

LAB 1 RESOURCES

ATTENTION TEACHERS:

Please have your students know how to use a pipette before proceeding to do this lab!

LAB SUPPLIES/EQUIPMENT/REAGENTS CHECKLIST


| LAB 1 KIT ITEMS | LABELS | VOLUMES |
|--------------------------|-----------|--|
| Agarose | | Weigh out 1.44g agarose and add 180mL of 1X SB |
| 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.

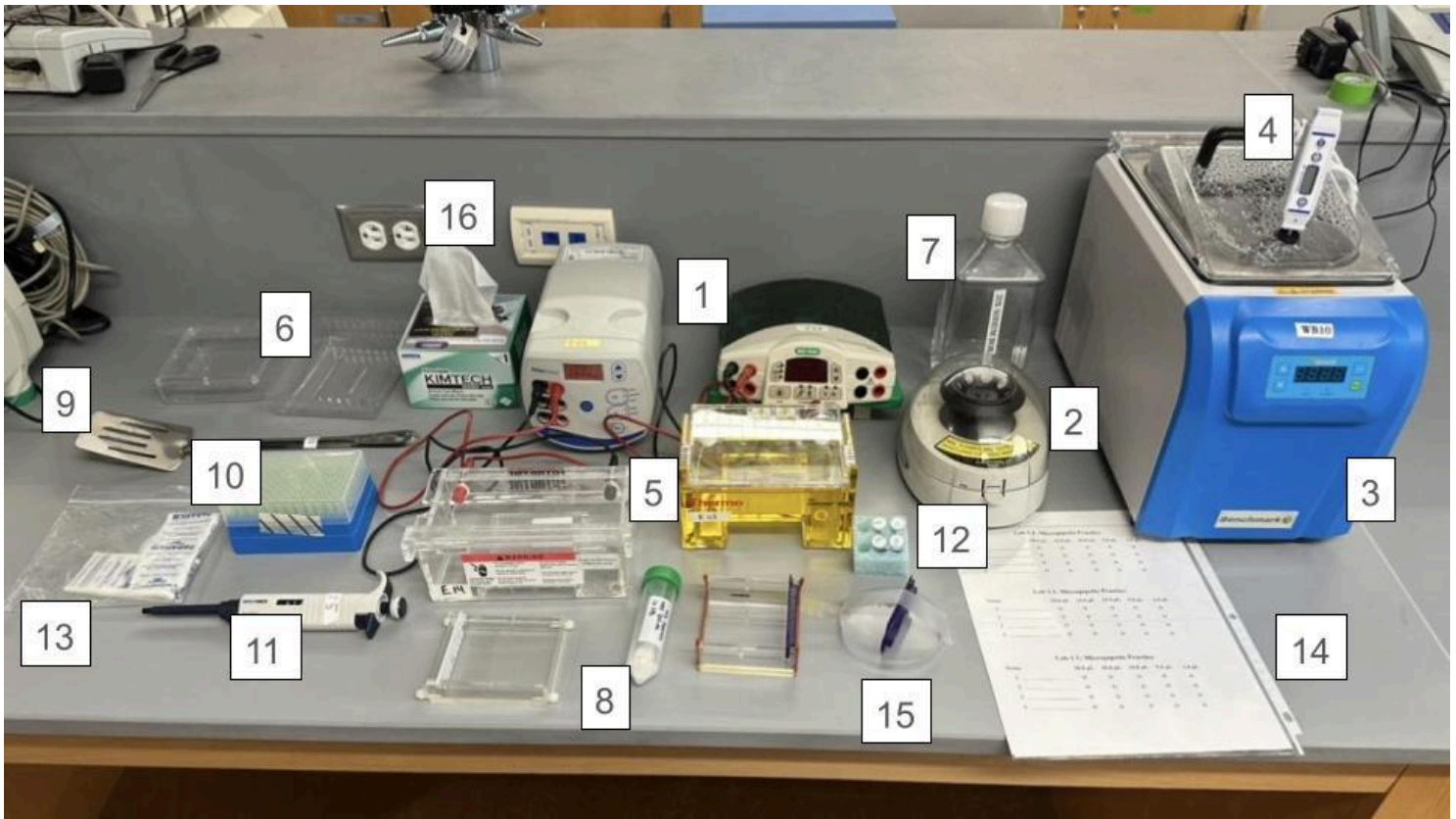
NEW Take a look at the worksheet on how to read the gels, it has a picture of what the gel should look like in color.

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 (2015) on page OV-30 or pg 33 in 2019 teacher Guide. Also refer to picture guide on Gel Making.

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

Thank you 

Lab 1.2

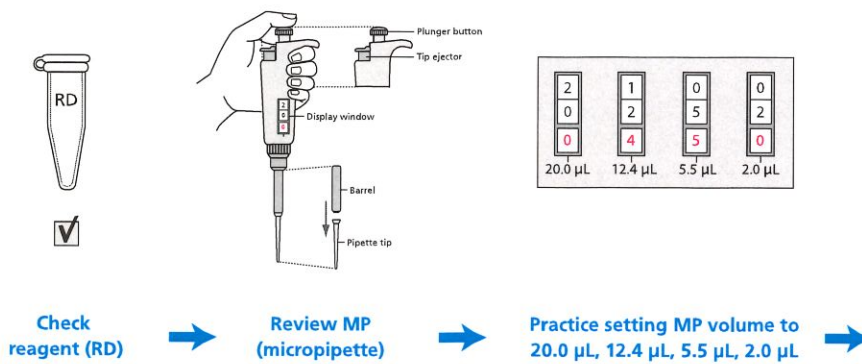


1. Electrophoretic power packs
2. Mini-micro centrifuge
3. Water bath
4. Thermometer
5. Gel electrophoretic apparatuses w/ tray and comb
6. Staining trays
7. 20x SB buffer
8. Agarose
9. Spatula
10. P20-200 pipette tips
11. P2-20 pipette
12. Solution 1,2,3 & red dye
13. Parafilm
14. Practice sheet
15.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

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.

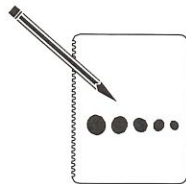
Laboratory 1.1 Flowchart



Micropipette Practice:

| Name | 20.0 µL | 15.0 µL | 10.0 µL | 5.0 µL | 2.0 µL |
|----------|---------|---------|---------|--------|--------|
| 1. _____ | ○ | ○ | ○ | ○ | ○ |
| 2. _____ | ○ | ○ | ○ | ○ | ○ |
| 3. _____ | ○ | ○ | ○ | ○ | ○ |
| 4. _____ | ○ | ○ | ○ | ○ | ○ |

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



Draw sizes of RD amounts in notebook

Lab 1.1: Micropipette Practice

| Name | 20.0 μL | 15.0 μL | 10.0 μL | 5.0 μL | 2.0 μL |
|----------|--------------------|--------------------|--------------------|-------------------|-------------------|
| 1. _____ | 0 | 0 | 0 | 0 | 0 |
| 2. _____ | 0 | 0 | 0 | 0 | 0 |
| 3. _____ | 0 | 0 | 0 | 0 | 0 |
| 4. _____ | 0 | 0 | 0 | 0 | 0 |

Lab 1.1: Micropipette Practice

| Name | 20.0 μL | 15.0 μL | 10.0 μL | 5.0 μL | 2.0 μL |
|----------|--------------------|--------------------|--------------------|-------------------|-------------------|
| 1. _____ | 0 | 0 | 0 | 0 | 0 |
| 2. _____ | 0 | 0 | 0 | 0 | 0 |
| 3. _____ | 0 | 0 | 0 | 0 | 0 |
| 4. _____ | 0 | 0 | 0 | 0 | 0 |

Lab 1.1: Micropipette Practice

| Name | 20.0 μL | 15.0 μL | 10.0 μL | 5.0 μL | 2.0 μL |
|----------|--------------------|--------------------|--------------------|-------------------|-------------------|
| 1. _____ | 0 | 0 | 0 | 0 | 0 |
| 2. _____ | 0 | 0 | 0 | 0 | 0 |
| 3. _____ | 0 | 0 | 0 | 0 | 0 |
| 4. _____ | 0 | 0 | 0 | 0 | 0 |

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.

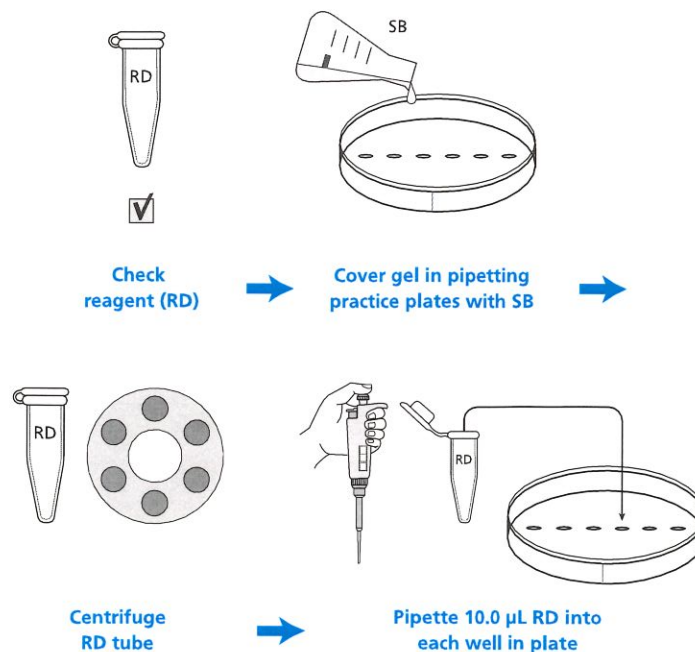


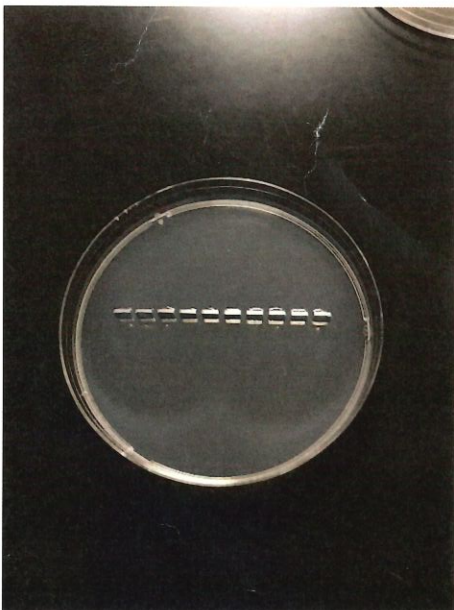
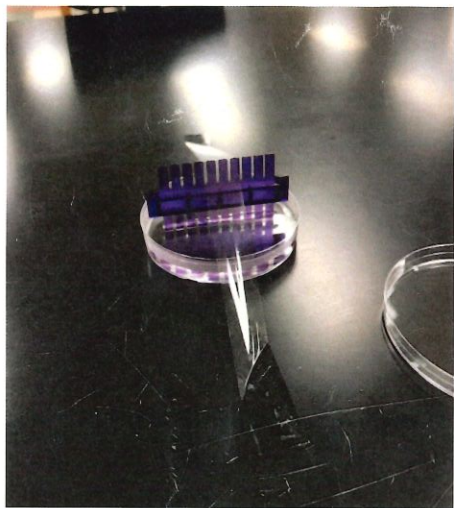
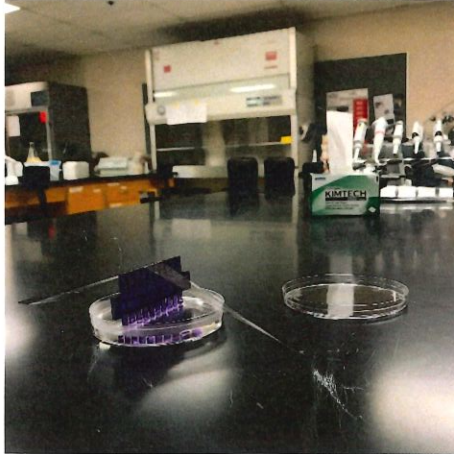
Strategy: For the lab, you may want to show students the sample flowchart rather than have them create their own.

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





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 make these plates.

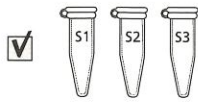
You will need:

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

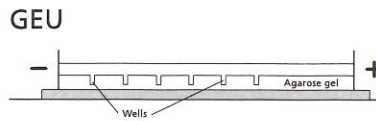
Steps:

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.

Laboratory 1.2, Part B Flowchart

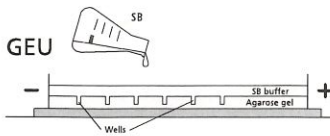


Check reagents (S1, S2, and S3)

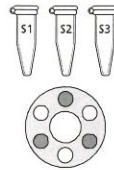


Find out which wells our group will use

Review gel electrophoresis unit (GEU)



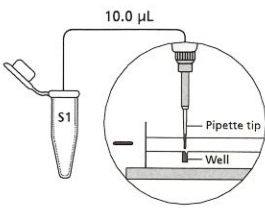
Cover gel in GEU with SB



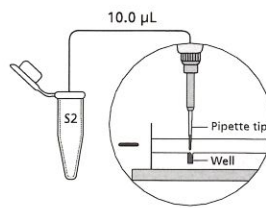
Centrifuge S1, S2, and S3



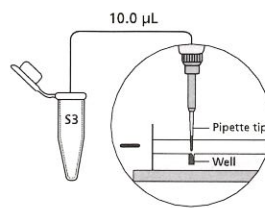
Draw location of each well, and label its solution in notebook



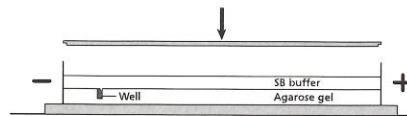
Pipette 10.0 µL S1 into GEU well



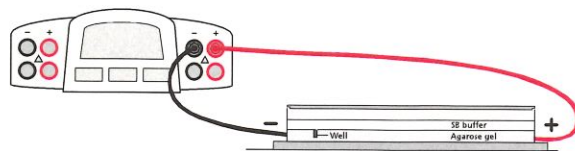
Pipette 10.0 µL S2 into GEU well



Pipette 10.0 µL S3 into GEU well



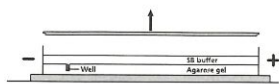
Place cover on GEU



Connect leads to power supply

Turn on power supply and set voltage to 130–135 V

Run gel for 10 minutes



Remove cover from GEU



Draw the relative location of the bands and their colors in notebook

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

