2014 BME 150L Lab Partners

Tues Lab Section Lab Partners
Kenny Chan/Alex Ng
William Cheung/Charles Warren
Kierstin Gray/Matt Reissman
Max Hogan/Royce York
Carolina Morales/Tyler Ortega
Gerarado Perez/Juan Saludes
Jeff Chung/Garrett Graham
Nikita Divekar/Prachi Dhavalikar
Beau Norgeot/Janani Ravikrishnan

Wed Lab Section Partners
Christa Cheung/Emilio Feal
Megan Der/Francesca Shadeed
Lucas Duong/Christopher Keh
Kendy Hoang/Kaylee Walker
Sonya Pita/Arjun Sandhu
Anna Postlethwaite/Cody Wong
Issa Delkaninia/Adam Fulton
Jason Fernandez/Jaron Nazaroff
Du Linh Lam/Abigail Rodriguez
Ardalan Monshi/Sarah Padiernos
BME 2014 150L Week 3 PCR Lab

Before Lab
1st Choose a time to start your PCR reaction on the day of lab. Times are 10:00 AM, 11:00 AM, 12:00 AM, or 1:00 PM. Make sure one or both lab partners work together to get this done.

2nd Set up your PCR reactions (15 minutes). After everyone has their PCR reactions set up, the TAs will demonstrate programming and running the PCR machine and assist you in starting the PCR reaction running. PCR reactions take ~3.5 hrs and will be put on ice and waiting for you when you arrive in lab at 5 PM.

During Lab
3rd Groups need to pour an agarose gel for purification of the PCR products. It takes 15-20 minutes or more to cool the gel to the point that you can load it with your samples and then another hour for the gel to run. You need to get the gel made, loaded and started running as your first task.

4th While the gel is running, you will work on designing the primers for the protein your group is working with. Please bring your laptop for this.

5th After you finish primer design, you will finish purification of your PCR product from the gel and then cleanup.

Supplies-
- lab coat, goggles, gloves
- Test tube rack “brick”
- Erlenmeyer flask
- Pipetors
- Disposable Pipet tips
- Ice bucket with ice
- Marking Pen
- Cardboard Freezer box (retrieve from the freezer)
- PCR tubes
- PCR tube rack

Reagents-
- PCR
- Phusion 2x master mix (keep on ice)
- Primer mix (keep on ice)
- Plasmid template

DNA Gel Electrophoresis-
- 1xTBE buffer
- Sea-Kem Agarose
- DNA ladder (pre- aliquoted in 1.5 ml tube) Should be in your freezer box
- DNA 6x loading buffer (pre- aliquoted in 1.5 ml tube) Should be in your freezer box
- MilliQ water (pre- aliquoted in 15 ml tube)
- Gel box w/power supply
- Gel purification spin column

Shared Common Equipment/Supplies
Balance
Incubator
Bench top Centrifuge
Microwave
Gel Boxes
Power supplies
Graduated cylinder
Gel Documentation
**METHODS - VECTOR PCR LINEARIZATION AND AMPLIFICATION: (BEFORE LAB)**

You need to setup 2 PCR reaction rxns (tubes)
50 ul total volume/rxn.

- 5 ul of ~10 ng/ul Template DNA
- 5 ul of 10 uM Forward Primer
- 5 ul of 10 uM Reverse Primer
- 10 ul of Water
- 25 ul of Phusion Master Mix

TAs will demostrate Use of the PCR Machine for you.

**PCR Temperature program:**

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<thead>
<tr>
<th>x</th>
<th>Temp</th>
<th>Time</th>
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<tbody>
<tr>
<td>1x</td>
<td>98 C</td>
<td>15 seconds</td>
</tr>
<tr>
<td>1x</td>
<td>98 C</td>
<td>10 seconds</td>
</tr>
<tr>
<td>14x*</td>
<td>64-57 C</td>
<td>10 seconds</td>
</tr>
<tr>
<td>14x</td>
<td>72 C</td>
<td>5 minutes 30 seconds</td>
</tr>
<tr>
<td>1x</td>
<td>98 C</td>
<td>10 seconds</td>
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<tr>
<td>14x</td>
<td>57 C</td>
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<tr>
<td>14x</td>
<td>72 C</td>
<td>5 minutes 30 seconds</td>
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<tr>
<td>1x</td>
<td>72 C</td>
<td>5 minutes</td>
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<tr>
<td>1x</td>
<td>4 C</td>
<td>Hold Indefinitely</td>
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</table>

**GEL PURIFICATION (DURING LAB):**

1. Prepare a 1% agarose gel in 1x TBE buffer. TAs will show you which gel boxes and combs to use.
2. Add 10 ul 6x gel loading buffer with cyber gold into PRC reaction, mix well.
3. Load all 60 ul of the PCR reaction + gel loading buffer into a single lane of a 1% Agarose gel.
4. Make sure to load a DNA ladder into the first lane of your gel (3 ul of Ladder, 2 ul of 6x gel loading buffer, 7 ul MilliQ water).
5. Run gel for 45 minutes at 105V.
6. After visualizing cut out the larger of the two solid bands. (TA’s will assist you in this).
7. Add gel slice into gel purification column and spin at 5000g for 10 minutes.
   a. Make sure all the Agarose has left the loading cone, spin for longer if it has not.
8. Using a pipette, estimate the total volume of flow through.
9. Add 5x that volume of DNA binding buffer and load into a DNA clean and concentrate column.
10. Spin at max speed for 1 minute.
11. Discard flow through and add 200 ul of zippy wash buffer and spin for 30 seconds.
12. Discard flow through and add 200 ul zippy wash buffer and spin for 30 seconds.
13. Move column to a fresh eppendorf tube and add 10 ul water, let sit for at least 30 seconds then spin at max speed for 30 seconds.
14. Nanodrop the flow through to determine DNA concentration and purity.
15. Label tube and store in class freezer box (TA will have the box for you).