion topics

PCR

Gel Electrophoresis

DNA Quantification

Blog Post

**Clean-Up Kit Protocol**

*This station was a disaster! Groups needed to be separated. There needs to be a centrifuge manager to get things in and out of there.*

*Students used the collection tubes in place of 1.5ul Centrifuge tubes*

*Students did samples that failed at the PCR step*

*Keeping student’s samples straight was a real problem*

*Students need to be assigned roles to streamline this and other processes*

*Students are bad at labeling stuff*

Buffering

Start by making sure your tube is labeled.

(you have ~ 40 uL of PCR product to clean up)

Add 80uL Buffer NTI to your PCR product

Mix by pipetting up and down a few times in the tube

Bind DNA to membrane

Put whole mixture in the spin column (Tube with a removable short tube in the center)

Label the cap and the tube

Centrifuge 30 sec at 11,000xg

Wash and Dry

Add 700uL buffer NT3 to spin column

Centrifuge 30 sec at 11,000xg

Dump out washed product (at the bottom of the long tube)

Centrifuge 1 min at 11,000xg

Wash and Dry...Again

Add 700uL buffer NT3 to spin column

Centrifuge 30 sec at 11,000xg

Dump out washed product (at the bottom of the long tube)

Centrifuge 1 min at 11,000xg

Elute

Move spin column to a NEW 1.5uL eppendorf tube (NOT A COLLECTION TUBE!!! When you finish, you will need to cap it. Once you remove the spin column from the collection tube, there’s no cap. Think people.)

Label your tube and cap

Add 20 uL NE buffer make sure the spin column is dry. you may want to centrifuge again for a minute in a collection tube.

Incubate at room temp for 1 min

Centrifuge 1 min at 11,000xg

Again, make sure everything is labeled.