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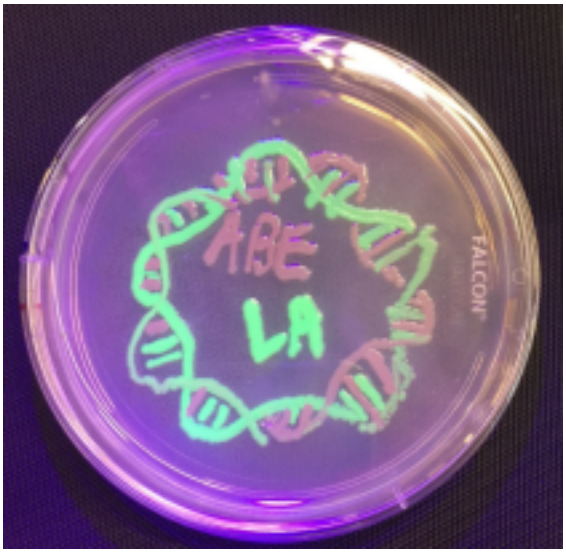
Scientific Discovery for the Classroom

Greater Los Angeles



ABE-LA PBC Kit Notebook

This guide is meant to be used as a supplement to the Amgen Biotech Experience Teacher's Guide with information specific to the ABE-LA PBC Distribution Site including adapted lab protocols which differ slightly from the online ABE Student Guide and helpful tips for each lab to ensure your students' success.

ABE PBC Distribution Center Contact Information:

Samantha Leano, ABE-LA PBC Site Coordinator
2265 E Foothill Blvd
Pasadena, CA 91107
(818) 859-3096

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ABE Curriculum Guides And Lab Series Resources

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ABE Complete GE Student Guide 2023	ABE Abridge GE Student Guide 2023	ABE Focus Lab Series Student Guide
ABE Complete GE Teachers Guide 2023(M3rlot!)	ABE Abridge GE Teacher Guide 2023(M3rlot!)	ABE Focus Lab Series Teacher Guide 2023(M3rlot!)
Blank Flow Charts for students	Blank Flow Charts for students	Blank Flow Chart for students
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Scheduling and Ordering of Kits and Supplies

The PBC Distribution Center has teachers complete all necessary forms online. These can all be accessed via the **ABE-LA PBC Center webpage** →

The current **PBC Kit/Supply Schedule** will be posted on the webpage every year in March. Teachers who have completed an ABE training workshop should check the schedule for available time slots. Each school is allowed one 3-week time period per year to use a kit therefore only a single teacher per school needs to complete the reservation form. Multiple teachers at a single school must be creative in scheduling the labs as they will be sharing one kit. Schools who have their own equipment and only need supplies have more leeway in their scheduling. Time slots will be assigned by the PBC Site Coordinator based on when the requests are received and kit availability. Teachers are encouraged to complete the Reservation Request Form as soon as possible once the form is opened up for the year. Teachers should mark the assigned pickup and return dates in their calendars as those dates are strictly adhered to.

KIT/SUPPLY RESERVATION (FORM A)	KIT/SUPPLY ORDERS (FORM B)	TEACHER POLICIES (FORM C)	LAB RESULTS SURVEY (FORM D)	HOW TO COMPLETE FORMS

How to Complete the ABE-LA PBC Supply Order Form - Quick Guide

Deadline: Submit 4 weeks before your pickup date. Each teacher must complete it every school year.

Purpose:

1. Calculate needed lab supplies
2. Collect data for grant reporting

What you'll need to provide:

Basic Info:

- Contact, school, and reservation details
- Materials requested (full kit, reagents, equipment)
- ABE participation (first year or not)

Student & Course Info:

- Total # of students, classes, and courses
- For each course: name, labs used, # or students
    - *Example: Honors Bio, Focus Series (no Lab 6), 80 students*

Supplies Section:

Only answer for the lab series you're doing:

Complete Series:

Students, groups, transformation date, groups doing protein lab & PCR

- *Example: 40 students, 10 groups*

Abridged or Focus Series:

- Same info as above (Skip info not applicable)

Protein Lab Options:

- Choose: Supernatant, Pre-lysed cells, or Grow your own

ABE-LA PBC Kit and Supply Pick-Up & Return Policies

Schedule

- Pick-Up: Wednesdays
- Returns: Tuesdays
    - Note: Dates may vary – always check the kit schedule and record your assigned dates.

Appointment Scheduling

- Use Sign Up Genius to schedule pick-up/return appointments →
    - Kit: 30-minute time slots
    - Supplies-only: 30-minute time slots (usually scheduled via email with the site coordinator)
- Invitations are sent about one month in advance of your date.
- You'll also get an email when the invitation is created – check spam folders if needed.
- Scheduling is first come, first served.

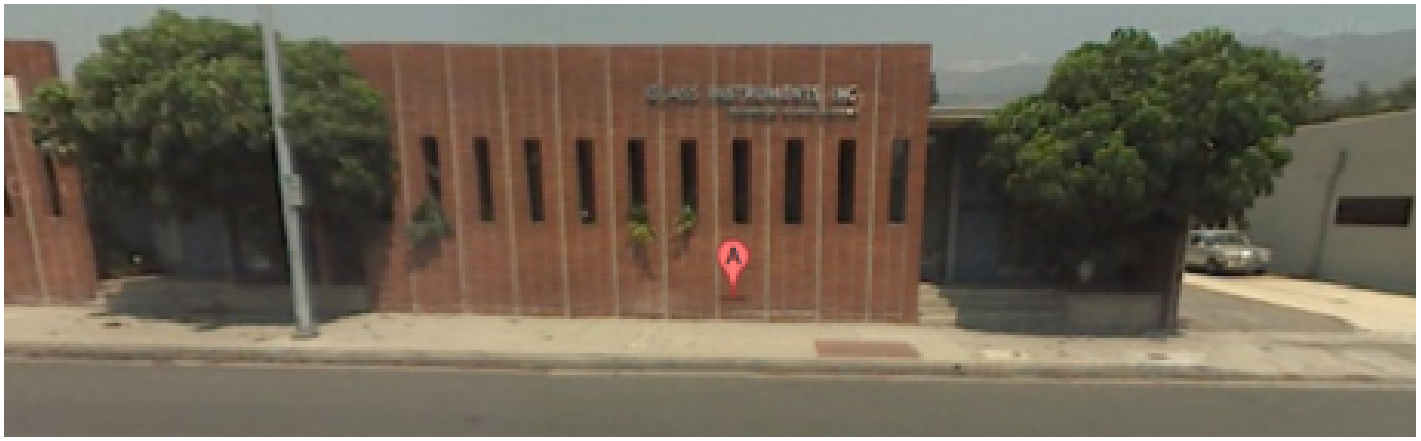
Important Notes

- Only one teacher per school will receive the Sign Up Genius invite.
    - If someone else is handing pick-up/return, forward the invite to them.
    - Be sure to provide the correct name and cell number for each appointment.

Location and Procedure

- Pick-Up/Return Location:
    - Pasadena Bio Collaborative Incubator
      2265 E Foothill Blvd, Pasadena, CA 91107
    - Park along the left wall in the back lot
    - Go to the last door (farthest from driveway)
    - Call (818) 859-3096
- We will complete the supply checklist together:
    - Once at pick-up to confirm items received
    - Again at return to confirm all items are returned

PBC located on Google Maps



Supply and Reagent Storage

Supply Spreadsheet

- You'll receive a digital copy of your supply spreadsheet when picking up your supplies or kit.
- This document lists reagents volumes and is color-coded by storage temperature:
    - Red – Freezer (in Isofreeze unit)
    - Blue – Freezer
    - Green – Refrigerator
    - Black – Room temperature
- Review your spreadsheet carefully and report any discrepancies to your site coordinator immediately

Storage Guidelines

- Reagent storage information is also summarized in the Quick Supply/Reagent Sheet.
- Store reagents promptly and correctly upon returning to your school.

Return Requirements

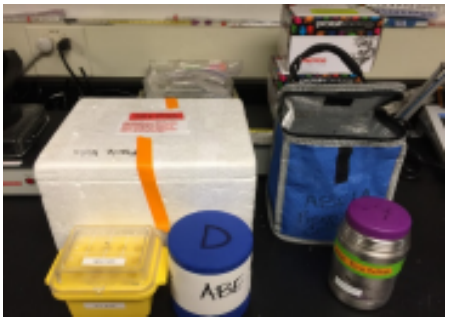
- All unused reagents and consumables must be returned, including tubes and bottles.
    - This allows for quality testing if experiments fail and supports our waste reduction efforts by reusing containers.

Site Visits

During your 3 - week time slot, teachers typically visit the PBC site three time:

1. To pick up the kit and supplies
2. To pick up labs 5 and above materials (i.e competent cells, plates, and protein purification supplies)
3. To return all kits and supplies

Quick Supply/Reagent Storage Summary Sheet



FREEZER (PUT IN ASAP!)

Thermos containing: Plasmids, Bam/Hind, 2.5XB, Ligase, 5XB, DNA Marker, Master Mix, primers.

Isofreeze Unit Containing: Competent cells and lab 6 cells

REFRIGERATOR
Sleeves of LB agar plates, tubes of LB and LBA broth, lysis buffer

ROOM TEMPERATURE:
General consumables like: 20X Sodium Borate Buffer

ROOM TEMPERATURE (IN THE DARK):
GGLD or GRLD*
Note: Some gels will come prestained with fluorescent

Teacher Task Checklist for the ABE labs

KIT Packing

Unpacking the Kit Equipment

Items Not Provided by ABE-LA PBC Site:
Schools are responsible for supplying the following:

- Waste containers
- Microwave oven
- Student PPE (Personal Protective Equipment)

Incubator and Water Needs:

- PBC has two incubators available for loan- request in advance.
- Schools should use deionized/distilled (DI) water in equipment and for final rinses; tap water is corrosive.
    - The site can provide up to 2 gallons of DI water if needed.
    - Return the Bottles after use, as they are refilled and reused

Kit Equipment Checklist

Repacking And Returning Kits and Supplies

General Tips

- Repack items as you complete each lab to avoid last-minute stress.
- Follow the content list and packing photos on each box– kits include different models, so items must be packed in a specific order.
- Kit Checklists are available online or through the QR code on this page.

Before Repacking

- Follow the waste disposal protocol →
- Refill tip boxes and rinse the following with distilled water, then air dry:
    - Gel boxes
    - Gle trays and combs
    - Casting units
    - Melting Flasks
- Empty the 1X Sodium Borate Buffer Bottle in the sink
- No liquids should be stored in kit boxes unless sealed in secondary sleeves.
- Keep the isofreeze unit, thermos, and ice packs frozen. If thawed, refreeze before return.

Don't Forget to Check

- Your preproom, freezer, refrigerator, and classroom for:
    - Gel trays and combs
    - Thermos insulated bags
    - Empty DI water bottles

Damage or Lost Equipment

- Label damage items clearly (e.g., "pipette not aspirating").
- Notify your site coordinator promptly
- Return any broken parts, especially acrylic gel tray chips – we may be able to repair them.
- See the ABE Kit Price List for replacement cost →

Preparing And Aliquoting Reagents

General Preparation Steps

- Before aliquoting, always:
  - Thaw, spin down, and gently mix reagents (using pipette or vortex followed by spin).
  - Store aliquots in the freezer until needed.

Special Handling

- Restriction enzymes, Ligase, and PCR Master Mix:
  - Contain glycerol to prevent damage from freeze-thaw cycles.
  - Must be kept on ice while aliquoting.
  - Gently mix before use.
- Competent Cells (Labs 5/5A/5B):
  - Thaw in a cup crushed ice until “slurpy” in texture.
  - Resuspend by pipetting up and down.
  - Aliquot:
    - 100uL per tube (1.5 mL, pre-chilled)
    - Or 50uL into two pre-chilled tubes labeled P+/ P-
  - Only thaw cells needed for each class period
  - Refreeze immediately after aliquoting– transformation efficiency decreases the longer cells stay thawed.

Aliquoting Guide

- Refer to the Aliquoting Guide for specific volumes needed per student group.

Centrifuges

- Mini centrifuges should be placed in accessible areas for students to use during labs.
- High-speed centrifuge is needed to pellet cells in Lab 6.

Safety & Disinfection

- Safety Data Sheets (SDS) for all reagents are available:
  - In the ABE-LA Teachers General Resources Folder
  - On the thumb drive in each kit’s notebook
- Follow your school/district PPE requirements, including:
  - Safety goggles
  - Gloves
  - Disinfection protocols

Spill Cleanup

- For bacterial spills:
  - Cover with paper towels
  - Saturate with 10% bleach or 70% isopropyl alcohol
  - Let sit for 20 minutes
  - Dispose of according to school policy
- Do NOT use bleach on equipment –only use the supplied 70% isopropyl alcohol

ABE Reagent Recipes for Teachers →

ABELA Aliquoting Guide

Lab 1 ***Some tools of the trade***

Red Practice Dye (**RD**) and Solutions **S1, S2, S3**. **Distilled water**:
*A class set (12 tubes each) has been pre-aliquoted and can be found in one of the equipment bins. When these solutions get low, please inform your site coordinator. ** dH_2O is used in several labs.

Lab 2 ***Preparing to clone the RFP gene: digesting the pKAN-R and pARA plasmids***

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	**A**	pARA (80 ng/uL)	10uL	8uL
1.5 mL	**K**	pKAN-R (80 ng/uL)	10uL	8uL
1.5 mL	**RE**	*Bam*H I and *Hin*d III	5uL	4uL
1.5 mL	**2.5xB**	2.5x restriction buffer	20uL	16uL
1.5 mL	**dH_2O**	Distilled water	**	**

** dH_2O is used in several labs and comes in pre aliquoted tubes

Lab 2A ***Preparing to verify the RFP gene: digesting the pARA-R plasmid***

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	***RP***	*pARA-R (**70** ng/uL)	10uL	8uL
1.5 mL	**RE**	*Bam*H I and *Hin*d III	3uL	2uL
1.5 mL	**2.5xB**	2.5x restriction buffer	12uL	8uL
1.5 mL	**dH_2O**	Distilled water	**	2uL

*There are two concentrations of pARA-R for the AbridgedSeries Labs: **Lab 2A** uses the **70 ng/uL pARA-R**. Check the reagent tube labels carefully. ** dH_2O is used in several labs and comes in pre aliquoted tubes

Lab 3 ***Building the pARA-R plasmid***

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	**5xB**	5x ligation buffer	4uL	3uL
1.5 mL	**LIG**	T4 DNA ligase	2uL	2uL
1.5 mL	**dH_2O**	Distilled water	**	**

*students will add their A+/K+ to their Lig tube ** dH_2O is used in several labs and comes in pre aliquoted tubes

Lab 4 ***Verification of restriction and ligation using gel electrophoresis***

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	**M**	1 kb DNA ladder/marker	10uL	8uL
1.5 mL	**LD**	50XGelRed or 50X GelGreen loading dye	14uL	12uL

Lab 4A ***Verification of the recombinant plasmid using gel electrophoresis***

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	**M**	1 kb DNA ladder/marker	10uL	8uL
1.5 mL	**LD**	50XGelRed or 50X GelGreen loading dye	8uL	6uL

Lab 5 *Transforming bacteria with the ligation products*

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	**LB**	Luria broth	350uL	300uL
1.5 mL	**CC**	*Competent cells	100uL	100uL

*Do not aliquot the competent cells until class time, 15 minutes before students begin the lab.

Lab 5A/5B *Transforming bacteria with recombinant plasmids*

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	**LB**	Luria broth	320uL	300uL
1.5 mL	**RP**	**pARA-R (**10** ng/uL)	12uL	10uL
1.5 mL	**CC**	*Competent cells	100uL	100uL

*Do not aliquot the competent cells until class time, 15 minutes before students begin the lab. **There are two concentrations of pARA-R for the AbridgedSeries Labs: **Lab 5A** uses the **10 ng/uL pARA-R**.. Check the reagent tube labels carefully.

Lab 6 *Purifying the fluorescent protein* Part A

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	EC	LB/amp/ara culture of *E. coli*	*2 x 1mL	2mL
1.5 mL	LyB	Lysis buffer	155uL	150uL
1.5 mL	EB	Elution buffer	**	150uL

*Each group will centrifuge a *total* of 2mL of the *E coli* culture. They will first spin down 1 ml, decant, then add a second mL to the same tube, spin down and decant again. ****You will be provided with either sets of 15mL tubes or 30ml bottles of each buffer. Give one set to each group. Before returning, columns should be filled with 20% ETOH.

Lab 6 *Purifying the fluorescent protein* Part B

*Size of tube	Label tube	Contents of tube	*Aliquot	Actually used
1.5 mL	Super**	supernatant**	200uL	200uL
15mL	BB	Binding buffer 4M $(NH_4)_2SO_4$	*	200uL
15ml	WB	Wash buffer 1.3M $(NH_4)_2SO_4$	*	1mL
15mL	EB	Elution buffer 10mM TE	*	2mL
15mL	CEB	Column equilibration buffer 2M $(NH_4)_2SO_4$	*	2mL

*You will be provided with either 15mL tubes or one large bottle of each buffer. If you have the tube sets, give one set to each group. If you have the larger bottles, divide each buffer into a set of flasks that can be shared by two groups. Before returning, columns should be filled with 20% ETOH. **if not doing Lab 6A but starting with 6B, aliquot supernatant.

Colony PCR

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5mL	PCR	Colony PCR Master Mix**	96uL	92uL
1.5mL	+	pARA-R 0.025ng/uL	3uL	2uL
1.5mL	-	pARA 0.025ng/uL	3uL	2uL
1.5mL	M	1 kb DNA ladder/marker	10uL	8uL
1.5mL	LD	50XGelRed or 50X GelGreen loading dye	14uL	10uL

** 53µL of One Taq MM is mixed with 21.5µL of Forward and 21.5µL of Reverse Primers for each student group. Mix these together for the entire class (plus one extra group) and then aliquot 100µL per group. See "Preparing PCR Mix" for complete directions on how to mix PCR Master Mix.

How to Prepare PCR Master Mix: Teacher Instructions

ABELA is now supplying teachers with *One Taq 2X Master Mix with Standard Buffer* aka "**One Taq MM**". This contains: taq polymerase, dNTPs, the Mg2+ enzyme cofactor, buffer and glycerol. It may be *clear or green* colored. For the PCR amplification to occur, specific forward and reverse primers must be added to the MM prior to adding the template DNA. In order to limit primer dimer formation, each primer and the MM will be provided in three separate tubes. Teachers will need to mix their **"PCR"** Master Mix prior to aliquoting for their students.

Keep all tubes on ice during preparation. Mix primers with MM just prior to class.
Gently mix MM with F and R primers , then aliquot per group.

Colony PCR Master Mix Instructions

Colony PCR Reagents	One Taq MM (MM)	col PCR Forward primer (cF)	col PCR Reverse primer (cR)	Working PCR MM (PCR) total
1 student group	52µL**	22µL**	22µL**	**96µL****
10 student groups	520µL	220µL	220µL	**960µL**
12 student groups	624µL	264µL	264µL	**1,152µL**

when using the Eppendorf Repeater Plus: 55µL One Taq + 23µL F +23µL R for 101µL PCR mix
when using the Corning Step R: 55µL One Taq + 23 µL F + 23µL R for 101µL PCR mix

Colony PCR TIPS:

When picking cells from the LBaa plates, try not to gouge the agar and do not add so many cells that the PCR mix becomes cloudy or pink. The LB agar will dampen amplification. If too many cells are added, the genomic DNA will inhibit amplification of the desired segments of DNA and produce a large DNA band that never leaves the well during electrophoresis.

EPM/PTC Master Mix Instructions

EPM/PTC PCR reagents	One Taq MM (MM)	PTC PCR Forward primer (pF)	PTC PCR Reverse primer (pR)	Working PTC PCR MM (PCR) total
1 student	**12.5µL**	**5.25µL**	**5.25µL**	**23**
10 students	**125µL**	**52.5µL**	**52.5µL**	**230**
20 students	**250µL**	**105µL**	**105µL**	**460µL**

Do not premix the MM and primers until the day you plan on doing the lab!

Unused **PCR** mixture can only be used for that specific PCR cycle (Colony versus EPM) and only for a short time. Only mix as much as you are *sure* you will use that day and store in the *freezer* until the lab period.

Return any remaining unused MM and primers frozen in their separate tubes.

Remember that the ABELA site puts the DNA stain (Gel Green or Gel Red) into the Loading Dye, so students must add GGLD or GRLD to all of their samples including the marker prior to loading the wells, regardless of whether the MM is green or clear colored..

Preparing 1X Sodium Borate (SB) Electrophoresis Buffer

To save time between classes, it is recommended that teachers prepare large volumes of 1X SB buffer in advance. This buffer is used both for casting agarose gels and in electrophoresis chambers (e.g OWL and MiniOnes, which hold ~100-300mL each).

Dilution Instructions (From 20X stock)

The SB buffer is supplied at 20X concentration**.** To dilute it to 1X, use the formula:

$$C_1V_1 = C_2V_2$$

C = Concentration

V = Volume

Example:

To make 180mL of 1X SB buffer for 6 gels (30mL each):

$(20X)(V_1) = (1X)(180\ mL) \rightarrow V_1 = 9\ mL$

Add 171 mL of distilled water to 9mL of 20X SB to get 180 mL of 1X SB buffer.

Quick Reference Table:

Final Volume (1X SB)	Volume of 20X SB	Volume of Water
1000 mL	50 mL	950 mL
500 mL	25 mL	475 mL
200 mL	10 mL	190 mL
150 mL	7.5 mL	142.5 mL

Tips:

- Store diluted buffers in 500 mL containers for easy student use.
- The same dilution method applies to 10X TBE buffer (adjust values accordingly).
- Prepared 1X SB buffers can be reused for multiple class periods.

Preparing Agarose Gels for Electrophoresis

Agarose gels are made by dissolving agarose powder in a 1X SB running buffer, typically using a vented 250 mL Erlenmeyer flask (included in your kit). Only melt up to 200mL at a time to avoid overflow during boiling. You will be making a **0.8% gel** for larger DNA fragments (~1000bp) – used in Labs 1.2, 4/4a, and colony PCR.	YouTube Tutorial (1:33)

Gel and Buffer Volume Guide by Unit		
Electrophoresis Unit	**Gel Volume**	**Buffer Tank Volume**
OWL (Thermo Fisher)	30 mL	300 mL
Enduro Gel XL	32 mL	300 mL
MiniOne	12 mL	140 mL
BlueGel	20 mL	30 mL

Example Preparation

For 6 OWL gels:

- Total needed: 6 × 30 mL = **180 mL**
- Round up to **200 mL** to allow for loss.

To make 200 mL of 0.8% agarose:

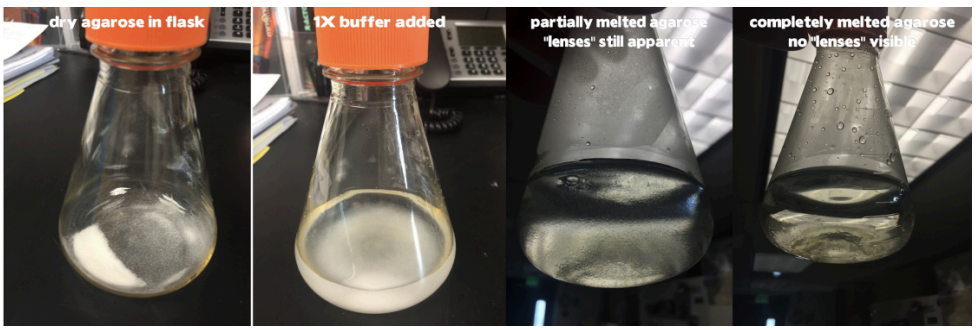
- Use the formula:
  Agarose (g) = % concentration × volume (mL)
  → 0.008 × 200 = **1.6 g agarose**

Quick Steps for Preparing Agarose Gels

Gel recipe (0.8%): 100mL → 0.8g, 150mL → 1.2g, 200mL → 1.6 g

1. Cool agarose to 55-60 celsius before pouring to avoid tray damage or leaks.
2. Set up trays while agarose cools; allow extra cooling for Enduro, MiniOne, or Bluegel systems.
3. Our gel using a Falcon tube; pop bubbles with a pipette tip
4. Insert combs and let gels solidify for 20-30 minutes
5. Remove combs gently once gels are firm and slightly opaque
6. Use or store gels with buffer and laminated cards; refrigerate if no stain was added to agarose.

Note: At ABE-LA, stain is added to the agarose. Please store gel at room temp in the dark.



NEW GEL ABELA GREEN/GEL RED PROTOCOL STARTING 5.2025

Starting in May 2025, ABELA will provide teachers with concentrated DNA Stain (Gel Green or Gel Red) to be added directly to the molten agarose prior to casting gels for Labs 4/4A and PCR only. Students will add Loading Dye to each sample prior to loading their wells.

The concentrated DNA stain will be provided in the tall black container with instructions for how to use it. The Loading Dye will be provided in the short black container, also with instructions.

Protocol for teachers to add the DNA stain to the molten agarose

Prepare agarose for Labs 4/4A and PCR in the gel melting flask as normal. After the agarose is melted in the microwave, allow it to cool to approximately 50°C. Once cooled, add the concentrated Gel Green (or Gel Red) to the molten agarose and gently swirl to mix. Then cast the gels are usual.

Add 1µL of GG/GR for every 20mL of agarose.

Gels containing DNA stain should be stored in the gel storage box with a splash of 1X buffer and kept in a drawer as they are light sensitive.

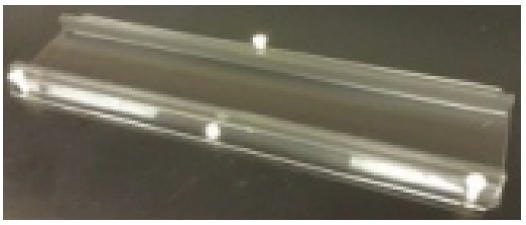
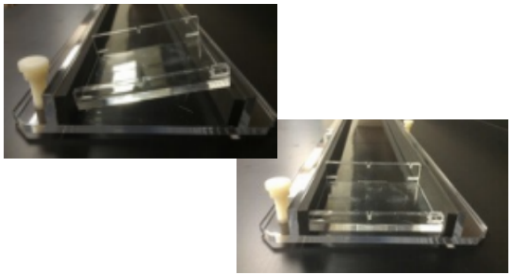
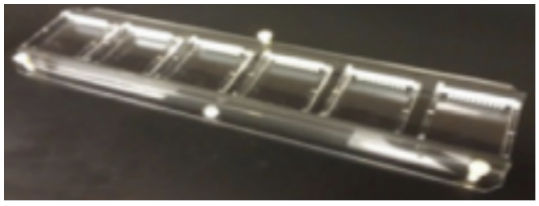
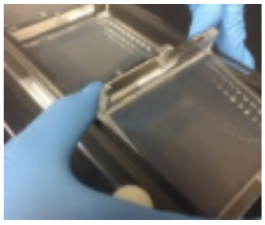
All extra Gel Green and Gel Red should be returned to your ABELA center.

There are no changes to the students' lab protocol checklists as those instruct students to add LD to each of their samples.

By putting the DNA stain into the agarose and having students add LD to their samples, ABELA will now adhere to the ABE Student Guide protocols *with one exception*. ABELA will continue to send out Marker without LD added to it so it will be clear.

Students will still need to add 2µL of LD to their Marker prior to loading it into the wells.

Quick Steps for Casting OWl Gels

1. **Prepare Buffer and agarose**: Please look at the two previous pages (pg. 12-13) to get detailed instructions on how to dilute a buffer and prepare agarose. 2. **Set Up Board**: Place the casting board on a **flat surface** with bubble level closest to you.	
3. **Insert Gel Trays:** Wet tray edges with water and **insert carefully** between rubber gaskets– avoid tearing the rubber. 4. **Load All Trays:** Repeat until all 6 trays are inserted, leaving space to lift them later.	
5. **Insert Combs**: Place combs evenly in each tray. Choose from **6,8, or 12 wells**; double combing is optional.	
6. **Level the Board**: Adjust white knobs until the bubble is centered in the level.	
7. **Pour Agarose**: Add **30 mL per tray**, filling halfway up comb teeth. Pop bubbles with a pipette tip. 8. **Solidify & Remove**: Once gels are slightly opaque, gently remove combs and lift trays straight up– **do not slide**.	

ABE-LA PBC Waste Disposal & Sanitation Protocol

Waste Disposal

1. **Labs 1-4:** Tips, tubes, and gels → regular trash.
2. **Labs 5-6 & Colony PCR:** Waste that has touched bacterial (plates, tips, tubes, gloves, etc.) → Clear autoclave bags (fill only to printed line, twist tie closed).

   Do not: Overfill bags or include regular trash!

3. **Student Waste Bins:** Keep bins empty during labs to prevent spills. Afterward, empty into autoclave bags, disinfect with 10% bleach (20 minutes), rinse, and air dry.
4. **Return Reagents:** All tubes, bottles, and unused supplies must be returned, even if partially used.
5. **1X SB Buffer:** Discard down the drain; return empty bottle in the kit.
6. **Liquid Waste Handling:** All bottles (e.g., column buffer) → return in baggies or cryovial boxes. No loose liquids in kit boxes.
7. **20X SB Buffer:** Return bottles in their original baggies and cardboard box.
8. **Agar Plates:** Do not bleach and trash. Return in the autoclave bag to PBC.

If you have any questions, please contact Samantha Leano

Equipment Sanitation

- Limit student contact with shared equipment (e.g., kit boxes, heat blocks, centrifuges)
- Sanitize with 70% isopropyl alcohol only using paper towels (no spraying into ports).
- Wipe down: Micropipettes, tube racks, weight boats, templates, etc.
- Allow all surfaces to air dry before returning equipment.

Teacher Prep Sheet

Lab 1.1, 1.2A, & 1.2B

Materials Needed:

All Labs Require:

- Aliquot box of Red Dye, Water, and solutions 1,2, & 3
- P20 Micropipette
- P200 Tip Box
- Waste Container

Lab 1.1

- Red Practice Dye
- Laminated Pipette Practice sheet

Lab 1.2A

- Red Practice Dye
- Silicone Practice Gel

Lab 1.2B

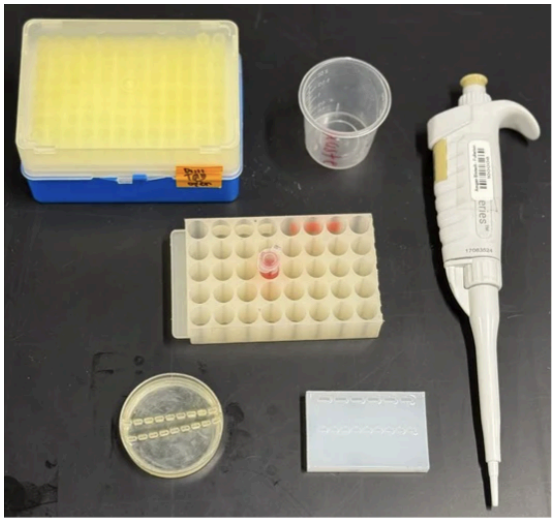
- 0.8% Agarose Gels
- 20X SB Buffer or dilute 1X SB Buffer
- Gel Electrophoresis System
- Funnel
- Solution 1,2, & 3

Lab 1.1 Setup:

Suggested Videos:

How to Use a Micropipette

Lab 1.2A Setup:



Lab 1.2B Setup:

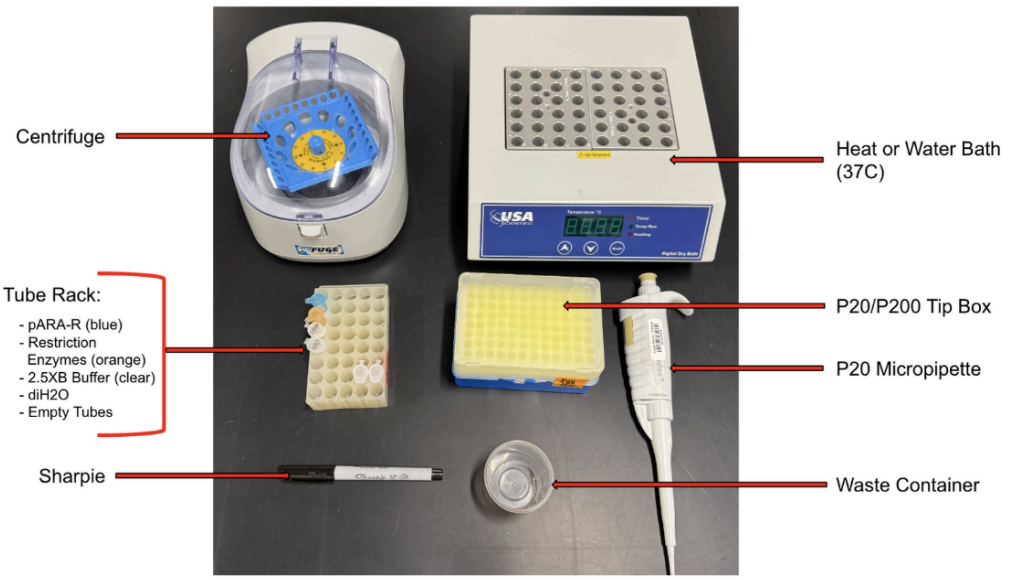
Teacher Prep Sheet

Lab 2/2A - Digesting your Plasmids

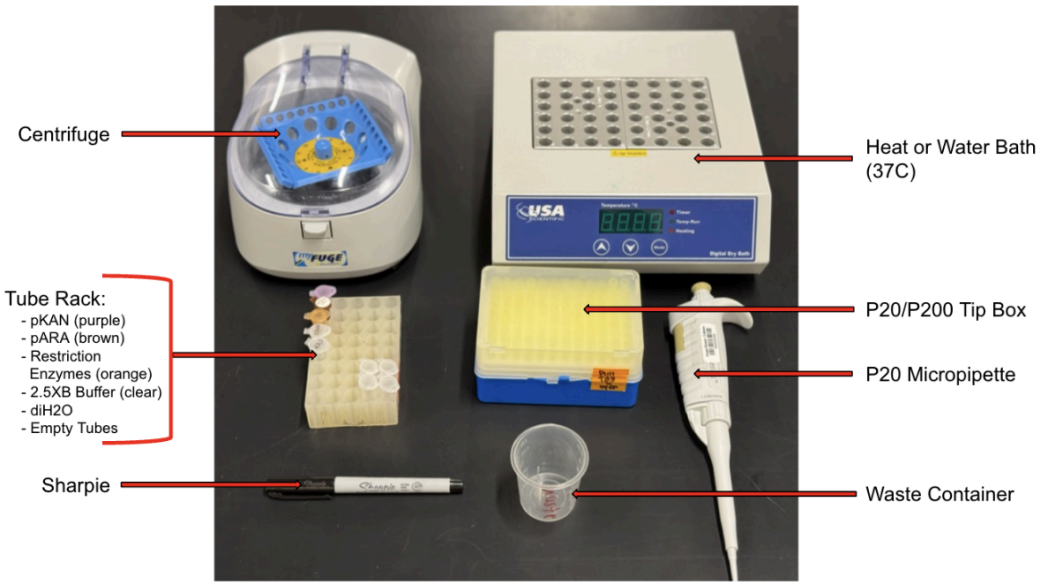
Materials Needed:

- Microcentrifuge
- Dry Heat/ Water Bath (Set to 37C)
- p20 Micropipette
- P200 Micropipette Tips
- Waste Container
- Sharpie
- Microfuge Tube rack
- Plasmids (Look at pictures for further insight)
- Restriction Enzymes
- 2.5XB Restriction Buffer
- DI H2O
- Empty Microfuge Tubes

Lab 2A Setup:



Lab 2 Setup:



Reminders:

- **Dry Heat Bath:** Turn on 30 minutes ahead of the lab.
- **Waste Disposal:** Regular Trash
- Spin down thawed reagents in centrifuge before using. **NOTE: pipette up and down your restriction enzymes**.
- Remind students to look at pipette tip before dispensing.

Suggested Video:

Lab 2A Restriction Digest (Student POV)

Lab 2 Restriction Digest (Student POV)

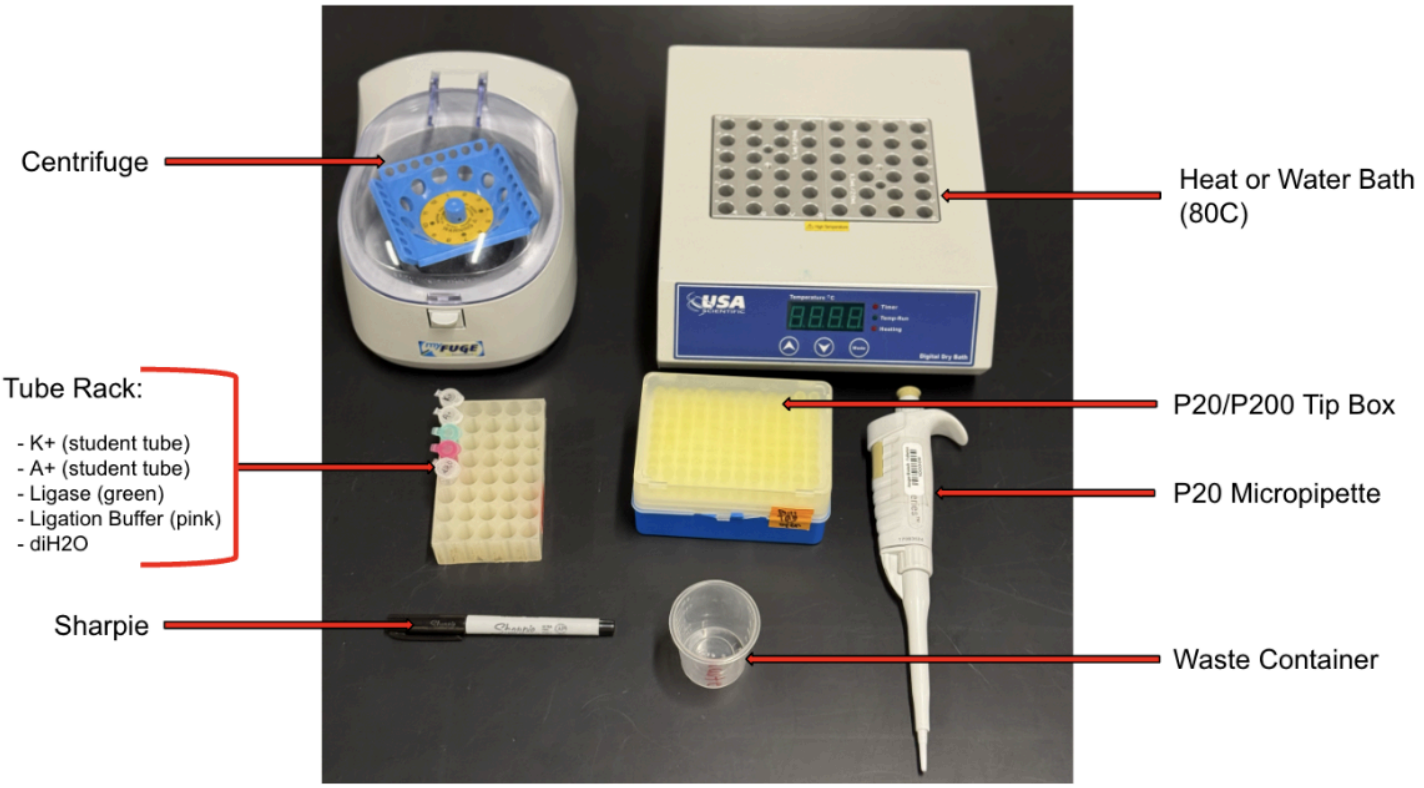
Teacher Prep Sheet

Lab 3 - Ligating your Plasmids (Complete Series ONLY!)

Materials Needed:

- Microcentrifuge
- Dry Heat/ Water Bath (Set to 80C)
- p20 Micropipette
- P200 Micropipette Tips
- Waste Container
- Sharpie
- Microfuge Tube rack
- K + (student tube)
- K - (student tube)
- Ligase tube (already has 2ul of DNA ligase)
- Ligation buffer
- DI H2O

Lab 3 Setup:



Reminders:

- **Dry Heat Bath:** Turn on 30 minutes ahead of the lab.
- **Waste Disposal:** Regular Trash
- Spin down thawed reagents in centrifuge before using. **NOTE: pipette up and down your restriction enzymes**.
- **Ligation:** Takes 20-30 minutes at room temperature

Suggested Video:

Lab 3 Ligation (Student POV)

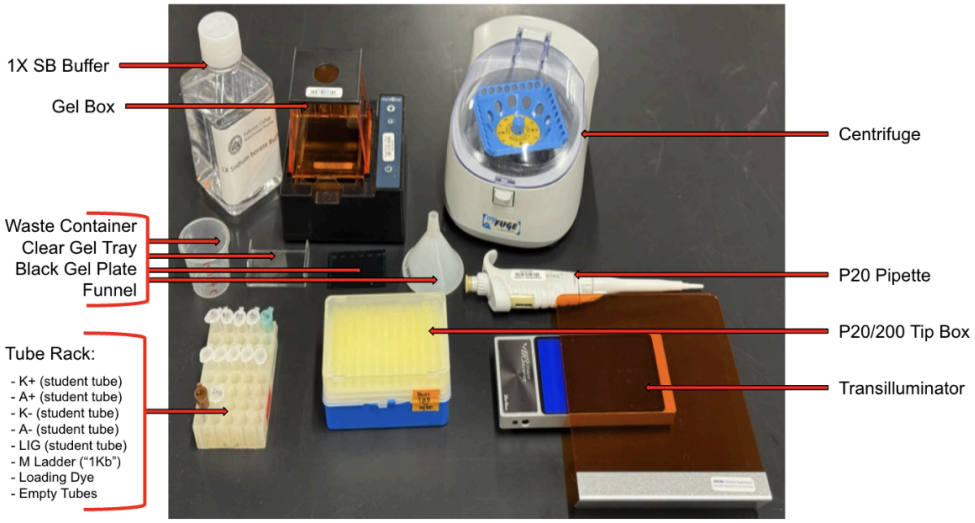
Teacher Prep Sheet

Lab 4/4A - Verifying your Plasmids

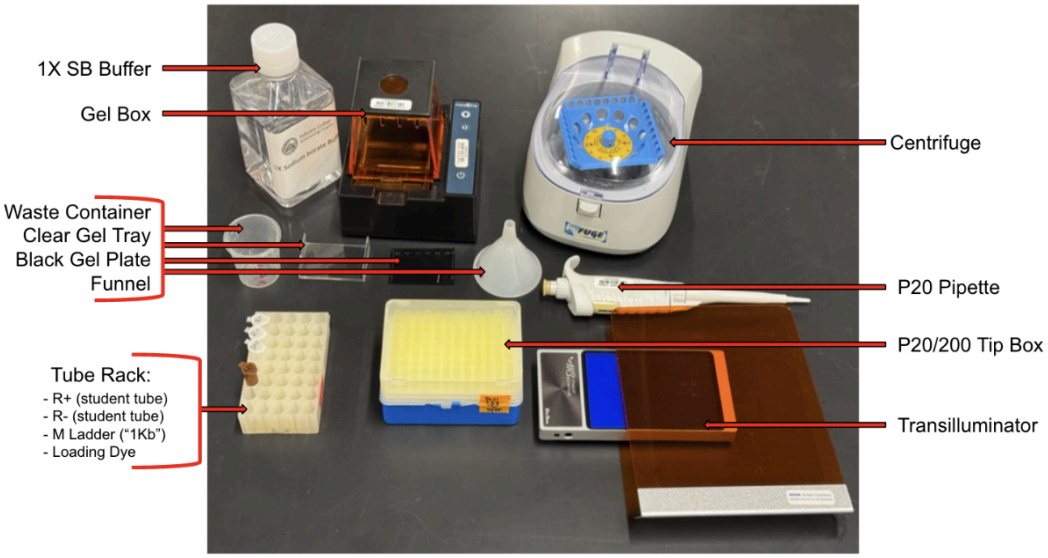
Materials Needed:

- Microcentrifuge
- Gel Electrophoresis System
- Blue Light Transilluminator
- p20 Micropipette
- P200 Micropipette Tips
- Waste Container
- Microfuge Tube rack
- Plasmids (Look at pictures for further insight)
- M Ladder (Labeled “1 KB”)
- Loading Dye + Gel Green (brown/black tube)*
    - **Note**: Starting 2025 and onward, we will be pre staining dye and we will provide agarose that will have the gel green fluorescent dye

Lab 4A Setup:



Lab 4 Setup:



Reminders:

- One gel can be shared between two groups OR one class can run samples on the left side of the gel while another class runs on the right.
- **If making your own gels,** be sure to make them ahead of the actual lab! See how to make agarose on pages 13-14

Suggested Video:

Lab 4A DNA Gel Electrophoresis (Student POV)

Lab 4 DNA Gel Electrophoresis (Student POV)

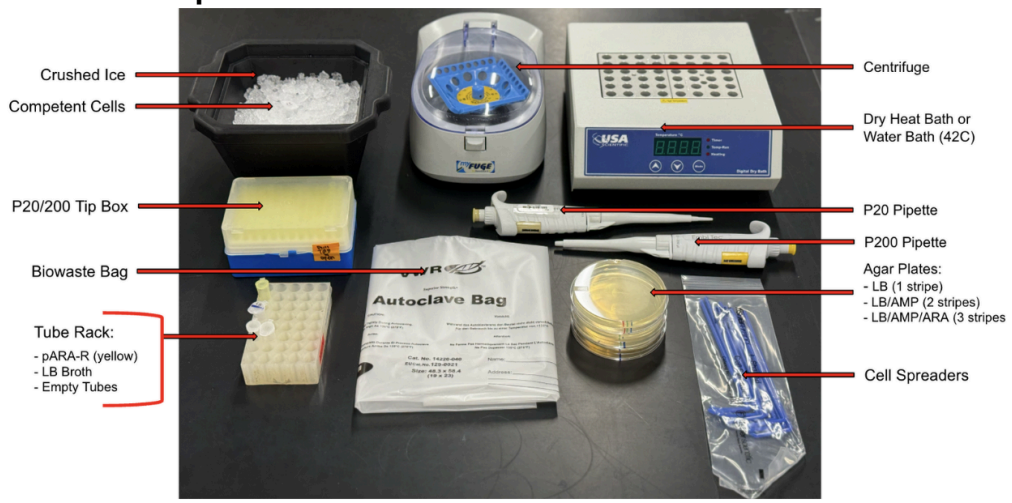
Teacher Prep Sheet

Lab 5/5A/B - Transforming Bacteria with your Plasmid

Materials Needed

- Microcentrifuge
- Dry Heat Block (42C)
- Blue Light Transilluminator
- P20 Micropipette
- P200 pipette Tip Box
- Waste Container
- Sharpie
- Crushed Ice
- Competent Cells (thawed on ice in slushie state!)
- Plasmids (Look at pictures for further insight)
- LB Broth
- Empty tubes
- Agar Plates (LB, LB/AMP, LB/AMP/ARA)
- L Shape Spreaders
- Autoclave Biowaste Bag

Lab 5A/B Setup:



Lab 5 Setup:

Reminders:

- **Competent Cells:** Thaw on ice 15 minutes before the lab.
  Aliquot 100uL Comp. Cells per group
- **Incubator:** Set to 37C the morning of lab
- **Dry Heat Block:** Turn on 30 minutes before the lab.
- **Waste Disposal:** Any materials that come into contact with Comp. Cells MUST go in a biowaste bag. DO NOT ADD BLEACH.

Suggested Video:

Lab 5A/B Transformation (Student POV)

Lab 5 Transformation (Student POV)

Growing a Culture of Transformed E. Coli for Lab 6 Protein Purification

If Growing Your own Culture:

- Start at least 5 days before Lab 6A
- Store culture in the fridge and supernatant in the freezer

Materials Needed:

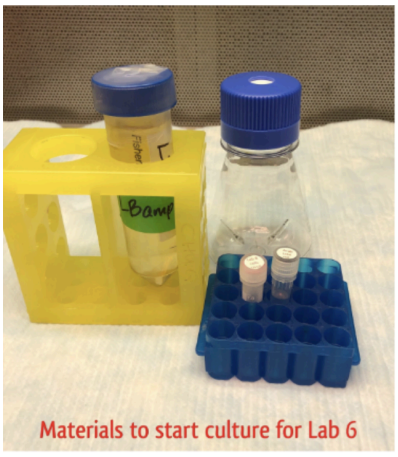
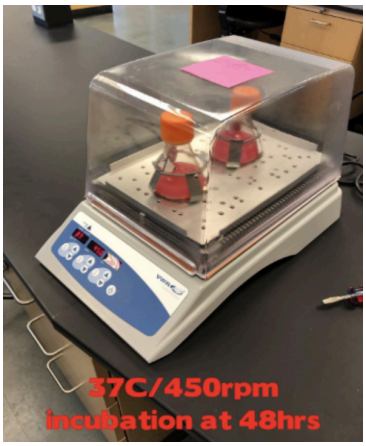
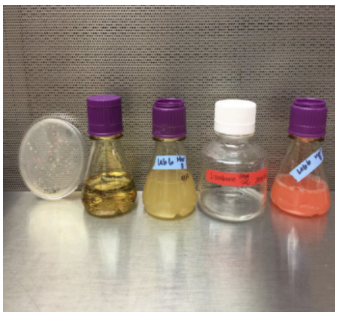
Equipment:

- Shaking incubator
- Sterile, vented culture flask
- P1000 micropipette and tips

Reagents:

- LB/AMP broth
- 1 vial Lab 6 cells (labeled as “TCC” or pink colony from LBAA plate
- 1 vial arabinose

Culture Protocol

1. Set Up culture (Preferably in the morning): a. Aseptically pour room-temp LB/AMP broth into flask b. Add entire tube of thawed Lab 6 cells (TCC) or several pink colonies	2. Incubation (2-3 hours): a. Shake at 37C, 350-450 rpm b. Avoid splashing; leave shaking until broth turns turbid	3. Add Arabinose: a. When turbidity reaches MacFarland 3 (mid-log phase) b. Stop shaker, add entire arabinose tube aseptically c. Resume shaking overnight i. A Word of Advice: The Earlier you Induce the arabinose the better (2 hours max)	4. Next Morning Check: a. Hot Pink = ready → Store in fridge b. Salmon/ beige = shake up to 24 more hours c. Still not pink after 48 hours? Contact Samantha in advance.
			

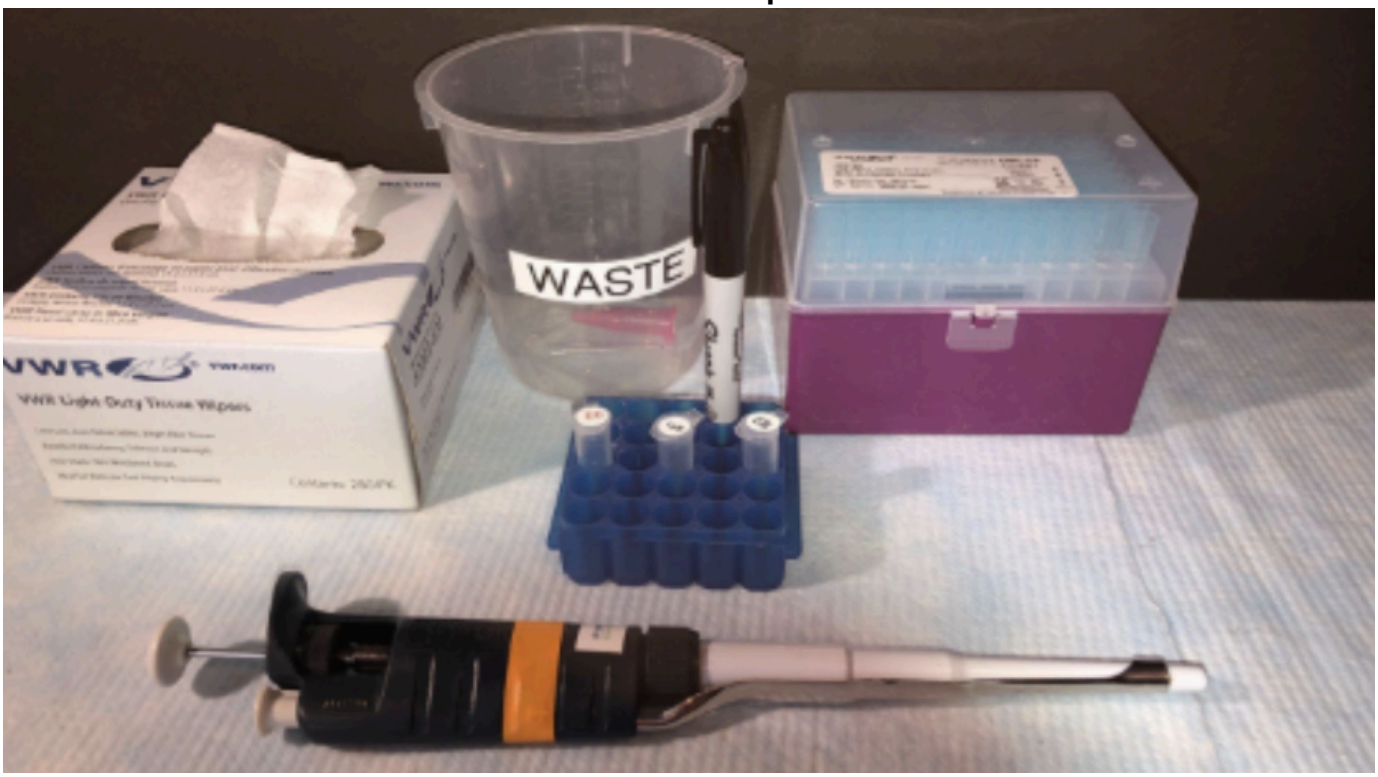
Teacher Prep Sheet

Lab 6A - Purifying your rfp Protein by lysing the cells

Materials Needed:

- Pre-grown Lab 6 culture
- Label and aliquot tubes (i.e. Lysis Buffer, Elution Buffer, and Lab 6 culture
- P1000 micropipette
- P1000 tip box
- Sharpie
- Waste beaker
- Microfuge Tube rack
- High speed microcentrifuge
- Autoclave bag

Lab 6A Setup:



Reminders:

- Always balance the high speed microcentrifuge
- **Waste Disposal:** Any materials that come into contact with Comp. Cells MUST go in a biowaste bag. DO NOT ADD BLEACH.

Suggested Video:

Lab 6A Protein Purification (Student POV)

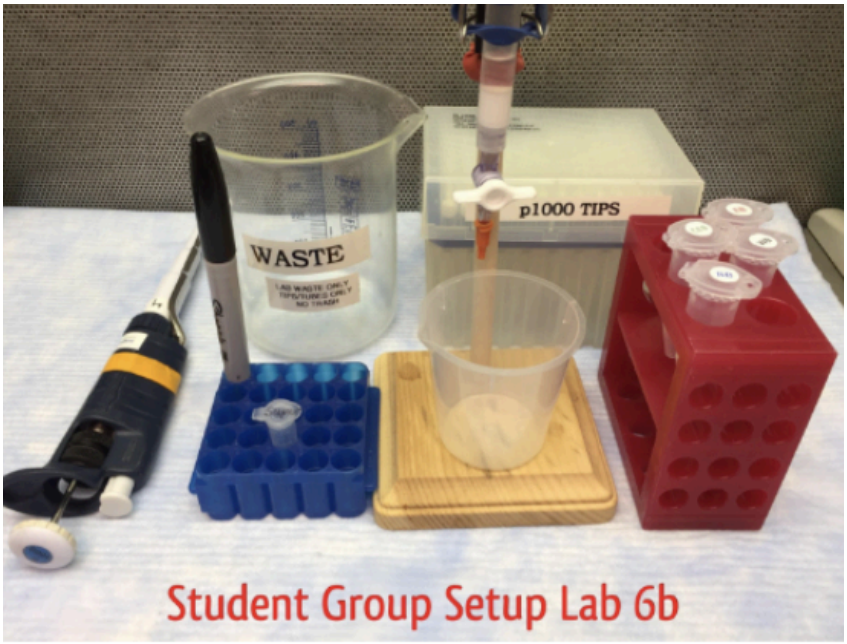
Teacher Prep Sheet

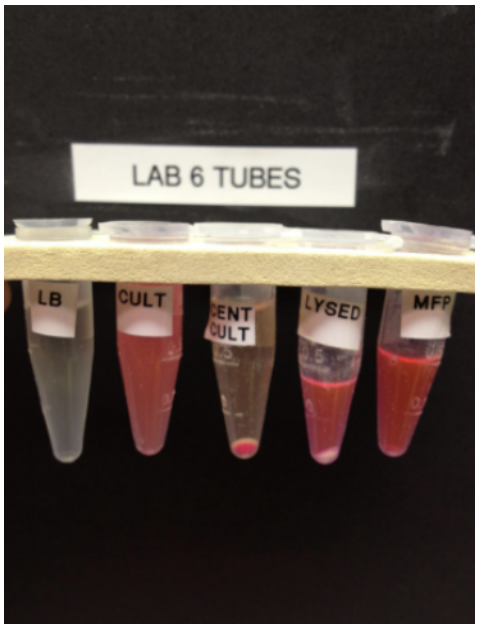
Lab 6B - Purifying your rfp Protein by lysing the cells

Materials Needed:

- Column stands with their respective columns
- Students' tubes of lysed Lab 6 cultures OR supernatant
- Waste Beaker
- Sharpie
- P1000 micropipette
- P1000 tip box
- Microfuge Tube rack
- Tubes of Binding buffer (BB), Column Equilibrium Buffer (CEB), Wash Buffer (WB), and Elution Buffer (EB)
- High Speed Microcentrifuge
- Blue light Transilluminator and beaker of boiling water (optional)

Lab 6B Setup:





Reminders:

- **Using the HIC-Protein Columns:** Never let the column resin dry out and always drip the buffers slowly down the column to not disturb the resin bed.
- **Waste Disposal:** Any materials that come into contact with Comp. Cells MUST go in a biowaste bag. DO NOT ADD BLEACH.
- Follow the PINK!

Suggested Video:

Lab 6B Protein Purification (Student POV) 0:53

Care and Maintenance of HIC-Purification Columns

The HIC-columns are a 3cc syringe barrel with luer lock stocklock, end cap (top), and tip cap (bottom). All resin is stored in 20% ethanol.

Set-Up for Use:

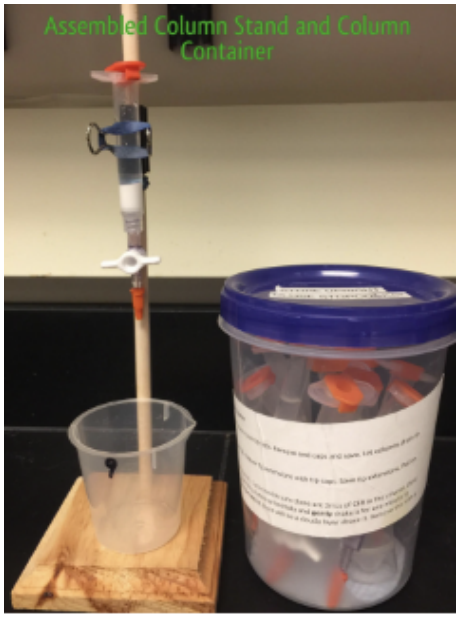
1. Remove tip and end caps (save for storage)
2. Drain ethanol to 2mm above resin bed
3. Add 1-2 mL of CEB, flush resin
4. Let drain to 2mm above resin bed → Column is ready to use

Repacking for Storage:

1. Drain to 2mm above resin
2. Add 2mL of 20 % ethanol
3. Close stocklock, replace caps (tip + end)
4. Stand upright in canister for return

Fixing Slow Flow (Clogged Frit):

1. Cap and gently shake column sideways to re-suspend resin (do not create more fines, i.e. cloudy layer)
2. Set upright to allow settling
3. Remove cloudy layer (fines) above dense resin using pipet
4. Add 1mL of CEB → Column should flow normally



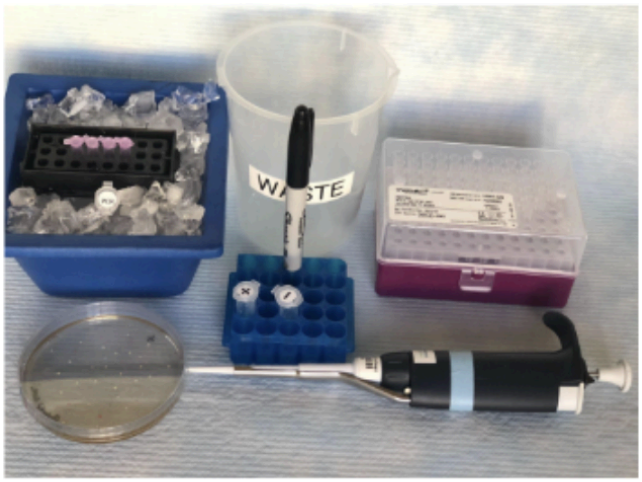
Teacher Prep Sheet

Lab 7A - Colony PCR

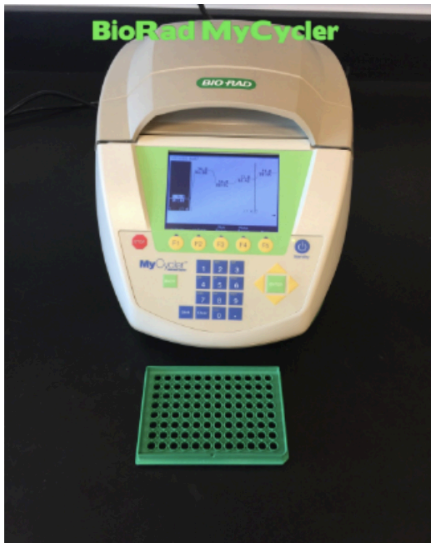
Materials Needed:

- Thawed Once taq MM and Colony PCR Primer Mix in crushed ice
- Tubes that are labeled: Master Mix, pARA-R 25pg, pARA 25pg
- Students Lab 5 LBAA plates from the refrigerator
- BioRad Thermal Cycler (pre set)
- P20 micropipette
- P200 tips
- PCR tube rack set in crushed ice
- Waste container
- Strip of 4 PCR tubes
- Sharpie
- Autoclave bags

Lab 7A Setup:



Colony PCR Part A Set Up



Reminders:

- **Using the PCR MM/primers:** Only mix up enough colony PCR MM/primers for one class period at a time
- Keep EVERYTHING on ice!
- **Waste Disposal:** Any materials that come into contact with Comp. Cells MUST go in a biowaste bag. DO NOT ADD BLEACH.
- Students should just barely touch the colonies when picking up cells and twirl the end of the tip in the MM several times. It should NOT appear cloudy

Suggested Video:

Lab 7A Protein Purification (Student POV)

BioRad MyCycler -Quick Start Guide

Before Starting:

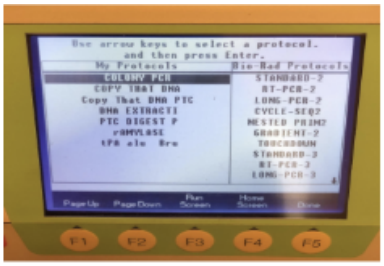
- Keep PCR tubes on crushed ice
- Spin down tubes to collect contents
- MyCycler includes a 4C pre- and post-hold – start program early to prechill tubes

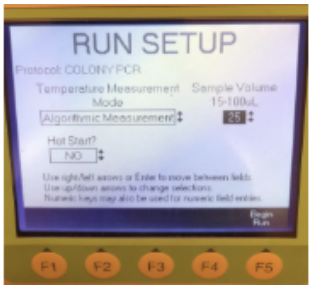
Set-Up Steps:

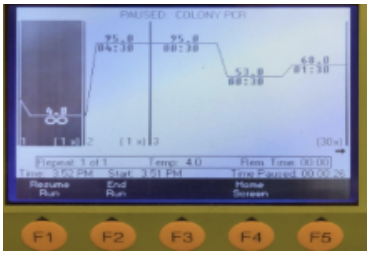
1. Plug in and press STANDBY
2. Wait for self-check to complete
3. Press F1 for Protocol Library
4. Ensure green tube grid is oriented with A1 in top left
5. Use grid template to label student tubes

Running the Protocol:

6. Use ARROW keys and ENTER to choose protocol from My Protocols
7. At “Choose Operations” screen:
    a. Press ENTER to begin
    b. Confirm: 25uL, NO hot start
    c. Press F5 to Begin Rub → unit chills to 4C
8. Load samples, lock lid, and press F1 to resume
    a. Confirm heating has begun

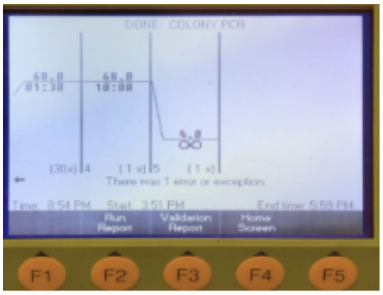






After the Run:

- Run lasts ~2.5 hours, can be left overnight
- Final screen shoulds errors (if any)
    - Press F3 to generate report if needed
- Press F4 to end
- Hold STANDBY to turn off
- Unplug and pack, include green tube grid



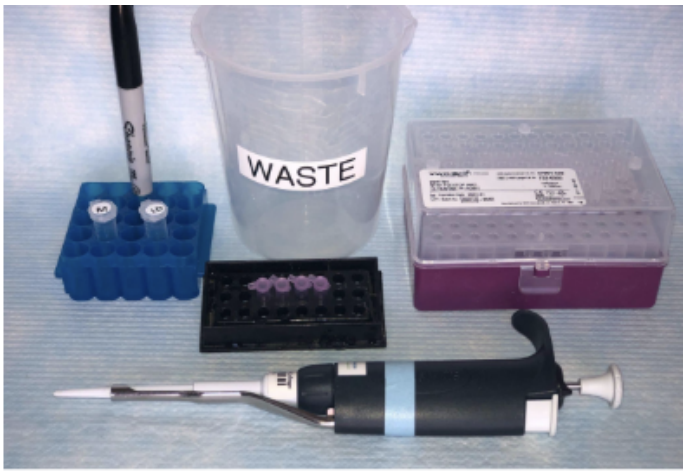
Teacher Prep Sheet

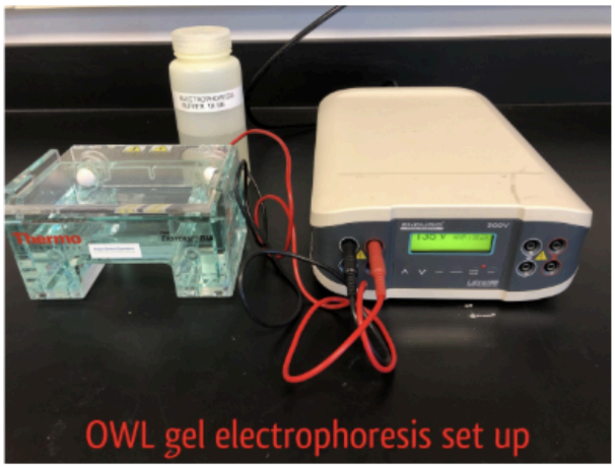
Lab 7B - Gel Electrophoresis

Materials Needed:

- Thawed Once taq MM and Colony PCR Primer Mix in crushed ice
- Tubes that are labeled: Master Mix, pARA-R 25pg, pARA 25pg
- Students Lab 5 LBAA plates from the refrigerator
- BioRad Thermal Cycler (pre set)
- P20 micropipette
- P200 tips
- PCR tube rack set in crushed ice
- Waste container
- Strip of 4 PCR tubes
- Sharpie
- Autoclave bags

Lab 7A Setup:





Reminders:

- **Using the PCR MM/primers:** Only mix up enough colony PCR MM/primers for one class period at a time
- Keep EVERYTHING on ice!
- **Waste Disposal:** Any materials that come into contact with Comp. Cells MUST go in a biowaste bag. DO NOT ADD BLEACH.
- Students should just barely touch the colonies when picking up cells and twirl the end of the tip in the MM several times. It should NOT appear cloudy

Suggested Video:

How to load samples into a gel electrophoresis chamber