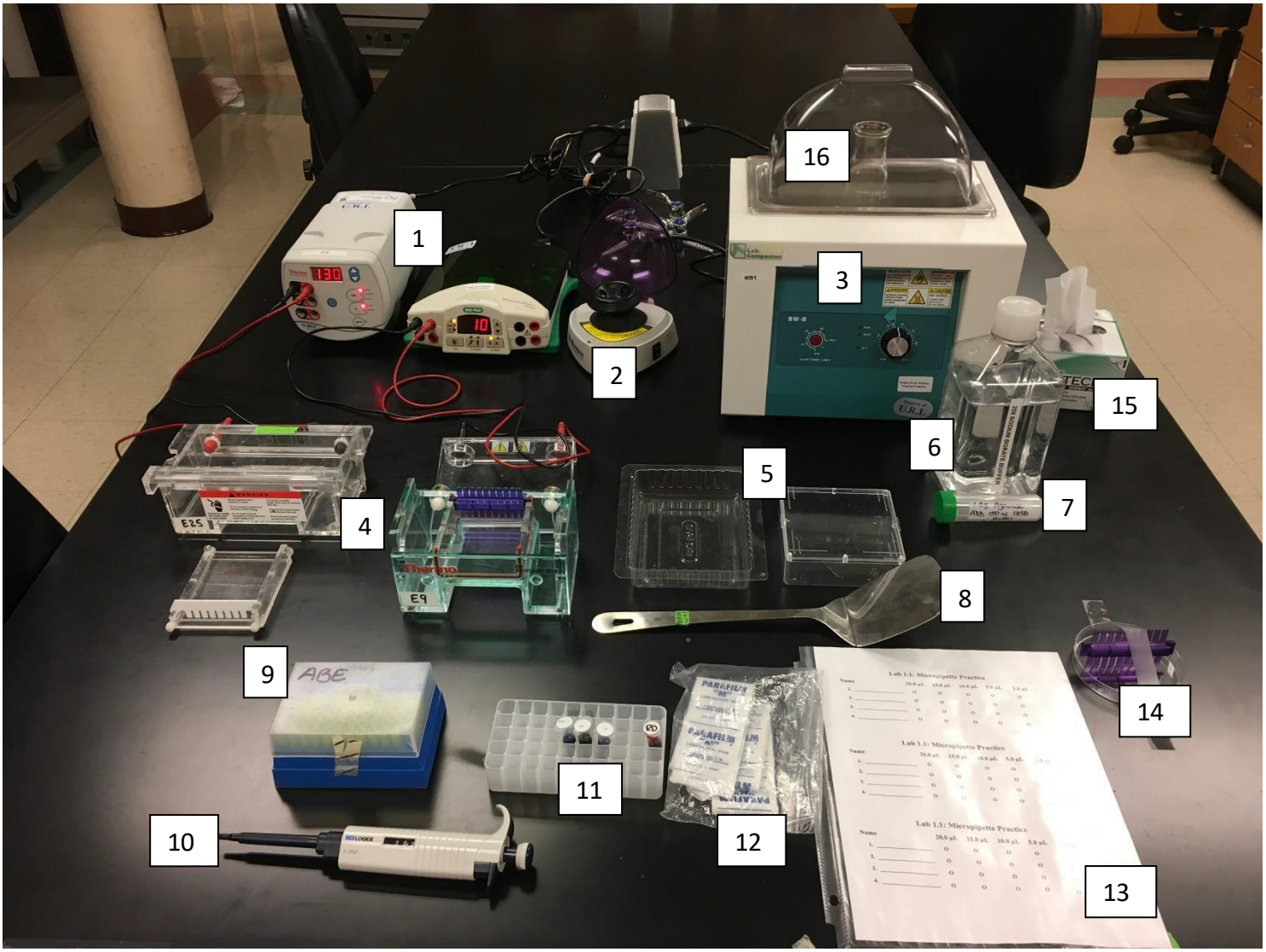
Pictorial Manual

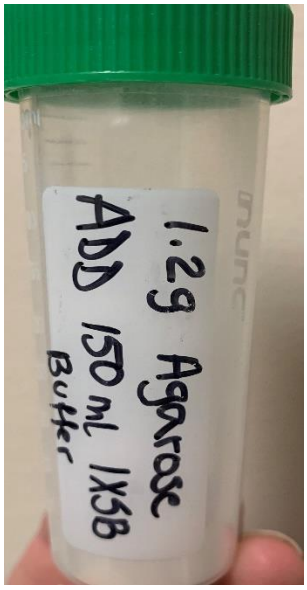
Amgen Biotech Experience

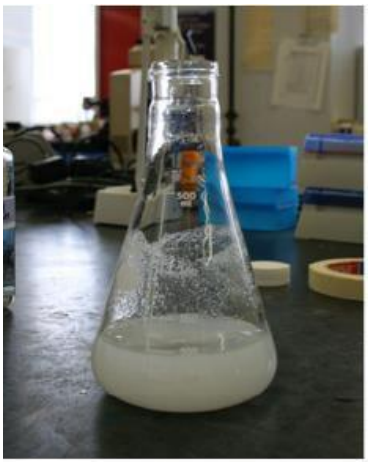
Scientific Discovery for the Classroom

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





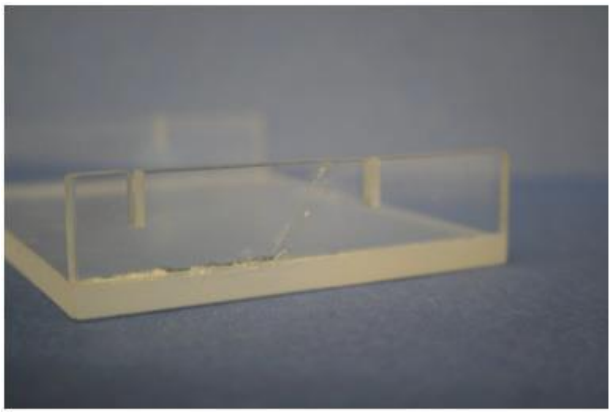
Kit Materials:

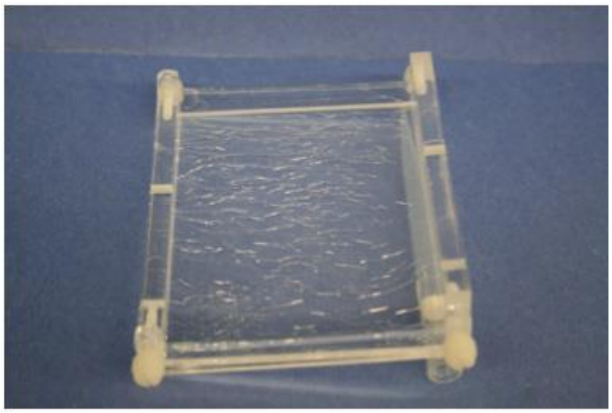
agarose, 20x Sodium Borate (SB) buffer, gel trays, combs, electrophoresis chamber, power supply, Solutions #1, 2, 3 (store at RT), red dye, P-20 micropipette and tips

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.





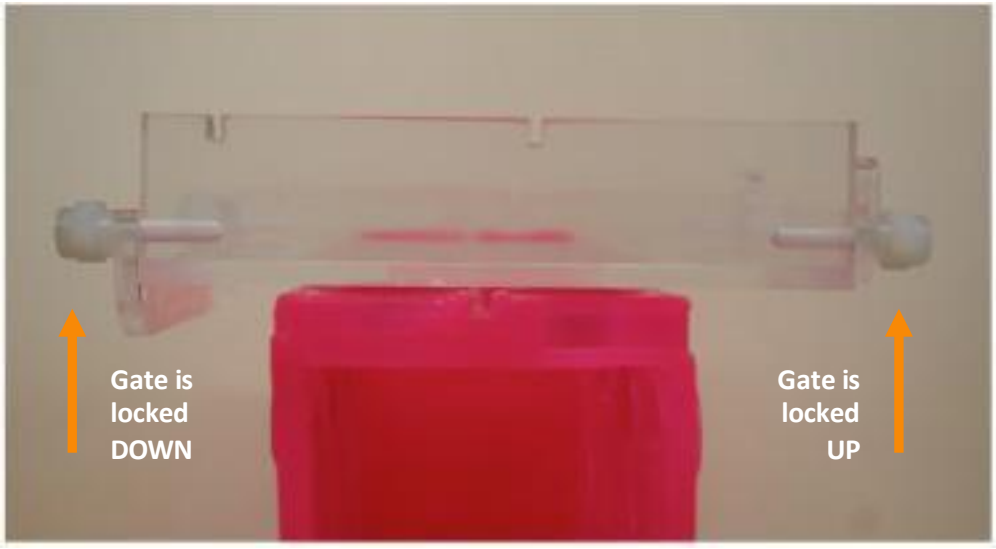
Examples of cracked/warped trays as a result of pouring hot agar solution

- 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)

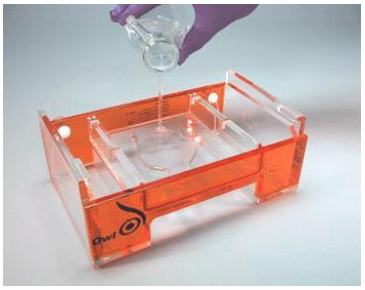
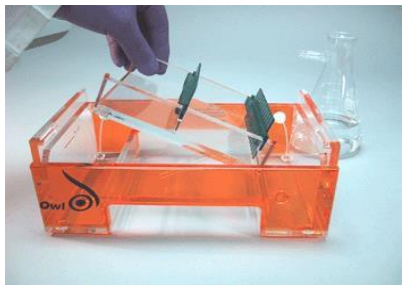
- **Preparing Trays**

  - 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.



Alternative Gel Apparatus

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**

Lab 2A

Kit Materials:

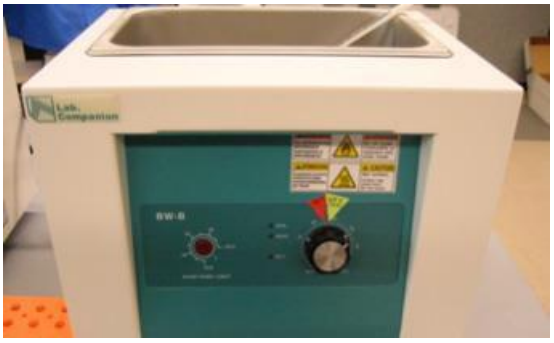
p-ARA plasmid (store in freezer), restriction enzymes BamHI and HindIII (store in freezer), 2.5x restriction buffer (store in freezer), dH20, water bath, thermometer, green, blue or white float, P-20 micropipette and tips

Aliquoting:

Items: plasmid (p-ARA), enzymes (BamHI, HindIII), 2.5x restriction buffer, water

- Vortex and spin enzyme mix and 2.5x restriction buffer before aliquoting the tubes for student groups. If you do not have a vortex, finger flick the tube several times to mix and then spin down in the centrifuge.

Label Tube	Contents	Aliquot	Actually Use
2A	pARA	10 µL	8 µL
RE	BamHI and HindIII	3-4 µL (equal amts of each i.e. 1.5 -2µL of each)	2µL
2.5xB	2.5x Restriction buffer	12 µL	8 µL
dH20	Distilled water	1000 µL	2 µL

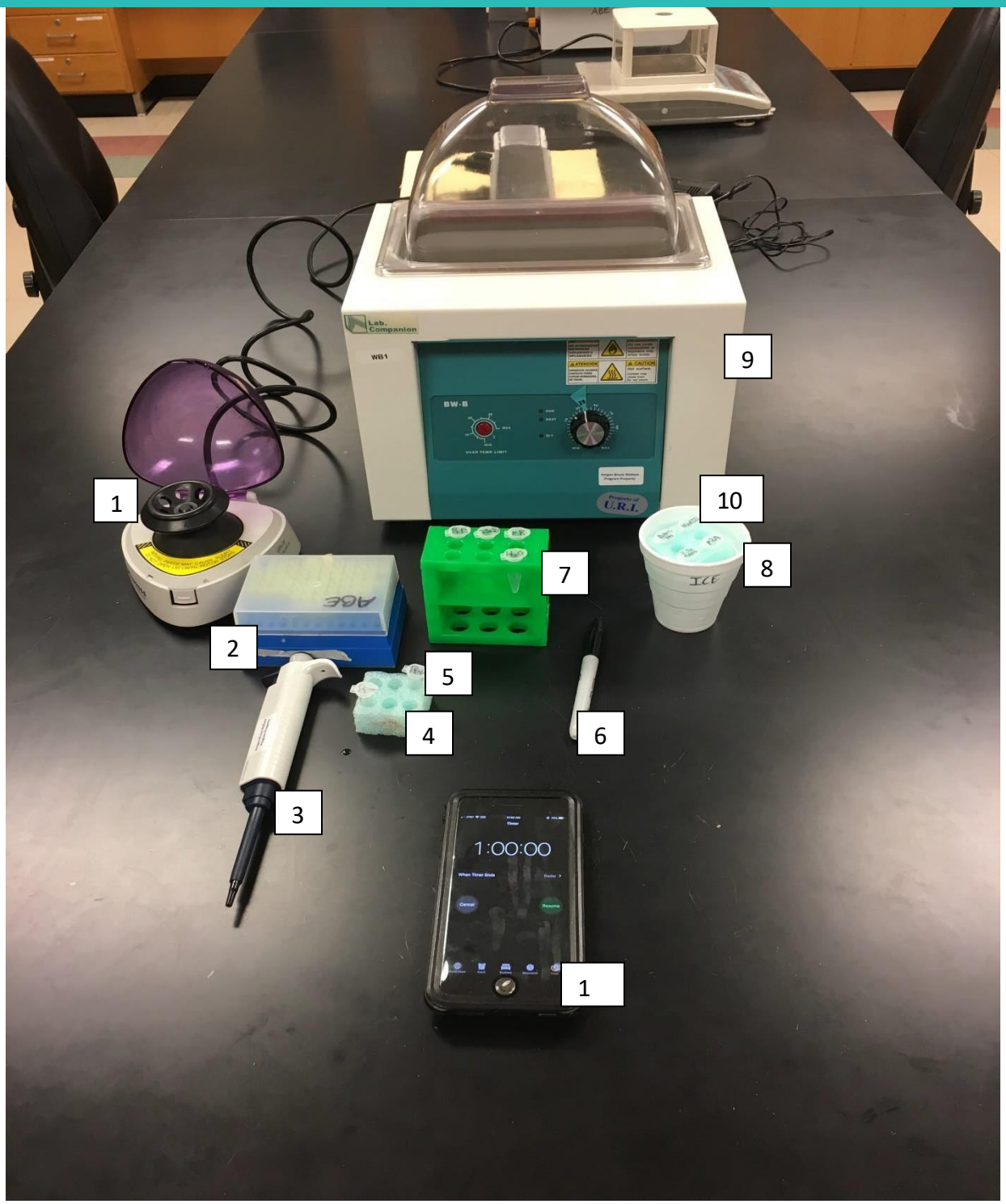


Restriction Digest

Items: water bath, thermometer, float, samples to be digested

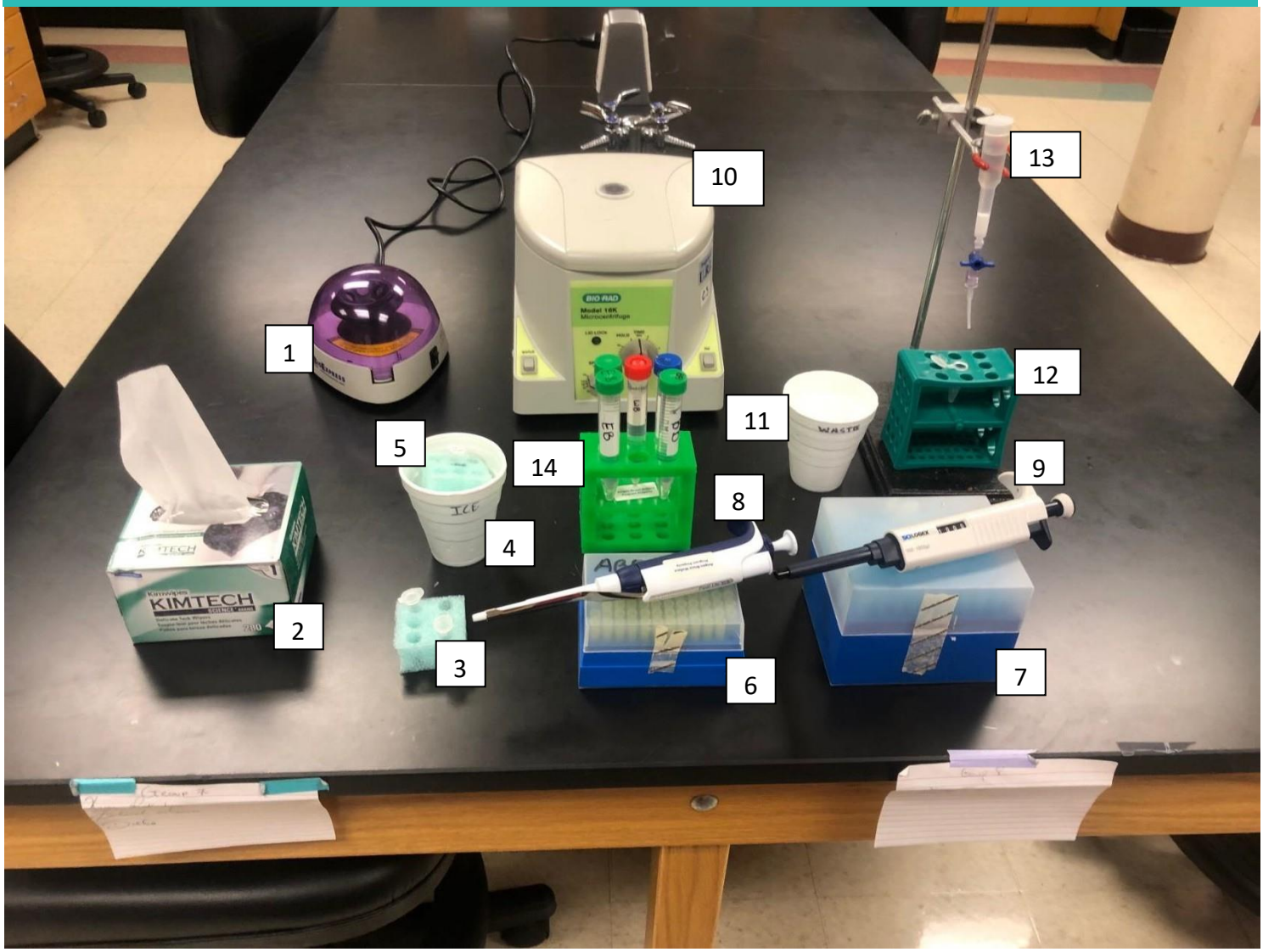
- Calibrate water bath to 37˚C the day before the lab to ensure temperature is correct for the restriction digest of student samples (60 minutes).
- Do not leave the digest in the water for over 2 hours, as BamHI will begin to cut DNA randomly.
- Store digested samples at -20˚C until you are ready to run the gel electrophoresis (lab 4A)..
- Please empty out and wipe down the water baths before returning.

Lab 2A

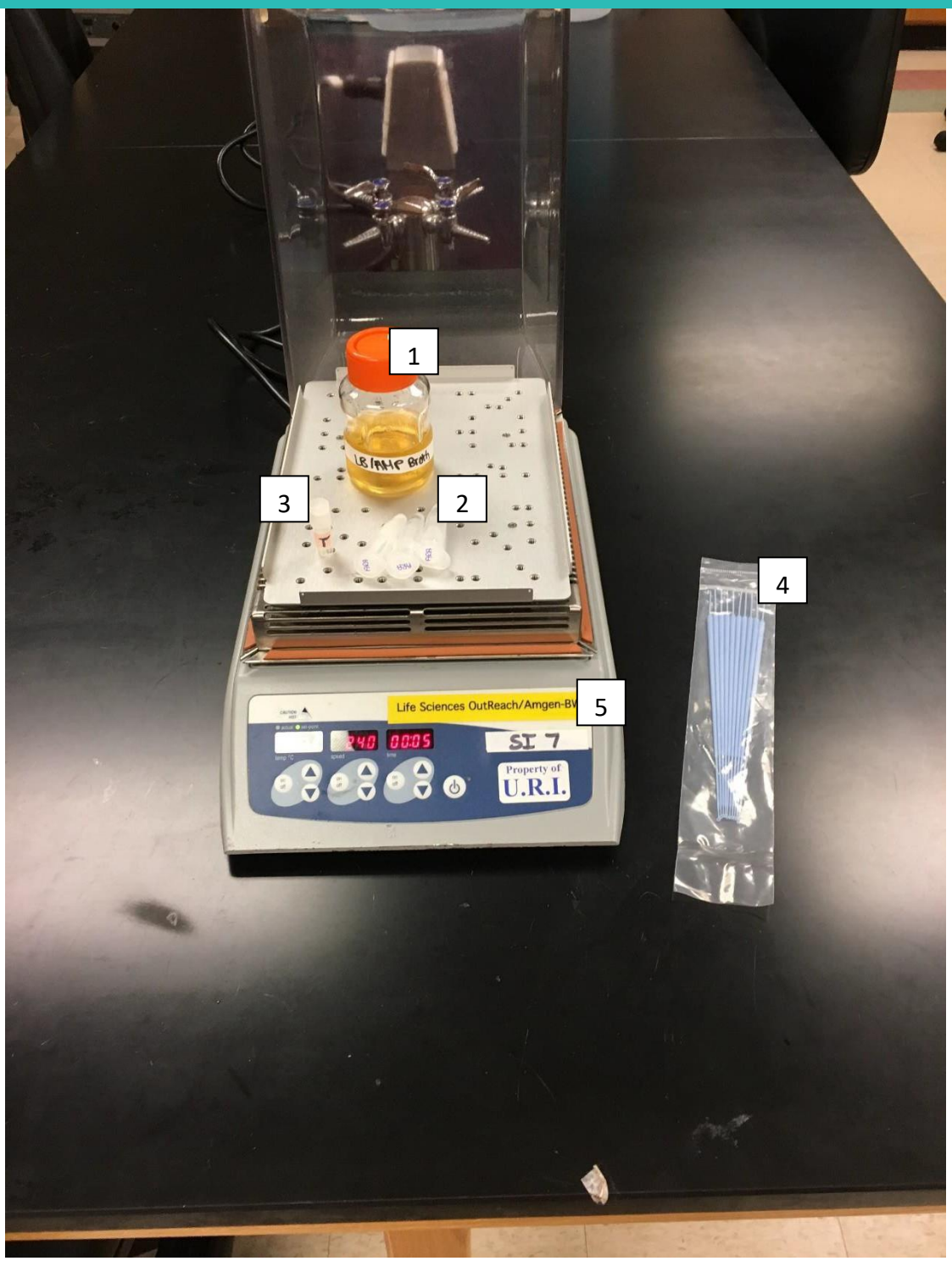


1. Mini-centrifuge
2. P20-200 pipette tips
3. P-20 pipette
4. Green microfuge tube float holding tubes labeled **R+** and **R-**
5. Microfuge tubes
6. Sharpie marker
7. Microfuge tube rack holder holding tubes labeled **2.5xB**, **pR**, **RE**, **dH$_2$O (** should be aliquoted by **teacher**)
8. Cup to hold ice
9. Water bath (set to 37°C for this specific experiment)
10. Microfuge tubes that **must** be on ice containing either of the following: **BamH1, HindIII, pARA 2A & 2.5x restriction buffer**
11. Timer **(WE DO NOT PROVIDE!)**

Lab 6

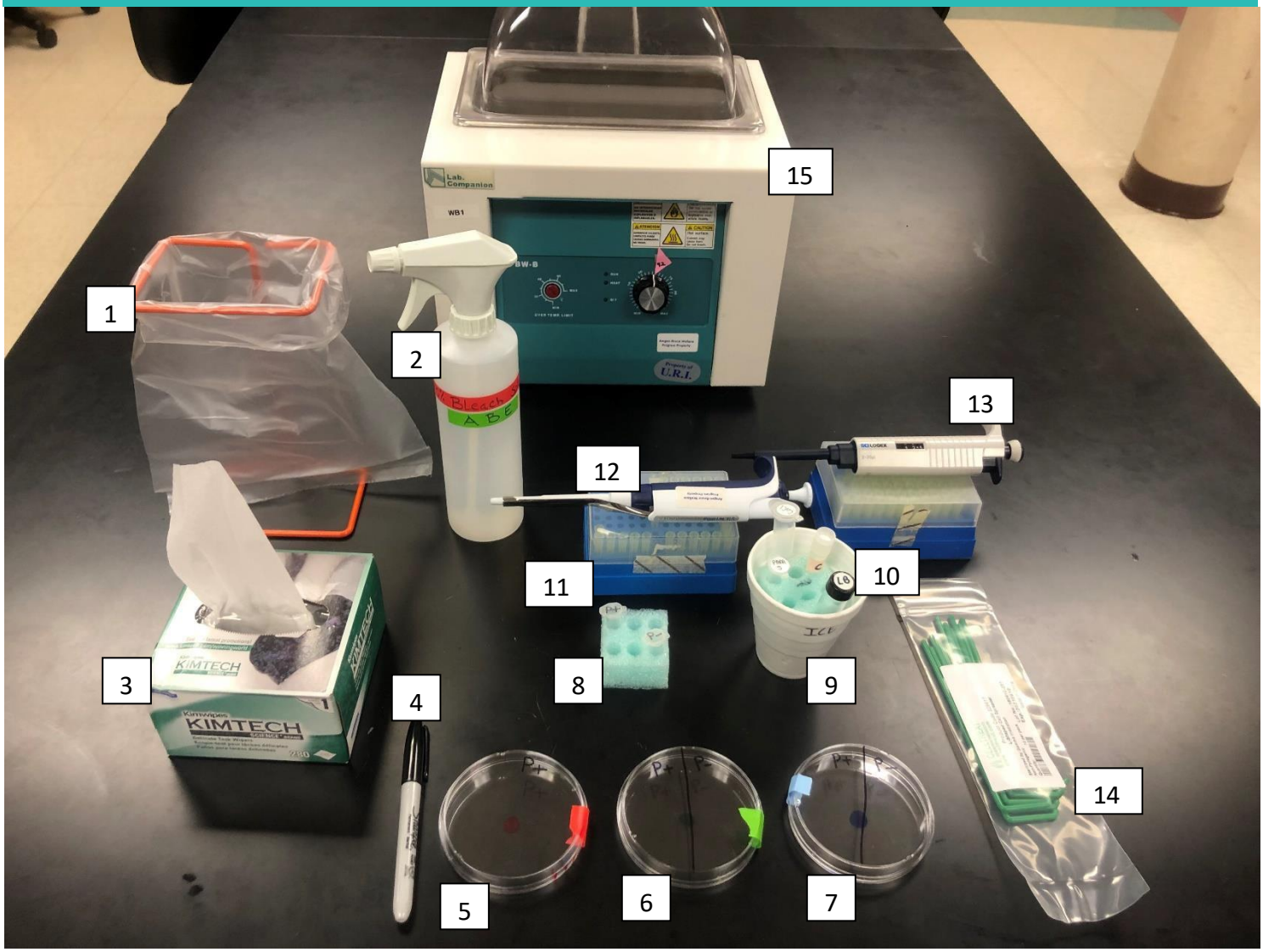


1. Mini centrifuge
2. Kimwipes
3. Greet float tube holder: holds tubes throughout the experiment
4. Ice cup
5. Tube labeled **LYS**; the Lysis buffer tube should be on ice
6. P20-200 pipette tips
7. P100-1000 pipette tips
8. P20-200 pipette
9. P100-1000 pipette
10. Large centrifuge
11. Waste cup
12. Microfuge tube rack holder: holds the **RFP** tube the collects sample
13. Column
14. Microfuge tube rack that holds: elution buffer (**EB**), binding buffer (**BB**), wash buffer (**WB**), column equilibration buffer (**CEB**), and **20% ethanol**



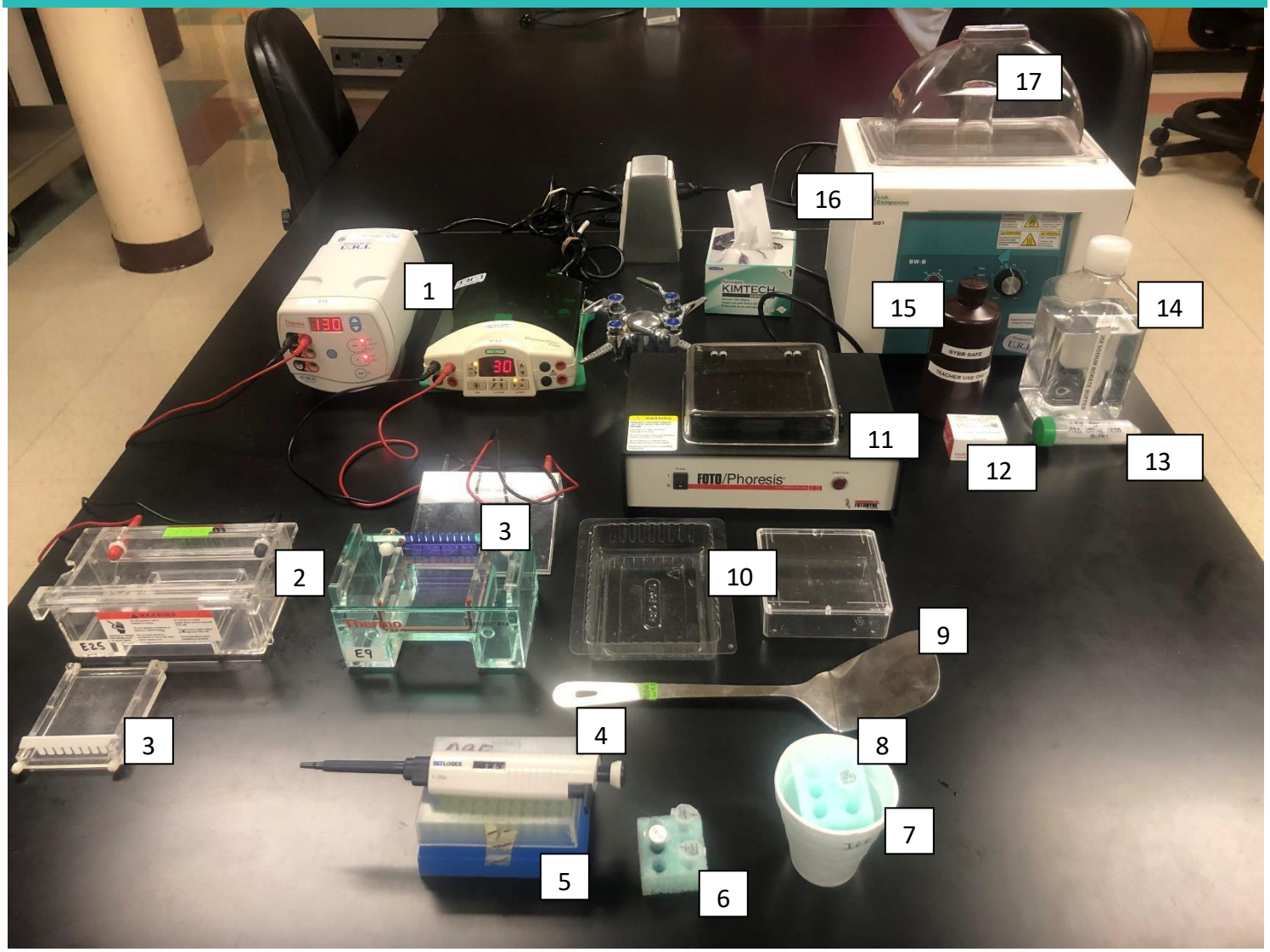
1. 100 mL sterile Lb broth
2. 3 tubes of arabinose
3. 1mL tube of **Transform** cells
4. Inoculating loops
5. Shaker/ incubator

Lab 5



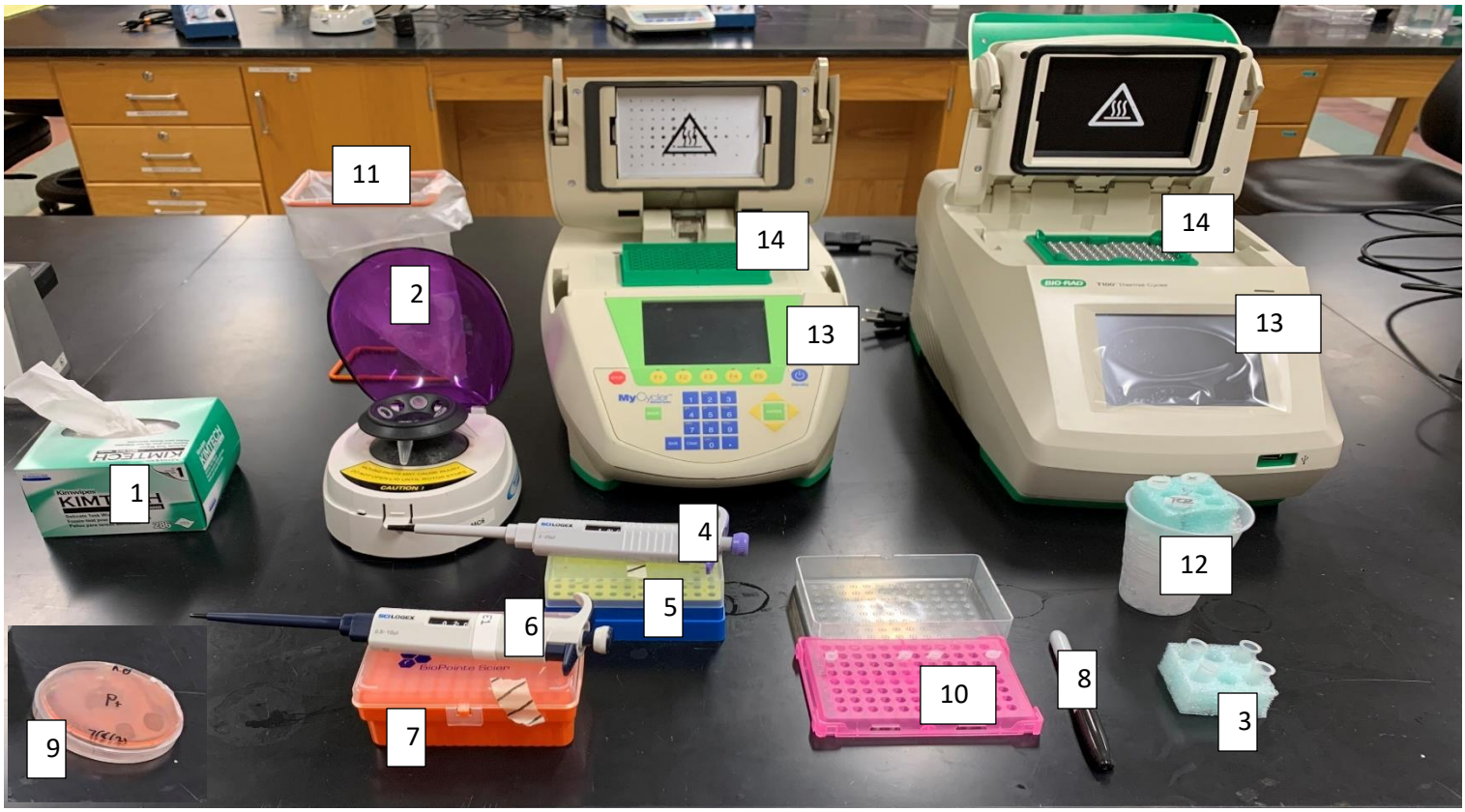
1. Biohazard bag and waste stand
2. 10% bleach bottle
3. Kimwipes
4. Sharpie marker
5. LB/AMP/ARA plate (marked by **3 red** stripes)
6. LB/AMP plate (marked by **2 green** stripes)
7. LB plate (marked by **1 blue** stripe)
8. Green float: used to hold tubes marked **P+ & P-**
9. Ice cup
10. **LB glass vial, competent cells, pARA 5a and Calcium chloride (CaCL) should all be on ice**.
11. P20-200 pipette tips
12. P20-200 pipette
13. P2-20 pipette
14. Spreaders
15. Water bath (should be set at 42°C for this specific experiment)

Lab 4A



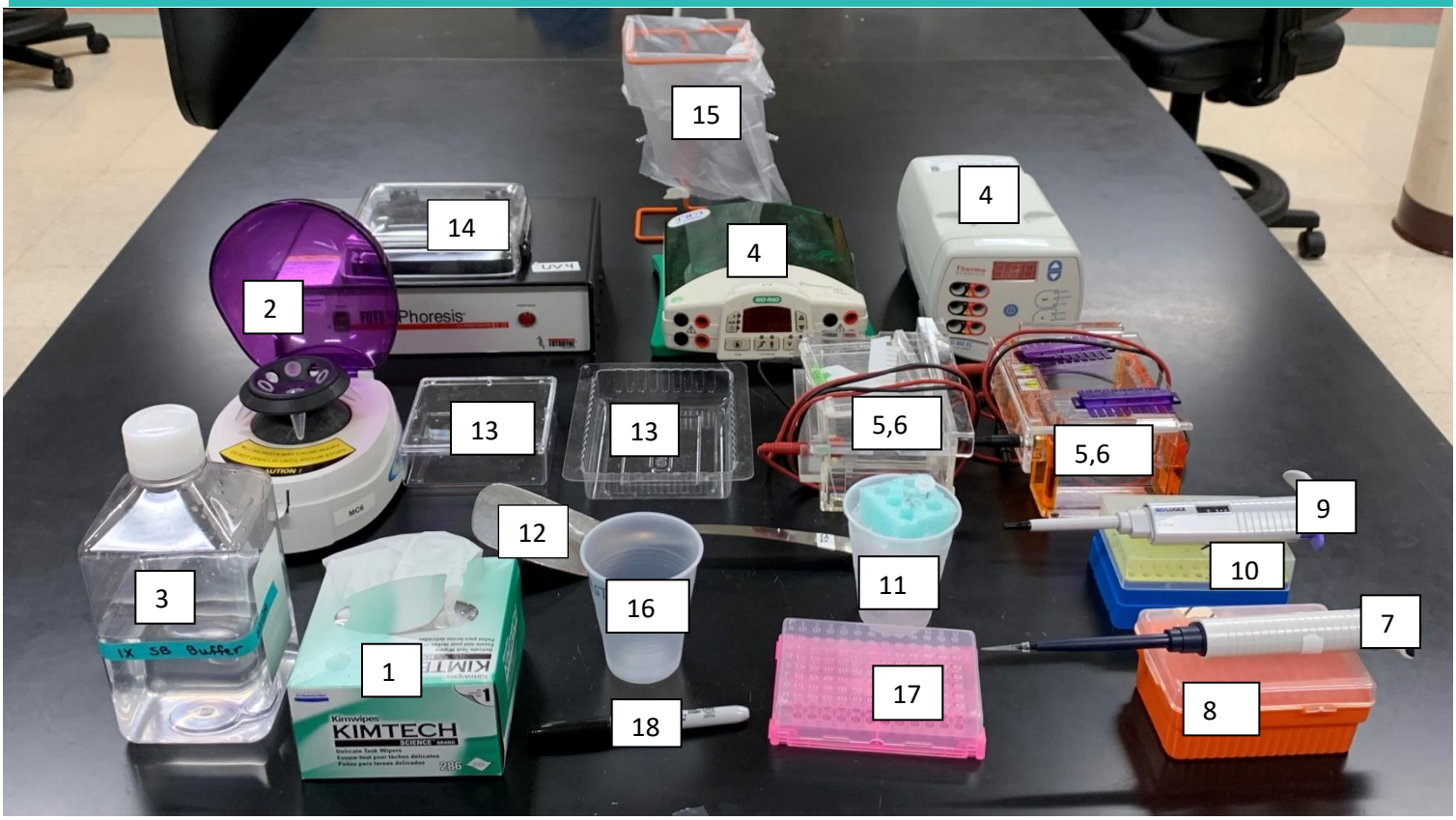
1. Electrophoresis power packs (both types that may be provided in kits are being displayed)
2. Electrophoresis chambers (both types that may be provided in kits are being displayed)
3. Trays and combs (both types that may be provided in kits are being displayed)
4. P2-20 micropipette
5. P20-200 micropipette tips
6. Green float tube holder- should be used to hold tubes labeled: **R+, R-,** and 5x loading dye (**LD**); it is the same as Sol. 2.
7. Ice cup
8. Tubes that should be on ice: **1Kb Ladder**
9. Spatula
10. Gel trays (both types that may be provided in kits are being displayed)
11. UV trans illuminator
12. SybrSafe tube
13. 1.2g of agarose tube
14. 20x SB buffer
15. SybrSafe Post Stain Bottle
16. Water bath
17. Hot agarose in a flask should be placed in water bath to cool down (make sure to set water bath to **55-60°**)

Lab Colony PCR Part I (A)



1. Kimwipes
2. Mini centrifuge
3. Cap-less tubes & forceps & green foam float rack: **cap-less tubes** should be used to keep small PCR tubes safe when centrifuging, **forceps** should be used to help retrieve PCR tubes and cap-less tubes from either the mini centrifuge or the large centrifuge.
4. P0.5-10 micropipette
5. P10 pipette tips
6. P20 micropette
7. P20-200 pipette tips
8. Sharpie marker
9. Lab5 Transformed P+ colonies the arabinose/ampicillin plate: “Pink colonies”
10. PCR tube holder with 4 sample tubes from each group
11. Waste Bag and holder
12. Ice cup: should hold tubes labeled: Positive (+), Negative (-), and PCR (contains TAQ and Primers)
13. Thermocycler (PCR)- both types that may be provided in kits are being displayed
14. PCR green adapter- both types that may be provided in kits are being displayed

Lab Colony PCR Part II (B) Gel Electrophoresis



1. Kim wipes
2. Mini centrifuge with capless tubes
3. 1X Sodium Borate Buffer (Made up earlier from 20X Sodium Borate stock).
4. Electrophoresis power packs (both types that may be provided in kits are being displayed)
5. Electrophoresis chambers (both types that may be provided in kits are being displayed)
6. Trays and combs (both types that may be provided in kits are being displayed)
7. P0.5-10 pipette
8. P-10 pipette tips
9. P20 micropette
10. P20-200 pipette tips
11. Ice cup: holding the 100bp ladder: (M-100) and green float
12. Spatula
13. Gel trays (both types that may be provided in kits are being displayed)
14. UV trans illuminator
15. Waste receptacle
16. Waste cup
17. PCR tube holder (with PCR samples 4 per group)
18. Sharpie marker

Not Shown but needed for Gel preparation

1.2g of agarose tubes

20x SB buffer

Sybrsafe in tube (keep away from light)

Water bath (used to cool down the agarose- set temp at 55-60°C)

Flask containing the hot agarose should be placed in the water bath to cool down before being poured.