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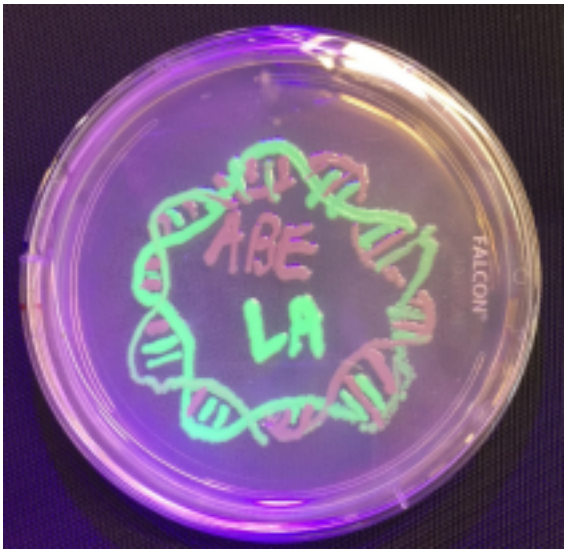
Scientific Discovery for the Classroom

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 abbreviated Student Manual. Please read through this entire guide to familiarize yourself with the actual supplies/equipment unique to our site and helpful tips for each lab to ensure your students' success.

ABE PBC Distribution Center Contact Information:

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Table of Contents

ABE-LA PBC Scheduling and Ordering of Kits and Supplies

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

A link to the current PBC Kit/Supply Schedule will be posted on the webpage every year in May. Teachers who have completed an ABE training workshop should check the schedule for available time slots before completing the PBC Kit/Supply Reservation Form. 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.

Once a teacher is assigned a time slot, they should download, sign and return a copy of the ABELA Teacher's Expectations and Policies to the site coordinator. This form must be completed by *every* teacher who will do any of the ABE labs *every* year.

At least four weeks prior to the scheduled pick up date, every teacher doing the labs must also complete the PBC Supply Order Form. This form collects data required by the national ABE Program Office for our grant funding as well as calculates the volumes of reagents and supplies needed. A Supply Spreadsheet will then be generated for each teacher. The number of students and groups should be entered accurately in the Supply Order Form and not “bumped up” as the spreadsheet formulae already calculate extra reagents and supplies.

Example Supply Spreadsheet

HOW TO COMPLETE THE ABELA PBC SUPPLY ORDER FORM Complete the

PBC Supply Order Form

The online order must be completed 4 weeks prior to your scheduled pick up date. It must be completed by each teacher who plans on running the labs and every semester.

The purpose of the order form is twofold: 1) to collect the data needed for our annual grant funding report and 2) to calculate the amount and type of supplies you will need for the labs. Because of this it may seem as though the form is asking the same questions multiple times.

Questions that collect data for annual grant report:

-Complete the following information:

Distribution site (PBC), reservation date, your first name, last name, main email, alternate email, the school name, type of school (public vs private), Title 1 school or not, school address, city, state, zip code, school phone #, your cell phone #, district, is this your first year doing ABE (yes or no), is this your school's first year doing ABE (yes or no), what materials will you need (full or partial kit, reagents only, or equipment specific to one lab ie a thermal cycler)

-Then answer the questions regarding the number of students in your classes and what labs they will be doing:

Total # of students doing any of the ABE labs

Total # of classes or sections

Total # of different courses

These answers should be *numbers*.

FOR EXAMPLE: if you have 80 students (arranged into 20 groups) in *two* classes of Honors Bio who will be doing the Focus on Bacteria Series of Labs without the protein lab or Colony PCR and 40 students in one class of AP Bio who will be doing the Complete Series of labs *with* the Protein lab and Colony PCR

Your answer would be: # Students=120, # classes =3, # of courses= 2

-Then list the name of Course # 1 and what labs you will do in that class and total # students doing them:

IN THIS EXAMPLE: Course # 1 is Honors Bio and will be doing the Focus on Bacteria without lab 6 and there are 80 students total doing those labs

Your answer would be: *Honors Bio, Focus Series without Lab 6, 80*

-Then list the name of Course #2 and what labs you will do in that class and total # students:

IN THIS EXAMPLE: Course # 2 is AP Bio doing the Complete Series of labs 1-6 with PCR and there are 40 students doing those labs

Your answer would be: *AP Bio, Complete series with Lab 6 and PCR, 40*

-Then list the name of Course #3 and what labs you will do in that class and total # students: IN THIS

EXAMPLE: you would not answer anything for these questions as you did not have a third course

Continue to answer in this manner for a fourth and fifth course if you have them.

Questions that calculate the volume and type of supplies you will need:

-Then answer the total # of students and groups doing the COMPLETE SERIES in all of your classes, the date you plan on running transformation, # groups doing protein lab and # groups doing PCR with the Complete Series. (SKIP these questions if none of your students will be doing the Complete series.)

IN THIS EXAMPLE your answer would be: 40, 10, 10, and 10

-Then answer the total # students doing the ABRIDGED SERIES in all of your classes, the date you plan on running transformation, # groups doing protein and # groups doing PCR with the Abridged Series. (Skip these questions if none of your students will be doing the Abridged Series.)

IN THIS EXAMPLE: you would enter nothing here since none of your classes are doing the Abridged Series.

-Then answer the total # of students doing the FOCUS ON BACTERIA SERIES in all of your classes, date for transformation, # groups doing protein with the Focus Series (if they are not doing the protein lab, skip this question.) (SKIP these questions if none of your students will be doing the Focus on Bacteria Series.)

IN THIS EXAMPLE: 80

-Then answer the total # of students doing the

-Then answer how you would like your supplies for the PROTEIN LAB. (We offer the supplies to grow your own culture for the protein lab, pre-lysed cells or supernatant.) (SKIP these questions if none of your students are doing the protein lab.)

Enter # groups that would need either SUPERNATANT, already grown culture for lab 6 (CELLS FOR LYSIS) or LIQUID CULTURE (you plan on growing your own culture)

IN THIS EXAMPLE: 10 groups for liquid culture

-Then answer # students/groups doing Introduction to Biotech *ONLY* (lab 1 is included in all the series of labs so you do not need to add it for students that you list for any of the other series of labs)(SKIP these questions if none of your students will be doing Lab 1 ONLY.)

IN THIS EXAMPLE: you would answer 0

-Then list the # student manual sets needed (these come in classroom sets of 12), any specific equipment needed (if other than a full kit), what gel box and transilluminator your school has (this tells us what type of DNA stain to send you) and any other comments. NOTE: ABELA PBC has a limited number of extra equipment, not all requests for partial equipment may be met. Any questions can be directed to karins@lapromisefund.org.

ABELA PBC Kit and Supply Pick Up and Return Policies

At the Pasadena Site, teachers are scheduled to pick up kits and supplies on Wednesdays and return them on Tuesdays but be sure to check the kit schedule as some pick-up and drop-off days may vary. Remember to record pick-up and return dates in your calendar as soon as they are assigned.

To coordinate the borrowing of kits between teachers, an online scheduling program, Sign Up Genius, is used to schedule pick up and return appointment times. Each time slot for a kit pick-up or return is 60 minutes long. Those for supplies-only are 30 minutes and are typically scheduled via email with the site coordinator.

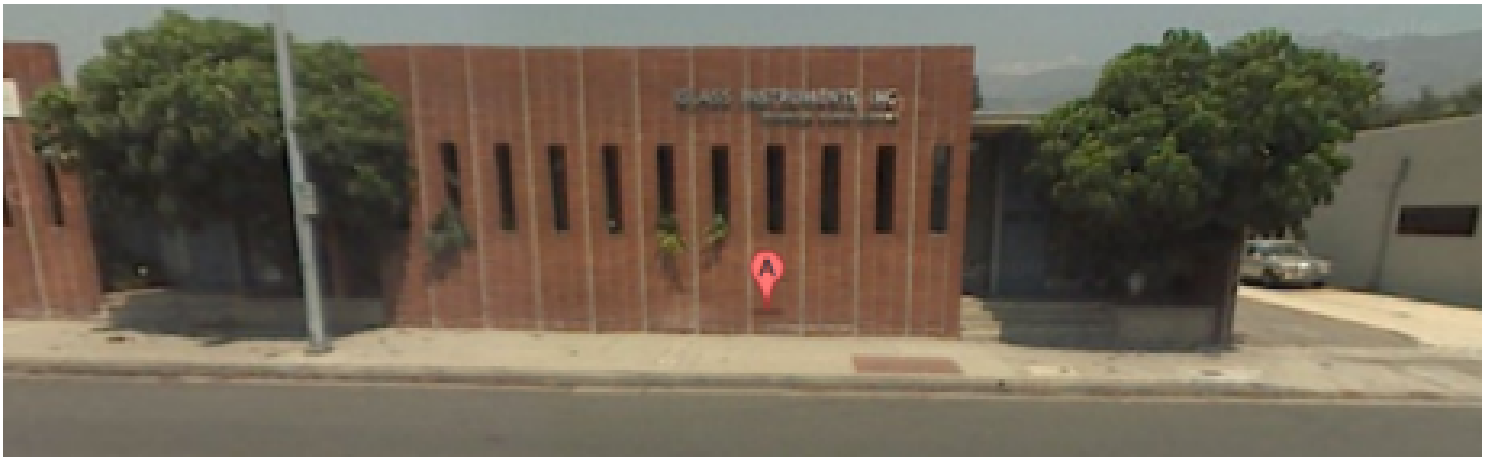
Sign Up Genius invitations are sent out approximately one month prior to the scheduled pick-up or return date. There are separate Sign Up Genius schedules for each date. An email will also be sent out to inform teachers that the Sign Up Genius invitations have been created as some school districts' email program block Sign Up Genius. *Be sure to check your spam folders as time slots are scheduled on a "first come, first served" basis.*

Important information regarding parking, contact phone numbers, etc. are included in the Sign Up Genius invitations. Please read them.

Only one teacher per school will be sent an invitation. They should forward the invitation to another teacher if they are not the one who will physically be the person picking up or returning the supplies. The teacher's name and cell phone number are required to schedule an appointment. So, if one teacher is picking up and a different teacher is returning, they need to enter the correct information for each time slot.

Kit pick-up will occur in the parking lot behind the Pasadena Bio Collaborative Incubator: **2265 E Foothill Blvd, Pasadena, California 91107**

When you arrive to pick up your equipment, park along the left wall of the back parking lot. I am located behind the third door that you see, in the back (the one farthest from the driveway that you just drove down. Call (609) 516-1556 and RING THE BELL of the last door. I will come and open the door for you. We will need to complete a supply check-list together to ensure you receive all your equipment. When you return your equipment, we will complete the same checklist again to ensure all supplies borrowed are returned.



Supply and Reagent Storage

When you pick up your supplies and/or kit, you will receive a hard copy of your Supply Spreadsheet which lists the volume of every reagent and consumable provided. This spreadsheet is color coordinated to indicate the correct temperature each reagent should be stored at. This spreadsheet will be emailed to you to confirm your order. Please review it and contact your site coordinator as soon as possible if there are any errors.

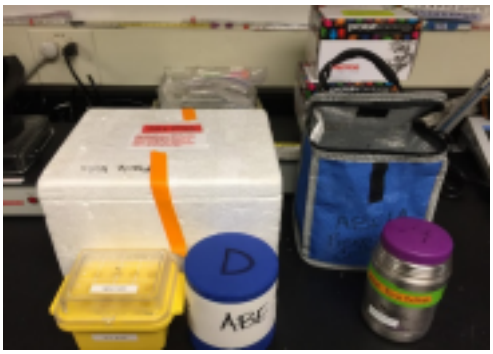
Items in **red** are stored in the freezer inside an isofreeze unit.
Items in **blue** are stored in the freezer.
Items in **green** are stored in the refrigerator.
Items in **black** are stored at room temperature.

Where to store all the reagents and supplies is also summarized in the Quick Supply/Reagent Sheet. Be sure to properly store all reagents as soon as you return to your school.

Please note that all tubes and bottles of extra reagents and supplies must be returned to the ABELA PBC Site. If your students did not get the expected results, we will need to test those remaining reagents. We also reuse many plastic containers to limit waste.

Typically a teacher needs to come to PBC three times during their scheduled 3-week time slot: 1. To pick up the kit and supplies
2. To pick up the transformation (competent cells and plates) and protein lab supplies
3. To return the kit and supplies

Quick Supply/Reagent Storage Summary Sheet



Put in freezer ASAP.

Thermos containing:
Plasmids, Bam/Hind, 2.5XB, Ligase, 5XB, marker, Master Mix, primers

Isofreeze unit containing:
Competent cells, lab 6 cells, super

Put in refrigerator:

Sleeves of LB agar plates, tubes of LB and LBamp broth, lysis buffer

Keep at **room temperature:**

Cardboard box containing 20X sodium borate buffer, agarose powder, tips, tubes, etc

Store at **room temperature in the dark**:

GRLD or GGLD

Unpacking the Kit Equipment

The following items are needed to perform the ABE labs but are not provided by the ABELA Pasadena site: waste containers, microwave oven, and PPE required for the students. Schools are responsible for these items.

The Pasadena site has one incubator which can be loaned out to schools. **Teachers should also purchase distilled (DI) water to use in the equipment and for final rinsing as tap water is corrosive.** The Pasadena site can provide 2 gallons of DI water to schools if necessary. The bottles must be returned as they are refilled and reused.

The Pasadena ABE kits (Purple Kit, Green Kit, Blue Kit, and Silver Kit) are all very similar, consisting of 6 tubs of supplies per kit. A kit equipment check-list (example below) will be completed at kit pick up and return to ensure each teacher receives and returns all the equipment.

Pasadena ABE Kit

Teacher Name: _____ Check-Out Date _____

School Name: _____ Check-In Date _____

Check-Out	Check-In	TUB 1 (LAB 1-3)
		(1) Teacher Guide
		(6) student manuals
		(1) Kit Notebook
		(1) Student Lab Ckechlist
		Lab 1.1 box (Green Dye, practice sheet, practice plates, tape)
		Lab 1.2 box (Solution 1-3)
		(2) Boxes of sharpies
		(12) P-20 Micropipettes
		(12) P-20/200 tip boxes
		(3) Floating Racks
		(3) Multisize racks
		(12) microtube racks
		(1) Vortex Machine

Check-Out	Check-In	TUB 2 (LAB 3-4)
		(1) Centrifuge
		(2) Microcentrifuges
		(1) Dry heat block
		(1) Digital Thermometer
		(1) Bag of cords for all electronic equipment
		(1) Casting tray
		(3) Power Boxes

Check-Out	Check-In	TUB 3 (LAB 4-6)
		(2) Cell Spreader bags
		(1) Digital scale
		(1) Transiluminator with hood + SOP
		(6) Combs (6, 10, 12)
		(1) Agrose flask with vent. cap
		(1) Roll of parafilm
		(6) Electrophoresis Chambers
		(12) p1000 pipettes
		(12) p1000 tip boxes
		(12) p200 pipettes
		(1) Funnel
		(1) Graduated cylinder
		(1) Flask with no vent. cap

Check-Out	Check-In	TUB 4 (LAB 6, PCR)
		(1) Shaking Incubator
		(1) Chromatography column with stands
		(13) Clips and Rubber bands
		(13) Waste cups
		(13) Protein columns
		(1) Hydrophobicity ball

Check-Out	Check-In	REAGENT BOX/BAG
		(1) BB, CEB, WB, EB, LyB
		LB, LB/amp, and LB/amp/ara plates ___ ea type
		LB amp broth
		Isofreeze unit/ styrofoam cooler
		(1) RP,K, A, RE, 2.5B, LIG, 5XB, M
		Ice bricks # _____
		A bag of P-20/200 tips
		A bag of P-1000 tips
		A bag of microfuge tubes
		Agarose Powder __ g
		20X SB buffer
		Competent cells*
		Autoclave bags + twisties

I agree to be financially responsible for any lost or broken equipment from this kit as per ABE policies

Signature Date

* You have the decision to check it out the first day of pick up or come back for it later and check it out then

Kit Checklist

Repacking and Returning the Kit and Supplies

It is advisable to repack equipment as you finish the labs instead of all at the end in a rush. Since every kit has different models of equipment, they must be packed in a specific order for everything to fit inside the main boxes. Please use the content list and packing photos on each box as a guide. The kit checklists can be found here.

Prior to repacking and returning the kit:

- Refer to the waste disposal protocol on page 21 of this notebook
- Sanitize equipment using the supplied 70% isopropyl alcohol (also page 21)
- Refill all tip boxes
- Rinse gel boxes, gel trays, combs, casting units and melting flasks with distilled water. Allow to *air dry* prior to repacking.
- Empty the 1x buffer bottle and return it to Box 3
- *No liquids should be stored in the kit boxes unless they are in secondary containers such as baggies or cryovial boxes. Spilled liquids can corrode equipment.*
- Keep the isofreeze unit, thermos and ice packs in the freezer. If you let them thaw, be sure to refreeze them before returning.
- Be sure to check your preproom, freezer and refrigerator as well as your classroom for items often forgotten such as gel trays and combs, thermos insulated bags and empty DI water bottles.
- Complete the online PBC Lab Results Survey and upload your gel and transformation photos to the PBC Results Photos Folder prior to returning the kit and/or supplies. (Please create a folder with your last name, school and the year)

DAMAGED OR LOST EQUIPMENT: If a piece of equipment is damaged please label it with a piece of masking tape stating the problem. For example on a pipette, “not accurately aspirating”. Contact your site coordinator as soon as possible. Be sure to gather and return any pieces of an item that may have broken off. The acrylic OWL gel trays will chip when dropped. We can sometimes glue that piece back on. Refer to the ABE Kit Price List for estimated replacement costs for each item.

Preparing and Aliquoting Reagents

Before aliquoting reagents for any lab be sure to thaw, spin down and then gently mix them using a pipette. Alternatively, they can be vortexed and then spun down. Aliquoted tubes can be stored in the freezer.

Restriction enzymes, Ligase and PCR Master Mix contain glycerol to keep them from going through repeated freeze and thaw cycles. These should be kept on ice while aliquoting and should also be gently mixed before aliquoting.

When aliquoting competent cells for Labs 5/5A/5B, thaw the cells in a cup of crushed ice. Once the cells have reached a “slurpy” consistency, resuspend the cells by pipetting several times and then aliquot 100µl into a pre-chilled 1.5ml tube (or 50µl into two prechilled tubes labeled P+/P-). Only thaw and aliquot comp cells needed for each class period. Aliquots of competent cells can be refrozen however transformation efficiency decreases the longer cells remain thawed. So if pre-aliquoting for your classes, refreeze the tubes immediately.

The Aliquoting Guide which follows shows how much of every reagent each student group will need.

Position the small microcentrifuges in easily accessible areas of the classroom so that students can spin down their tubes when needed. The larger high speed microcentrifuge is used for pelleting cells in Lab 6.

The Safety Data Sheets for every reagent used in the ABE labs can be found in the ABE-LA Teachers General Resources Folder and also on the thumb drive contained in each kit’s notebook. Be sure to follow your school and district’s safety regulations regarding the use of PPE, including safety goggles and gloves, and disinfection. Please refer to the ABELA PBC Waste Disposal Protocol in this notebook. If any bacterial spills occur on the bench top place paper towels over the spill and saturate with either freshly prepared 10% bleach solution or 70% isopropyl alcohol. Please refer to your school's regulations for approved disinfectants. Allow to sit for 20 minutes before disposing. Bleach should not be used on the equipment itself. Only the 70% isopropyl alcohol provided should be used on equipment (refer to Sanitizing equipment at the end of this notebook)

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Alternate Aliquoting Guide (Rev. October 2020)

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 boxes in a plastic microfuge tube box. When these solutions get low, please inform your site coordinator. ** dH_2O is used for 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 **	4uL

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 different tubes of pARA-R for the Abridged Genetic Engineering Sequence, Lab 2A uses the **70**ng/uL pARA-R. Check the reagent tube labels carefully.

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 **	2uL

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 8uL	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 8uL	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 *Transforming bacteria with recombinant plasmids*

Size of tube	Label tube	Contents of tube Aliquot	Actually used
1.5 mL	LB	Luria broth 350uL	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 different tubes of pARA-R for the Abridged Genetic Engineering Sequence, Lab 5A uses the **10** ng/uL pARA-R. Check the reagent tube labels carefully.

Lab 5B *Transforming bacteria with recombinant plasmids*

Size of tube	Label tube	Contents of tube Aliquot	Actually used
1.5 mL	LB	Luria broth 350uL	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

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 160uL	150uL
1.5 mL	EB	Elution buffer 200uL	150uL

*Each group will centrifuge a total of 2mL of the LB/amp/ara culture, but they will have to receive 2 aliquots of 1mL as 2mL will not fit into a 1.5mL microfuge tube.

Part B

*Size of tube	Label tube	Contents of tube *Aliquot	Actually used
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 sets of 15mL tubes of each buffer or one large container of each buffer. If you have the 15mL tubes, one can be given to each group. If you have the larger container, divide each buffer into a set of flasks that can be shared by two groups.

Colony PCR

Size of tube	Label tube	Contents of tube Aliquot	Actually used
1.5mL	PCR	One Taq 2X Master Mix + primer mix 96uL	92uL
1.5mL	+	pARA-R 0.025ng/uL 3uL	2uL
1.5mL 1.5mL 1.5mL	- M LD	pARA 0.025ng/uL 3uL 1 kb DNA ladder/marker 9uL 50XGelRed or 50X GelGreen loading dye 12uL	2uL 8uL 10uL

To save time between classes, it is advised that teachers dilute large volumes of 1X Sodium Borate buffer which will be used for making the agarose gels as well as in the electrophoresis chamber. The directions for diluting 20X Sodium Borate buffer follow. Both the OWL and Enduro gel boxes hold approximately 300ml of running buffer. The same formula can be used to dilute 10X TBE buffer to 1X if necessary.

20X Sodium Borate Buffer Dilution

1X Sodium Borate Buffer Preparation

The Sodium Borate electrophoresis solution is sent to you at higher concentration (*stock concentration 20X*) than the concentration students actually use (*working concentration 1X*). To calculate the dilution from a *stock* concentration to a *working* concentration use the following method.

- Determine how much of the working solution you will need.
- Then use the formula $C_1V_1 = C_2V_2$to determine the volume of stock solution (V_1) you will need to dilute to prepare the final volume of working solution (V_2). (C=concentration, V=volume)

Example. You need to prepare 1x SB buffer (electrophoresis buffer) for 6 agarose gels. Each gel needs about 30 mL of this buffer. Amgen supplies the SB buffer, but its concentration is 20x. 30 mL x 6 gels = 180 mL

Apply the formula $C_1V_1 = C_2V2$

$(20x)(V1) = (1x)(180mL)$

$(V1) = \frac{(1x)\ (180\ mL)}{20x}$

$(V_1) = 9mL$

To 9.0 mL of 20x SB, add 171.0 mL of dH2O (deionized or distilled) to make 180 mL of 1x SB electrophoresis buffer.

1XSB electrophoresis buffer is needed to run the gel in the electrophoresis chamber as well as to prepare the gels. Time can be saved by preparing a large volume of 1xSB buffer prior to making the gels using the formula above, or by using the table below.

For Total Volume of 1X SB	Use 20X SB	Use Water
1000 mL 1X SB	= 50 mL 20X SB	950 mL
500 mL 1X SB	= 25 mL 20X SB	475 mL
200 mL 1X SB	= 10 mL 20X SB	190 mL
150 mL 1X SB	= 7.5 mL 20X SB	142.5 mL

Store the diluted buffer in several 500 mL containers to make it easy for students to pour the buffer into their electrophoresis chambers. This buffer can remain in the gel chambers and be used for several classes.

Preparing Agarose Gels

Agarose gels are prepared by melting agarose powder in 1X running buffer. This is done in a large vented microwave safe container. A vented 250ml erlenmeyer flask is provided in each kit for this purpose. Only melt 200ml at a time in this flask to allow plenty of headspace in case the agarose boils up. The concentration of agarose can vary with higher concentration like 2% being used to separate smaller bands (200bp) and lower concentration such as 0.8% being used to separate larger bands (1000bp). Labs 1.2, 4/4a and Colony PCR all utilize 0.8% agarose gels.

Use the following chart to calculate the volume of agarose and 1X buffer to prepare. Always make slightly more.

Electrophoresis Unit	Gel volume Buffer tank volume
Themo Fisher OWL B1A	30ml 300ml
Enduro Gel XL	32ml 300ml
MiniOne	12ml 140ml
BluGel	20ml 30ml

For example, 6 OWL gels would require 180 mL of agarose solution. To allow for some loss due to solution staying in the flask, round up to 200 mL. **(Note: each MiniOne gel requires approximately 12 mL of a 0.8% agarose solution. Use this number when calculating the amount of agarose solution needed for multiple MiniOne gels.)**

0.8 = x grams of agarose This is the basic formula, where ? is the volume of agarose solution desired
100 ?mL of 1X buffer

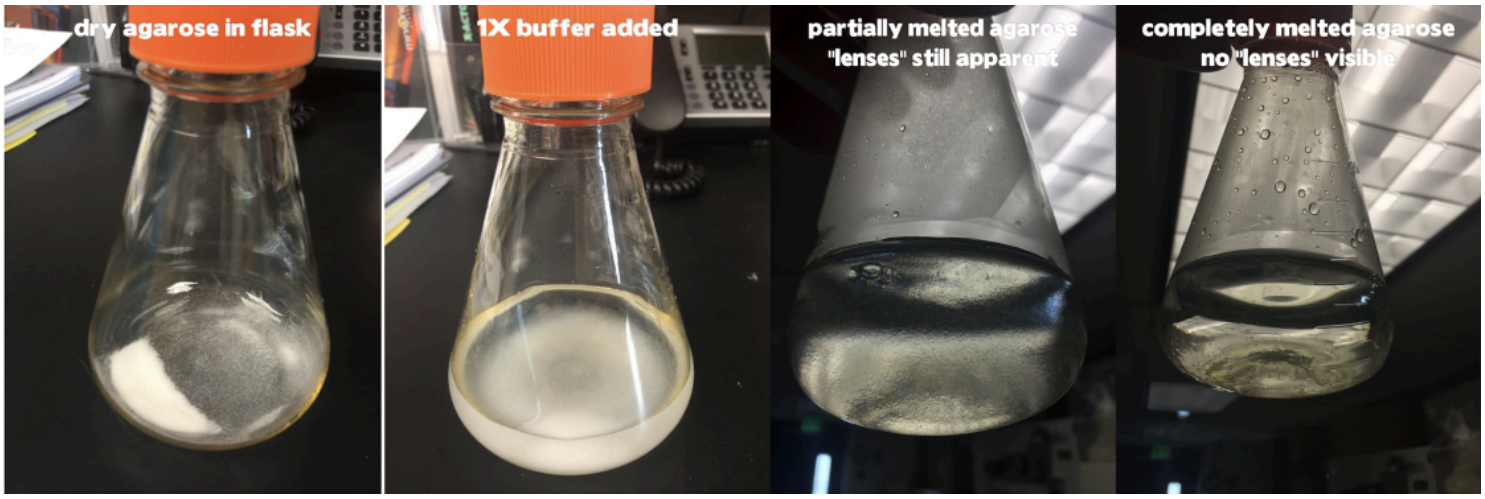
0.8 = x grams of agarose
100 200mL of 1X buffer

.

(200) (0.008) = 1.6 grams of agarose (this percentage is weight of agarose over volume of 1xSB buffer)

• Place 1.6 grams of agarose into a 500 mL flask. Add 200 mL of 1x SB
• Cover the flask opening with plastic wrap. Use a pipette tip to poke a small hole in the plastic wrap. • Place the covered flask in a microwave. Set the microwave for 1 minute on high. (A hot plate can also be used to melt agarose but you will need to use a double-boiler.)
• As soon as boiling starts, *gently* swirl the flask with a gloved hand. Continue this procedure, reducing the time on the microwave (5 ± 15 seconds), until all of the agarose has been dissolved. *To check this, hold the flask to the light and swirl the solution. Look carefully for “lenses” of agarose crystals suspended in the liquid. If no lenses are visible, the agarose is dissolved. See figure below.

*If your students will pour the gels, transfer 30 mL melted agarose into 50 mL conical tubes, cap, and Keep in a 60°C water bath.



Melting Agarose Powder

- Cool the agarose to 55-60°C before pouring so as *not to warp the gel trays*. It is cool enough when you can comfortably hold the bottom of the flask in your hand. While the agarose is cooling, set up the OWL gel trays within the casting board or the Enduro Gel XL casting units. When pouring Enduro, Mini One or Blugel gels the agarose should be allowed to cool down more (55°C) to limit leakage of molten agarose beneath the gel trays. Any agarose which does leak under the gel trays can be saved and remelted for new gels.
- Use a 15 or 50ml falcon tube to measure out the agarose for each gel tray. Pop any bubbles with a pipette tip.
- Place the gel combs inside the gel trays and allow the agarose to solidify. This takes about 20-30 minutes. The agarose will turn from completely clear to slightly opaque and should feel firm to the touch.
- Once solidified, gently remove the combs from the gels by pulling them straight up. The gel trays can be placed directly into a gel box or the gels may be removed from the trays and stored in a **gel storage box**. Each layer of gels should be separated by one of the laminated cards. A few mls of 1X buffer should be added to the box before tightly sealing and storing in the refrigerator.
- **NOTE**: Here at ABELA, the DNA stain (Gel Green or Gel Red) is added to a Loading Dye which is then added to all samples prior to loading the wells. However the DNA stain can be added to molten agarose prior to casting. If your site coordinator asks you to use this method, the gels should be stored at *room temperature in the dark.*

For Total Volume Use Agarose Use 1X SB

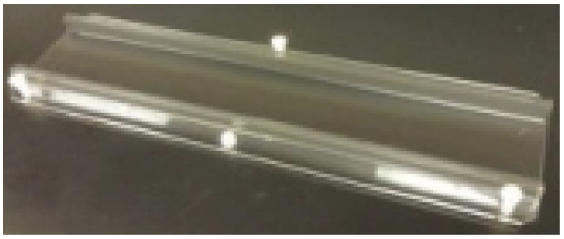
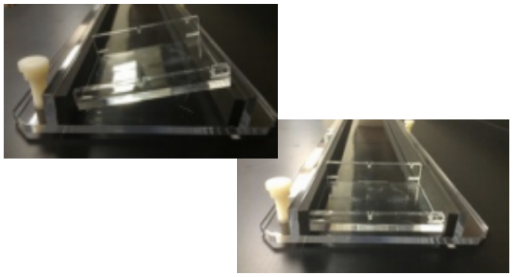
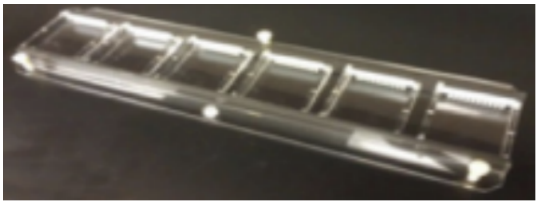
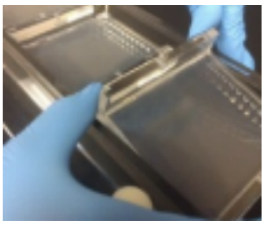
100 mL 0.8% Agarose gel= 0.8 gram in 100 mL 1X SB

150 mL 0.8% Agarose gel= 1.2 gram in 150 mL 1X SB

200 mL 0.8% Agarose gel= 1.6 gram in 200 mL 1X SB

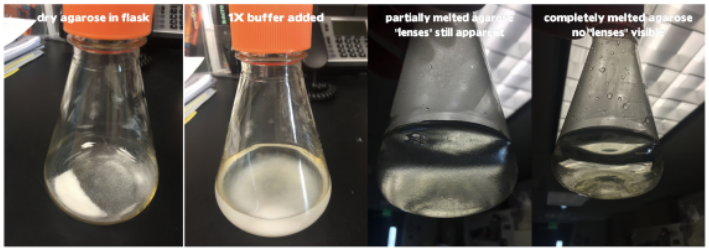
Casting OWL Gels With the Multi-Gel Casting Board

The Gel Casting Board is designed to cast six OWL gels simultaneously

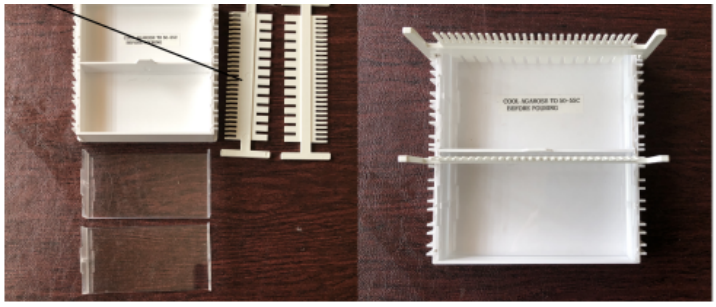
1. Prepare 1X buffer (25ml 20X buffer with 475ml water) 2. Prepare 200ml molten agarose solution and let cool to 55°C (0.8g agarose in 100ml 1X buffer. Repeat in another flask) 3. Place the Gel Casting Board on a flat lab bench with the bubble level closest to you.	
	4. Use your finger to wet both edges of the gel tray with water. Insert it between the rubber gaskets of the Gel Casting Board by pushing one edge against one side. Then *gently* push the other end down into the board so that the gel tray is all the way down. Be careful - the rubber will tear.
5. Repeat with the remaining 5 gel trays. The casting board will hold 6 gel trays.	6. Be sure to leave enough space between each gel tray for your fingers. You will need to lift the gel trays out.
7. Insert the gel combs into the gel trays. Make sure they are inserted evenly. There are 3 well sizes to choose from: 6, 8 and 12. Gels may also be double combed.	
	8. Adjust the two white knobs so that the bubble in in the center of the black circle in the level.
9. Once the agarose is cool, pour 30ml per gel. The agarose should come halfway up the height of the comb teeth. Remove any bubbles with a pipette tip. Once the agarose has solidified (turned slightly opaque) gently remove the combs. Lift the gel trays up and out of the casting board. DO NOT SLIDE THEM OUT.	

Casting Gels for the Enduro Gel XL

1. Prepare 1X sodium borate buffer. (eg. Add 100mL 20X buffer to 1900mL DI water.)
2. For six gels, prepare and melt 210mL agarose solution.. [For 0.8% agarose solution mix 1.68g powdered agarose in 210mL 1X buffer. This may need to be done in two vented flasks. Microwave to melt.]
3. Let the molten agarose cool to between 50-55°C.

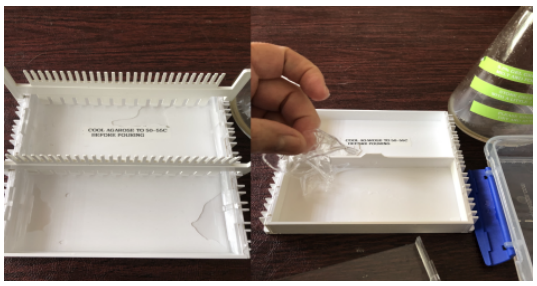


Melting Agarose Powder



1. While the agarose is cooling, set up the Enduro Gel XL casting units. Place the casting units on a level surface and insert the 14-well side of the comb into each gel tray. [Each casting unit casts two gels, set up at least three units at one time.]

1. Once the agarose has cooled (it should feel warm but not hot when held in your hand), pour 32mL into each gel tray.
2. Pop any bubbles which form with a pipette tip.
3. The hotter the agarose is when poured, the more will leak under the gel trays. Hot agarose permanently warps and bows the gel trays making it impossible to cast gels with appropriately sized wells.
4. Each gel can serve at least 2 student groups.



1. Allow the gels to cool for at least 20 minutes. They should feel *firm* when touched.
2. Gently remove the combs by pulling them straight up. 3. Pull the gel trays from the casting unit and remove any extra agarose. Any extra agarose that leaked under the trays can be remelted.

1. Either place the gel tray into the Enduro buffer tank to run or slide the gel from the tray into the Gel Storage Box for storage.
2. Separate layers of gels in the box with a laminated card and add a splash of 1X buffer before sealing the box and placing in the refrigerator.

ABE-LA PBC Waste Disposal Protocol

1. All tips, tubes and agarose gels from Lab 1-4 can go into your regular trash.

2. Plates, tips, tubes, kimwipes, cell spreaders, and gloves from lab 5, 6 and Colony PCR should go in the clear Autoclave Bags that are provided. The bags should only be filled up to the level where “Autoclave bag” is printed and then twist tied closed. Do not overfill the bag as it needs to fit into a rigid bin for processing at PBC . Do not fill the bags with regular trash such as water bottles and cups. Do not tape the bags shut as they need to be opened and have a small amount of water added prior to autoclaving. *Do not use red biohazard bags* to put your waste in. PBC can not legally process these without paying a hazmat disposal fee. It is advised that the waste containers used by the students in labs 5, 6 and Colony PCR remain empty on the bench top as students frequently knock them over. Once the labs are finished, they can be emptied into the autoclave bags and then disinfected by filling with freshly prepared 10% bleach, allowed to sit for 20 minutes and finally rinsed with water and air dried.

3. Please return all reagent tubes, bottles and unused supplies. There should be a small amount of plasmids, buffers and enzymes left in the tubes and we will need those to test if there is any problem with your results.

4. Discard your remaining 1X Sodium Borate buffer down the drain and return the *empty* 1X bottle in the kit.

5. The column buffer bottles should be returned in the bags that they went out in. NO bottles or tubes of liquids should be placed directly into a kit box. They should all be in secondary containers such as cryovial boxes or baggies. When liquids spill they can corrode the equipment.

6. Return the 20X Sodium borate buffer bottles inside their baggies in the cardboard box they came in .

7. No, you can’t just bleach your plates and dispose of them in your school trash. You must return them in the autoclave bags to PBC .

8. If you have any questions please contact your site coordinator

Equipment Sanitation Between Students

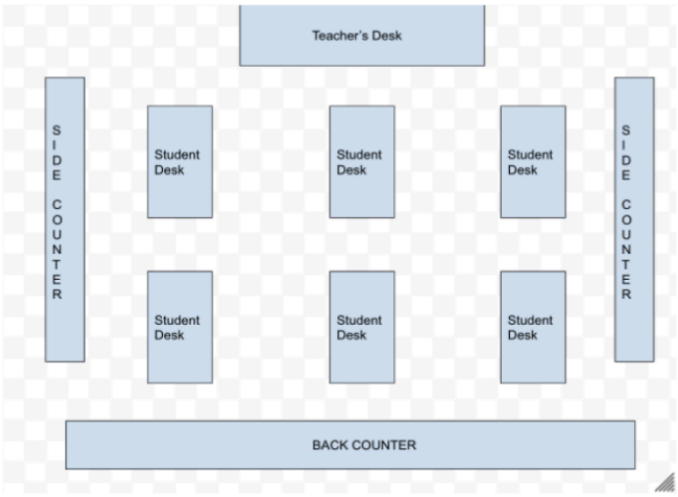
Teachers will need to sanitize equipment between students. In order to minimize the time needed to sanitize, teachers should limit the amount of equipment handled by the students. Students should be discouraged from handling shared equipment such as the kit boxes themselves, water baths or heat blocks, the high speed microcentrifuge and incubators. Spray bottles of 70% isopropyl alcohol will be provided. Do not use anything other this 70% isopropyl alcohol to sanitize any of the ABELA equipment. Teachers should use paper towels sprayed with 70% isopropyl alcohol to wipe down the surfaces commonly touched by the students such as the micropipettes, tube racks, Lab 1.1 weighboats, templates, touch pads on power supplies etc and tablet screens. It should not be sprayed into any electrical ports. Allow the equipment to air dry. All equipment should be wiped down prior to returning the ki

LAB SET UP GUIDE

All Amgen Biotech Experience Labs are to be performed using all appropriate personal protection equipment (PPE) and precautions required in a California Science laboratory classroom. Please follow your school and district's safety rules. The laboratory curricula can be downloaded from the National ABE website website.

The following Set Up Guide lists the equipment and reagents required for each lab, photos of student group set ups, preparation information, helpful videos and tips for successful results. A partial list of video resources can be found here. An example for classroom equipment placement is shown below.

EXAMPLE ABE CLASSROOM SET UP DIAGRAM



Side counters: shared equipment: microcentrifuges, autoclave bags, water bath/heat block
Student desks: student groups set ups: tube racks. micropipettes, tip boxes, waste beakers, gel boxes, columns, etc
Teacher's Desk: isofreeze unit with comp cells, any extra reagents
Back Counter: Larger shared equipment: high speed microcentrifuge, transilluminator with hood, vortexer, thermal cycler, shaking incubator, etc

Lab Protocol Checklists are provided with each kit. There are 12 copies of each lab. They are inside page protectors so that students may use their sharpie to check off steps they have completed. These should be wiped down with 70% isopropyl prior to kit return.

Preparation:

☐Spin down the tubes of Red Dye [RD] provided in the Lab 1.1 box, refill from the stock tube if necessary.

Equipment Needed:

Each student group:

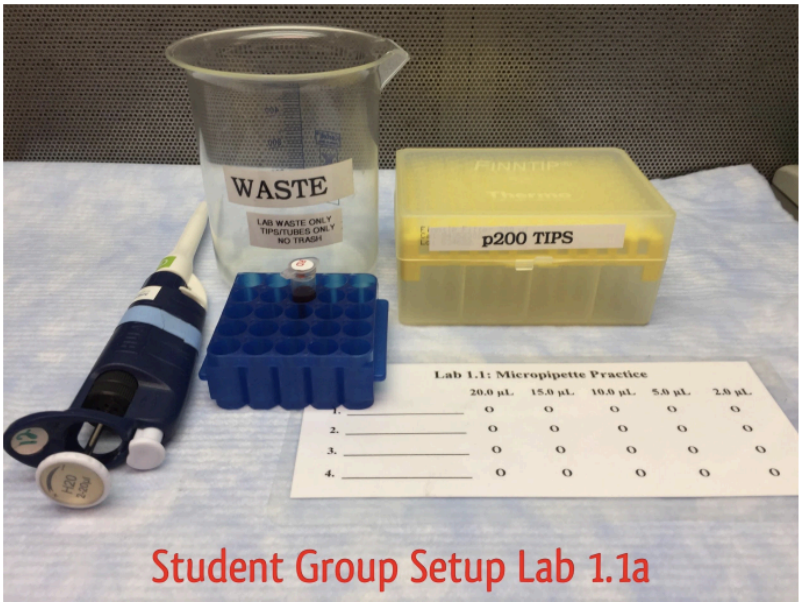
☐p20 micropipette ☐box of p200 tips
☐small microfuge tube rack ☐waste container
☐Lab 1.1 practice template ☐ paper towels for easy clean up

TIPS:

Here is a good video on how to use a micropipette. Be sure to spend time practicing how to use the micropipette with the students. Every other ABE lab depends on students mastering this skill. Have students first practice pushing the plunger to the first and second stops before ever adding a tip. Use easily remembered phrases such as “first stop: aspirate, second stop: dispense” and “Never Dip Without a Tip”.

Waste Disposal:

All used tips and paper towels can go in the regular trash.



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Lab 1.2 Part A: Practice Pipetting Into Wells

Preparation:

☐Spin down the tubes of Red Dye [RD] provided in the Lab 1.1 box, refill from the stock tube if necessary.

☐Set practice gels in weigh boats.

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips ☐waste container ☐small microfuge tube rack ☐practice gel and black weigh boat

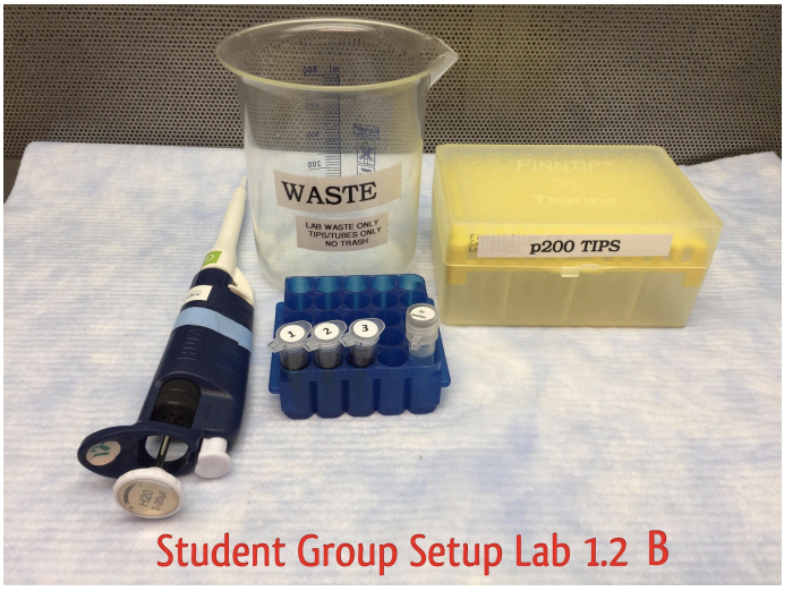
☐small beaker of DI water ☐paper towels for easy clean up

TIPS:

Be sure to have the students fill the practice gels with distilled water before loading RD into the wells, otherwise they may permanently stain. After filling the practice gels with distilled water, bubbles can form in the wells. These need to be removed prior to loading the wells with RD. Use a pipet tip to gently pull the bubbles from the wells. Wash out the gels *immediately* after each class by swishing them around upside down in a tub of distilled water. The students really get a feel for loading actual agarose gels when these practice gels are made from agar agar. If you have time, you can melt powdered agar agar in water and cast practice gels. After use these can be left in a tub of water until the dye diffuses out of them and then reused.

Waste Disposal:

All tips can be disposed of in the regular trash.



Lab 1.2B: Separating Dyes With Gel Electrophoresis

Preparation:

☐Dilute 1X Sodium Borate buffer (see page 16)
☐Prepare 0.8% agarose gels (see pages 17-20)
☐Spin down the S1-3 and DI water tubes from the Lab 1.2 box (1 set per group) *OR* Dog Drool Dye set (A, B, C, D and suspect) *OR* Kitten Paternity set (P-V)
☐Set up gel electrophoresis units

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips ☐waste container ☐small microfuge tube rack ☐set of S1-3, DI tubes

Shared Equipment:

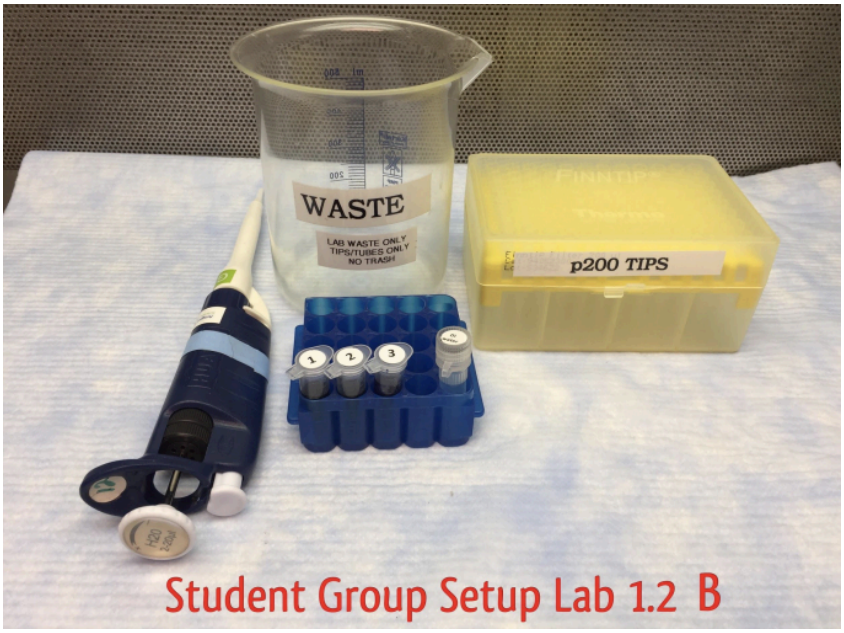
☐gel electrophoresis units ☐minifuges

TIPS:

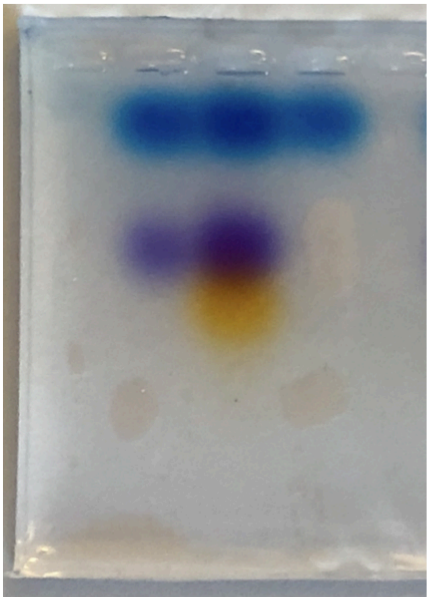
Be sure to place the agarose gels right side up and with the wells nearest the black (negative) electrode. Replace the buffer after two runs if using larger gel tanks such as the OWL or Enduro. Replace after every run if using small gel tanks such as the Blugel or MiniOne. The Dog Drool Lab may be run instead of Lab 1.2B. Make sure that the students *do not discard* any of the tubes of Solution 1-3, DI water, Dog Drool or Kitten Paternity samples . Here is a video on gel electrophoresis.

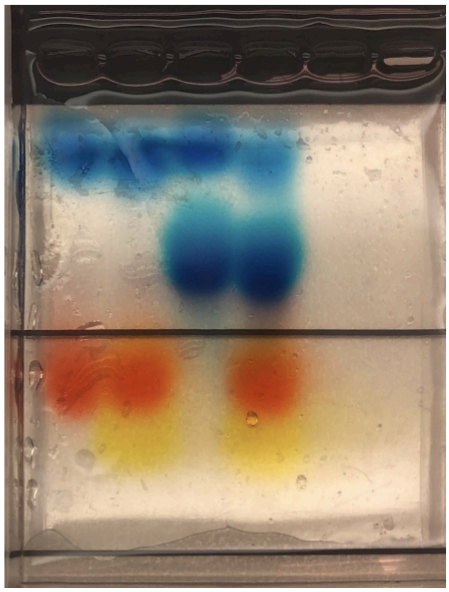
Waste Disposal:

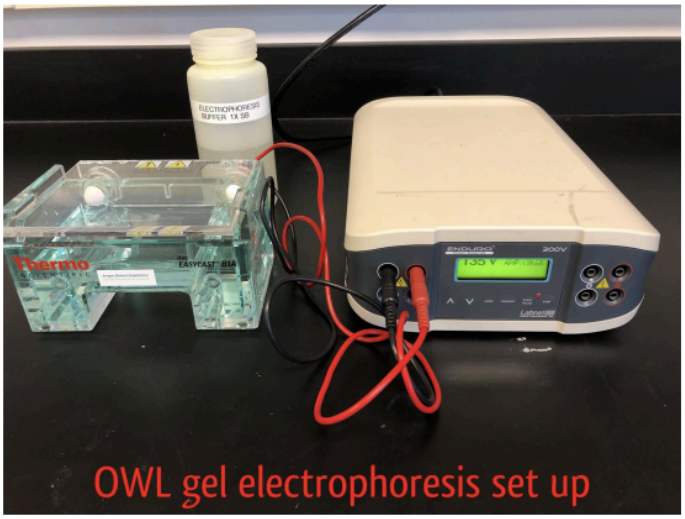
All tips and gels can be disposed of in the regular trash. Pour used 1X Sodium Borate buffer down the sink.

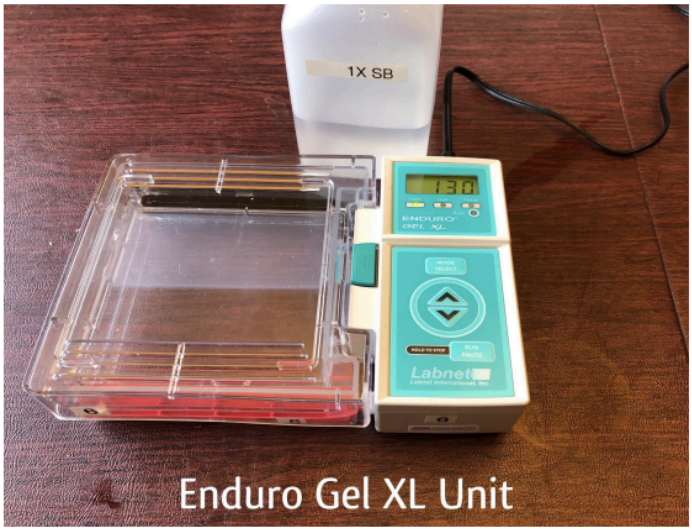


Lab 1.2B: Sol 1, 2, 3 Dog Drool Dyes: A, B, C, D









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Lab 2: Digesting the pKAN-R and pARA Plasmids

Preparation:

☐Label and aliquot tubes (1 each per student group) Can aliquot and store in freezer until day of lab.
DI water is provided in 1ml aliquots and can be found in the Lab 1.2 Dye Box.

☐restriction enzymes [RE] 5µl ☐pARA plasmid 80ng/µl [A] 10µl
☐restriction buffer [2.5xB] 20µl ☐pKAN-R plasmid 80ng/µl [K] 10µl

☐Calibrate the water bath or heat block to 37°C the day before and then turn on again first thing on the morning of the lab.

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips
☐small microfuge tube rack ☐waste container
☐sharpie marker ☐four clean 1.5ml tubes

Shared Equipment:

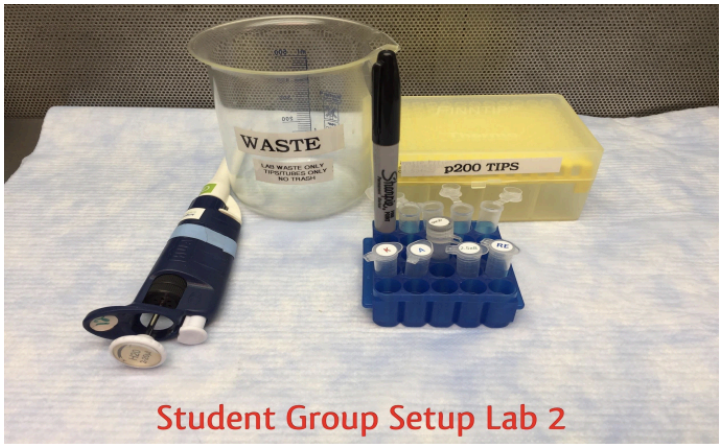
☐ minifuges
☐thermometer
☐heat block set at 37°C

TIPS:

Spin down all reagent tubes prior to setting out for students. Once students have added K, A, 2.5xB and RE have them spin the tubes to pool reagents. Digestion only takes 15 minutes at 37°C but tubes can be left in for up to 60 minutes. Be sure to not leave tubes at 37°C overnight as the DNA may degrade. Once the students have completed the lab store their tubes in the freezer until Lab 4a. Here is a nice summary video of Lab 2/2a. Here is a video on using restriction enzymes to clone a gene of interest. Here is an excellent video on pipetting small volumes of liquids.

Waste Disposal:

All tips and tubes can be discarded in the regular trash.



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Lab 2A: Digesting the pARA-R Plasmid

Preparation:

☐Label and aliquot tubes (1 each per student group) Can aliquot and store in freezer until day of lab. DI water is provided in 1 ml aliquots and can be found in the Lab 1.2 Dye Box.
☐restriction enzymes [RE] 3µl
☐pARA-R plasmid 70ng/µl [R] 10µl
☐restriction buffer [2.5xB] 12µl

☐Calibrate the water bath or heat block to 37°C the day before and then turn on again first thing on the morning of the lab.

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips
☐small microfuge tube rack ☐waste container
☐sharpie marker ☐two clean 1.5ml tubes

Shared Equipment:

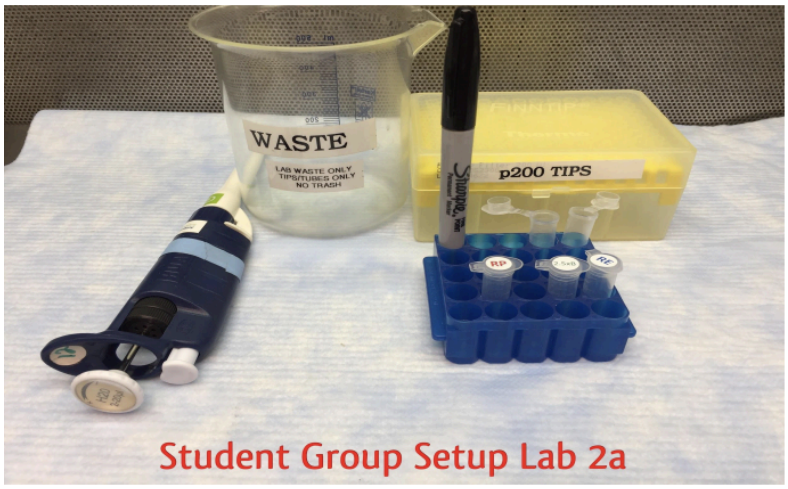
☐minifuges
☐thermometer
☐heat block set at 37°C

TIPS:

Spin down all reagent tubes prior to setting out for students. Have students add R and 2.5xB to the RE tube then spin tubes to pool reagents. Digestion only takes 15 minutes at 37°C but tubes can be left in for up to 60 minutes. Be sure to not leave tubes at 37°C overnight as the DNA may degrade. Store the students' tubes in the freezer until Lab 4a. Here is a nice summary video of Lab 2/2a. Here is a video on using restriction enzymes to clone a gene of interest.

Waste Disposal:

All tips and tubes can be discarded in the regular trash.



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Lab 3: Building the pARA-R Plasmid (Ligation)

Preparation:

☐Label and aliquot tubes (1 each per student group). Aliquots can be stored in freezer until the day of lab. DI water is provided in 1 ml aliquots and can be found in the Lab 1.2 Dye Box.

☐ligase [LIG] 2µl

☐ligation buffer [5xB] 4µl

☐Remove students' Lab 2 tubes from the freezer and spin down to pool reagents

☐Calibrate the heat block to 80°C the day before then turn off overnight. Turn on again first thing the morning of the lab. CAUTION: 80°C is very hot. Warn your students not to touch the heat block or water. Provide forceps to remove tubes.

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips

☐small microfuge tube rack ☐waste container

☐sharpie marker ☐ tubes from lab 2

Shared Equipment:

☐thermometer

☐heat block set at 80°C

☐minfuges

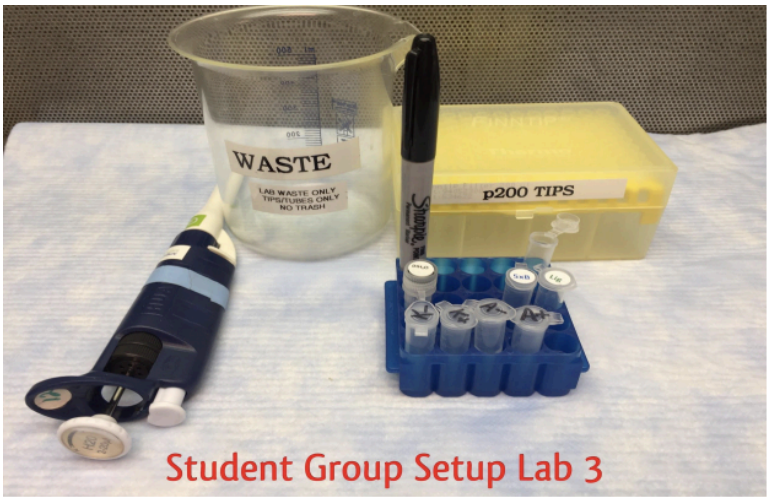
TIPS:

Be sure to have students spin down their tubes following the heat inactivation step as condensation in the lid occurs. Have students add A+, K+, 5xB and DI water to the LIG tube and then spin down the tubes to pool the reagents. Ligation takes at least 20-30 minutes at room temperature. Complete ligation can be ensured by leaving the tubes overnight.

Here is a video on Lab 3. Here is a PBS video which mentions ligase.

Waste Disposal:

All tubes and tips can be discarded in the regular trash.



Lab 4: Verification of the Recombinant Plasmid Using Gel Electrophoresis

Preparation:

☐Label and aliquot tubes (1 each per student group). Store GGLD/GRLD in the dark at room temp, store the DNA Marker in the freezer.

☐GRLD or GGLD[LD] 14µl ☐DNA Marker [M] 8µl

☐Dilute 1X Sodium Borate buffer (volume depends on gel box used, see pg 17)

☐Cast 0.8% agarose gels (volume of agarose depends on gel box used, see pg 17)
☐Tubes from Lab 2 and 3

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips ☐small microfuge tube rack ☐waste container ☐sharpie marker ☐four clean 1.5ml tubes
☐Laminated Lab 4/4a modified protocol

Shared Equipment:

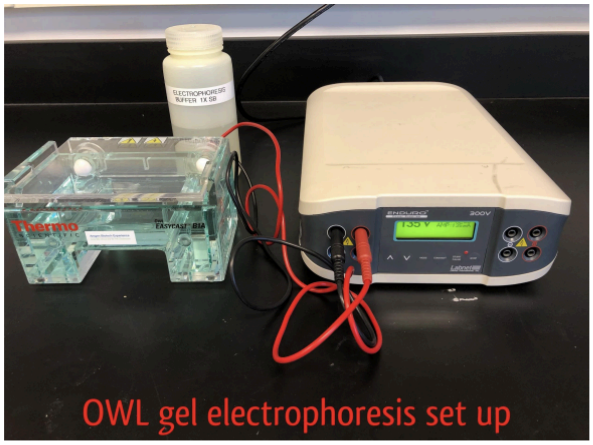
☐UV or Blue light transilluminator ☐hood with digital camera or phone platform ☐gel box set up ☐minifuges

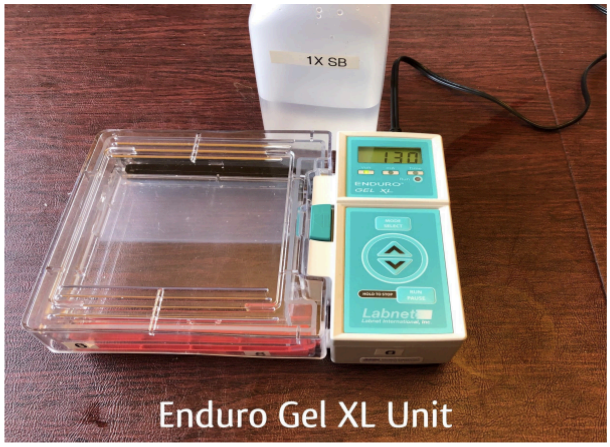
TIPS:

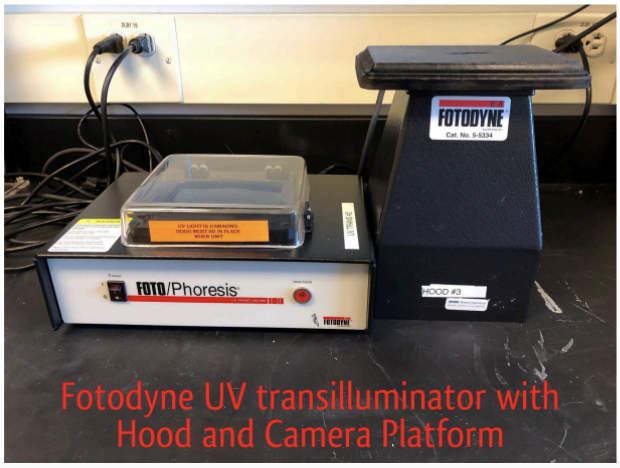
GG and GR will settle in the LD. Have students gently pipette up and down to mix it before adding 2µl to each sample and the marker. If the students do not have enough time to run the gels for at least 20 minutes then run them for 2 minutes to lock the DNA into the agarose, place the gels into ziplock baggies labeled with the group/period # and store in the refrigerator until after school when you can run them to completion. However the DNA in gels stored for more than one day diffuses a great deal. Teachers sometimes mistakenly give out Sol 2 instead of GGLD or GRLD. In which case the DNA will not be stained. If that happens once the gels are finished running, store them in baggies in the refrigerator and call your site coordinator. We may be able to give you a post stain to use. When using a Blue light transilluminator, the orange filter must be in place in order to visualize the bands. Gel Green can photobleach if the light is left on during a run in the Mini One or Blugel units. When using either of those units keep the light turned off. Only turn it on every five minutes to take a photo of the gel. Here is a video on gel electrophoresis. Here are the modified protocols for using GGLD or GRLD in Labs 4/4A.

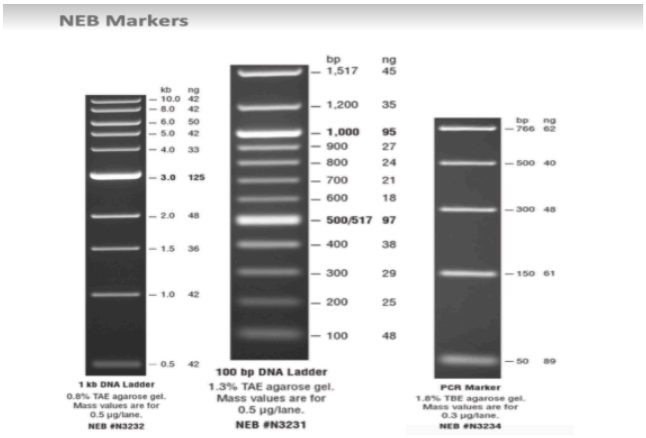
Waste Disposal:

All tips, tubes and gels can be discarded in the regular trash. Pour used 1 X running buffer down the sink.









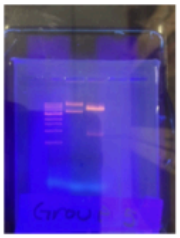
Various NEB DNA Markers
Lab 4/4a uses the 1kb marker in the center.

Tips for Optimizing Gel and Plate Photos

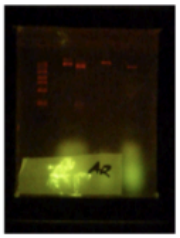
Tips for Photographing Gels

Quick photographs of gels and plates can be taken by the students with their phone cameras, however several tips can be used to optimize these photos so that the images can be used in presentations. Remember that you are required to upload at least one gel photo and one transformation plate photo to the ABE-LA Lab Results Photos Folder every year that you do the ABE labs. These photos help us to ensure the quality of our program.

- Try turning off the classroom lights when taking gel photos without the use of an opaque hood. If the classroom lights can not be turned off, try closing the window blinds or using a cardboard box over the transilluminator to cut down on extraneous light.
- Gels can be labeled in the photo by writing the group # in a piece of scotch tape and placing it on the lowest portion of the gel. (see image below)
- When using a blue light transilluminator (ie. mini one or blugel units or Sapphire transilluminator) always make sure that the orange hood is in place to filter out extraneous blue light and optimize visualization of the bands.
- When using an ultraviolet transilluminator, be sure to use the black hood which contains a special filter to optimize visualization of the bands.



UV transilluminator without filter



UV transilluminator with filter

Tips for taking photos using phone cameras:

- If not using a hood, stabilize the phone over the gel and gently touch the screen where the faintest bands are before taking the photo. This will set the exposure and focus properly.
- When using a hood, place the lens of the phone camera on the top of the hood. This will ensure that the proper focal distance is achieved. Again, touch the screen where the weakest bands are to set the exposure and focus.

Tips for using the digital camera/hood combos in the kits:

- Be sure to check out the user manual for the specific camera in your kit
- If not already set, use the following settings:

Program Mode	
Macro Mode	
No Flash	

- Place the gels on the transilluminator so that the *DNA bands are in the center of the camera field* so that the camera will be able to set the exposure and focus on them. The camera focuses on the center of the field and if the DNA bands are to the left or right of that it will not focus properly.

Tips for Photographing Plates:

- Place the plates on a dark background to highlight the bacterial colonies.
- Turn the flash off on the camera
- LB amp ara plates can be viewed and photographed on the transilluminator if desired.
- If using one of the digital camera/hood combos in the kit, disconnect the camera from the hood before taking a photo of the plates, otherwise the filter inside the hood will affect the photo. There is a screw on the bottom of the camera which attaches it to the metal adaptor on the hood.

Lab 4A: Verification of the Recombinant Plasmid Using Gel Electrophoresis

Preparation:

☐Label and aliquot tubes (1 each per student group). Store GGLD/GRLD in the dark at room temp, store the DNA Marker in the freezer.

☐GGLD or GRLD [LD] 8µl ☐DNA Marker [M] 8µl

☐Dilute 1X Sodium Borate buffer (volume depends on gel box used, see pg 17)

☐Cast 0.8% agarose gels (volume of agarose depends on gel box used, see pg 17)

☐Tubes from Lab 2A

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips ☐small microfuge tube rack ☐waste container ☐sharpie marker ☐two clean 1.5ml tubes

Shared Equipment:

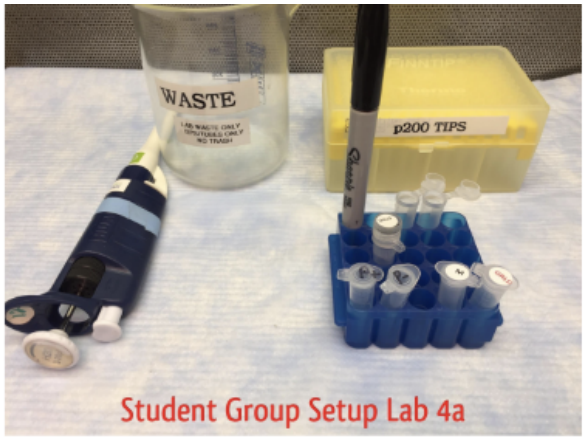
☐UV or Blue light transilluminator ☐hood with digital camera or phone platform ☐gel box set up ☐minifuges

TIPS:

GG and GR will settle in the LD. Have students gently pipette up and down to mix it before adding 2µl to each sample and the marker. If the students do not have enough time to run the gels for at least 20 minutes then run them for 2 minutes to lock the DNA into the agarose, place the gels into ziplock baggies labeled with the group/period # and store in the refrigerator until after school when you can run them to completion. However the DNA in gels stored for more than one day diffuses a great deal. Teachers sometimes mistakenly give out Sol 2 instead of GGLD or GRLD. In which case the DNA will not be stained. If that happens once the gels are finished running store them in baggies in the refrigerator and call your site coordinator. We may be able to give you a post stain to use. When using a Blue light transilluminator, the orange filter must be in place in order to visualize the bands. Gel Green can photobleach if the light is left on during a run in the Mini One or Blugel units. When using either of those units keep the light turned off. Only turn it on every five minutes to take a photo of the gel. Here is a video on gel electrophoresis. Here are the modified protocols for using GGLD or GRLD in Labs 4/4A.

Waste Disposal:

All tips, tubes and gels can be discarded in the regular trash. Pour used 1 X running buffer down the sink.



Lab 5: Transforming Bacteria with the Ligation Products

Preparation:

☐Label and aliquot tubes (1 per student group)
☐ [LB] 350µl
☐competent cells [CC] 100µl (or two 50µl tubes per group) DO THIS ON ICE!
☐pull students' [LIG] tubes from freezer
☐Set water bath or heat block to 42°C, set incubator to 37°C
☐Set plates out to warm up [LB], [LBA], [LBAA] (1 each per student group)

Equipment Needed:

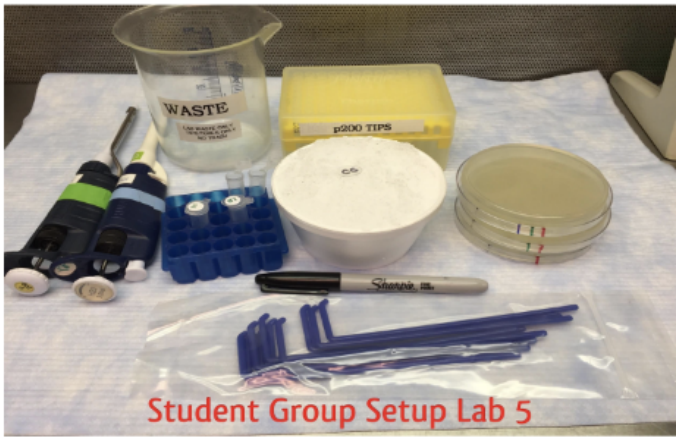
Each student group:
☐p20 and p200 micropipette ☐box of p200 tips ☐waste container ☐small microfuge tube rack ☐sharpie marker ☐two clean 1.5ml tubes ☐cell spreaders (2 per student group) ☐autoclave bags ☐appropriate PPE Shared Equipment:
☐heat block set at 42°C ☐incubator set at 37°C

TIPS:

The teacher can pre-label P+/P- tubes, prechill them in crushed ice and aliquot the 50µl of CC in each tube for the students. Have a flowchart on the board for the students to use. Have students delegate jobs prior to this lab. For example: one student labels plates, another does the heat shock, another keeps time and another spreads the cells. Be sure that students only hold the CC tubes by the rim. As the cells warm up they lose competency. Make sure students use one cell spreader for the P- samples and one for the P+ samples. They should first spread the LB plate, then the LBa and finally the LBaa. This lab can be split into two consecutive lab sessions by putting the stopping at step 10 the first day (cells incubating on ice in LB post heat shock) and storing the tubes in the refrigerator. Then spreading the cells the next day. Here is a video on bacterial transformation. Here is another. *If doing the Colony PCR lab, save students' LBAA plates in the refrigerator to inhibit growth of satellite colonies.*

Waste Disposal:

All tips, tubes, cell spreaders,gloves and plates go into the autoclave bag and returned to PBC . See the PBC Waste Disposal Protocol.



NOTE: +/- tubes should pre-chill in a cup of crushed ice

Lab 5A/B: Transforming Bacteria with p-ARAR

Preparation:

☐Label and aliquot tubes (1 per student group)

☐ [LB] 350µl

☐competent cells [CC] 100µl (or two 50µl tubes per group) DO THIS ON ICE!

☐p-ARAR 10ng/µl [RP] 12µl

☐Set water bath or heat block to 42°C, set incubator to 37°C

☐Set plates out to warm up [LB], [LBA], [LBAA] (1 each per student group)

Equipment Needed:

Each student group:

☐p20 and p200 micropipette ☐box of p200 tips ☐waste container ☐small microfuge tube rack ☐sharpie marker ☐two clean 1.5ml tubes ☐cell spreaders (2 per student group) ☐autoclave bags ☐appropriate PPE Shared Equipment:

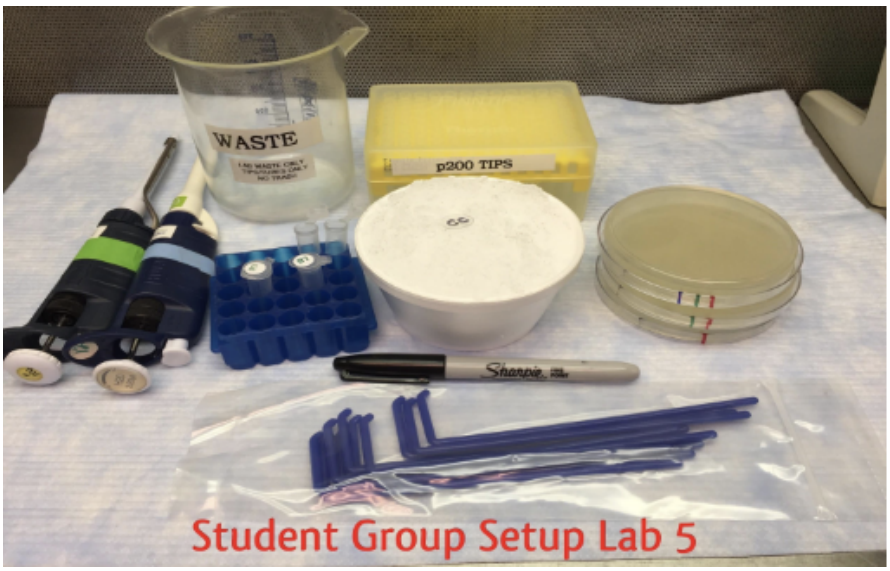
☐heat block set at 42°C ☐incubator set at 37°C

TIPS:

The teacher can pre-label P+/P- tubes, prechill them in crushed ice and aliquot the 50µl of CC in each tube for the students. Have a flowchart on the board for the students to use. Have students delegate jobs prior to this lab. For example: one student labels plates, another does the heat shock, another keeps time and another spreads the cells. Be sure that students only hold the CC tubes by the rim. As the cells warm up they lose competency. Make sure students use one cell spreader for the P- samples and one for the P+ samples. They should first spread the LB plate, then the LBa and finally the LBaa. This lab can be split into two consecutive lab sessions by putting the stopping at step 10 the first day (cells incubating on ice in LB post heat shock) and storing the tubes in the refrigerator. Then spreading the cells the next day. Here is a video on bacterial transformation. Here is another. If doing the Colony PCR lab, save students' LBAA plates in the refrigerator to inhibit growth of satellite colonies.

Waste Disposal:

All tips, tubes, cell spreaders,gloves and plates go into the autoclave bag and returned to PBC . See the PBC Waste Disposal Protocol.



NOTE: +/- tubes should pre-chill in a cup of crushed ice

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

Teachers coming to the PBC ABE-LA site have a choice of either growing up their own Lab 6 culture, using a pre-grown culture or starting with supernatant in their classes. The online Supply Order Form has teachers choose which of the three.

Teachers who decide to grow their own culture should start it at least 5 days prior to starting Lab 6A. This way if the culture does not turn bright pink, the site coordinator can be contacted and hopefully have time to grow a replacement culture up. Store Lab 6 culture in the refrigerator and supernatant in the freezer. Here is a video on growing up the Lab 6 culture.

Equipment Needed:

Shaking incubator, sterile vented culture flask, p1000 micropipette, p1000 tip box

Reagents Needed:

LB broth, vial of ampicillin, vial of Lab 6 cells or pink colony from LBAA plate, vial of arabinose

Protocol:

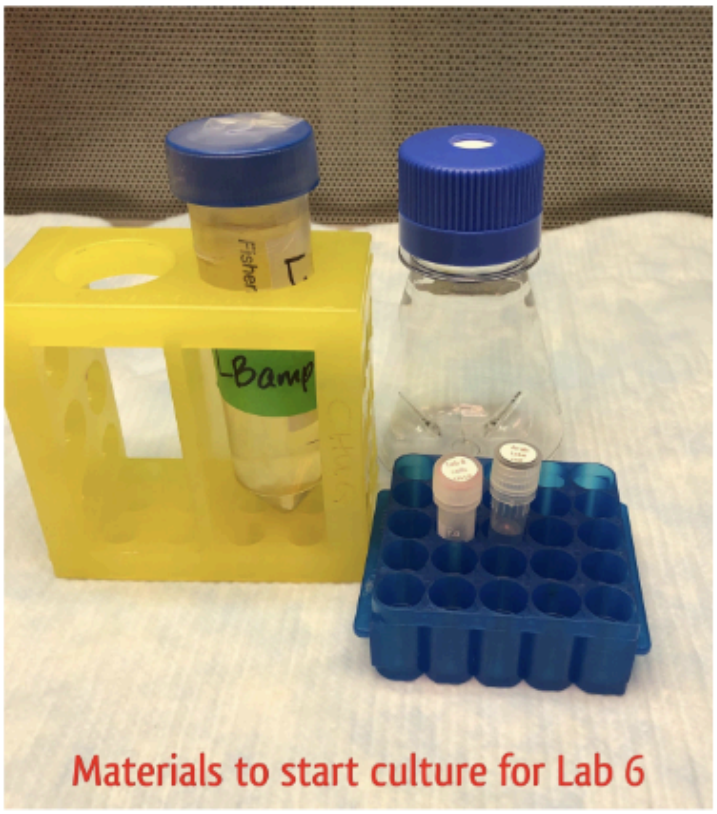
- In the morning, aseptically pour the room temperature LB broth into the sterile, vented culture flask. Add one vial of ampicillin per 50ml of LB broth. No more than 100mls should be added to each 125ml flask to allow headspace during shaking.

- Thaw and pipet the entire volume of Lab 6 cells into the flask. One tube of cells per flask. (If plucking a colony, use a sterile loop to pluck several pink colonies so that your inoculum is large enough.)

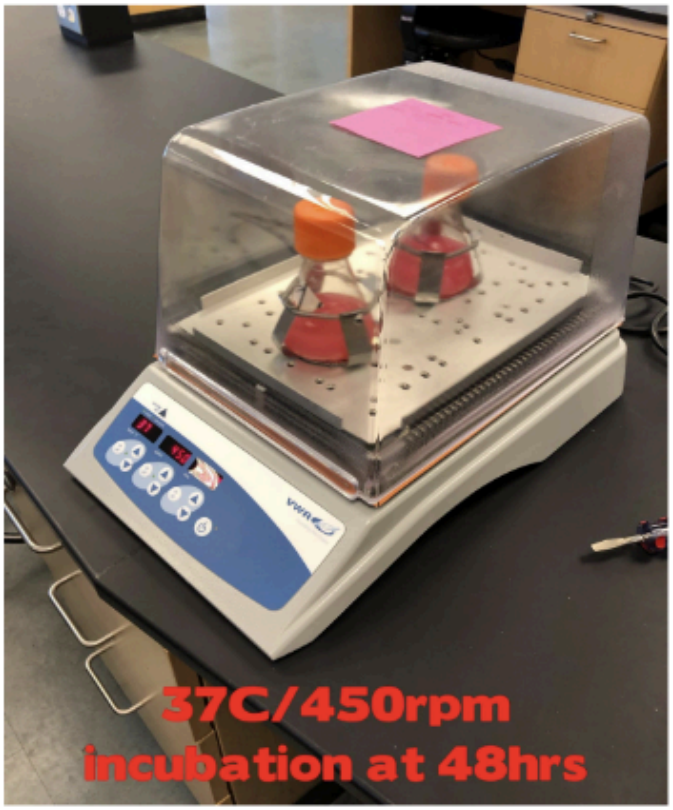
- Place the flask into the shaker clamp and set the shaking incubator for 350-450rpm, 37°C with no time limit. Shake the flask at the highest speed without having the broth splash up onto the lid. Leave the culture to shake for 2-3 hours.

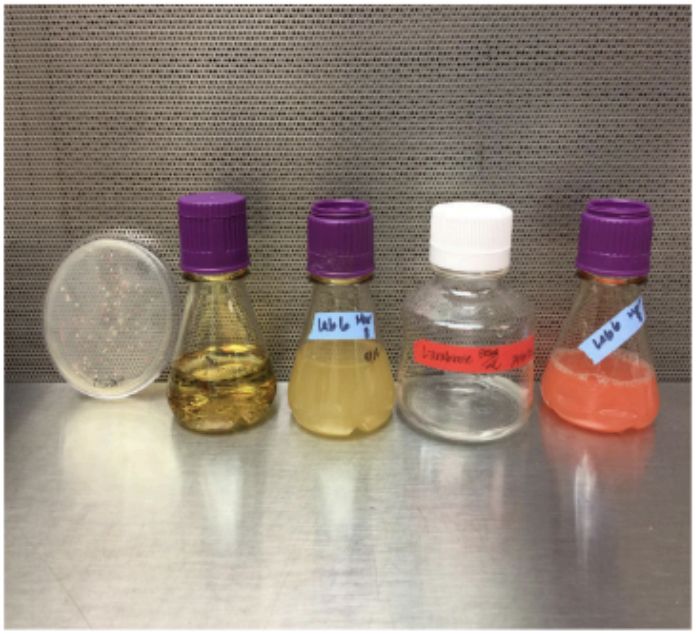
- After 2-3 hours the broth should be turbid or cloudy. The arabinose should be added once the culture reaches mid-log phase. Refer to the turbidity reference photo below and on the lid of the shaking incubator. Once the culture has reached mid-log phase as indicated by the turbidity of the tube second from the right (MacFarland 3), stop the shaker and aseptically pipet the entire tube of arabinose into the flask. Replace the vented lid and continue shaking overnight. It is better to add the arabinose too soon rather than too late. Adding arabinose to a culture that has gone beyond mid-log will most likely not lead to a pink culture as too many of the cells are in senescence.

- If the culture is HOT PINK the next morning, place it in the refrigerator until needed for Lab 6A. If it is salmon or beige colored, let it continue to shake for another 24 hrs. If it has not turned pink after 48 hours of incubation, contact your site coordinator.



Materials to start culture for Lab 6





Lab 6A: Purifying the Fluorescent Protein, Lysing the Cells Grown in the Shaker

Preparation:

☐Grow up the Lab 6 culture on the shaking incubator 5 days prior and store in the refrigerator* ☐Label and aliquot tubes (1 per student group):

☐Lysis Buffer [LyB] 160µl ☐Elution Buffer [EB] 150µl

☐E coli culture [EC] 1ml

Equipment Needed:

Teacher:

☐p1000 micropipette ☐ p1000 tip box

Each student group:

☐p1000 micropipette* ☐p1000 tip box* ☐sharpie marker

☐waste beaker ☐small microfuge tube rack

Shared Equipment:

☐high speed microcentrifuge ☐autoclave bag ☐appropriate PPE

TIPS:

NOTE: See modified Lab 6A Flowchart. *DO NOT HAVE STUDENTS FOLLOW the directions in Step 8 of the student guide! Pipettes should never be used in an inverted position.* Teachers can request either to grow their own Lab 6 culture, get pre-grown culture or receive supernatant from the PBC site. Students who start with supernatant will skip Lab 6A. To ensure students have a bright pink supernatant for the column chromatography in Lab 6B, teachers can pre-aliquot 1ml EC, spin down, decant and then add 1ml of EC for the students to start at step 1 in Lab 6A. This way they end up with a 3 ml cell pellet instead of a 2ml at the end of step 8. *If you intend to do this, please notify your site coordinator so that they can recalculate your supply volumes*. Students can tap their EC tube onto a small piece of paper towel or kim wipe to get any remaining supernatant off their cell pellet. The piece of paper can be discarded in the autoclave bag. Here are some videos on: Growing bacterial cultures, Lysing the bacteria, Lab 6. Reminders: Always balance the high speed microcentrifuge!

Waste Disposal:

All tips, tubes, decanted supernatant, gloves and used kim wipes go in the autoclave bag and returned to PBC . See the PBC Waste Disposal Protocol.

Lab 6B: Purifying the Red Fluorescent Protein Using Column Chromatography

Preparation:

☐Set up the column stands and columns

☐Prepare the columns by draining the 20% ethanol, adding 2 ml of Column Equilibrium Buffer [CEB] and then draining that down to 2mm above the resin bed.

☐Students' tubes of lysed [EC]

☐Or if starting with supernatant [SUP] provided by your ABE site, label and aliquot 200µl per group

Equipment Needed:

Each student group:

☐column stand, column, waste beaker ☐sharpie marker

☐p1000 micropipette ☐p1000 tip box

☐small microfuge tube rack ☐two clean 1.5ml tubes

☐tubes of [BB], [CEB], [WB], [EB]

Shared equipment:

☐high speed microcentrifuge ☐appropriate PPE

☐UV or blue light transilluminator ☐beaker of boiling water (optional)

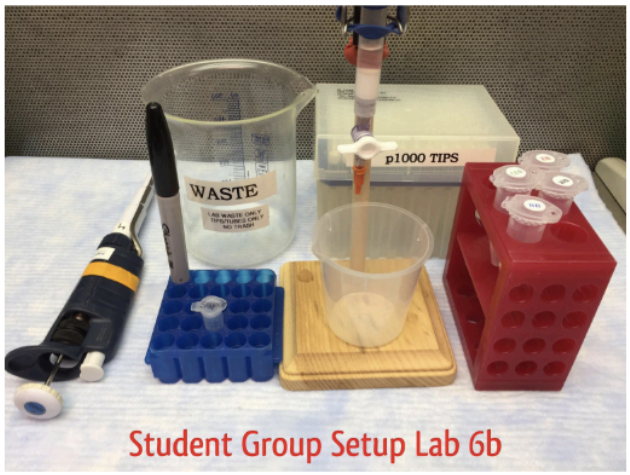
TIPS:

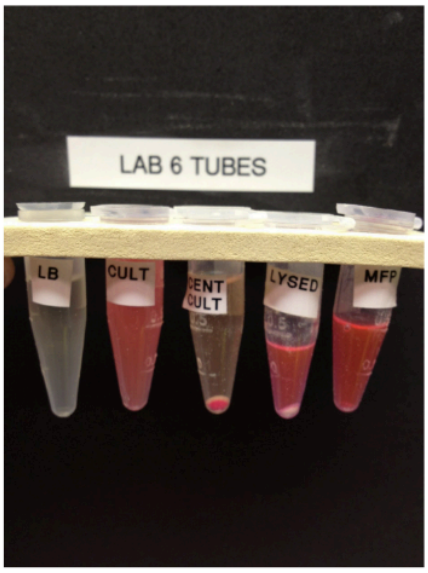
Remind students to 1) Never let the column resin dry out 2) always drip the buffers slowly down the side wall of the column so as to not disturb the resin bed and 3) remember to catch their RFP eluate when it drips from the stopcock. This video is a good summary of Lab 6 parts A and B. Here is a very good video for your students on using the columns. This is a good video summarizing the principle of HIC. The RFP is pink in the visible spectrum but fluoresces neon orange on a UV transilluminator. Your students could have a contest at the end of Lab 6B on who obtained the most RFP. Tubes can be visualized on the transilluminator. Have your students discuss the parameters of the contest. In other words, should they collect as much pink eluate as they can or should they only collect the concentrated fractions? These are questions that manufacturing has to solve. The RFP will denature when exposed to high temperatures. Float a tube of the purified RFP in a beaker of boiling water and see what happens. If the tube is removed before the protein is completely denatured (becomes colorless) it will renature once the temperature drops. Before packing the columns back in the kit, drain the remaining buffer and add 2ml of 20% ethanol before tightly capping. This prevents mold from growing in the resin.

In industry these columns are usually run using vacuum pressure. In the classroom we have to rely on gravity so as the level of buffer lowers, the columns run slower. If you find that a column is running unusually slow, the frit (sieve) at the bottom is probably clogged with fines (small pieces of broken resin beads). Here is a video on unclogging the frits.

Waste Disposal:

All tips, tubes, supernatant, gloves and used kit wipes go into the autoclave bag which is returned to PBC . See the PBC Waste Disposal Protocol.


WASTE
p1000 TIPS
Student Group Setup Lab 6b


LAB 6 TUBES
LB
CULT
LYSED
MFP

Care and Maintenance of mFP Purification Columns

The ABE-LA Protein Columns for Lab 6 consist of a 3cc syringe barrel with a luer lock into which the stopcocks screw in. On top they have an end cap which forms a seal to prevent evaporation of the column resin and at the bottom there is a tip cap.

To Set Up the Columns for Use:
Replace the tip and end caps. Be sure to save them for storage. The columns should be filled with 20% ethanol when you receive them. Drain the columns to just 2mm above the resin bed and then add 1-2mL of CEB. Flush the resin bed with the CEB and let it drain to just 2mm above the resin bed. The columns are now ready to use.

Repacking the Columns in the Kit:
Before repacking the columns into the kit, drain the columns to 2mm above the resin bed and then add 2ml of 20% ethanol. Make sure the stopcocks are closed. Replace the tip caps and tightly seal the end caps before standing the columns upright in the canister.

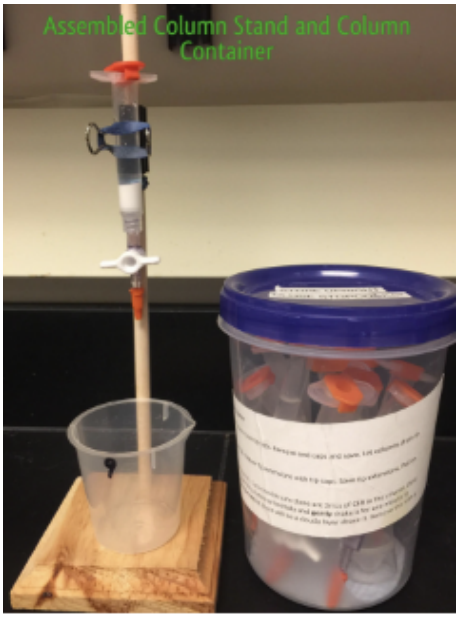
Slow Running Columns?
If your columns are running slowly, it's probably the result of *fines* (small pieces of broken resin beads) blocking the *frit* (sieve) positioned near the bottom of the column. In order to get the column to flow properly, these fines must be removed from the frit. To do this, follow these steps:

1) Cap the column and hold the capped column sideways (horizontal). Gently shake the column to re-suspend the resin in the *Column Equilibration Buffer*. This will take a few minutes. Shaking gently will help to prevent creating additional fines. The shaking will eventually dislodge the fines from the frit.

2) Once the resin is completely re-suspended and the fines, clogging the frit, have been suspended in the buffer, set the column in a vertical position to allow the resin to settle in the column.

3) After a few minutes, you will notice a relatively dense layer of resin above the frit, at the bottom of the column. These are unbroken resin beads; the fines take longer to settle. The fines will form a less dense and somewhat cloudy layer above. Take a disposable pipet or P-1000, and carefully remove this cloudy layer. These are the resin fragments that are clogging the frit.

4) Add 1 mL of CEB to the column. The column should be ready to use now.



Colony PCR Lab Part A: PCR

Preparation:

☐Thaw the One Taq MM and Colony Primer Mix in crushed ice. Follow the "Instructor Protocol for Using One Taq 2X Quick Load Master Mix" pg 42

☐Label and aliquot tubes (1 per student group):

☐Master Mix [PCR] 94µl ☐p-ARAR 0.025ng/µl [+] 3µl ☐p-ARA 0.025ng/µl [-] 3µl

☐Pull students' Lab 5 LBAA plates from the refrigerator

☐Set up the thermal cycler. If using the mini pcrs, be sure to charge the tablets overnight.

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips ☐PCR tube rack set in cup of crushed ice ☐waste container ☐strip of 4 PCR tubes ☐sharpie marker

☐appropriate PPE

Shared Equipment:

☐thermal cycler(s) (see pgs 43-44 for set up) ☐minifuges

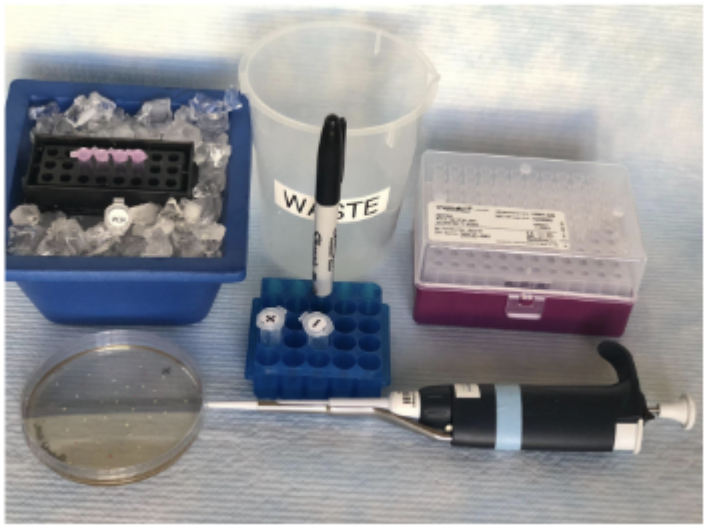
☐autoclave bags

TIPS:

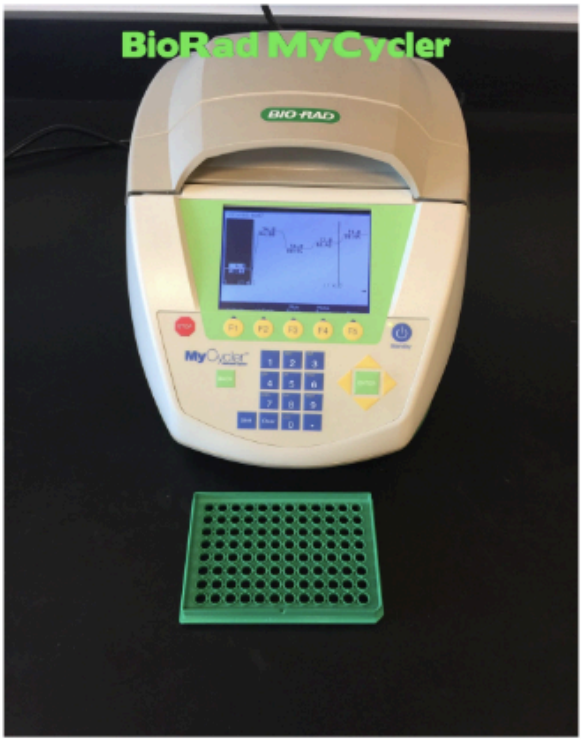
Only mix up enough colony PCR MM/primers [PCR] for one class period at a time. Keep it on ice. Leave any remaining MM and primers unmixed and return frozen. Prechill the students PCR tubes. Students should label the PCR tubes on their sides near the lid as markings will be cooked off the lids themselves. It is easier to have students hold the pipette tip with their gloved fingers while picking up cells rather than to try manipulating the tip at the end of a pipette. Students should just barely touch the colonies when picking up cells and twirl the end of the tip in the MM several times. The PCR MM should not appear cloudy once they have added the cells have been added. The addition of too many cells will inhibit the primers from binding to the template DNA and huge bands of genomic DNA will be the only ones seen on the gels. *Quickly* spin students' PCR tubes before putting them into the thermal cycler. If using the BioRad Mycycler, multiple classes can be run at the same time. When combining runs like this, the first period's tubes should be placed in the *freezer immediately* following the mixing of MM and template DNA to discourage the formation of primer dimers. When the run is finished, store tubes in the freezer. Here is a video on PCR. Here is an overview of the Colony PCR Lab.

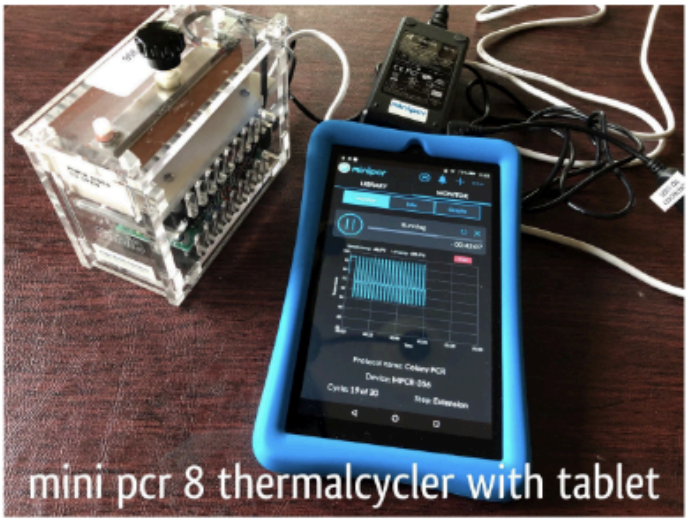
Waste Disposal:

All tips, tubes, gloves and plates go into the autoclave bag and returned to PBC . See the PBC Waste Disposal Protocol.



Colony PCR Part A Set Up





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	53µL	21.5µL	21.5µL	**96µL**
10 student groups	530µL	215µL	215µL	**960µL**
12 student groups	636µL	258µL	258µL	**1,152µL**

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

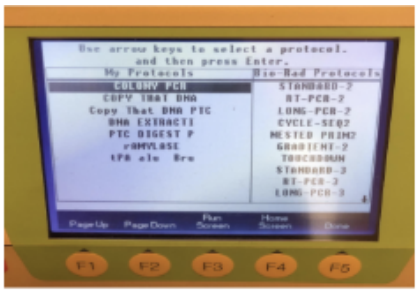
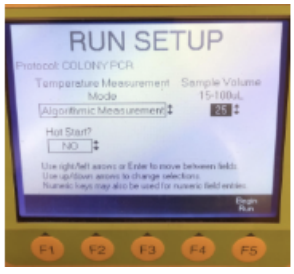
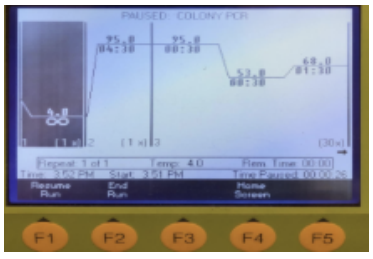
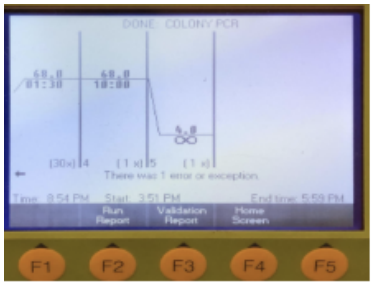
EPM/PTC PCR reagents	One Taq MM (MM)	PTC PCR Forward primer (pF)	PTC PCR Reverse primer (pR)	Working PTC PCR MM (PCR) total
1 student	**12.5µL**	**5.25µL**	**5.25µL**	**23**
10 students	**125µL**	**52.5µL**	**52.5µL**	**230**
20 students	**250µL**	**105µL**	**105µL**	**460µL**

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 prior to loading the wells, regardless of whether the MM is green or clear.

Bio Rad MyCycler Quick Start Guide

-Keep PCR tubes in crushed ice while setting up the reactions.
-Quickly spin down PCR tubes prior to run to ensure all reactants are in the bottom of the tubes. -The Mycycler PCR programs include a 4°C indefinite hold at the beginning and end. Start the run to prechill the tubes before setting up the PCR reactions.

Plug the unit in, press STANDBY and let it complete the self check. Press F1 for the Protocol Library. Make sure the green plastic tube grid is oriented so tube A1 will be in the top left corner. Have students identify their tubes using the grid template.	
Choose the protocol you wish to run from those listed under My Protocols. Use the yellow ARROW keys followed by the green ENTER key to select the desired program.	
From the choose operation screen press the ENTER key to start the run. Make sure that the sample volume is 25µl with NO hot start and press F5 to Begin Run. The unit will prechill to 4°C and hold.	 
Load the samples, lock the lid and press F1 to resume the run and start heating the tubes. Confirm that the tubes are heating before leaving the unit. A run can be stopped at any time by pressing the red STOP key.	
The run will take about 2.5 hours and can be left overnight. The end screen will state whether any errors occurred during the run. If there were, a validation report can be generated by pressing F3. To end the run press F4. When the run is finished, turn off the unit by pressing STANDBY for several seconds. Unplug and pack into the box. Don't forget the green tube grid.	

Colony PCR Part B: Gel electrophoresis

Preparation:

☐Prepare 1X sodium borate buffer and 0.8% agarose gels. (see pg 17)

☐Label and aliquot tubes (1 per student group) Store GGLD/GRLD in the dark at room temp, store the DNA Marker in the freezer.

☐ GGLD or GRLD [LD] 14µl ☐ DNA Marker [M] 9µl

☐Thaw PCR tubes from part A

Equipment Needed:

Each student group:

☐p20 micropipette ☐box of p200 tips ☐waste container ☐small microfuge tube rack ☐PCR tube rack

Shared Equipment:

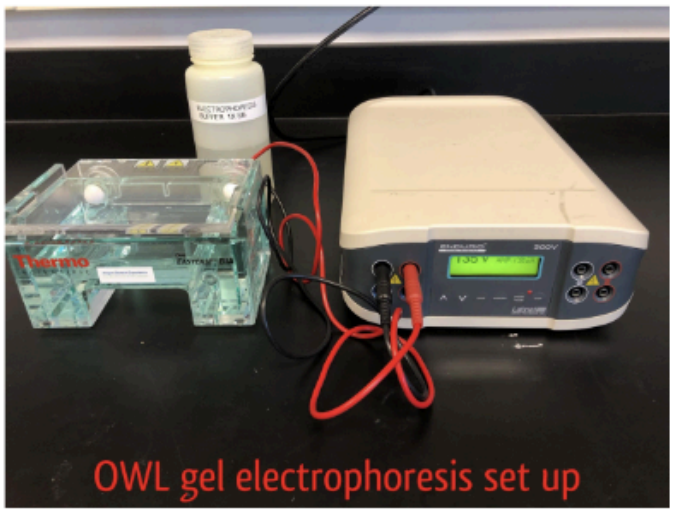
☐UV or Blue light transilluminator ☐hood with digital camera or phone platform ☐gel box set up ☐minifuges

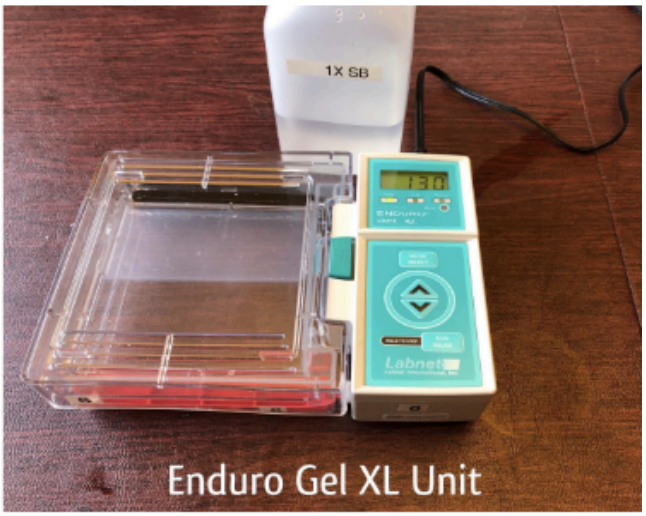
TIPS:

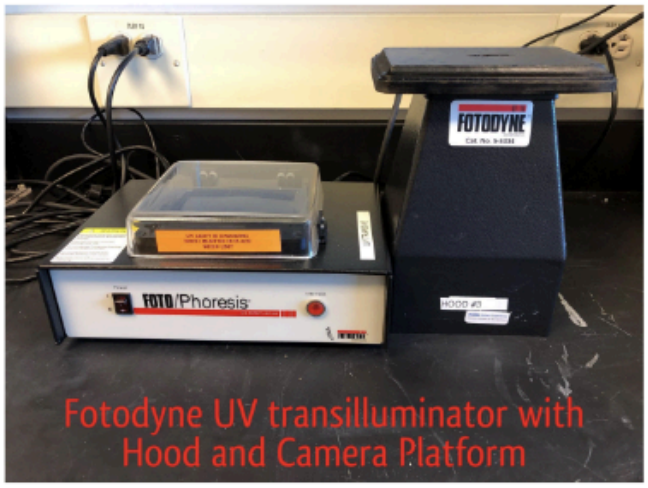
Students may not not have gotten the bacterial cells down into the MM and so no amplification could occur. As long as a red colony, a white colony and the +/- controls are amplified by at least one student group, then you know that the MM and the PCR cycle worked. Some student groups may not obtain amplification in every tube. The amplified region from the white colony and the "-" control is 662bp and should be easily visible. If not visible in a Mini One or Blugel, be sure to place those gels onto a separate stronger transilluminator such as the Prep One Sapphire. Here are the modified protocols for using GGLD or GRLD. If using agarose gels containing GG then follow the Student PCR protocol by having students add simple LD to all of their samples prior to loading the wells.

Waste Disposal:

All tips, tubes and gels can be discarded in the regular trash. Pour used 1 X running buffer down the sink.


OWL gel electrophoresis set up


1X SB
Enduro Gel XL Unit


FOTODYNE
FOTO/Phoresis
Fotodyne UV transilluminator with
Hood and Camera Platform


PrepOne Sapphire
blue light transilluminator

Appendix

Information regarding Troubleshooting the ABE labs can be found online in the shared ABE-LA Teacher's General Resources Folder. Contact your site coordinator regarding any problems or issues that come up.

The Master ABE SDS List contains the links to Safety Data Sheets for all reagents used in the ABE labs. Individual SDS sheets can be found in this folder.

The Checklists for every kit can be found online in the PBC ABELA Teacher's Resources Folder. Use these to make sure that you pack and return everything.

Per the ABELA Teacher Expectations and Policies, teachers and schools may be charged for any lost or broken equipment. Please refer to the ABELA Kit Price List for the replacements costs of most items.

Kit Equipment Sanitation/Disinfection Protocol

In response to the need for increased sanitation due to the Corona Virus Outbreak, the ABELA distribution sites will be sanitizing the kit equipment between schools. However, teachers will need to sanitize the equipment in between their students. In order to minimize the time needed for disinfection, teachers should limit the amount of equipment handled by students. Students should be discouraged from handling shared equipment such as the kit boxes themselves, waterbaths or heat blocks, high speed centrifuges, and incubators. Spray bottles of 70% isopropyl alcohol will be provided. DO NOT use anything other than 70% isopropyl alcohol to disinfect the ABELA equipment as other chemicals can be corrosive.

In between classes or groups, teachers should use paper towels generously sprayed with 70% isopropyl alcohol to wipe down all surfaces commonly touched by students such as: the micropipettes, tube racks, Lab 1.1 weigh boats and templates, the outside surfaces of: waste containers, gel boxes (gel trays if handled), tip boxes, sharpie markers, the touch pads on: power supplies, minifuges, Mini One, Enduro and MyGel units, mini pcrs, Mini One PCR machines, and tablet screens. Isopropyl alcohol should not be sprayed into any electrical ports. Equipment can be allowed to air dry as the alcohol will evaporate.

All equipment should be wiped down prior to repacking the kit.