LAB 1 RESOURCES

LAB SUPPLIES/EQUIPMENT/REAGENTS CHECKLIST

LAB 1 KIT ITEMS	LABELS	VOLUMES
Agarose		1.2g agarose to 150mL dH_2O
Solution #1	**S1**	-
Solution #2	**S2**	--
Solution #3	**S3**	--
Red Practice Dye	**RD**	--
20x SB buffer		--
Extra microfuge tubes		--
P-20 micropipette		--
P-20 pipette tips		--
Electrophoresis chambers		--
Power Supply		--
Gel trays/combs		--
Spatula		--
Gloves		--
Microfuge tube racks		--
Parafilm		
Sharpie markers		--

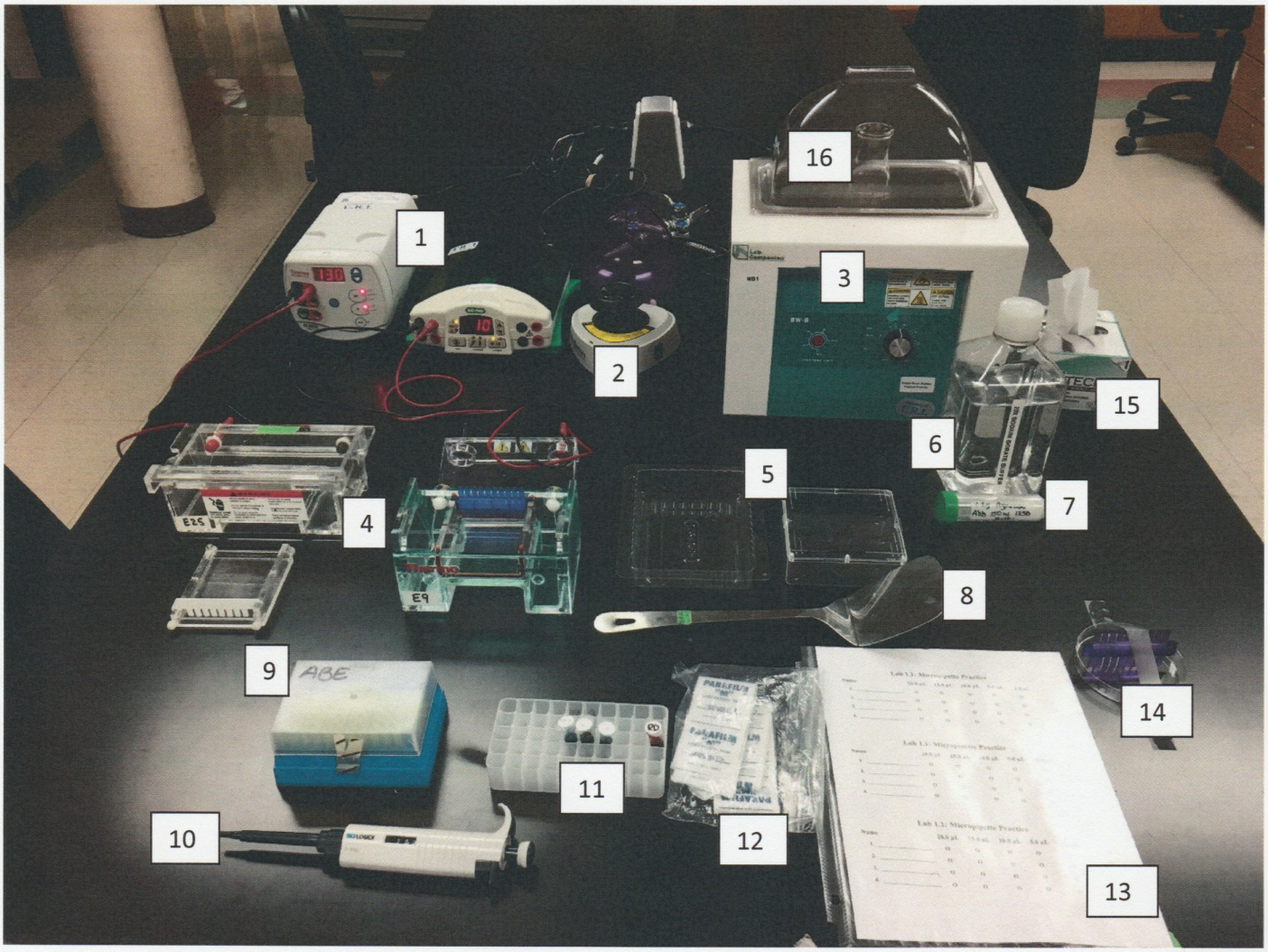
Notes: Lab 1.2: Be sure to use a CLEAN flask before melting your agarose. You can make up all your gels early and store them in zip lock baggies. (Make sure to add SB buffer to zip lock baggies so the gels doesn't dry out)
A gel tray uses about 30mls of agarose.

NEW Take a look at the worksheet on how to read the gels, it has a picture of what the gel should look like in color.

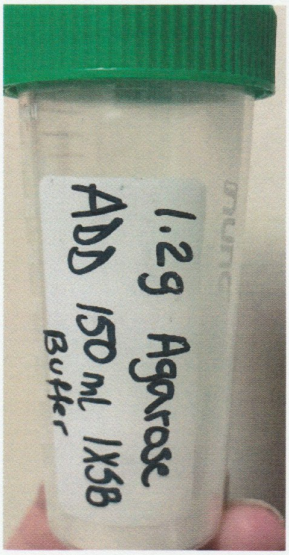
Diluting 20x SB Buffer to 1x SB buffer---- Mix 9mLs of 20x SB Buffer with 171 mLs of deionized water
You can find this in the ABE Teacher Guide on page OV-30

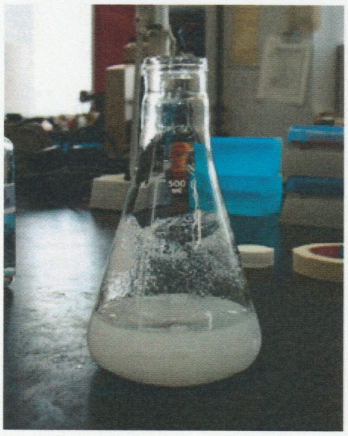
P-20, P-200, and P-1000 pipettes may contain locks on them: Please **UNLOCK** the pipette when adjusting the measurement

Lab 1.2



1. Electrophoretic power packs
2. Mini-micro centrifuge
3. Water bath
4. Gel electrophoretic apparatuses w/ tray and comb
5. Staining trays
6. 20x SB buffer
7. Agarose
8. Spatula
9. P20-200 pipette tips
10. P2-20 pipette
11. Solution 1,2,3 & red dye
12. Parafilm
13. Practice sheet
14. Practice petri dish
15. Kim wipes
16. Flask in water bath to cool agarose





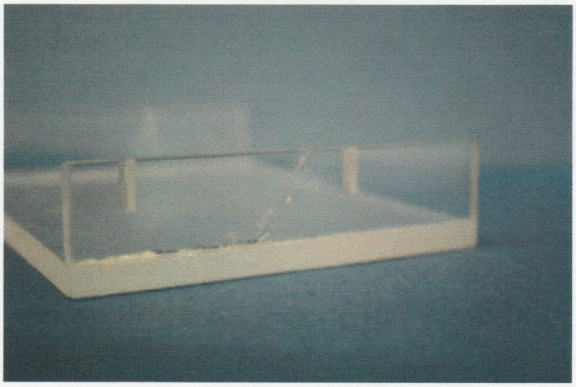
Kit Materials:

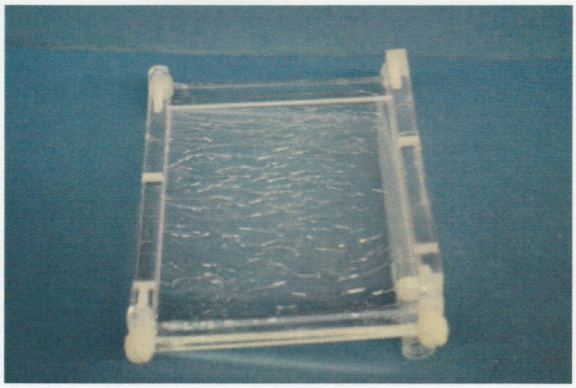
agarose, 20x Sodium Borate (SB) buffer, gel trays, combs, electrophoresis chamber, power supply, Solutions #1, 2, 3 (store at RT), red dye, P-20 micropipette and tips

Gel Preparation:

Items: Agarose, 20x Sodium Borate (SB) buffer (to be diluted to 1x SB buffer), gel trays, combs

- **Melting Agarose**
    - To prepare 0.8% agarose gel, add 150mL of 1x SB buffer to 1.2g (already measured in conical tubes unless otherwise noted) of agarose into a 500mL flask.
    - Place the covered flask in a microwave. Set the microwave for 1 minute on high. With a gloved hand, (it's hot) gently swirl the flask.
    - Place the covered flask in a microwave. Set the microwave for 1 minute on high. With a gloved hand, (it's hot) gently swirl the flask.
    - Continue this procedure, reducing the time on the microwave (5 – 15 seconds), until all of the agarose has been dissolved and the solution is clear.
    - Let the agarose cool until the flask is warm to the touch. Pouring hot liquid will warp the trays, resulting in poor electrophoresis results.





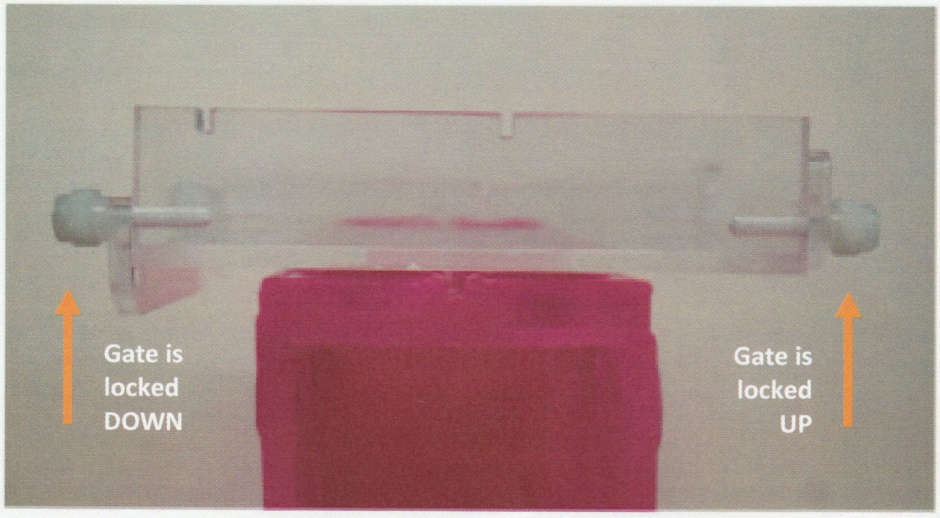
Examples of cracked/warped trays as a result of pouring hot agar solution

- You can keep melted agarose in a 60°C waterbath if there is a delay before pouring the gels or if they are having students pour the gels)

- **Preparing Trays**

  - Prepare the trays for casting by pushing "up" the gates on the ends of each tray then tightening the screws enough so the gates seal and stay up, as well as inserting the desired number of combs.

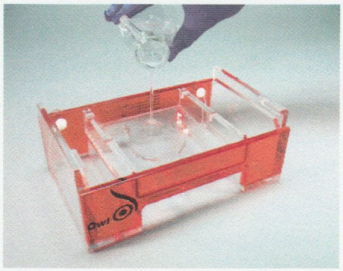
  - Pour about 30mL of the solution into each tray, covering about 2mm of the comb.



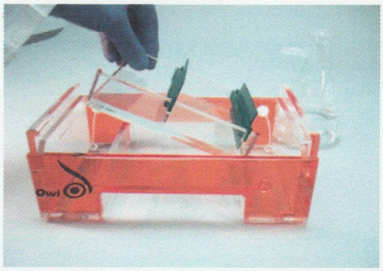
Thermo Scientific B1A EasyCast Mini Gel System
Casting with Owl's Gel Casting System

1. Place UVT gel tray in buffer chamber in the casting position. Gaskets will form a seal against the walls of the chamber.

2. Pour warm (<60°) agarose onto tray and set combs in the desired comb slot(s).



3. Once solidified, turn the tray 90 degrees to the running position, remove combs, add buffer, load samples and run the gel.



>> **For more information, visit: www.thermoscientifc.com/owlsci**

P-20, P-200, and P-1000 pipettes may contain locks on them: Please **UNLOCK** the pipette when adjusting the measurement!

Thank you 

Lab 1.1: Micropipette Practice

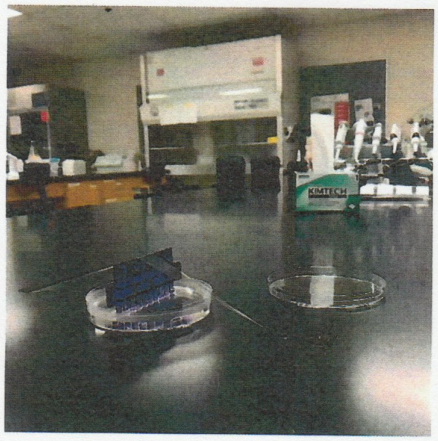
Name	20.0 µL	15.0 µL	10.0 µL	5.0 µL	2.0 µL
1. _____	O	O	O	O	O
2. _____	O	O	O	O	O
3. _____	O	O	O	O	O
4. _____	O	O	O	O	O

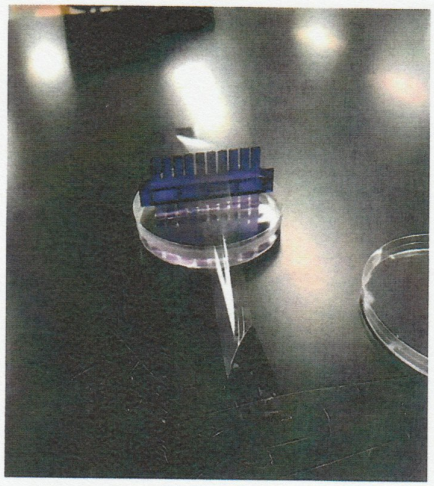
Lab 1.1: Micropipette Practice

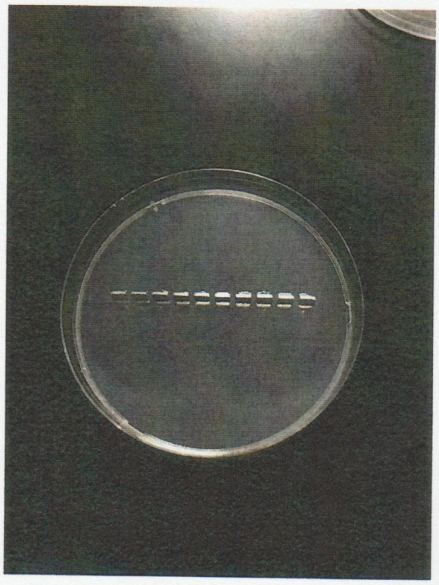
Name	20.0 µL	15.0 µL	10.0 µL	5.0 µL	2.0 µL
1. _____	O	O	O	O	O
2. _____	O	O	O	O	O
3. _____	O	O	O	O	O
4. _____	O	O	O	O	O

Lab 1.1: Micropipette Practice

Name	20.0 µL	15.0 µL	10.0 µL	5.0 µL	2.0 µL
1. _____	O	O	O	O	O
2. _____	O	O	O	O	O
3. _____	O	O	O	O	O
4. _____	O	O	O	O	O







LAB 1:

We have provided you with some extra petri dishes if you would like to make some practice plates for the pipetting lab. You can use any leftover agarose to makes these plates.

You will need:

- Electrophoresis comb
- 1X SB buffer
- Tape
- Empty Petri Dish
- 15 mLs of Agarose

Steps:

1. Use a piece of tape to hold comb upright in petri dish. (As shown on the left)
2. Add about 15 mLs of agarose to dish.
3. Once the agarose solidifies remove the comb.
4. To store plates add 1X SB Buffer and store in the refrigerator.

LAB 1.2 Gel Electrophoresis

Solutions

Dyes

Orange G 408.40 au

Bromophenol 699.98 au

Xylene cyanole 538.62 au

Heavier molecules move slower

Solution 1: bromophenol blue, xylene cyanole, glycerin and water

Solution 2: bromophenol blue, xylene cyanole, orange G, glycerin and water

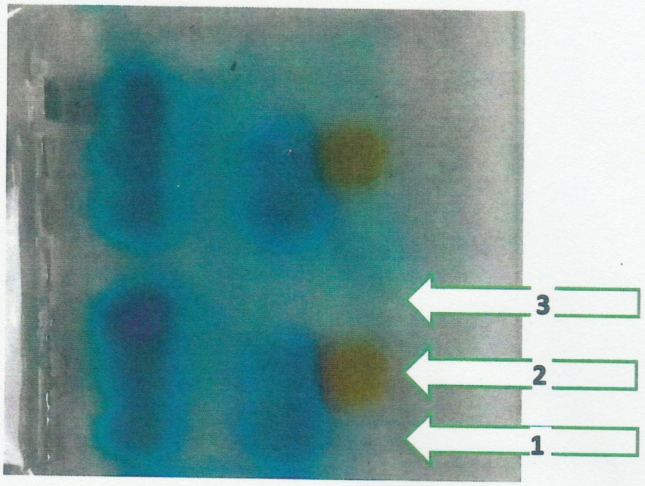
Solution 3: Xylene cyanole, glycerin and water.

Reading the Gel:

Bromophenol blue will appear purple

Xylene cyanole will appear blue

Orange G will appear orange/yellow

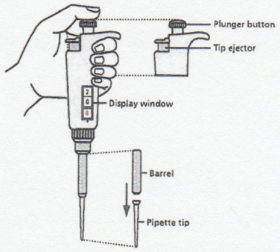


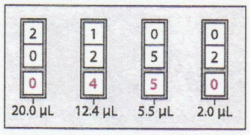
Possible answers:

1. Why do you think it is necessary to use very small and exact volumes of reagents in biotechnology? *In this field you would use very small amounts of the reagents and the correct measurements of reagent amounts is necessary for procedures to be successful.*

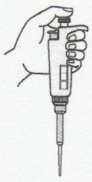
2. Read through the Methods section on pages 21 through 23 [of the Student Guide] and briefly outline the steps, using words and a flowchart.

***Laboratory 1.1* Flowchart**





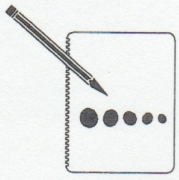
Check reagent (RD) → Review MP (micropipette) → Practice setting MP volume to 20.0 µL, 12.4 µL, 5.5 µL, 2.0 µL →



Micropipette Practice:

Name	20.0 µL	15.0 µL	10.0 µL	5.0 µL	2.0 µL
1. _____	o	o	o	o	o
2. _____	o	o	o	o	o
3. _____	o	o	o	o	o
4. _____	o	o	o	o	o

Pipette 20.0 µL RD onto laminated sheet → Pipette other amounts of RD onto laminated sheet →



Draw sizes of RD amounts in notebook

SESSION 2

Key ideas: **Those who carry out genetic engineering use very specific tools and have well-honed laboratory skills. Gel electrophoresis allows for the visualization of minute amounts of DNA. Using this technique, scientists can separate and identify pieces of DNA they are working with.**

Have students complete *Laboratory 1.2*. During the lab, have students share their answers to the Before the Lab and the STOP AND THINK questions and explain their thinking. (35 min.)

Have students share their answers to the Before the Lab questions with the class.

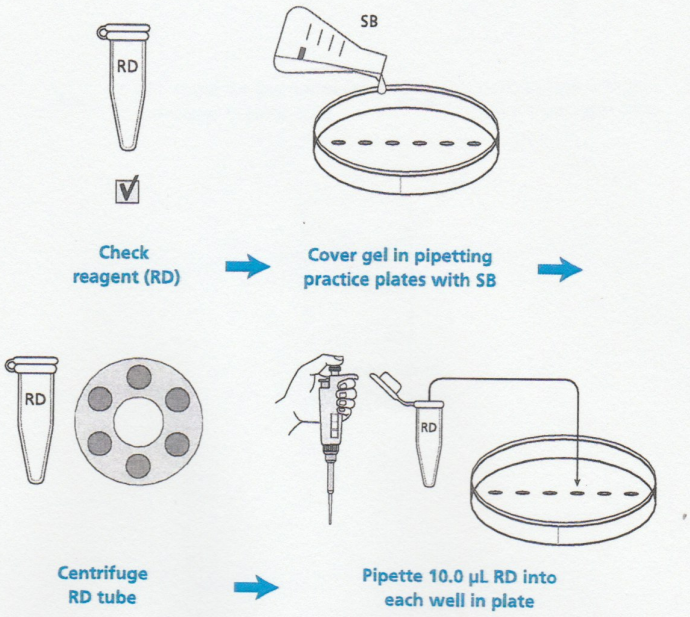
Strategy: **For the lab, you may want to show students the sample flowchart rather than have them create their own.**

Possible answers:

1. In what circumstances might it be important to use gel electrophoresis to separate and identify plasmids and short linear pieces of DNA? *This would be important if you are making a recombinant plasmid and have to verify that you have been successful.*

2. Read through the Methods section on pages 28 through 31 [of the Student Guide] and briefly outline the steps for *Part A* and for *Part B*, using words and a flowchart.

***Laboratory 1.2, Part A* Flowchart**



Laboratory 1.2, Part B Flowchart