ABE-Fullerton College KIT BINDER (2019)

Amgen Biotech Experience
Scientific Discovery for the Classroom
Greater Los Angeles

CONTENTS of KIT BINDER

ABE-Fullerton College KIT BINDER (updated June 2019)

NOTE from Jo Wu:

I am sure you understand the value of the ABE program, in saving money of reagent costs for your school and providing meaningful labs for your students. As an educator, I applaud the extra effort that you are doing to provide students with hands-on, relevant science lab experience. As there is so much preparation to doing wet labs, I am trying to give you guidance and simplify your work. But **you will need to be organized, review the materials, and plan ahead!**

Please recognize that my staff and I put in a tremendous amount of work to prepare the materials for you, and communicate early if you have a change in plans, student numbers and lab dates.

CONTACT INFORMATION:

- ☐ Jo Wu, ABE-FC Coordinator, jwu@fullcoll.edu, 949-872-4087
- ☐ Jessica Wu-Woods, ABE-FC Lab Technician for Reagents, amgenbwoc@gmail.com, 949-887-6075
- ☐ Sherry Tsai, Co-Director of ABE-Greater LA Region, sherrymtsai@gmail.com

ORDERING Information:

- ☐ Reserve the ABE equipment by looking at the annual Reservation Schedule (see abe-la.org/fullerton).
- ☐ At least one month before your reservation slot, fill out the online order and be specific about
  - Class names, # students, # groups, which labs for each class.
  - If you do not hear a confirmation from Jessica, be sure to email her to ask.
- ☐ Schedule pick up and drop off appointments online (Signupgenius) or by email.
  - You will pick up transformation reagents separately (a few days before lab) unless you live far away. Competent cells are best if kept frozen in isofreeze box and used within the week.
- ☐ Ask by email for any special arrangements, eg. crushed ice, want Jessica to come to your school.

KIT INFORMATION AND REAGENT STORAGE:

- ☐ At pickup, allow 30 minutes to ensure that you are receiving everything on the checklist/spreadsheet
- ☐ Kit Boxes Checklist shows all of the equipment that you received
- ☐ Materials Spreadsheet shows the list of reagents and supplies
  - Follow the color-coding on the materials spreadsheet for storage.
    - **FREEZE:** DNA, enzymes, supernatant, PCR reagents, cold packs, comp cells (in isofreeze box)
    - **REFRIGERATOR:** LB broth, agar plates (agar side down), agarose gels (keep container flat)
    - **ROOM TEMP:** Loading Dye, SB Buffer, Chromatography buffers
  - **LIGHT SENSITIVE:** Loading Dyes, Chromatography Columns

UNPACKING OF THE KIT AT YOUR SCHOOL:

- ☐ See video
- ☐ Take photo of the entire kit, so that you remember what should be returned.
- ☐ Freeze the isofreeze box (with reagents) and *all cold packs*. Keep Styrofoam box and cooler bag to return to FC.
- ☐ Refrigerate agarose gels (keep flat, away from freezer), agar plates, and LB Broth.

ABE-FC Spreadsheet of Reagents and Equipment								
Reservation Date								**EQUIPMENT**
School								**Kit #**
Teacher								Teacher box #
# students total								Box of 2 Minifuges
								Lab 1 Practice Gels
FROZEN REAGENTS			Aliquots		**Intro to Biotechnology**	**#**		Box of Soln 123, RD
yellow (RP) pARA-R (10ng/µL)	0	µL	**0**		# students Lab 1			Box of CSI dyes
blue (pR) pARA-R (70ng/µL)	0	µL			# groups Lab 1			Box of Paternity dyes
purple (K) pKAN-R (80ng/µL)	0	µL			# groups CSI (uses Lab 1 ge			Microplate Art Design Kit
tan (A) pARA (80ng/µL)	0	µL			# groups Paternity			Deionized Water
orange (RE) BamH I + Hind III (5U/µL)	0	µL	**0**		# Microplate Art			Water Bath for Labs 2 ,3, 5
clear (2.5xB) 2.5x restriction buffer	0	µL	**0**					Incubator for Lab 5
pink (LIG) DNA ligase (0.5U/µL)	0	µL			**Abridged G E Sequence**			
green (5xB) 5x ligase buffer	0	µL			# students Abridged GE			Lab 5 bags, tape
clear (M) 1KB DNA marker (25ng/µL)	0	µL	**0**		# groups Abridged GE			Transilluminator, Blue
(CC) Competent cells in Isofreeze Box	0	mL	**0**		Abr with Lab 6			Transilluminator, uV
Lab 6 supernatant aliquots	0	tubes	**0**		Abr with cPCR			Lab 6 Columns / Buffers
(EC) Lab 6 pellet of Cultured cells	0	mL			Date of Cells			Lab 6 Pipettes/Tips
(Lab 6) Frozen Lab 6 cells to culture	0	tubes						Lab 6 Sterile Flask
OneTaq Master Mix 2x for cPCR	0	µL			**Complete G E Sequence**			Lab 6 Vortexer
Primer Mix (F+R) for cPCR	0	µL			# students Complete GE			Lab 6 High Speed Centrifuge
pARA (24pg/uL) for cPCR	0	µL			# groups Complete GE			Lab 6 Shaking Incubator
pARA-R (24pg/uL) for cPCR	0	µL			Complete GE w Lab 6			Thermocycler
					Complete GE w cPCR			Manuals, Student
REFRIGERATED REAGENTS					Date of Cells			Pipettes
Pre-made Mini One Agarose gels	0		**0**					
Agar Plates: LB, LB/amp, LB/amp/ara	0	each	**0**		**Focus on Bacteria**			**REQUESTS**
(LB) LB broth for Lab 5	0	mL	**0**		# students FB			School Equipment Owned
Lab 6 Demo					# groups FB			
(LyB) Lysis Buffer for Lab 6	0	uL	**0**		Focus w Lab 6			
LB/amp broth for Lab 6	0	mL			Date of Cells			
Arabinose for Lab 6	0	tube/flask						**Special Requests**
					Lab 6 Purifying Protein			
STORE at ROOM TEMP					#group HIC total	**0**		
(LD) 50x GRLD or GGLD loading dye	0	µL	**0**		Supernatant	**0**		
Tips P20-200	0	tips			Prepared Overnight Culture			
Tips P1000	20	tips			Growing Overnight Culture			**Comments**
Tubes: 1.5 mL	0	tubes						
Cell spreaders	0	packs			**Colony PCR Sequence**			
Agarose	0	g			# groups PCR total	**0**		
20x SB buffer	0	L						
Tubes: 0.25mL PCR	0	tubes			**ABE 2.0 Precision Medicine**			
					# groups 2.0 PCR total			
					# groups 2.0 Digest total			Submitted ABE Policies
								Lab Results Survey
								Lab Photos

INSERT Photo of Reagent Box

FULLERTON REAGENT PREPARATION and TECHNICAL SUPPORT:

☐ Teachers are invited to come to the Fullerton Reagent Prep days to ask questions, to aliquot reagents and to prepare your agarose gels ahead of time. If Jo Wu has enough assistants to help with lab reagent preparation, we will be helping you by:

- ***Casting the 0.8% agarose gels*** (in SB Buffer) for use with the Mini One gel system. These gels will be stored flat on laminated sheets inside a plastic box that MUST be stored flat and in refrigerator. You would use the provided laminated "gel spatula" to transfer the gel onto a casting tray or directly onto the gel plate. If using only dye samples, then use a white or gray gel plate. If using DNA samples with gel green stain, use a black gel plate.

- ***Aliquoting reagents for student groups***. Please return all labeled tubes, tube holders, square cardboard boxes. Separate unused tubes into one row in box. Discard any contaminated tubes. If many teachers do not return these labeled tubes, then we will discontinue this huge, extra effort.

☐ For new teachers, Jessica or another assistant may be available to come assist your classes for the transformation lab, if schedules work out and if Jo has enough funding. You would need to provide any necessary permission/paperwork at your school to have them come visit.

TEACHER PREPARATION:

☐ Follow the suggested lesson planning schedules provided by ABE master teachers (in Dropbox).
☐ Go back through the powerpoints provided at the ABE Teacher Training.
☐ Read the teacher manual, to review key points.
☐ Follow the Teacher Prep Sheets in this binder.
☐ Gather materials not provided in kit: glassware, gloves, crushed ice, disinfectant wipes.
☐ Email or text questions to Jo Wu or ABE-FC lab technicians as needed.
☐ Attend a Fullerton Reagent Production Day to get specific help from Jo Wu.

BEST PRACTICES FOR LAB EXPERIMENTS:

1) Teach the overview of ABE labs and molecular biology concepts. Videos, sample teaching powerpoints, puzzles, quizzes are provided at ABE-LA.org/Resources link.
2) Students should read the ABE Student manual (hard copy or pdf files) to fill out the unlabeled FLOW CHARTS as homework, or write down the protocols in their lab notebook.
3) Have students do "Air Biotech" (like Air Guitar), and do a dry run of the experimental protocol (without reagents).
4) When doing actual lab experiments, provide the one page STUDENT PROTOCOLS (in plastic sheets). Students can check off as they do the steps.
    a. Note: Wipe off plastic Protocol sheets for use by the next class.
    b. **Expo** markers wipe off with dry paper towel.
    c. **Sharpie** markers require isopropanol or baby wipes.
5) **Take lab result photos and send to amgenbwoc@gmail.com**. This is a requirement to be able to reserve the ABE kit next year.

TROUBLESHOOTING:

- ☐ If a lab does NOT work, please send a detailed email to amgenbwoc@gmail.com with subject line (**URGENT – ABE LAB problem**) ASAP and describe as much detail as you can and include photos.
    - Keep all of the reagent tubes (original and any leftover aliquots) in the freezer until kit return, so that we can run the troubleshooting experiments at FC.
    - Please tape a big sign on the reagent box (To TROUBLESHOOT)
- ☐ Submit lab results photos to Jessica. Even if the students' results are not perfect, we can use the photos for troubleshooting powerpoints.

EQUIPMENT PROBLEM:

- ☐ If a piece of equipment does NOT work or got contaminated,
    - please email with subject line (**Urgent – ABE LAB problem**) ASAP and describe the details of the issue. Indicate if you need a replacement equipment by a specific date.
    - If small item, place in a baggie with **PROBLEM** label, name, date, and detailed problem. E.g. "does not work" is not detailed. Better description is "pipettor does not stay at one volume, and seems to slip downward"
    - If large equipment, place a colored tape with **PROBLEM** label and date.

REAGENT DISPOSAL: see powerpoint for more detail

- ☐ **Labs 1,2,3,4, 6B:** Used agarose gels, tubes, pipet tips, gloves can be discarded in regular school trash. You can use the 1X SB buffer for two to three gels. Used 1X SB Buffer can be poured down the drain. If you have unused SB buffer, please return (but do not mix 1X and 20X stock bottles).
- ☐ **Labs 5, 6A, Colony PCR:** anything that touched or could grow bacterial cells (spreaders, pipet tips, tubes) must be discarded in the designated biowaste bag …. And return to FC.
- ☐ **BIOWASTE BAG in Plastic Bin: Clear** Biowaste Bag should contain ONLY the used tubes, pipet tips, gloves from Labs 5, 6 and Colony PCR. We would prefer the used cell spreaders in their own biowaste bag. Twist tie (or masking tape) the biowaste bags closed, and place in plastic bin. The USED agar plates should be placed in sleeves and taped tight, and return taped bags in the plastic bin. Note: we are now providing smaller clear biowaste bags (instead of the red biohazard bags).

CLEANING and PACKING:

- ☐ Rinse the gel buffer tanks, practice gel dishes in distilled water and air dry.
    - DO NOT wipe them dry with paper towel or ethanol or other solvent.
- ☐ Wipe off laminated sheets and protocol sheets.
- ☐ Refill pipet tip boxes.
- ☐ Each teacher should fill out the **online LAB results survey** and submit photos to amgenbwoc@gmail.com (or share your Google Drive or Dropbox folder).
- ☐ Make appointment to drop off kit at FC at tinyurl.com/ABEFCAppt OR write amgenbwoc@gmail.com email to ask for alternative date/times.

RETURN TO DISTRIBUTION CENTER:

- ☐ ALL tubes that have typed labels (***used or not***), but they must be kept in appropriate storage temperature until you bring them back.
    - Note: We expect students to use the Lab 3 labeled Ligase tubes and discard these.

- ☐ All UNUSED reagents, supplies, empty bottles.
    - Unused Agar plates of all types can be stacked together into one plastic sleeve, tape the sleeve to close, and label UNUSED. Keep in refrigerator until returning to FC.

- ☐ USED agar plates should be stacked together and put into plastic sleeves and taped closed. Keep in refrigerator until returning to FC. Place sleeves and biowaste bags in the gray plastic bins "Biowaste", to preventing dripping inside your car.

- ☐ Pack all kit boxes, according to the specific box checklist and photos.

- ☐ Use Parafilm to cover 2 transformation (or any problem) agar plates closed, and put into a sandwich baggie. Label with your name, school, and date.
    - This is proof that you had successful transformation, which is required in order to do colony PCR lab next year (with training).
    - Jo Wu will need these cells, in case we need to do any technical troubleshooting.

ABE-Fullerton Equipment List (for MiniOne Kits)

Teacher Box
- 1 P20 Micropipette
- 1 P200 Micropipette
- 1 P1000 Micropipette
- 1 Box of p20/200 tips
- 1 Box of p1000
- 1 Large Microfuge Tube Rack
- 1 Digital Balance AWS-100
- 1 Flask for melting agarose
- 6 Tubes for holding melted agarose
- 3 Casting Stands, 3 Combs, 6 Casting Trays, 3 lids
- Agarose, spatula, weigh boats
- Plasmid DNA model

Box #1
- 4 Student Station Boxes
- 4 p20/p200 tip boxes
- 2 1X SB buffer bottles
- 4 Floating racks (foam circles)

Box #2
- 4 Student Station Boxes
- 4 p20/p200 tip boxes
- 2 1x SB buffer bottles
- 4 Floating racks (foam circles)

Box #3
- 4 Student Station Boxes
- 4 p20/p200 tip boxes
- 2 1x SB buffer bottles
- 4 Floating rack (foam circles)

Box #4
- 2 Microcentrifuges
- 2 Power cords

Box #5
- Blue LED Transilluminator
- Orange filter
- Power cord

Box #6
- Vortexer
- High Speed Microcentrifuge
- 2 Power Cords

Box #7
- Waterbath
- Waterbath Lid
- Thermometer
- Thermometer Sleeve

Box #8
- 12 Columns for chromatography
- 12 wooden bases
- 12 wooden OR metal dowels
- 6 boxes with 4 buffer bottles each
- Extra buffer bottles
- 20% Ethanol bottle
- Buffer transfer pipettes
- Binder clips/rubber bands or Orange cable clips
- Bag of tips for columns
- Box of columns (keep upright)
- Color switch ball
- Box with 3D RFP Model

Box #9
- 12 P1000 Micropipettes
- 6 boxes of P1000 tips

3 Boxes of Thermocyclers
- 2 Embitec PCR units
- 2 tablets
- all power cords
- PCR Ice Bath boxes

Boxes of Student Guides

Deionized Water Gallon Jugs

Incubator for plates
Shaking Incubator

Kit Binder
Protocol Binder

Box of Agarose gels
Cooler Bag
- Reagent Aliquot boxes
- Ice packs

Box of Dry supplies
- Bag of p200 tips
- Bag of p1000 tips
- Bag of tubes
- Bag of transformation supplies (spreaders, biowaste bags, twist ties, masking tape)
- Box of Lab 1: Solutions 123, Red Dye, water
- Box of Dog Drool CSI dyes
- Box of Kitten Paternity dyes

Transformation Supplies
- LB agar plates
- LB/amp agar plates
- LB/amp/ara agar plates
- LB broth aliquot tubes
- Plastic bin

Styrofoam Box
- Isofreeze box with Comp cells
- Ice packs

Box Microplate Art
- 96 well plate
- 15 mL tubes of various dyes
- instruction cards
- laminated sheets

EACH STUDENT STATION BOX
- 1 MiniOne Hood
- 1 MiniOne Carriage
- 1 MiniOne Buffer Tank
- 1 MiniOne Power Supply
- 1 Funnel
- 1 p20 Micropipette
- 1 p200 Micropipette
- 1 Microfuge Tube Rack
- 1 Resin Practice Gel
- 4 Practice Placemat
- 1 Laminated Sheet
- 1 Sharpie
- 1 Waste cup
- 1 transfer pipette
- 1 Black plate
- 1 Grey plate

ABE-Fullerton Equipment List (for Carolina and BioRad Kits)

Teacher Box
- 1 P20 Micropipette
- 1 P200 Micropipette
- 1 P1000 Micropipette
- 1 Box of p20/200 tips
- 1 Box of p1000
- 1 Large Microfuge Tube Rack
- 1 Digital Balance AWS-100
- 1 Flask for melting agarose
- 6 Tubes for holding melted agarose
- Agarose, spatula, weigh boats
- Plasmid DNA model

Box #1
- Box of 12 p20 micropipettes
- 12 p20 tip boxes
- 12 tube racks
- 12 Floating racks (foam circles)

Box #2
- 6 Electrophoresis chambers
- 6 casting trays
- 6 combs
- Masking tape

Box #3
- 3 Power supplies, cords

Box #4
- 2 Microcentrifuges
- 2 Power cords

Box #5
- uV Transilluminator system
- gel doc hood

Box #6
- Vortexer
- High Speed Microcentrifuge
- 2 Power Cords

Box #7
- Waterbath
- Waterbath Lid
- Thermometer
- Thermometer Sleeve

Box #8
- 12 Columns for chromatography
- 12 wooden bases
- 12 wooden OR metal dowels
- 6 boxes with 4 buffer bottles each
- Extra buffer bottles
- 20% Ethanol bottle
- Buffer transfer pipettes
- Binder clips/rubber bands or Orange cable clips
- Bag of tips for columns
- Box of columns (keep upright)
- Color switch ball
- Box with 3D RFP Model

Box #9
- 12 P1000 Micropipettes
- 6 boxes of P1000 tips

Box #10 Thermocycler
- Large Thermocycler
- PCR Ice Bath boxes
- Power Supply

Alt: 3 Boxes of small PCR
- 2 Embitec PCR units
- 2 tablets
- all power cords
- PCR Ice Bath boxes

Box #11
- 6 of 1X SB buffer bottles
- 1 of 20X SB buffer stock bottle

Box of Agarose gels

Boxes of Student Guides

Deionized Water Gallon Jugs

Incubator for plates

Shaking Incubator

Kit Binder
Protocol Binder

Box of Dry supplies
- Bag of p200 tips
- Bag of p1000 tips
- Bag of tubes
- Bag of transformation supplies (spreaders, biowaste bags, twist ties, masking tape)
- Box of Lab 1: Solutions 123, Red Dye, water
- Box of Dog Drool CSI dyes
- Box of Kitten Paternity dyes

Transformation Supplies
- LB agar plates
- LB/amp agar plates
- LB/amp/ara agar plates
- LB broth aliquot tubes
- Plastic bin

Styrofoam Box
- Isofreeze box with Comp cells
- Ice packs

Cooler Bag
- Reagent Aliquot boxes
- Ice packs

Box Microplate Art
- 96 well plate
- 15 mL tubes of various dyes
- instruction cards
- laminated sheets

ABE-Fullerton: One-Page Student Protocol Sheets with Checkboxes

- Teach the overview of ABE labs and molecular biology concepts.
- Have students read the ABE Student manual (pdf files) to fill out the boxes of the FLOW CHARTS as homework, ahead of time. Or have them rewrite the protocol steps in their lab book.
- When doing lab, provide the one-page Student PROTOCOLS (with checkboxes), so that students can check off as they do the steps.
- Note: If these Protocol sheets are placed in plastic protectors, they can be wiped off and reused by the next class.
    - **Expo** markers wipe off with dry paper towel.
    - **Sharpie** markers require isopropanol or wet wipe.

ABE-FC Student Protocol Sheets

- Lab 1 — Micropipette and Practice Gel Dish
- Alt Lab 1.2 — Gel Electrophoresis (with Dyes)
- Labs 2-3-4-5 — Complete Genetic Engineering Sequence
- Labs 2A-5A — Abridged Genetic Engineering Sequence
- Lab 5B — Focus on Bacteria
- Lab 6A — Overnight Culture of Bacteria
- Lab 6B — Column Chromatography from Part A
- Lab 6B –super — Column Chromatography from Supernatant
- Lab C-PCR — Colony PCR

FC Lab Extensions (sponsored by Jo Wu, not ABE)

- FC Kitten Paternity Case – Analysis by Gel Electrophoresis
    - Note that there are different dye versions, so that answers may vary from year to year.
- FC Dog Drool CSI Case – Analysis by Gel Electrophoresis
    - Note that there are different dye versions, so that answers may vary from year to year
- FC Microplate Art Design
    - Materials include 96-well microplates, colored dyes, art patterns, electronic scale.
    - Can be done for micropipetting practice or for micropipetting accuracy.
    - **Do NOT change pipet tips** when changing colors for this activity. Just tap on paper towel.
- FC ELISA Testing
- FC Laborador Coat Color Genetic Testing and Family Pedigree

Alternate Aliquoting Guide (updated June 2015 for LA site)

Note: FC will be providing these reagent aliquots for your students.

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

Size of Tube	Label tube	Contents of tube	*Aliquot	Actually used
1.5 mL	RD	Red practice solution		Varies
1.5 mL	S1	Solution 1 - Blue and purple dye		10uL
1.5 mL	S2	Solution 2 - Blue, purple, yellow dye		10uL
1.5 mL	S3	Solution 3 – Purple dye		12uL
1.5 mL	dH_2O	**Distilled water		varies

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

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

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

*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	Tube Storage	Aliquot	Actually used
1.5 mL	5xB	5x ligation buffer	Freezer	4 uL	3 uL
1.5 mL	LIG	T4 DNA ligase	Freezer	2 uL	2 uL
1.5 mL	dH_2O	Distilled water		*	2 uL

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

Size of tube	Label tube	Contents of tube	Tube Storage	Aliquot	Actually used
1.5 mL	M	1 kb DNA ladder	Freezer	8 uL	8 uL
1.5 mL	LD	Loading dye (GRLD or GGLD)	Must be Room Temp	14 uL	12 uL

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

Size of tube	Label tube	Contents of tube	Tube Storage	Aliquot	Actually used
1.5 mL	M	1 kb DNA ladder	Freezer	8 uL	8 uL
1.5 mL	LD	Loading dye (GRLD or GGLD)	Must be Room Temp	8 uL	6 uL

Alternate Aliquoting Guide (continued) (updated June 2015 for LA site)

Lab 5 *Transforming bacteria with the ligation products*

Size of tube	Label tube	Contents of tube	Tube Storage	Aliquot	Actually used
1.5 mL	LB	Luria broth	Refrigerator	350uL	300uL
1.5 mL	CC	*Competent cells	Freezer (in isofreeze box	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	Tube Storage	Aliquot	Actually used
1.5 mL	LB	Luria broth	Refrigerator	350uL	300uL
1.5 mL	RP	**pARA-R (**10** ng/uL)	Freezer	12uL	10uL
1.5 mL	CC	*Competent cells	Freezer (in isofreeze box	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	Tube Storage	Aliquot	Actually used
1.5 mL	LB	Luria broth	Refrigerator	350uL	300uL
1.5 mL	RP	pARA-R (**10** ng/uL)	Freezer	12uL	10uL
1.5 mL	CC	*Competent cells	Freezer (in isofreeze box	100uL	100uL

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

Lab 6 *Purifying the fluorescent protein*
Part A – Lysis of Bacterial Liquid Culture

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
1.5 mL	EC	LB/amp/ara culture of *E. coli*	*2 x 1 mL	2 mL
1.5 mL	LyB	Lysis buffer	160 uL	150 uL
1.5 mL	EB	Elution buffer	200 uL	150 uL

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

FULLERTON PROVIDES 6 station boxes with chromatography bottles (so 2 groups share one box of bottles, teacher does not need to aliquot for students). There is a bottle of 20% ethanol for long term storage.

Part B -- Column Chromatography

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

*You will be provided with either sets of 15 mL tubes of each buffer or one large container of each buffer. If you have the 15 mL 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.

ABE Introduction to Biotechnology Sequence

- ❖ Lab 1.1
- ❖ Lab 1.2
- ❖ Lab 1.2 Alternate
- ❖ Lab 1.3

Lab 1 Resources and Extensions

EXTENSION LABS

- Strawberry DNA Extraction
- CSI Case: Which Dog Ate My Homework?
- Paternity Case: Who is the Father of My Kittens?
- DNA Fingerprinting Cases
- Microplate Art Designs
- Pipetting by Design
- P20 and P200 Pipettor Practice
- Micropipettor Challenge

Possible Topics for Discussions:

- Biotechnology Careers
- Diabetes and Insulin Action
- DNA Profiling for CSI
- DNA Testing for Paternity
- Testing Inherited and Infectious Diseases
- Testing for Food-Poisoning
- Biotechnology Medicines
- Food Biotechnology
- Environmental Biotechnology

Videos for ABE Intro

http://players.brightcove.net/2710312605001/default_default/index.html?videoId=5184303556001

Videos for Lab 1: MICROPIPETTING

- ABE Lab 1. http://players.brightcove.net/2710312605001/default_default/index.html?videoId=5184280503001
- Intro to Micropipetting (1:29, University of Leicester, mov file). http://www2.le.ac.uk/projects/vgec/schoolscolleges/topics/recombinanttechniques/micropipette-1
- Setting the Volume of Micropipette (1:22, University of Leicester, mov file)
- Pipette Tips (0:46, University of Leicester, mov file)
- Pipetting Volumes (2:34, University of Leicester, mov file)
- Common Things to Avoid during Pipetting (2:23, University of Leicester, mov file)
- Loading a Micropipette and Common Mistakes (1:06, mp4 file)
- Working with Small Volume of Liquid (1:46, mp4 file)

Videos for Lab 1: GEL ELECTROPHORESIS

- Gel Electrophoresis (2:22, WGBH, mp4 file)
- Making an Agarose Gel (1:37, mp4 file)
- Using an Electrophoresis Box (1:05, mp4 file)
- Loading a Gel (1:44, mp4 file)
- Running a Gel (0:42, University of Leicester, wmv file)
- Use the New Mini-One Electrophoresis System. (OCBE, 5:28) https://www.youtube.com/watch?v=cscX8WaoEOg
- Setting up your MiniOne Gel Electrophoresis System (MiniOne, 5:40). https://www.youtube.com/watch?v=q3y1syrpmTw
- How to Use MiniOne Gel Cups and Casting System (The MiniOne, 5:12)
- Agarose Gel Electrophoresis (Bio-Rad Laboratories, 4:06). https://www.youtube.com/watch?v=vq759wKCCUQ
- Casting Agarose Gels – Owl system (Rahol Patharkar, 11:56). https://www.youtube.com/watch?v=zmb1dcQdJLs
- Fotodyne Gel Electrophoresis (OC Biotechnology Education, 7:14)
- Preparing an Agarose Gel. http://www.pbslearningmedia.org/resource/biot11.sci.life.gen.agarosemake/making-an-agarose-gel
- Agarose-Gel Electrophoresis. https://www.amgenbiotechexperience.com/curriculum/curriculum-resources/gel-electrophoresis-gel-analysis

Lab 1: TEACHER PREP

PLANNING (Teacher):

- ABE-FC will give you premade agarose gels (with 6 wells and 9 wells) for the Mini One system.
  - These Agarose gels are on laminated sheets in flat document plastic boxes….store flat in refrigerator. If gels are damaged/frozen, just leave in box to return, as we can remelt and recast.
  - One 6-well gel if doing only Lab 1.2 OR Alt Lab 1.2
  - Use one 9 well gel to run Lab 1 (or Alt Lab 1.2) and the Dog Drool CSI dye samples.
  - Use one 9-well gel to run Kitten Paternity dyes.
  - You can soak gels in 1X buffer overnight in different plastic box to diffuse out dyes to reuse, if desired. But students should tell you if they punctured the wells, so that you don't reuse these gels.
  - Buffer can be used for 2 gels and then discarded down the school sink

- PRACTICE GELS
  - Place resin practice gels into large black weigh boat. Fill with water, use transfer pipet to blow out bubbles inside wells.
  - Have students practice with 5 uL Red Dye multiple times.
  - Have them do it wrong to understand the rules: push to second stop and watch the sample spew out
  - Have them do it wrong: release thumb while in the buffer and check that liquid came back into tip.
  - The resin practice gels can give false sense of security, as the resin cannot be punctured. You could use food agar and water to make gels (use double comb) and have students practice loading these. Cut out the non-colored areas to remake new gels. But do NOT mix food agar with the actual agarose gels that are provided.

- If you are making your own agarose gels, be sure to make them day ahead of actual lab (need 20 minutes to solidify).
- Watch the Videos from Embitec:
- https://www.youtube.com/watch?v=9BIytMigOHc
- https://www.youtube.com/watch?v=s1gM7wkeb-8
-
  - 3 Mini One casting systems (for 6 gels) are provided in the Teacher box.
  - Decide on how many samples you want to use per gel, to decide on which comb to use for making gels.
- If you want your students to pour the 0.8% agarose gels, you may want to
  - prepare/mix the agarose and 1X SB solution in a **flask** (use 0.8 grams agarose in 100 mL 1X SB),
  - microwave to melt the agarose, pour melted agarose into 50 mL conical tubes,
  - cap tubes tightly and keep in 60°C water bath until ready to pour.
  - If agarose solidify inside tubes, remove or loosen caps, put tubes in beaker and remelt in microwave.
- Each Mini One gel requires 12.5 mL agarose solution, each casting tray holds two gels = 25 mL total. So 100 mL would make 8 gels. If you have leftover in the flask, just leave in refrigerator and return to FC to reuse. You can also add the next 100 mL buffer + 0.8 g Agarose to micromave and melt to cast more gels.

- Suggested Logistics:
- ❖ If doing Lab 1.2 only:
  - Class 1 use new buffer and left 3 wells of gel, pour buffer into marked beaker to cool,
  - Class 2 use new buffer and right 3 lanes, pour buffer into marked beaker to cool,
  - Class 3 use buffer from Class 1 and left 3 lanes on new gel
  - Class 4 use buffer from Class 2 and right 3 lanes
- ❖ If doing extensions:
- Run Lab 1.2 and Dog Drool CSI together: Use one new 9 well gel (for the 8 samples) for each group. Change buffers every 2 classes (though it is better to pour buffer into beaker to cool). Soak gel overnight and diffuse dyes so that you can reuse gels to run Kitten Paternity samples another day.

- ❖ For more pipetting practice before doing gel electrophoresis, do the Micropipet Art Designs activity.

PRE-LAB (Students):

- ❖ Read student guide, use info to Fill out the Student Flowchart (blank).

Materials Provided:

Store in Refrigerator

- FC provides premade 0.8% Agarose gels in SB
  - Keep box FLAT at all times
  - Do NOT store on the top shelf next to freezer, if using mini-fridge.

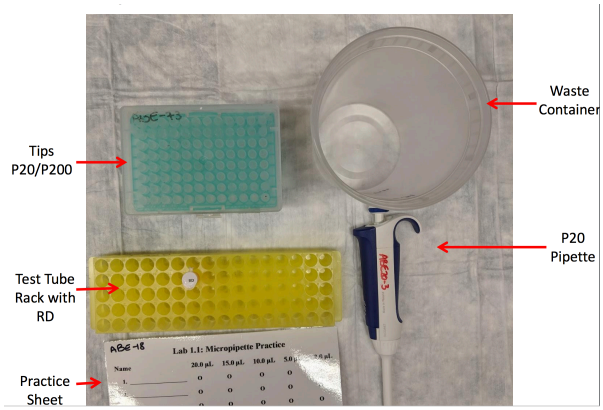
Store at Room Temperature:

- Note: Do NOT ever centrifuge any of the Class box of H_2O, Red dye, Soln 1,2,3 aliquots
- Class box of Dog CSI dye aliquots
- Class box of Kitten Paternity dye aliquots
- 1X SB Buffer bottles (premade at FC)
- 20X SB stock bottle (dilute to 1X SB as needed).
- Pipette Tip Boxes

Contents of ABE-FC Student Group Station Box:

- Micropipettes P20 and P200
- Microfuge Tube Rack
- Mini One System: Black Carriage, Buffer Tank, Gray & Black Plate, Orange Hood, Power Cord
- Casting System: Base, 2 acrylic trays, comb, lid
- Funnel
- Practice Plate (petri dish with polyurethane)
- large black weigh boat
- Transfer Pipet (plastic)
- Laminated white half-page sheet
- Laminated micropipette practice sheet
- Waste cup for pipet tips
- Sharpie permanent marker
- Dry Erase marker (NOT provided)

Lab 1 Setup for Practice Micropipette



Lab 1 Setup Using Mini One Gel System

DYES:

- Do NOT centrifuge the Class Set of Dye tubes – as they may not be the same volume or weight.
- Have students pull out their station microfuge tube rack out, so that you can pass out tubes of dyes.
- Pass out ONLY the dye tubes for the specific lab that your class will be using.
- Use Red Dye for practice pipetting on laminated practice sheet and practice plate.
- Do NOT use the CSI or Paternity dyes for practice pipetting (as these samples take much more lab technician time to prepare and quality test than the Red Practice Dye).
- **For Alt Lab 1.2:** Do have students centrifuge their total measured dyes (with 10 uL total volume), so that all of the liquid is at the bottom of the tube, before they load samples into the gel.

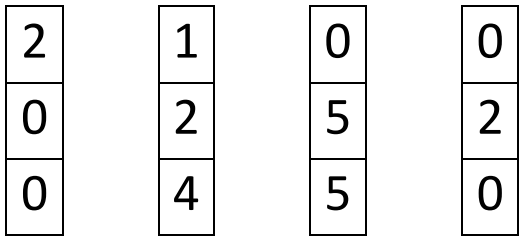
AGAROSE GELS – store FLAT in storage box in Refrigerator.

- Check that the gel buffer tank has the Gray plate in place (Black tray is used for DNA).
- Pour a small amount of 1X SB buffer into the top layer of pre-made Agarose gels in storage box.
- Gels are stored with the wells on the facing up. Use the laminated gel spatula to transfer gel onto the clear casting gel tray (or directly onto gray/white plate).
- Be sure the gel is in the correct orientation in the buffer tank, with the wells facing up and next to the negative electrode.
- After turning on power on gel boxes, have student look for bubbles forming on the negative electrode (to show electric current) and that dyes are moving toward the correct direction.
  If running the wrong way, wait until dyes are inside the agarose gel, then turn the gel 180^{o} and restart run.
- To take photos of the dyes in gel, use gel spatula to transfer gel from tank to laminated white sheet.
- To reuse gel, place in a plastic box with 1X SB Buffer overnight to diffuse the dyes. Gels without dyes can be reused directly (if gels are not damaged), or melted and recast into trays (or returned to distribution center to be recast).
- Gels with DNA bands cannot be reused, and can be discarded in regular trash.
- Keep all unused gels flat in box in the refrigerator. Return any unused gels (damaged or good) to Fullerton.

Protocol Sheet

Lab 1.1: How to Use a Micropipette

1. Unlock pipette to change volumes. Practice setting the p20 micropipette to the following volumes:
   - ☐ *20.0 µL, 12.4 µL, 5.5 µL and 2.0 µL.*



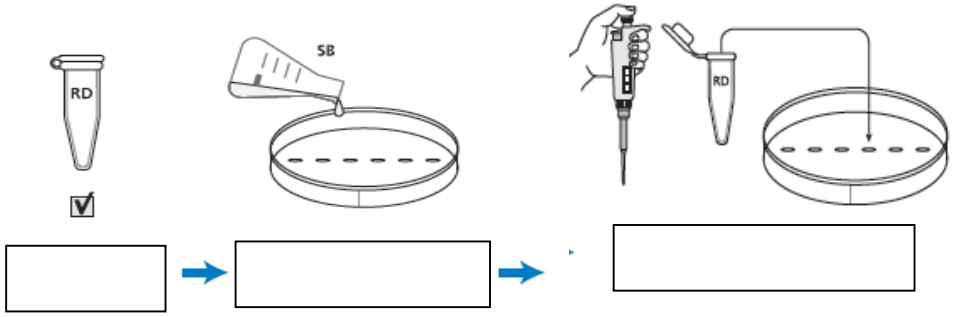
   - ☐ Using the p20 micropipette and tip, each student should pipet the following volumes of water or red dye solution onto the laminated sheet.
   - ☐ Record the size of the droplets.
   - ☐ Rinse the laminated sheet with DI water and dry.

Lab 1.2A: Practice Loading Dye into Practice Gel Dish

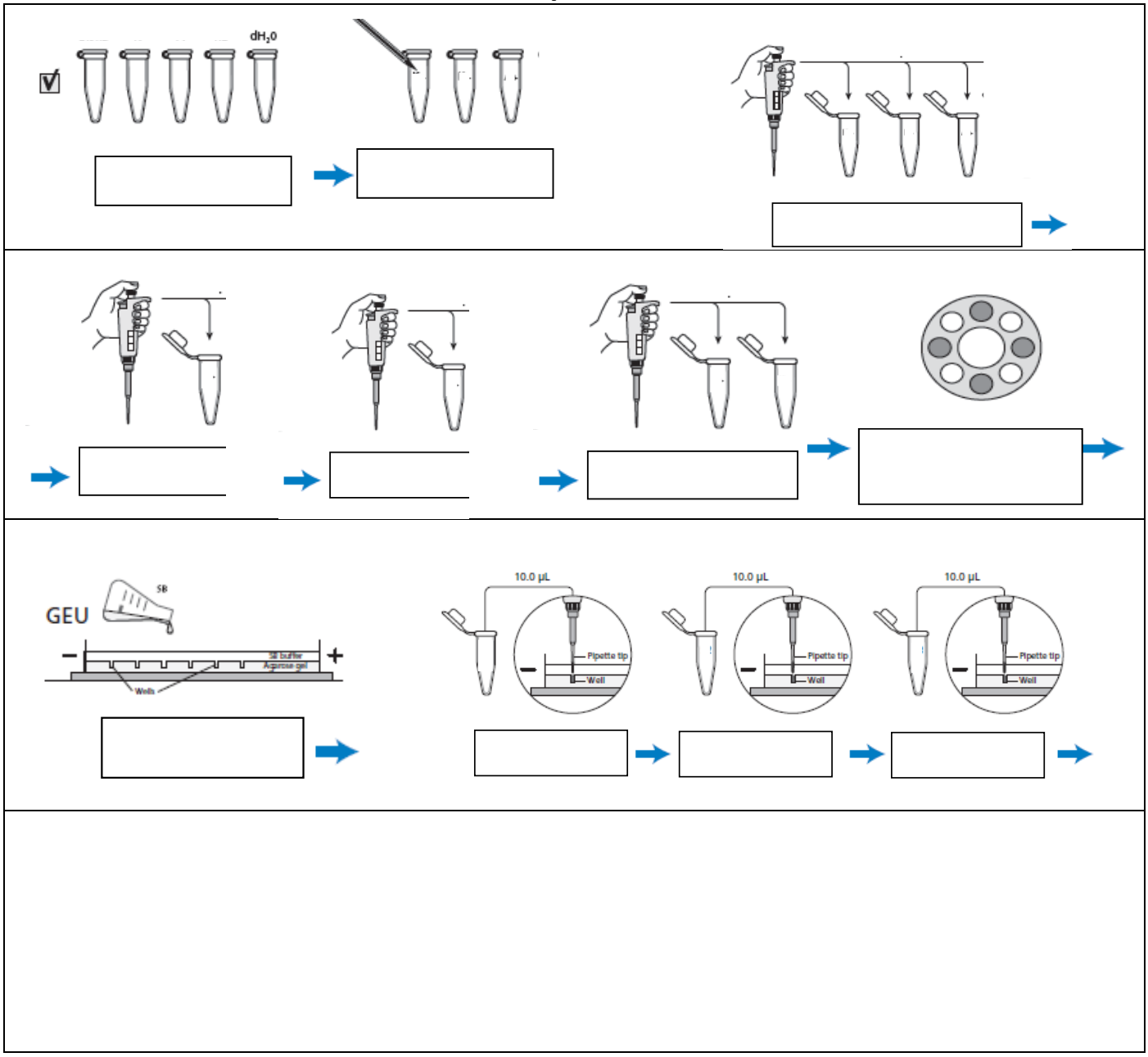
Each student should practice loading the wells of a urethane practice gel.

- ☐ Place a practice gel into a black weigh boat.
- ☐ Fill the practice gel with enough water to cover the wells completely.
- ☐ If there are any air bubbles in the wells, push down with thumb or use a transfer pipet to squirt water liquid to displace bubbles.
- ☐ Use the p20 micropipette, load *5 or 10 µL* of red dye solution into each well.
  - Pipette tip should be below water level, but right above the well.
  - Only push to the FIRST stop of pipette to dispense sample into the well.
- ☐ Do not change tip, practice loading into multiple wells.
- ☐ Rinse the gels with DI water and air dry.

Lab 1.2 Part A: Practice Loading Red Dye into Practice Gel Dish

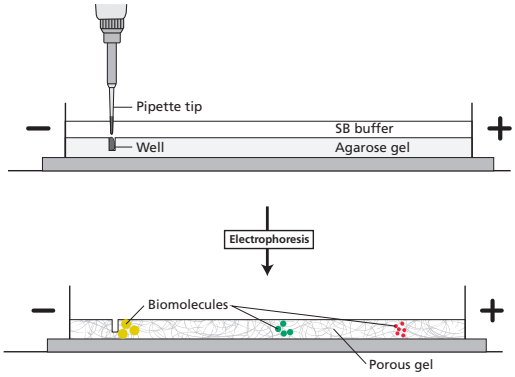


Alternate Lab 1.2 Part B Gel Electrophoresis



Alternate Lab 1.2 Part B Gel Electrophoresis Student Protocol (p1)

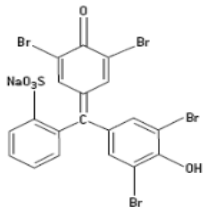
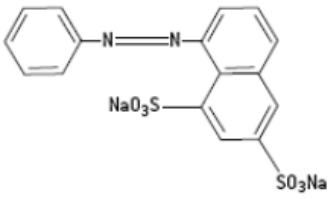
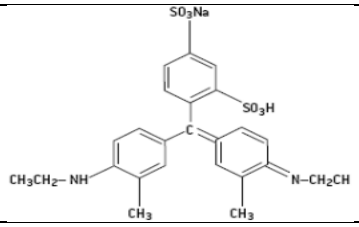
Introduction. The purpose of this laboratory is to give you experience with gel electrophoresis, which is used to separate and identify a mixture of biomolecules including DNA; the components of each mixture can then be identified by their location in the gel. Gel electrophoresis works based on the fact that biomolecules have a negative charge, which means that they will move in response to an electric charge. The biomolecules move through a gel, and their speed varies primarily according to their weight, although molecular shape and degree of charge also influence their movement. In the genetic engineering process, gel electrophoresis is used to separate and identify plasmids and short linear pieces of DNA.



The gel that the biomolecules move through is composed of *agarose*, a polysaccharide (complex sugar) found in seaweed. Its structure is a porous matrix (like a sponge) with lots of holes through which the solution and biomolecules flow. The electrophoresis setup consists of a box containing an agarose gel and two electrodes that create an electric field across the gel when the box is attached to a power supply. The negative electrode is black, and the positive electrode is red. Samples of biomolecules are pipetted into wells near the negative (black) electrode. The samples move through the gel toward the positive (red) electrode.

The agarose is mixed with an electrolytic solution called Sodium Borate (SB). This solution contains ions, which are electrically charged atoms. These ions help conduct the electrical current through the gel. As the molecules are drawn toward the positive electrode, the smaller molecules are able to move in and around this agarose network much more quickly than the larger molecules.

The dyes that you will separate by gel electrophoresis are shown in the table below.

Dye	Bromophenol Blue	Orange G	Xylene Cyanol
Color Band	purple	yellow	blue
Molecular Weight	670	452	539
Shape			
Charge			
Relative Migration			
Found in Tube #			

Analysis. Look at the colored bands in your gel and decide which dye(s) were found in Tubes A B C.

What electrical charge did the three dyes carry? _____ How do you know? _____

_____

Compare the molecular weight of the dyes to the migration pattern.

Did the dyes move according to their sizes? _____

What other factors may have affected the migration rate? _____

Alternate Lab 1.2 Part B Gel Electrophoresis Student Protocol

☐ 1. Use a permanent marker to label three microfuge tubes A, B and C.

☐ **2.** Use a new tip on the P-20 micropipette to dispense 2 µL dH_2O into tubes A, B and C.

☐ **3.** Change volume to 4 µL. Dispense 4 µL of Solution 1 into tube A.

☐ **4.** Eject the tip into the plastic waste container and replace with a fresh tip.

☐ **5.** Dispense 4 µL of Solution 3 into tube A and change the tip.

☐ **6.** Dispense 8 µL of Solution 2 into tube B and change the tip.

☐ **7.** Dispense 8 µL of Solution 3 into tube C.

Tube	dH_2O	Solution 1	Solution 2	Solution 3	Total volume
A	2 µL	4 µL	–	4 µL	10 µL
B	2 µL	–	8 µL	–	10 µL
C	2 µL	–	–	8 µL	10 µL

☐ 8. This table summarizes the contents of each tube.

☐ 9. To ensure that all of the liquid falls to the bottom of the tubes, you can close the tubes tightly and tap bottoms onto bench several times.
OR centrifuge for ten seconds, making sure that the tubes are arranged in a balanced orientation (either 2 tubes directly across OR 3 tubes in equilateral triangle).


Centrifuge

☐ 10. Obtain and set up an 0.8% agarose gel made with 1X SB Buffer onto a gray tray or white tray in the buffer tank of a MiniOne Gel Electrophoresis system.

☐ 11. Using a funnel, pour 1X SB Buffer until the gel is completely immersed.

☐ 12. Carefully place the buffer tank straight into the black cartridge, and check that the electrodes touch. Do not force this – call for help if needed.

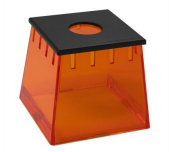
☐ 13. Plug in the power cord, and turn on light (light bulb icon) as desired. Place the gel box where you are comfortable to load samples, as you cannot move the gel box after the first sample goes in. If you share the gel, decide on the loading order of the gel lanes.

☐ 14. Pipet 10 uL from tube A into one well of the gel (remember to only go to the **FIRST** stop).

☐ 15. Change the tip and load 10 uL of tube B into a new well.

☐ 16. Change the tip and load 10 uL of tube C into a new well.

☐ 17. Place the orange hood over gel box and push the power (should see green light indicator on) to start the electrophoresis. Watch for bubbles on negative electrode and that dye is moving towards the positive electrode.



☐ Note: if the hood is moved, the power will turn off automatically. Check that the green light indicator stays on during the gel run.

☐ 18. Run gel until the colored bands separate. Turn off before any bands run off the gel.

☐ 19. Place cell phone camera on top of the hood to take a photo (without using flash).
OR Draw, label and color the band pattern in your lab notebook.

Fullerton College Lab Extension (Can run on same gel as Lab 1)

Name: _____

CSI Case: WHICH DOG ATE MY HOMEWORK?

SCENARIO. Jane had been diligently studying and doing her biology homework all day, before going to a party Saturday night. When she came back home, her homework was half chewed up. There was still a big puddle of drool on her desk. Jane knew it had to have been one of the dogs that did this dirty deed -- but which one? Her family has 2 dogs (suspects A and B). After questioning her brother, she also found out that the neighbors' two dogs (suspects C and D) had come over that night. So she took cheek swabs from her dogs and the neighbors' dogs to do a DNA fingerprinting analysis. Assume that the DNA samples from each suspect has already been isolated, amplified, and cut with restriction enzymes. You will be separating and analyzing the DNA fragments by agarose gel electrophoresis.

MATERIALS: P-20 micropipette, pipet tip box, waste cup, microfuge tube rack, tubes with CSI samples ABCDE, gel electrophoresis system, white laminated sheet

METHODS. Your teacher may have you run other samples in the same gel, listen to class instructions.

1. Your team will load 5 ul of each DNA sample into the 0.8% agarose gel.
2. Record how the DNA samples are loaded into the gel wells.
3. Run the gel for 10 - 20 minutes with Mini One gel system, and turn off before any dyes run off the gel. If using another brand for electrophoresis, run at 135 Volts for 15 - 30 minutes or until the colored samples are separated into distinct bands.
4. Remove the gel from the electrophoresis box and place on the white laminated paper. Mark your team name and the sample letters on the sheet for your data photo.

ELECTROPHORESIS of DNA SAMPLES - GEL LOADING ORDER

Lane	1	2	3	4	5	6	7	8	9
DNA Sample									

DATA. For each suspect and evidence, count the number of "bands" and list the different colors of the bands. Compare and record (draw) the location of the color bands of each lane in the gel, or attach photo.

DNA Sample	Dog Suspect A	Dog Suspect B	Dog Suspect C	Dog Suspect D	Homework Evidence
Draw Pattern of Colored Bands					

ANALYSIS. Explain which dog you suspect as the guilty dog. Justify your claim using evidence.

_____

_____

_____

_____

_____

Crime Scene Lab, Dr. Jo Wu and Matthew Emrick, Fullerton College, updated March 2016

PATERNITY CASE: WHO IS THE FATHER OF MY KITTENS?

Scenario:

Mary's white cat "Honey" was lost for 2 days about three months ago. She now has 4 kittens (see photo 1). Mary wants to know if the two neighboring cats ("Tom" in photo 2 and "Butch" in photo 3) could be the father. To analyze their DNA fingerprints, Mary has collected hair follicles from each adult cat and kitten, extracted DNA, amplified DNA using polymerase chain reaction.

You will be running the agarose gel electrophoresis on these "DNA" samples to determine the genetic father of each kitten.

Photo 1. Honey and kittens Cream, Molasses, Ginger, Sugar

Photo 2. Tom (male)

Photo 3. Butch (male)

HYPOTHESIS:

Looking at the photos, who might be the father of each kitten?

KITTEN	POTENTIAL DAD	EXPLAIN REASONING
Cream		
Molasses		
Ginger		
Sugar		

PROCEDURE:

NOTE: The following is completed when the electrophoresis chamber has been prepared with an 0.8% agarose gel and 1x SB buffer in the chamber.

1. Obtain the "DNA samples" - there are seven microfuge tubes labeled P- V.
2. Using a P-20 micropipettor and a pipet tip, measure 5 µL from Tube P and transfer into the first well of the agarose gel.
3. Using a new tip for each sample, transfer 5 µL of each sample into new wells of the gel.
4. Be sure to keep track of your sample loading, if you do not follow the table below. If there were any problems with the loading (punctured gel, not enough sample), be sure to write in the NOTE column.

PATERNITY CASE: WHO IS THE FATHER OF MY KITTENS?

GEL LOADING ORDER

Well	Tube	DNA Sample 5 uL	NOTES (problems, loss of samples, etc)
1	P	Tom (male)	
2	Q	Cream (kitten)	
3	R	Molasses (kitten)	
4	S	Honey (female)	
5	T	Ginger (kitten)	
6	U	Sugar (kitten)	
7	V	Butch (male)	

1. Run the gel for 15 minutes total if using a MiniOne electrophoresis system.
   If using another brand for electrophoresis, run at 135V for 30 minutes or until the colored bands are separated.
2. For best viewing the results, remove the gel from the electrophoresis box and place on the white laminated paper.
3. Take a photo with your cell phone every 5 minutes to preserve your results. This is to prevent loss of data if the gel should run too long.

ANALYSIS:

Use color pencils to record the band patterns (color the appropriate blocks) in the Data Table below.
Data Table. Colored "DNA" Bands Separated by Agarose Gel Electrophoresis

Tube	P	Q	R	S	T	U	V
Color Band	Tom Male	Cream	Molasses	Honey Female	Ginger	Sugar	Butch Male

1. Carefully consider each band of all four kitten samples and determine whether the band matches Tom, Honey or Butch. For the Kitten samples (columns QRTU) in the Data Table, write (on top of the colored blocks) who each band matches Tom, Honey, Butch.

2. Draw your conclusions based on the DNA evidence. Who was the father for each kitten based on looking at the kitten? Who is the father based on the DNA evidence? What is the specific evidence that justifies your claim for determining each kitten's father? Fill your responses to these questions in the table below.

KITTEN	FATHER based on Visual	FATHER based on DNA	Evidence
Cream			
Molasses			
Ginger			
Sugar			

Fullerton College Extension: Microplate Art Designs

(Jo Wu created these picture and alphabet designs, but the activity is adapted from the "Pipetting by Design" labs from University of Florida and University of Rhode Island)

Objectives: • Improve micropipetting skills and following directions carefully • Check for micropipetting accuracy • Allow students to be creative **To Teachers:** The directions about the proper use of micropipettes have not been included here. It is expected that all students would be taught the proper method to use micropipettes **PRIOR to this activity.** **Lab Prep:** Mix food coloring with water to get desired colors.	**Materials per student (or pair):** • Bottles of Colored solutions and water • 96 well plate (Test which minimum volume works best). • P20 or P200 Micropipette • Pipet tip box • Waste cup for tips **Cleanup:** • Fill tub of water. Submerge microplate into water completely and bring up. • Turn plate over and shake hard over a clear area in the sink. • Repeat 3 more times until there is no more color in the wells. • Air dry before storing and packing microplates.

As different microplates hold different volumes, you will need to test which minimum volume works best for your plates and tell your students (the volume is not written on the student design template cards). I suggest using 20 uL volumes with small volume microplates, so that students can use either p20 or p200 micropipettes. If working in pairs, have one student read aloud and check off the wells, while the other student dispenses liquid. Have students do all of one color at a time, to avoid wasting too many pipet tips. If you have large volume microplates, then students may need to dispense a minimum of 50 uL volumes per well.

Different ways (and difficulty levels) to use this lab activity:

- Level 1. Give each student one Picture Design template card, one microplate, and appropriate colors. Students should add the same volume into the indicated wells. There will be no mixing of colors in the wells. Provide the clue to the picture design as needed.

- Level 2. Have each student pipet their two initials on a microplate, using the Alphabet Design templates. They will need to follow the appropriate templates for the left side (first letter) and right side (second letter) of the microplate. Students may want to color a paper microplate diagram to understand the pattern before pipetting.

- Level 3. Have students create their own art design, using the microplate diagrams with color pencils. If you want students to mix colors, use the suggested proportions below (created for 100 uL well volume).

- Level 4. Check for pipetting accuracy.
- Note: Ensre that your pipettes have been validated.
  a. Measure Weight of empty microplate on electronic or analytical scale.
  b. Determine the total volume dispensed into the microplate. I have calculated the # wells for each FC design on the answer key below. But you will need to multiply by the volume students are dispensing to calculate total volume. For example, if students are dispensing 20 uL dye per well and there are 25 wells in their design, then their plate should contain 500 uL total.
  c. Determine the expected weight of the dispensed dye in plate. Assume that each mL of solution = 1 gram. So 500 uL dye would have 500 mg or 0.5 gram weight.

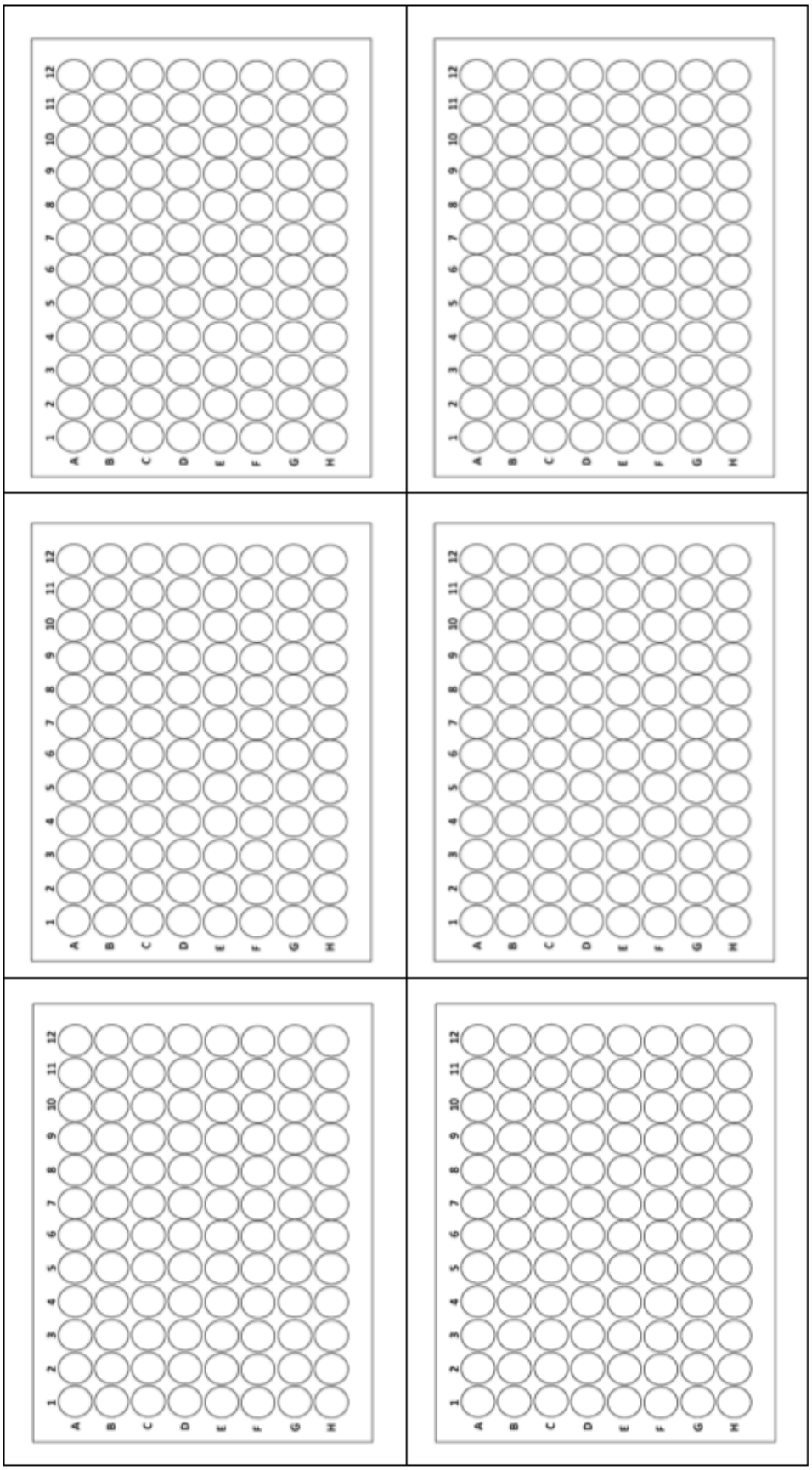
  d. Determine the percent error.

$$\text{percent error} = \frac{|\text{experimental value} - \text{accepted value}|}{\text{accepted value}} \times 100\%$$

Fullerton College Alphabet Letter Designs (pipet 2 initials onto one plate)

A (left)	A3, B2, B4, C1, C5, D1, D2, D3, D4, D5, E1, E5, F1, F5, G1, G5	**A** (right)	A10, B9, B11, C8, C12, D8, D9, D10, D11, D12, E8, E12, F8, F12, G8, G12
B (left)	A1, A2, A3, B1, B4, C1, C4, D1, D2, D3, E1, E4, F1, F4, G1, G2, G3	**B** (right)	A8, A9, A10, B8, B11, C8, C11, D8, D9, D10, E8, E11, F8, F11, G8, G9, G10
C (left)	A2, A3, A4, B1, B5, C1, D1, E1, F1, F5, G2, G3, G4	**C** (right)	A9, A10, A11, B8, B12, C8, D8, E8, F8, F12, G9, G10, G11
D (left)	A1, A2, A3, B1, B4, C1, C5, D1, D5, E1, E5, F1, F5, G1, G2, G3, G4	**D** (right)	A8, A9, A10, B8, B11, C8, C12, D8, D12, E8, E12, F8, F12, G8, G9, G10, G11
E (left)	A1, A2, A3, A4, B1, C1, D1, D2, D3, D4, E1, F1, G1, G2, G3, G4	**E** (right)	A8, A9, A10, A11, B8, C8, D8, D9, D10, D11, E8, F8, G8, G9, G10, G11
F (left)	A1, A2, A3, A4, B1, C1, D1, D2, D3, E1, F1, G1	**F** (right)	A8, A9, A10, A11, B8, C8, D8, D9, D10, E8, F8, G8
G (left)	A2, A3, A4, B1, B5, C1, D1, D2, D3, E1, E4, E5, F1, F5, G2, G3, G4	**G** (right)	A9, A10, A11, B8, B12, C8, D8, E8, E11, E12, F8, F12, G9, G10, G11
H (left)	A1, A5, B1, B5, C1, C5, D1, D2, D3, D4, D5, E1, E5, F1, F5, G1, G5	**H** right)	A8, A12, B8, B12, C8, C12, D8, D9, D10, D11, D12, E8, E12, F8, F12, G8, G12
I (left)	A1, A2, A3, A4, A5, B3, C3, D3, E3, F3, G1, G2, G3, G4, G5	**I** (right)	A8, A9, A10, A11, A12, B10, C10, D10, E10, F10, G8, G9, G10, G11, G12
J (left)	A1, A2, A3, A4, A5, B4, C4, D4, E1, E4, F1, F4, G2, G3	**J** (right)	A8, A9, A10, A11, A12, B11, C11, D11, E8, E11, F8, F11, G9, G10
K (left)	A1, A4, B1, B3, C1, C2, D1, E1, E2, F1, F3, G1, G4	**K** (right)	A8, A11, B8, B10, C8, C9, D8, E8, E9, F8, F10, G8, G11
L (left)	A1, B1, C1, D1, E1, F1, G1, G2, G3, G4, G5	**L** (right)	A8, B8, C8, D8, E8, F8, G8, G9, G10, G11, G12
M (left)	A1, A5, B1, B2, B4, B5, C1, C3, C5, D1, D5, E1, E5, F1, F5, G1, G5	**M** (right)	A8, A12, B8, B9, B11, B12, C8, C10, C12, D8, D12, E8, E12, F8, F12, G8, G12
N (left)	A1, A5, B1, B2, B5, C1, C3, C5, D1, D4, D5, E1, E5, F1, F5	**N** (right)	A8, A12, B8, B9, B12, C8, C10, C12, D8, D11, D12, E8, E12, F8, F12
O (left)	A2, A3, A4, B1, B5, C1, C5, D1, D5, E1, E5, F1, F5, G2, G3, G4	**O** (right)	A9, A10, A11, B8, B12, C8, C12, D8, D12, E8, E12, F8, F12, G9, G10, G11
P (left)	A1, A2, A3, A4, B1, B5, C1, C5, D1, D2, D3, E1, F1, G1	**P** (right)	A8, A9, A10, A11, B8, B12, C8, C12, D8, D9, D10, D11, E8, F8, G8
Q (left)	A2, A3, A4, B1, B5, C1, C5, D1, D5, E1, E4, E5, F1, F5, G2, G3, G4, G6	**Q** (right)	A9, A10, A11, B8, B12, C8, C12, D8, D12, E8, E11, E12, F8, F12, G9, G10, G11
R (left)	A1, A2, A3, A4, B1, B5, C1, C5, D1, D2, D3, E1, E2, F1, F3, G1, G4	**R** (right)	A8, A9, A10, A11, B8, B12, C8, C12, D8, D9, D10, D11, E8, E9, F8, F10, G8, G11
S (left)	A2, A3, A4, B1, B5, C1, C5, D2, D3, E4, F1, F5, G1, G5, H2, H3, H4	**S** (right)	A9, A10, A11, B8, B12, C8, C12, D9, D10, D11, E11, F8, F12, G8, G12, H9, H10, H11
T (left)	A1, A2, A3, A4, A5, B3, C3, D3, E3, F3, G3,	**T** (right)	A8, A9, A10, A11, A12, B10, C10, D10, E10, F10, G10
U (left)	A1, A5, B1, B5, C1, C5, D1, D5, E1, E5, F1, F5, G2, G3, G4	**U** (right)	A8, A12, B8, B12, C8, C12, D8, D12, E8, E12, F8, F12, G9, G10, G11
V (left)	A1, A5, B1, B5, C1, C5, D1, D5, E1, E5, F2, F4, G3	**V** (right)	A8, A12, B8, B12, C8, C12, D8, D12, E8, E12, F9, F11, G10
W (left)	A1, A5, B1, B5, C1, C5, D1, D5, E1, E5, F1, F3, F5, G1, G2, G4, G5	**W** (right)	A8, A12, B8, B12, C8, C12, D8, D12, E8, E12, F8, F10, F12, G8, G9, G11
X (left)	A1, A5, B1, B5, C2, C4, D3, E2, E4, F1, F5, G1, G5	**X** (right)	A8, A12, B8, B12, C9, C11, D10, E9, E11, F8, F12, G8, G12
Y (left)	A1, A5, B1, B5, C2, C4, D3, E3, F3, G3	**Y** (right)	A8, A12, B8, B12, C9, C11, D10, E10, F10, G10
Z (left)	A1, A2, A3, A4, A5, B5, C4, D3, E2, F1, G1, G2, G3, G4, G5	**Z** (right)	A8, A9, A10, A11, A12, B12, C11, D10, E9, F8, G8, G9, G10, G11, G12

Fullerton College Lab Extension: Microplate Art



TEACHER PREP for Lab 2A

PLANNING (Teacher):

- Water Bath or incubator – Turn 5-24 hours ahead at 37C. Check actual temperature.
- Digital heat block – turn 30 minutes ahead.
    - Squirt in water to get more even heating, if tubes are not perfect fit in wells.
- Thaw out frozen reagents, spin in centrifuge 10 seconds before dispensing to students.
- Teach how to use centrifuge
- Pacing: See Teacher guide and other teacher suggestions.
- Teach students to look at their pipet tip to see liquid after pick up and check that it is empty after dispensing liquid. Spin down.
- Digest should be stopped between 1-2 hours at 37C. Freeze tubes.
- Organize tubes by class in freezer box.

SUGGESTED videos:

- Genetic Engineering and Working with DNA (1:37, WGBH Digizyme)
    - http://www.pbslearningmedia.org/resource/biot11.sci.life.gen.genengdna/genetic-engineering-and-working-with-dna/
- Mechanism of Recombination (1:12, DNALC.org, YouTube)
    - https://www.youtube.com/watch?v=8rXizmLjegI
- Steps in Cloning a Gene (1:42, McGraw Hill)
    - http://highered.mheducation.com/sites/0072556781/student_view0/chapter14/animation_quiz_1.html
    - YouTube copy (lower quality). https://www.youtube.com/watch?v=KQ2exY26AJ
- Plasmid Cloning (WH Freeman)
    - http://www.sumanasinc.com/webcontent/animations/content/plasmidcloning.html

Materials:

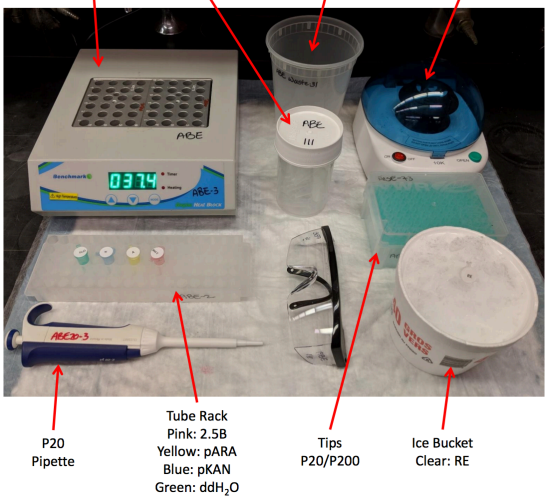
Equipment:

- Centrifuge(s)
- Water bath or Heat block
- Thermometer
- Floating microfuge tube rack

Student Station:

- P20 Micropipette
- Pipette Tip Box
- Microfuge Tube Rack
- 2 New tubes
- Tube of Distilled water (from Soln 123 dye box)
- Waste cup for pipet tips
- Sharpie permanent marker, Dry Erase marker
- Cup or container for crushed ice

Set Up:



Stored in Freezer

- Cardboard box with aliquots of:
    - pARA-R plasmid (RP) = blue tubes
    - 2.5X restriction buffer (2.5xB) = clear tubes with white labels
    - restriction enzymes BamHI and HindIII (RE) = orange tubes
- Crushed Ice

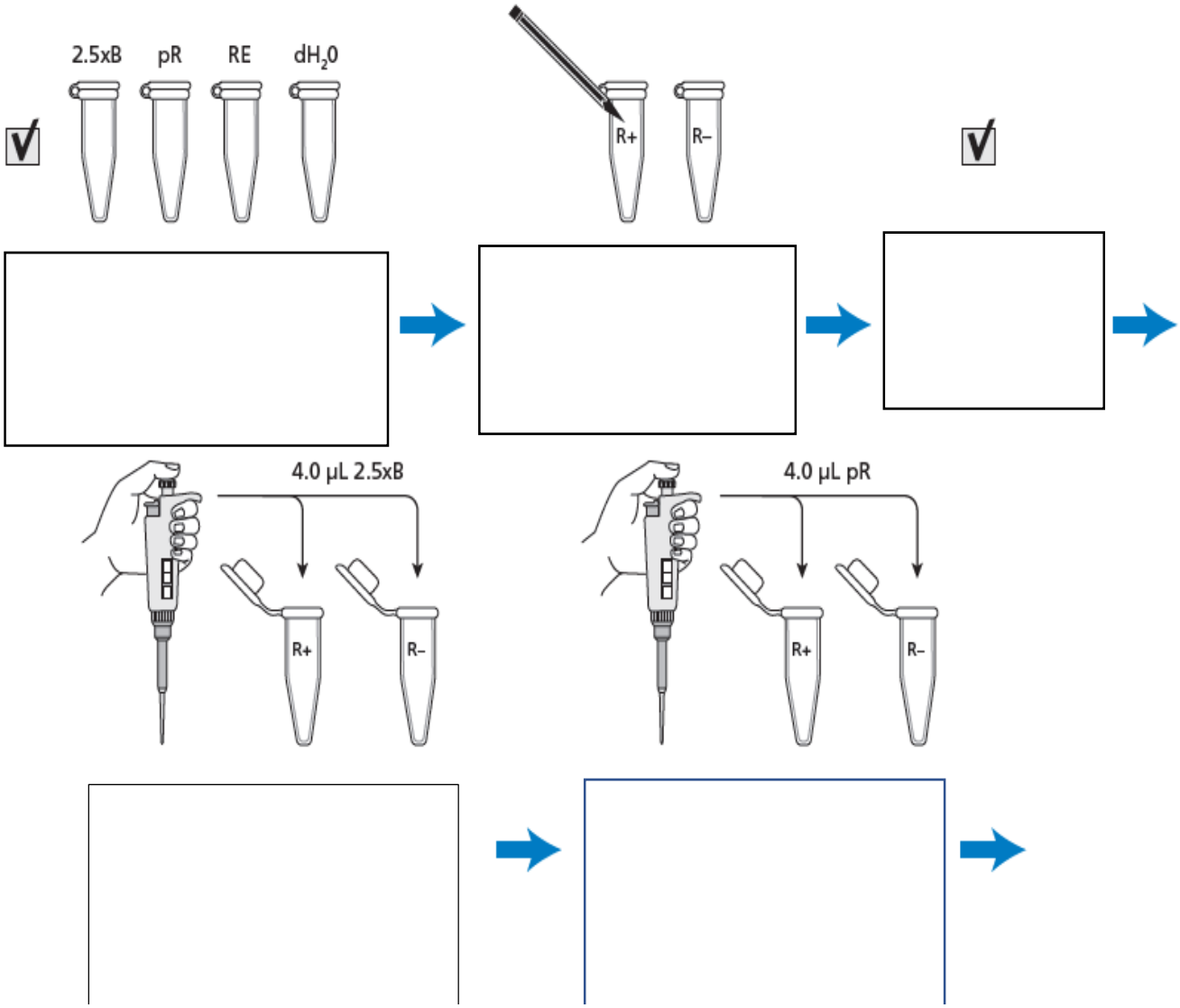
Name: _____ **Date:** _____

LABORATORY 2A: PREPARING TO VERIFY THE *rfp* GENE: Digesting the pARA-R plasmid Reference: ABE Student Guide pages C-15 to C-19

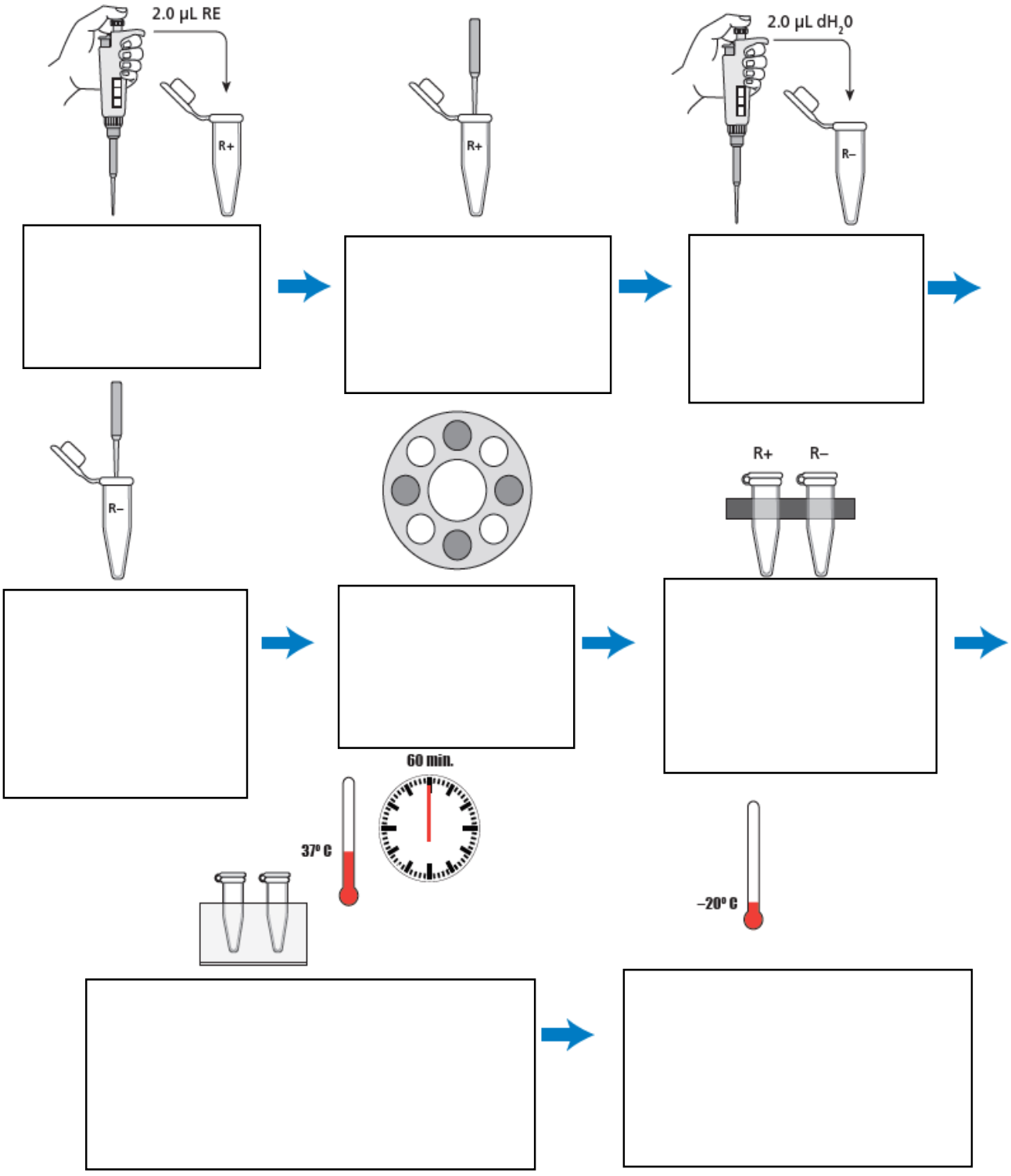
In your own words, describe the **Purpose** of Lab 2A**:**

Methods: Finish the flowchart for **Lab 2A** by describing the step for each of the diagrams below:

Laboratory 2A Flowchart



Lab 2A Flowchart (continued)



Lab 2A: Digesting the pARA-R Plasmid (Protocol Sheet)

Materials at Student Station:		
☐ P20 Micropipette ☐ Pipette Tip Box ☐ Microfuge Tube Rack ☐ 2 New tubes	☐ Waste cup for pipet tips ☐ Sharpie permanent marker ☐ Dry Erase marker - optional ☐ Paper towel - optional	☐ Container with crushed ice with: ☐ **RP** = pARA-R plasmid = blue tube ☐ **2.5xB** = 2.5X restrictibuffer = clear tube ☐ **RE** = BamHI and HindIII = orange tube ☐ **dH_2O** = distilled water = clear tube

☐ 1. Check that you have all materials. Ask if reagent tubes have been centrifuged.

☐ 2. Use a Sharpie to label two clean tubes the top and the side: **R+** and **R–**.
Include your group number and class period each tube.

☐ 3. Use new tips for different reagents. Add reagents to the solution at bottom of tube.
Check that your pipet tip is empty after dispensing.

☐ Add 4.0 µL of 2.5xB to the R+ and R– tubes. Change tip.

☐ Add 4.0 µL of RP to the R+ and R– tubes. Change tip.

☐ Add 2.0 µL of dH_2O to the bottom of the R– tube. Gently pump the solution in and out with the pipette to mix. Cap the tube when done.

☐ Add 2.0 µL of RE to the bottom of R+ the tube. Gently pump to mix. Cap tube.

☐ 5. Place two tubes (of equal volume) directly across from each other in the microcentrifuge.

☐ Spin for four seconds to pool the reagents at the bottom of each tube.

	R+ tube	R– tube
Step 4a: Restriction buffer (2.5xB)	4.0 µL	4.0 µL
Step 4b: pARA-R plasmid (RP)	4.0 µL	4.0 µL
Step 4c: Restriction enzymes (RE)	2.0 µL	
Step 4d: Distilled water (dH_2O)		2.0 µL

☐ 6. Place tubes into a floating rack in the 37°C water bath for one hour (but no more than two hours.)

☐ 7. After the incubation is complete, your teacher can freeze the tubes at -20°C. You will analyze the contents in Lab 4A by gel electrophoresis.

STOP AND THINK: You set up a tube without the restriction enzymes, BamHI and HindIII. What is the purpose of this step, and why is it important?

Why might the enzymes work best at 37°C? (Hint: The human body temperature is 37°C.)

Why should the enzymes then be placed in the freezer?

TEACHER PREP for Lab 4A

PLANNING (Teacher):

- It is better to use the 6 well gels, have two groups share a gel and run only one DNA Marker lane per gel.
- ABE-FC will give you premade agarose gels for the Mini One system.
    - You can share one gel between two groups. OR have one class run on left side wells, then the next class run on right side wells.
    - These Agarose gels are on laminated sheets in flat document plastic boxes….store flat at 4C.
    - If gels are damaged, just leave in box to return, as we can remelt and recast.

- If you are making your own agarose gels, be sure to make them ahead of actual lab.
    - 3 Mini One casting systems (for 6 gels) are provided in the Teacher box.
- If you want your students to pour the gels, you may want to
    - prepare/mix the agarose and 1X SB solution in a **flask** (use 0.8 grams agarose in 100 mL 1X SB),
    - microwave to melt the agarose, pour melted agarose into 50 mL conical tubes,
    - cap tubes tightly and keep in 60^{o}C water bath until ready to pour.
    - If agarose solidify inside tubes, remove or loosen caps, put tubes in beaker and remelt in microwave.
- Each Mini One gel requires 12.5 mL agarose solution, each casting tray holds two gels = 25 mL total.

PRE-LAB (Students):

- ❖ Read student guide pages, use info to Fill out the Student Flowchart (blank).
- ❖ Watch Video about using Mini One Gel Electrophoresis system

Materials Provided:

Equipment:

- Centrifuge
- Transilluminator
    - Blue LED for Gel Green dye
    - uV for Gel Red dye

Store in Freezer:

- Student R+ and R- tubes from Lab 2A
- DNA Ladder (M)

Store in Refrigerator

- premade 0.8% Agarose gels in SB – store flat

Store at Room Temperature:

- Loading dye (LD) aliquots (olive green plastic box)
- 1X SB Buffer bottles (premade at FC)
- 20X SB stock bottle (dilute to 1X SB as needed).

ABE-FC Student Group Station:

- Micropipettes P20 and P200
- Microfuge Tube Rack
- Mini One System: Black Carriage, Buffer Tank, Gray & Black Plate, Orange Hood, Power Cord
- Funnel
- Laminated white half-page sheet
- Laminated micropipette practice sheet
- Waste cup for pipet tips
- Sharpie permanent marker
- Dry Erase marker (NOT provided)
-

To practice loading gels:

- Practice Plate (petri dish with polyurethane)
- large black weigh boat
- Transfer Pipet (plastic)

Lab 4A : Verification of the Recombinant Plasmid Using Gel Electrophoresis - Student Protocol using Mini One Gel System

- ☐ 1. Add 2 uL LD (loading dye) each to the **R-** tube, **R+** tube, and **Marker** tube.
- ☐ 2. Place two tubes (of equal volume) directly across from each other in the microcentrifuge. OR place 3 tubes in equilateral configuration (see diagram).
- ☐ 3. Spin for 5-10 seconds to pool the reagents at the bottom of each tube.
- ☐ 4. Obtain and set up an 0.8% agarose gel onto a clear acrylic tray.
- ☐ 5. Set up the MiniOne Gel Electrophoresis system.
- ☐ Remove the gray (or white) plate from the buffer tank.
- ☐ 6. Put in the black plate instead, in the correct orientation.
- ☐ 7. Place the tray with gel into the buffer tank, with wells near negative electrode.
- ☐ 8. Using a funnel, pour 1X SB Buffer into the buffer tank until the gel is completely immersed (cannot see any gel dimples), but not above the line on buffer tank.

- ☐ 9. Carefully place the buffer tank ***straight down*** into the black cartridge, and check that the electrodes touch. Do not force this – call for help if needed.
  - ☐ If you spill buffer into the black cartridge, immediately remove buffer tank and wipe dry. The black cartridege is the power supply!
- ☐ 10. Plug in the power cord.
- ☐ 11. Place the gel box where you are comfortable to load samples, as you cannot move the gel box after the first sample goes in.
- ☐ 12. If you share the gel, decide on the loading order of the gel lanes.
- ☐ 13. Turn on light (light bulb icon) as desired, but do not leave on for too long.
- ☐ 14. Pipet 10 uL from tube **R-** into one well of the gel (remember to only go to the **FIRST** stop).
- ☐ 15. Change the tip and load 10 uL of tube **R+** into a new well.
- ☐ 16. Change the tip and load 10 uL of tube **M** into a new well.
- ☐ 17. Have other team load samples. Only NEED one Marker lane per gel.
- ☐ Place the orange hood over gel box and push the power (should see green light indicator on) to start the electrophoresis. Watch for bubbles on negative electrode and that dye is moving towards the positive electrode.

- ☐ If the hood is moved, the power will turn off. Check that the green light stays on during the gel run.
- ☐ 18. Run the gel until the colored bands separate. Turn off before any bands run off the gel.
- ☐ 19. Place cell phone camera on top of the hood to take a photo (without using flash). OR Draw, label and color the band pattern in your lab notebook.

Name: _____ **Date:** _____

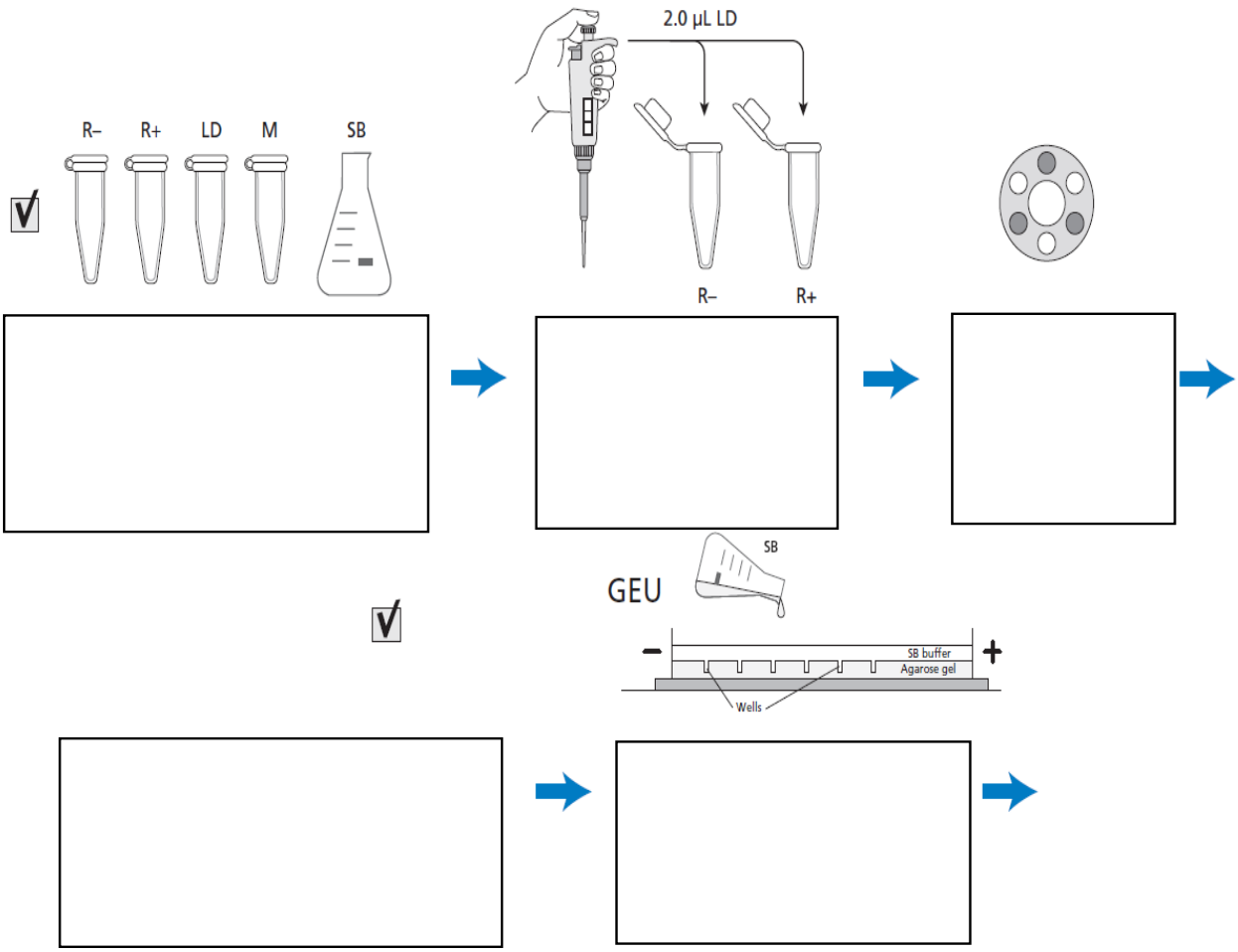
LABORATORY 4A: VERIFICATION OF THE RECOMBINANT PLASMID USING GEL ELECTROPHORESIS

Reference: ABE Student Guide pages C-31 to C-34

In your own words, describe the **Purpose** of Lab 4A:

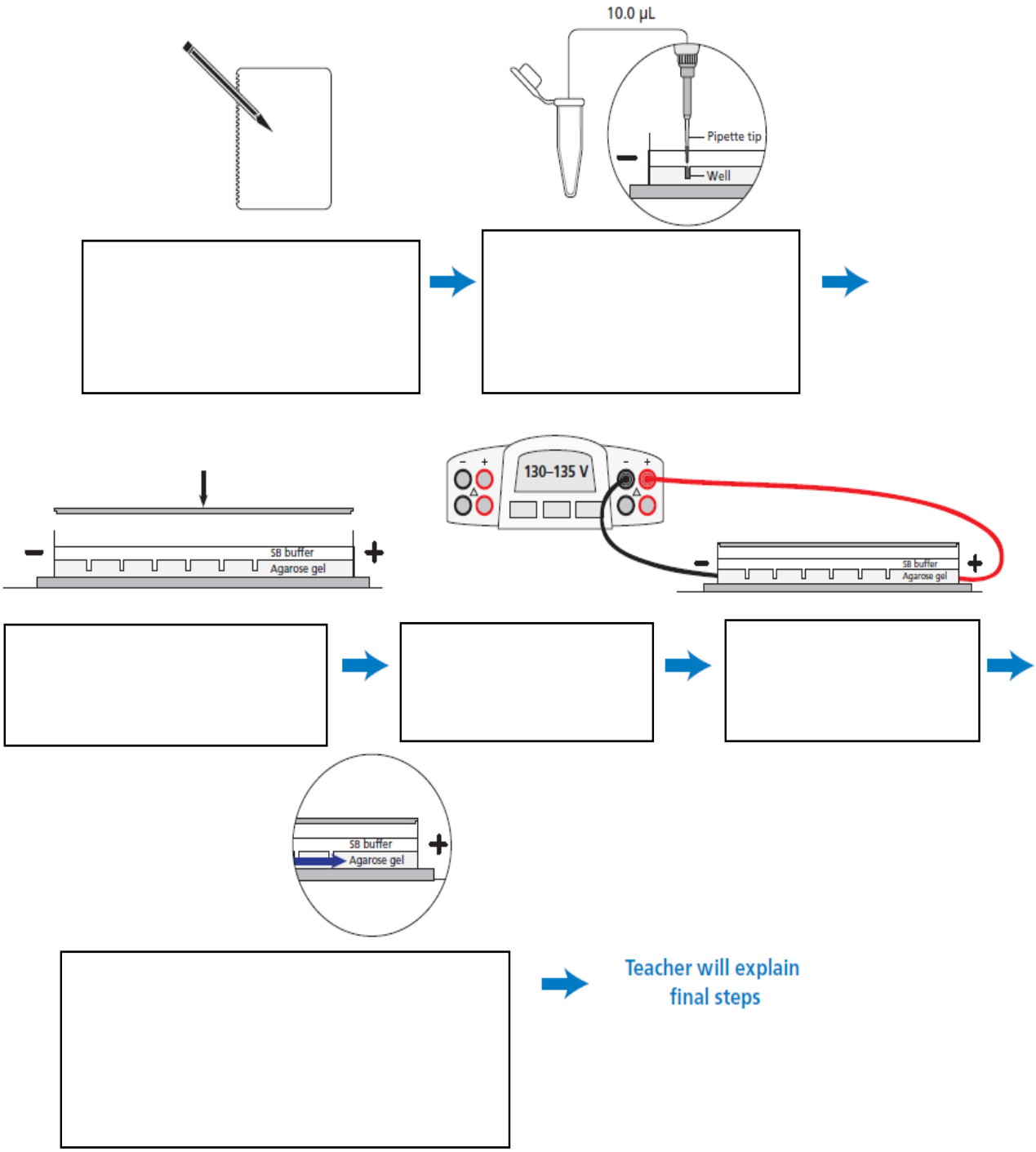
Methods: Finish the flowchart for **Lab 4A** by describing the step for each of the diagrams below:

Laboratory 4A Flowchart



(continued)

Laboratory 4A Flowchart (continued)



TEACHER PREP for Bacterial Transformation (Lab 5/5A/5B)

Proper Storage of Reagents:

STORAGE	REAGENT	NOTE
FREEZER	Frozen Competent cells (tubes) in isofreeze box pARA-R DNA : 10 ng/mL for Transformation in square reagent aliquot box	Pick up from Distribution Center few days before transformation lab. Leave tubes of cells in isofreeze box. Put entire isofreeze box in freezer. (pARA-R DNA tube that is 70 ng or 80ng/mL is for Restriction Digest Lab, NOT transformation lab)
Refrigerator	Agar Plates of LB (sleeved) Agar Plates of LB/Amp (sleeved) Agar Plates of LB/Amp/Ara (sleeved) LB Broth (tubes)	Store plates upside-down (agar side at the top) in sleeves. If needed, make shorter stacks in new bags. Bring to RT on morning of lab. Bring to RT on morning of transformation

TEACHER PREPARATION TIMELINE:

1-7 days ahead: pick up from FC: agar plates, competent cells, LB Broth

1 day ahead (or more):

- Download "colony counter app" for phone.
- Turn on and set incubator to 37°C.
- Label and Set up biowaste beaker (can add small amount of 10% bleach or disinfectant now or afterwards) for each group, and one biowaste bag for the class.

- Could have students do practice PLATE SPREADING. Use one of the extra LB plates and one spreader to have students practice clamshell method, pipetting water (or red dye) and spreading on top of the agar plate.

- Fullerton Center has prepared:
  - Aliquot 11 uL pARA-R DNA (10 ng/mL) for each group. Store in freezer.
  - Aliquot sterile LB broth. Store in refrigerator. Have groups share and finish one LB tube, before opening new LB tube.

Morning of TRANSFORMATION LAB DAY:

- Check incubator temperature. Place stacks of agar plates upside-down in 37C incubator (agar side at top).

15 minutes before Lab:

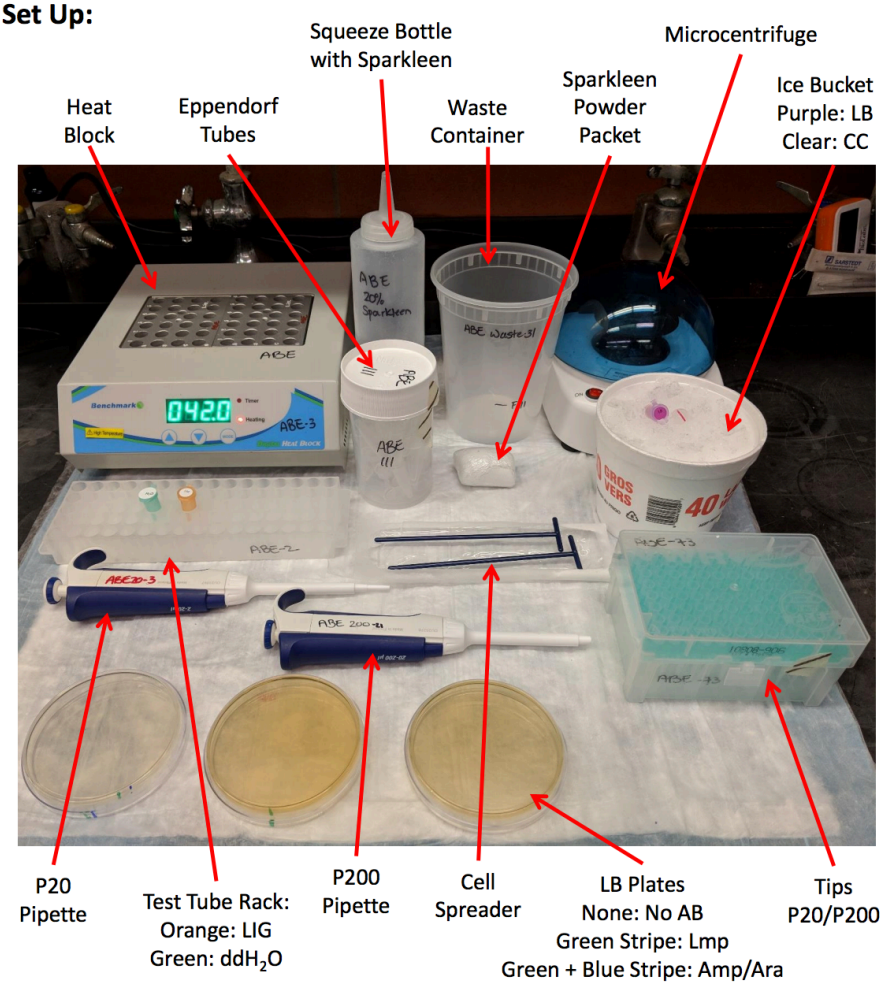
- Put crushed ice into Styrofoam cups.
- Thaw frozen competent cells ("Comp Cells") just before lab. Cells can NOT be refrozen for use.
- Aliquot 100 uL Comp Cells per student group and put into crushed ice immediately. Discard used pipette tip into biowaste beaker.

During Lab:

- Set up Practice PLATE SPREADING STATION (if not done the day before) while waiting for incubation.
- Bring LB Broth aliquots out of refrigerator and distribute to each group.
- Distribute 3 agar plates to each group. Have each group double-check that they have 1, 2, 3-striped plates.

- Collect all labeled pARA-R tubes to be returned to distribution center. Place all unused pARA-R aliquots and original tubes back into freezer.
- If leftover comp cells – you can use for other bacterial experiments, if you have broth or agar plates.

Set Up:



After Lab:

- Have students wipe off their lab work area and pipette body with Lysol disinfectant wipes.
- Dump biowaste beakers into class biowaste bag. Wipe outside of beaker with used desk wipe.
- Add disinfectant or 10% bleach into biowaste beakers, Sit for 10 minutes before washing in the sink.
- Students take off gloves in the proper way and discard into class biowaste bag. Then wash hands and arms carefully for 20 seconds, hum "Happy Birthday" song..

1-2 Day After Lab:

- Check agar plates for growth. Should see slight pink colonies in 3-striped agar plate by 24 hours.
- If many colonies, use "Colony Counter" app on cell phone, to get estimate of number of colonies. You may have to edit the captured photo in the app.
  - If pink colonies, put into refrigerator overnight. Colonies usually get darker pink.
  - If no pink colonies, then keep in 37C incubator longer.
- If incubated for 48 hours, usually will find numerous white satellite colonies.
- Take photos on cell phone, and send back to amgenbwoc@gmail.com (or share Dropbox or Google Drive).
- Wrap 2 best agar plates with parafilm, put into baggie, and return to FC. If anything unusual, also wrap and send back, so that we can troubleshoot.
- Stack all USED agar plates into sleeve, tape and mark USED. Place plate sleeve in refrigerator until return day.
- Sleeve UNUSED agar plates to return to center. Keep all agar plates in refrigerator until kit return day.

ON RETURN DAY to FC:

- Pack freezer items in cooler bag or foam box:
  - Isofreeze box with comp cells
  - Reagent aliquot box with tubes
- Pack Refrigerator items in gray biowaste bins: biowaste bags closed with bread ties
  - Sleeved agar plates, used and unused
  - LB Broth tubes in baggie

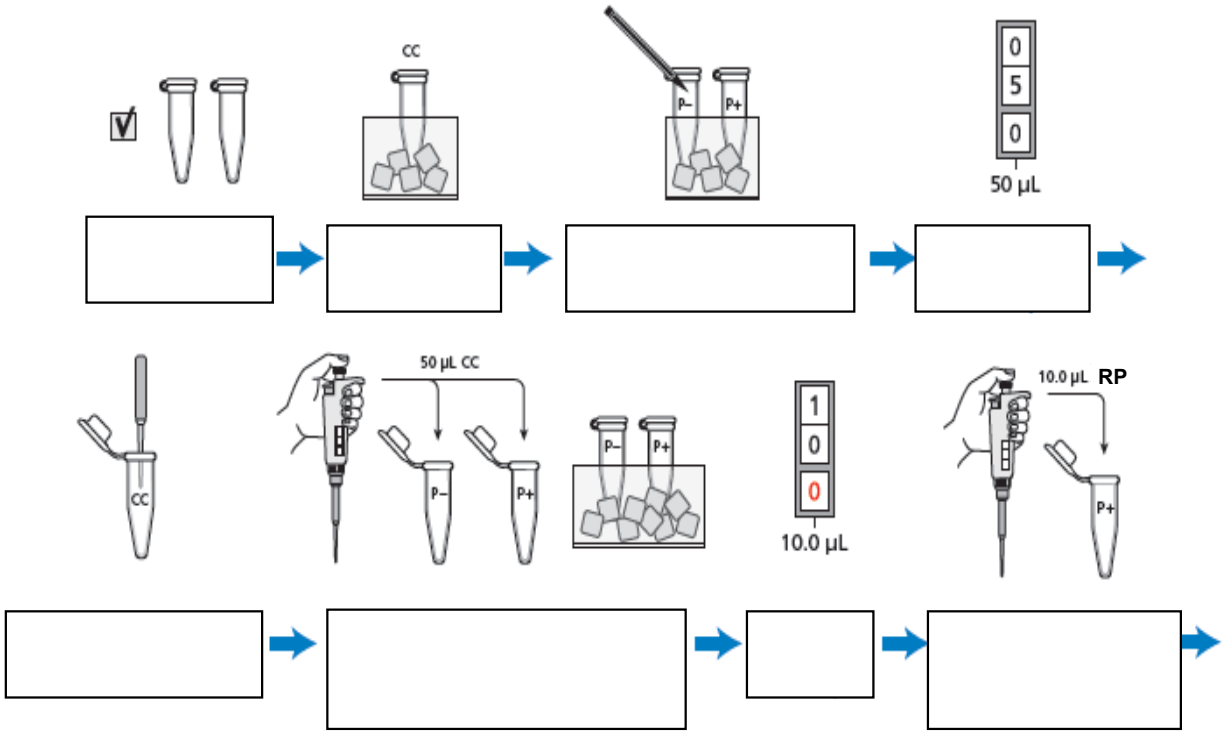
Name: _____ **Date:**_____ **Group:** _____

Laboratory 5B: Transforming Bacteria with a Recombinant Plasmid (pARA-R)

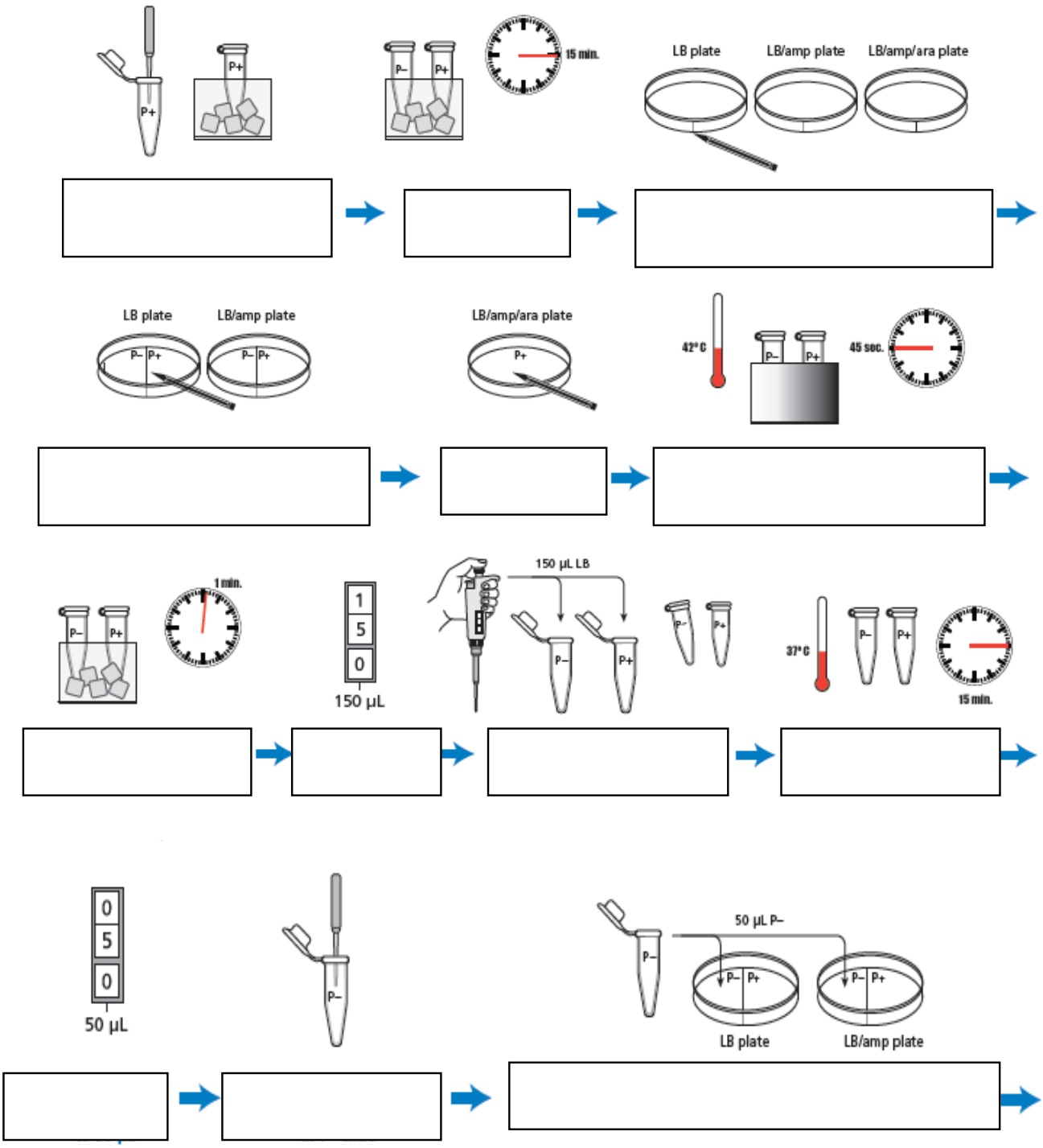
Purpose: In your own words, describe the Purpose of Lab 5B:

Materials for shared student stations:	**Materials at Teacher Station:**
☐ Pipettes and tip boxes: P20, P200 ☐ Microfuge rack: 2 new microfuge tubes, Sharpie, tube of LB Broth ☐ Cup of crushed ice: Tube of pARA-R Plasmid "RP" ☐ Beaker for collecting Biowaste ☐ Bag of cell spreaders ☐ Agar Petri plates (one set per group): ▪ one LB (1-stripe) ▪ one LB/Amp (2 stripe) ▪ one LB/Amp/Ara (3 stripe)	☐ Bucket of Crushed ICE: Tube of Competent E coli cells "CC" ☐ LB Broth tube ☐ Biowaste Bag ☐ Gloves **Equipment:** ☐ 42°C Water Bath ☐ 37°C Incubator

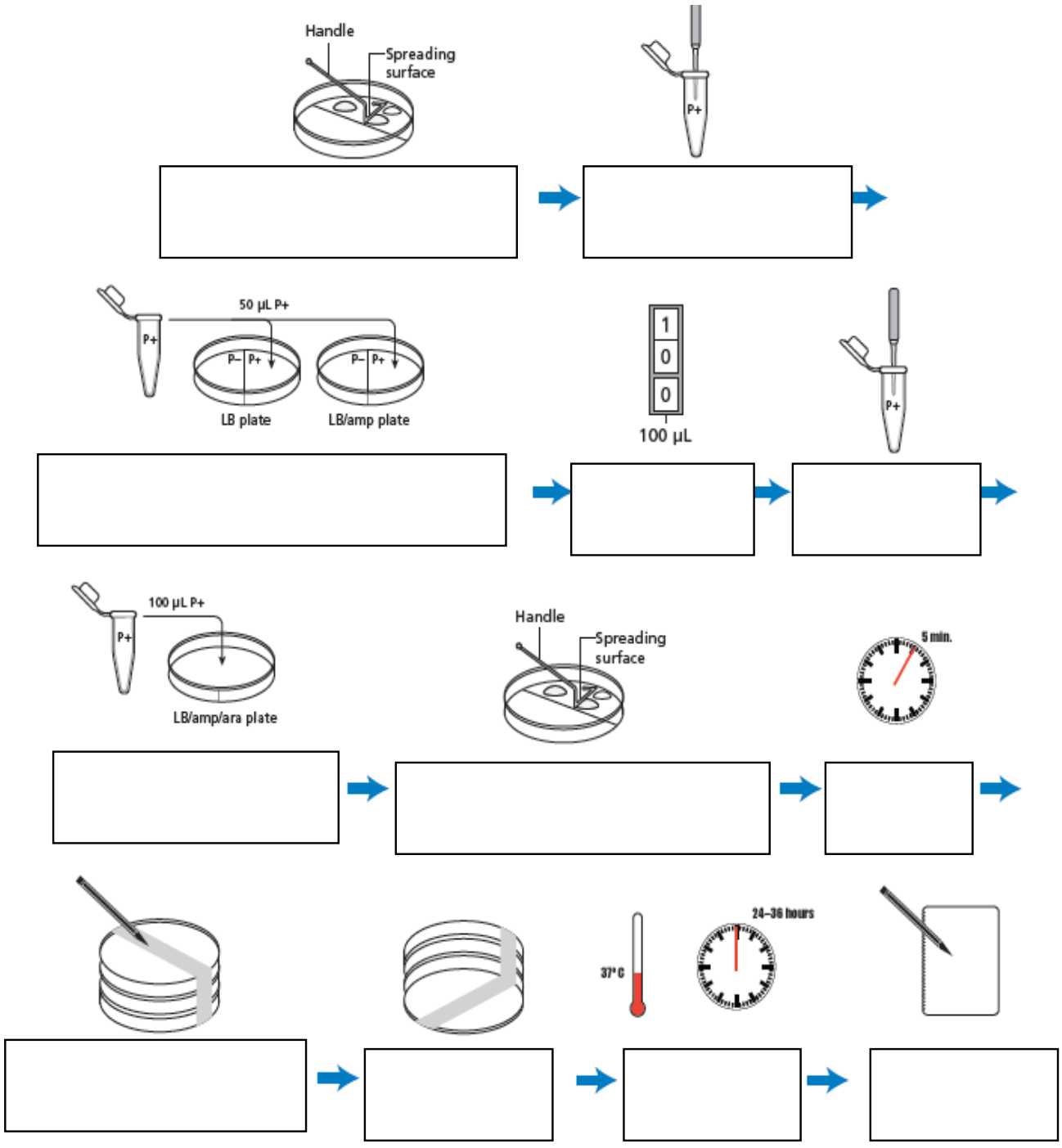
Methods: Fill out the Lab 5B Flowchart by describing each step on the diagram below:



Lab 5B Flowchart (page 2)



Lab 5B Flowchart (page 3)



BACTERIAL GROWTH PREDICTIONS

Predict how much bacterial growth you will see on each plate. Mark the plate/plate section with +++ (for high growth), ++ (for medium growth), + (low growth), or - (for no growth):

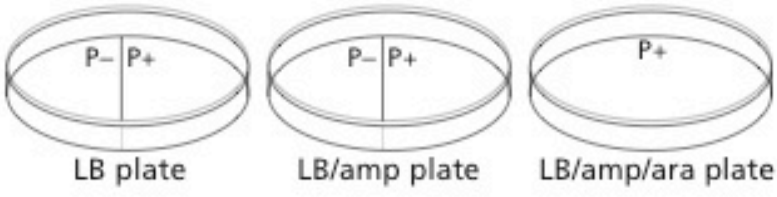


Table 1: P– Control Group (Non-Transformed Bacteria)

Plate Contains:	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)			
Luria Broth ampicillin (LB/amp)			

Table 2: P+ Experimental Group (Transformed Bacteria)

Plate Contains:	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)			
Luria Broth ampicillin (LB/amp)			
Luria Broth ampicillin arabinose (LB/amp/ara)			

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Complete Genetic Engineering Sequence includes

- Lab 1
- Lab 2
- Lab 3
- Lab 4
- Lab 5
- Lab 6 (optional)
    - can be ordered as a separate kit
- Colony PCR (optional)
    - Note that you must have
    - Attended training for cPCR lab
    - demonstrated prior successful transformation Lab 5 (send photos and 2 good agar plates back to the distribution center)

LABORATORY 2: PREPARING TO CLONE THE RFP GENE: DIGESTING THE pKAN-R AND pARA

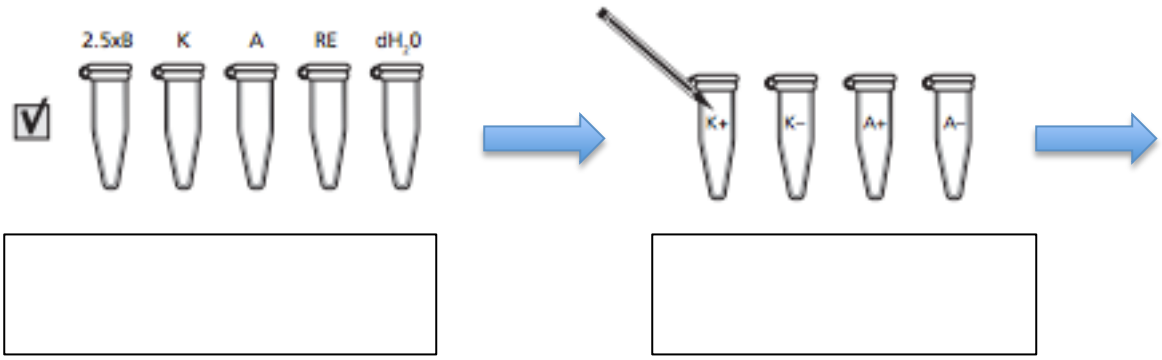
Reference On-line Student Guide (Complete Sequence) pages 46-50

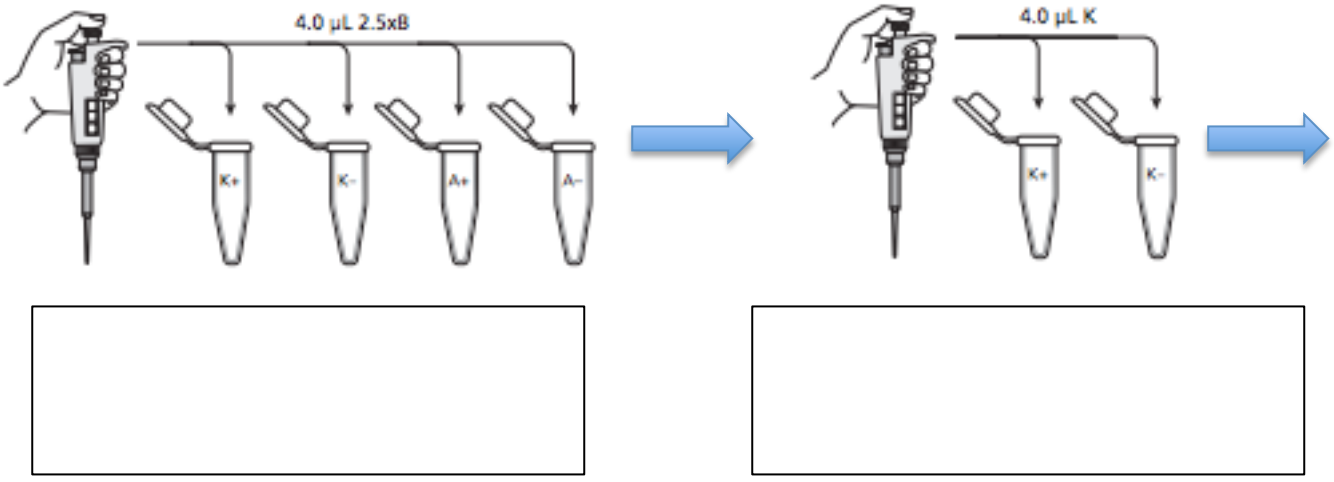
Prelab: Complete BEFORE doing the Lab

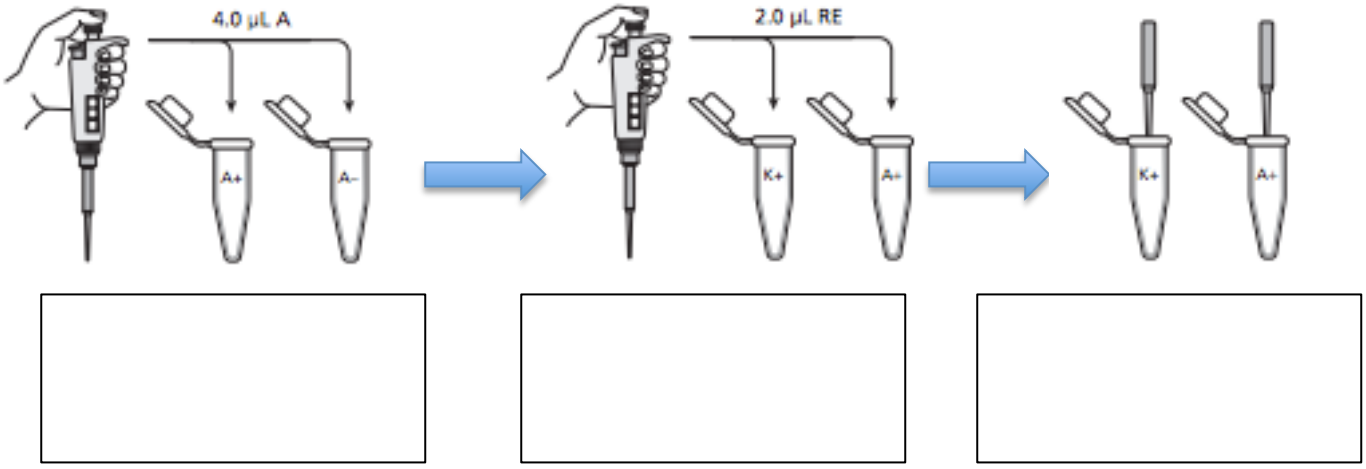
In your own words, describe the **Purpose** of Lab 2:

List the **Materials** (Reagents, Equipment, and Supplies) that you will need for Lab 2:

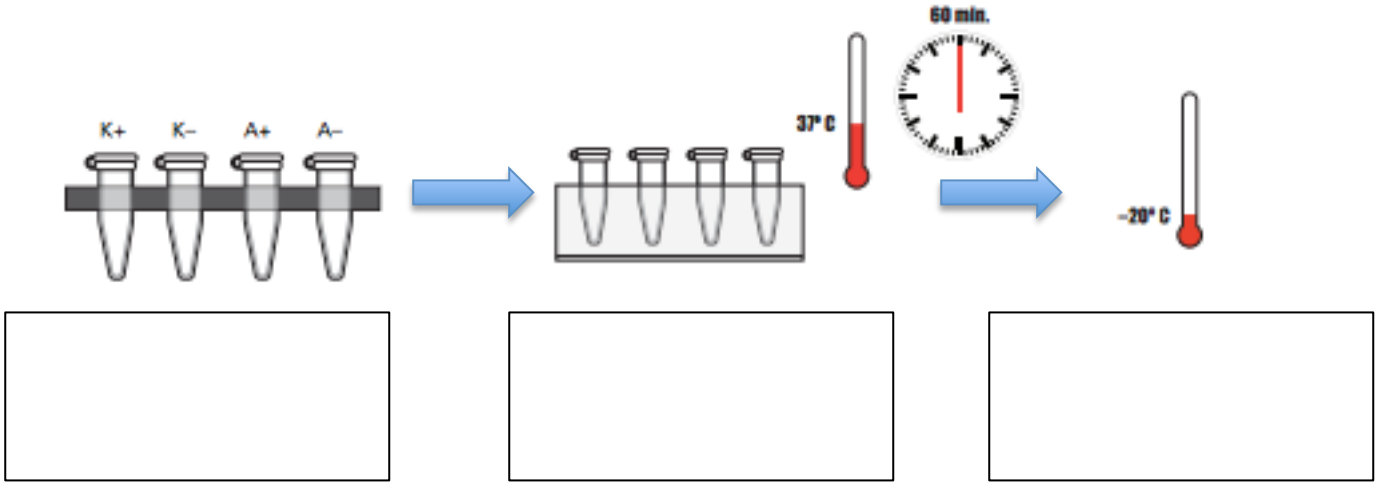
Methods: Finish the flowgram for Lab 2 by labeling each of the diagrams below:




4.0 µL 2.5xB
K+
K–
A+
A–
4.0 µL K
K+
K–


4.0 µL A
A+
A–
2.0 µL RE
K+
A+
K+
A+


2.0 µL dH2O
K–
A–
K–
A–



Fill in the pipetting matrix below:

	K+	K-	A+	A-
Step 4a:				
Step 4b:				
Step 4c:				
Step 4d:				
Step 4e:				

After the Lab Analysis Questions:

1. In step 4, you are asked to set up two tubes without the restriction enzymes, BamHI and HindIII. What is the purpose of this step, and why is it important?

2. Why might the enzymes work best at 37°C? Why should the enzymes then be placed in the freezer?

3. Explain the purpose of this lab in the genetic engineering process.

LABORATORY 3: BUILDING THE pARA-R PLASMID

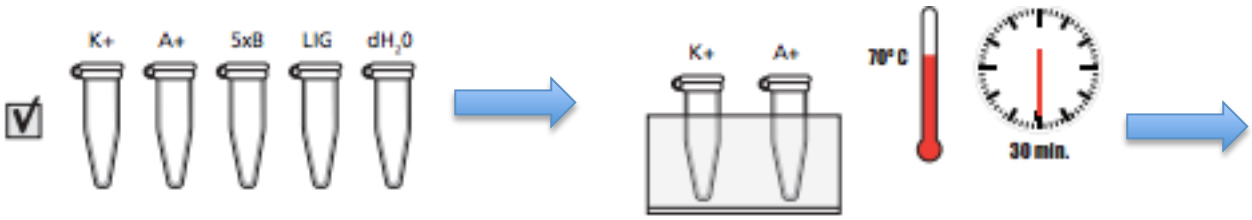
On-line Student Guide (Complete Sequence) pages 61-63

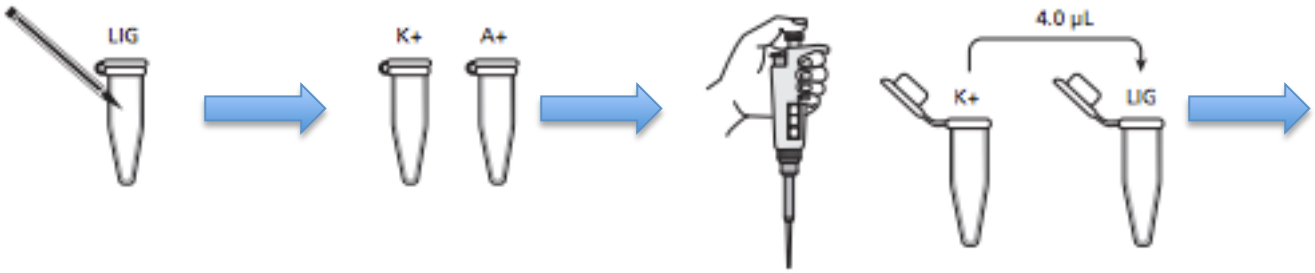
Prelab: Complete BEFORE doing the Lab

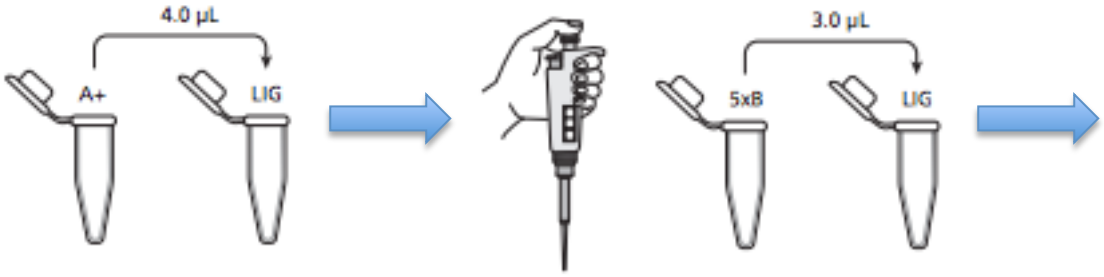
In your own words, describe the **Purpose** of Lab 3:

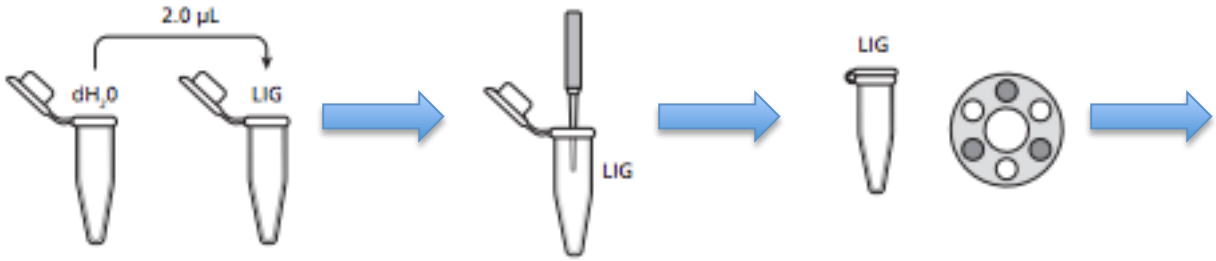
List the **Materials** (Reagents, Equipment, and Supplies) that you will need for Lab 3:

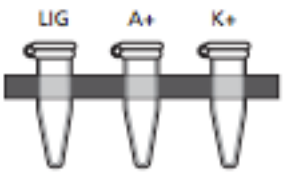
Methods: Finish the flowgram for Lab 3 by labeling each of the diagrams below:




LIG
K+
A+
4.0 µL
K+
LIG


4.0 µL
A+
LIG
3.0 µL
5xB
LIG


2.0 µL
dH2O
LIG
LIG
LIG


LIG
A+
K+

After the Lab Analysis Questions:

1. Why is it important to inactivate the BamHI and HindIII restriction enzymes before ligating the fragments? What might happen if you did not perform this step?

2. What properties of the DNA restriction fragments produced in Laboratory 2 enable ligation of these fragments?

3. Could two rfp fragments join to form a plasmid during the ligation? If not, what would prevent that? If so, what would be the result?

4. During ligation, both hydrogen and covalent bonds form. Which bonds form first? Why do both types of bonds need to form?

LABORATORY 4: VERIFICATION OF RESTRICTION AND LIGATION USING GEL ELECTROPHORESIS

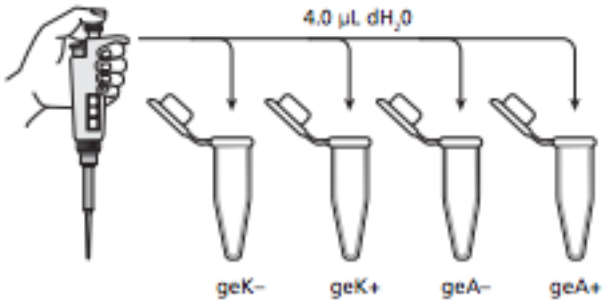
Reference: On-line Student Guide (Complete Sequence) pages 75-79

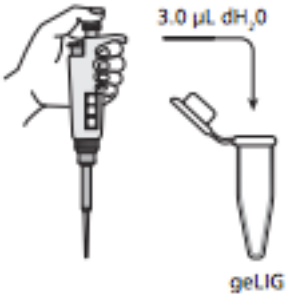
Prelab: Complete BEFORE doing the Lab

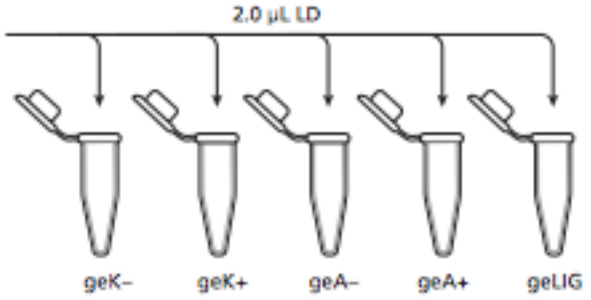
In your own words, describe the **Purpose** of Lab 4:

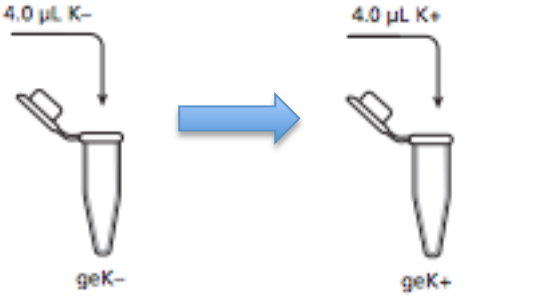
List the **Materials** (Reagents, Equipment, and Supplies) that you will need for Lab 4:

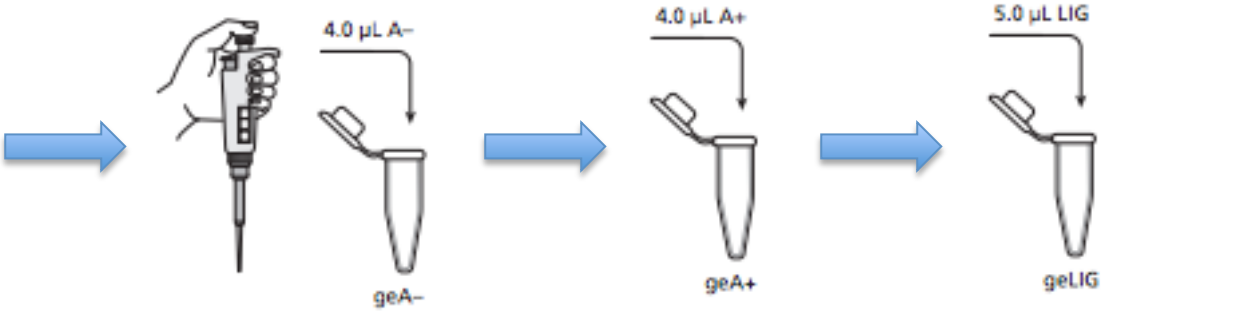
Methods: Finish the flowgram for Lab 4 by labeling each of the diagrams below:


4.0 µL dH2O
geK–
geK+
geA–
geA+

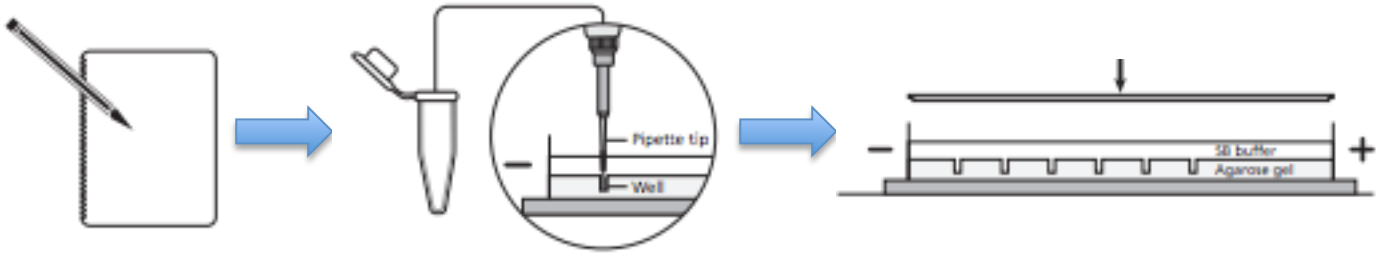

3.0 µL dH2O
geLIG

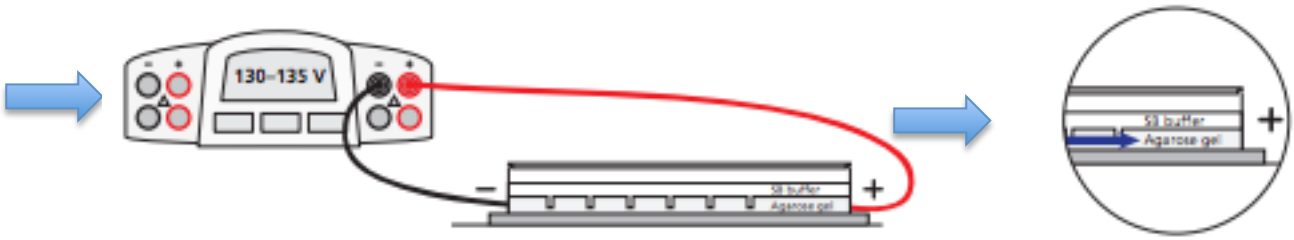

2.0 µL LD
geK–
geK+
geA–
geA+
geLIG


4.0 µL K–
4.0 µL K+
geK–
geK+


4.0 µL A–
4.0 µL A+
5.0 µL LIG
geA–
geA+
geLIG


LIG
GEU
SB
SB buffer
Agarose gel
Wells

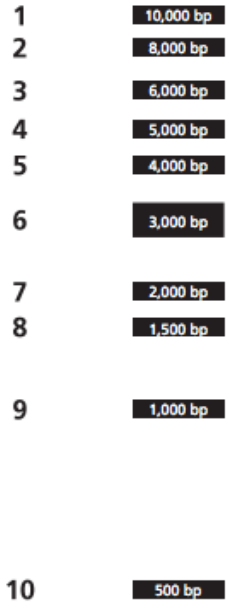

Pipette tip
Well
SB buffer
Agarose gel


130–135 V
SB buffer
Agarose gel

Fill in the pipetting matrix below:

Sequence and Reagent	geK-	geK+	geA-	geA+	geLig
Step 4 & 5:					
Step 6:					
Step 7:					
Step 7:					
Step 7:					
Step 7:					
Step 8:					

1. The DNA ladder serves as a standard because it contains a mixture of DNA molecules of known sizes. By running your samples and the DNA ladder side by side in your gel, you can estimate the actual size in base pairs of unknown DNA molecules. The DNA Ladder Diagram shows 10 DNA bands of known sizes. Using this information, predict the positions of DNA bands produced by the possible products found in the K–, K+, A–, A+, and LIG tubes by drawing their relative positions on the diagram.



After the Lab Analysis Questions:

1. Analyze your gel. Do you see any bands that are not expected? What could explain the origin of these unexpected bands?

2. Does the gel show that your restriction digest and ligation procedures were successful? Describe the evidence you used to make this assessment.

3. In the geK– and geA– lanes, do you see evidence of multiple configurations of plasmids? Explain your answer.

4. In the geK+ and geA+ lanes, do you see evidence of complete digestion? Explain your answer.

5. In which lane would you expect to find the rfp gene and the ampR gene in the gel photograph? Are you able to locate these two genes? Explain your answer.

6. Compare the lanes that have linear fragments with the lanes that have uncut plasmids. Is there a difference in the shape of the bands between these two DNA forms? Describe it.

7. In Laboratory 3, you described all the possible plasmids that you could make by ligating the digested fragments of the pKAN-R and the pARA plasmids. Two of the rfp gene fragments (807 bp each) may form a circularized fragment because each end of the fragments terminates in BamHI and HindIII sticky ends. Is there evidence of a circularized 1,614 bp fragment in the geLIG tube lane? Justify your answer.

8. Is there verification in the gel that you have successfully produced the plasmid of interest? Explain why or why not?

Lab 2 Teacher Prep (NOT FINISHED)

PLANNING (Teacher):

- ABE-FC will give you premade agarose gels for the Mini One system.
  - Use the gels with 6-wells, to get the best results.
  - You can share one gel between two groups. OR have one class run on left side wells, then the next class run on right side wells.
  - These Agarose gels are on laminated sheets in flat document plastic boxes….store flat at 4C.
  - If gels are damaged, just leave in box to return, as we can remelt and recast.

- If you are making your own agarose gels, be sure to make them ahead of actual lab.
  - 3 Mini One casting systems (for 6 gels) are provided in the Teacher box.
- Decide on how many samples you want to use per gel, to decide on which comb to use for making gels.
- If you want your students to pour the gels, you may want to
  - prepare/mix the agarose and 1X SB solution in a **flask** (use 0.8 grams agarose in 100 mL 1X SB),
  - melt the agarose, pour melted agarose into 50 mL conical tubes,
  - cap tubes tightly and keep in 60^{o}C water bath until ready to pour.
  - If agarose solidify inside tubes, remove or loosen caps, put tubes in beaker and remelt in microwave.
- Each Mini One gel requires 12.5 mL agarose solution, each casting tray holds two gels = 25 mL total.

PRE-LAB (Students):

- ❖ Read student guide pages, use info to Fill out the Student Flowchart (blank).
- ❖ Watch Video about using Mini One Gel Electrophoresis system

Materials Provided:

Equipment:

- Centrifuge
- Transilluminator
  - Blue LED for Gel Green dye
  - uV for Gel Red dye

Store in Freezer:

- Student R+ and R- tubes from Lab 2A
- DNA Ladder (M)

Store in Refrigerator

- premade 0.8% Agarose gels in SB – store flat

Store at Room Temperature:

- Loading dye (LD) aliquots (olive green plastic box)
- 1X SB Buffer bottles (premade at FC)
- 20X SB stock bottle (dilute to 1X SB as needed).

ABE-FC Student Group Station:

- Micropipettes P20 and P200
- Microfuge Tube Rack
- Mini One System: Black Carriage, Buffer Tank, Gray & Black Plate, Orange Hood, Power Cord
- Funnel
- Laminated white half-page sheet
- Laminated micropipette practice sheet
- Waste cup for pipet tips
- Sharpie permanent marker
- Dry Erase marker (NOT provided)
-

To practice loading gels:

- Practice Plate (petri dish with polyurethane)
- large black weigh boat
- Transfer Pipet (plastic)

Lab 2 Restriction Digest Student Protocol

☐ 1. Use a permanent marker to label the top of four clean microfuge tubes as follows: K+, K–, A+, and A–. (Also include your group number and class period on each tube.)

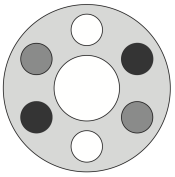
☐ 2. Dispense the reagents onto the wall of the tube, so that you can check you delivered the reagent (also check afterwards that your pipet tip is empty).

☐3. Use new tips for new reagents. Check off as you go, to ensure you added everything.

	K+ tube	K– tube	A+ tube	A– tube
Step 4a: Restriction buffer (2.5xB)	4.0 µL	4.0 µL	4.0 µL	4.0 µL
Step 4b: pKAN-R plasmid (K)	4.0 µL	4.0 µL		
Step 4c: pARA plasmid (A)			4.0 µL	4.0 µL
Step 4d: BamHI and HindIII (RE)	2.0 µL		2.0 µL	
Step 4e: Distilled water (dH_2O)		2.0 µL		2.0 µL

☐ 4. Close tubes tightly, flick tube with finger several times to mix.
OR gently pump the solution in and out with the pipette to mix the reagents.

LAB TECHNIQUE: Distribute the tubes evenly in the microcentrifuge so that two tubes with balanced weight are directly across from each other. Since all four tubes have the same volume, you do not need to weigh the tubes.



☐5. Spin the tubes in the microcentrifuge for 10 seconds to pool the reagents at the bottom of each tube.

☐6. Place tubes into a floating rack in the 37°C water bath for at least one hour, but no longer than two.

☐7. After the incubation is complete, continue to Lab 3 protocol OR your teacher can freeze tubes.

Lab 3 Teacher Prep (NOT FINISHED)

PLANNING (Teacher):

- ABE-FC will give you premade agarose gels for the Mini One system.
  - Use the gels with 6-wells, to get the best results.
  - You can share one gel between two groups. OR have one class run on left side wells, then the next class run on right side wells.
  - These Agarose gels are on laminated sheets in flat document plastic boxes….store flat at 4C.
  - If gels are damaged, just leave in box to return, as we can remelt and recast.

- If you are making your own agarose gels, be sure to make them ahead of actual lab.
  - 3 Mini One casting systems (for 6 gels) are provided in the Teacher box.
- Decide on how many samples you want to use per gel, to decide on which comb to use for making gels.
- If you want your students to pour the gels, you may want to
  - prepare/mix the agarose and 1X SB solution in a **flask** (use 0.8 grams agarose in 100 mL 1X SB),
  - melt the agarose, pour melted agarose into 50 mL conical tubes,
  - cap tubes tightly and keep in 60^{o}C water bath until ready to pour.
  - If agarose solidify inside tubes, remove or loosen caps, put tubes in beaker and remelt in microwave.
- Each Mini One gel requires 12.5 mL agarose solution, each casting tray holds two gels = 25 mL total.

PRE-LAB (Students):

- ❖ Read student guide pages, use info to Fill out the Student Flowchart (blank).
- ❖ Watch Video about using Mini One Gel Electrophoresis system

Materials Provided:

Equipment:

- Centrifuge
- Transilluminator
  - Blue LED for Gel Green dye
  - uV for Gel Red dye

Store in Freezer:

- Student R+ and R- tubes from Lab 2A
- DNA Ladder (M)

Store in Refrigerator

- premade 0.8% Agarose gels in SB – store flat

Store at Room Temperature:

- Loading dye (LD) aliquots (olive green plastic box)
- 1X SB Buffer bottles (premade at FC)
- 20X SB stock bottle (dilute to 1X SB as needed).

ABE-FC Student Group Station:

- Micropipettes P20 and P200
- Microfuge Tube Rack
- Mini One System: Black Carriage, Buffer Tank, Gray & Black Plate, Orange Hood, Power Cord
- Funnel
- Laminated white half-page sheet
- Laminated micropipette practice sheet
- Waste cup for pipet tips
- Sharpie permanent marker
- Dry Erase marker (NOT provided)
-

To practice loading gels:

- Practice Plate (petri dish with polyurethane)
- large black weigh boat
- Transfer Pipet (plastic)

Lab 3 Ligation Student Protocol

☐1. Obtain your Lab 2 tubes from freezer.|

☐ Place K+ and A+ tubes from *Lab 2* in a floating rack in the ***80°C water bath for 30 minutes.*** This heat exposure will *denature* (inactivate) the restriction enzymes.

☐2. Obtain a LIG tube (containing DNA Ligase). Label the LIG tube with your group number and class period.

☐3. After 30 minutes, remove the K+ and A+ tubes from the water bath and place them in your rack.

☐4. Using new tips for each reagent, add the following directly into the bottom of the LIG tube:

☐ 4.0 µL of A+	☐ 3.0 µL of 5xB
☐ 4.0 µL of K+	☐ 2.0 µL of dH_2O

☐ 5. Mix the reagents by gently pumping the solution in and out with the pipette.

☐ 6. Find a balancer tube (from another team) and spin the LIG tube in the microcentrifuge for 5-10 seconds to pool the reagents at the bottom of the tube.

☐ 7. Place your A+, and K+ tubes in the class tube rack, so your teacher can **freeze** them for use in Lab 4.

☐ 8. Place your LIG tube in the class rack, so that it can incubate at **room temperature** for 30 minutes. Your teacher will freeze the tubes afterwards until the next class.

Lab 4 Teacher Prep (NOT FINISHED)

PLANNING (Teacher):

- ABE-FC will give you premade agarose gels for the Mini One system.
  - Use the gels with 6-wells, to get the best results.
  - You can share one gel between two groups. OR have one class run on left side wells, then the next class run on right side wells.
  - These Agarose gels are on laminated sheets in flat document plastic boxes….store flat at 4C.
  - If gels are damaged, just leave in box to return, as we can remelt and recast.

- If you are making your own agarose gels, be sure to make them ahead of actual lab.
  - 3 Mini One casting systems (for 6 gels) are provided in the Teacher box.
- Decide on how many samples you want to use per gel, to decide on which comb to use for making gels.
- If you want your students to pour the gels, you may want to
  - prepare/mix the agarose and 1X SB solution in a **flask** (use 0.8 grams agarose in 100 mL 1X SB),
  - melt the agarose, pour melted agarose into 50 mL conical tubes,
  - cap tubes tightly and keep in 60^{o}C water bath until ready to pour.
  - If agarose solidify inside tubes, remove or loosen caps, put tubes in beaker and remelt in microwave.
- Each Mini One gel requires 12.5 mL agarose solution, each casting tray holds two gels = 25 mL total.

PRE-LAB (Students):

- ❖ Read student guide pages, use info to Fill out the Student Flowchart (blank).
- ❖ Watch Video about using Mini One Gel Electrophoresis system

Materials Provided:

Equipment:

- Centrifuge
- Transilluminator
  - Blue LED for Gel Green dye
  - uV for Gel Red dye

Store in Freezer:

- Student R+ and R- tubes from Lab 2A
- DNA Ladder (M)

Store in Refrigerator

- premade 0.8% Agarose gels in SB – store flat

Store at Room Temperature:

- Loading dye (LD) aliquots (olive green plastic box)
- 1X SB Buffer bottles (premade at FC)
- 20X SB stock bottle (dilute to 1X SB as needed).

ABE-FC Student Group Station:

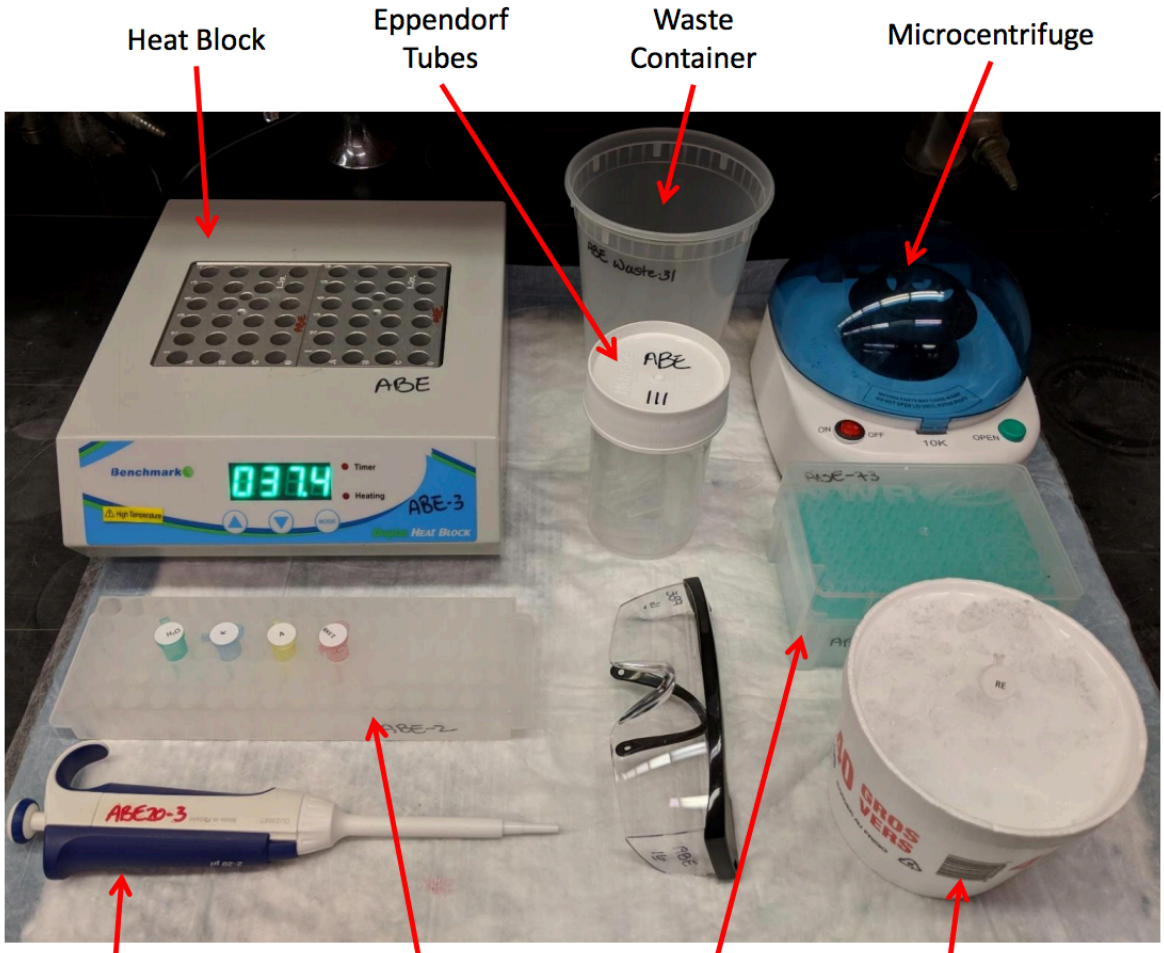
- Micropipettes P20 and P200
- Microfuge Tube Rack
- Mini One System: Black Carriage, Buffer Tank, Gray & Black Plate, Orange Hood, Power Cord
- Funnel
- Laminated white half-page sheet
- Laminated micropipette practice sheet
- Waste cup for pipet tips
- Sharpie permanent marker
- Dry Erase marker (NOT provided)
-

To practice loading gels:

- Practice Plate (petri dish with polyurethane)
- large black weigh boat
- Transfer Pipet (plastic)

Lab 4

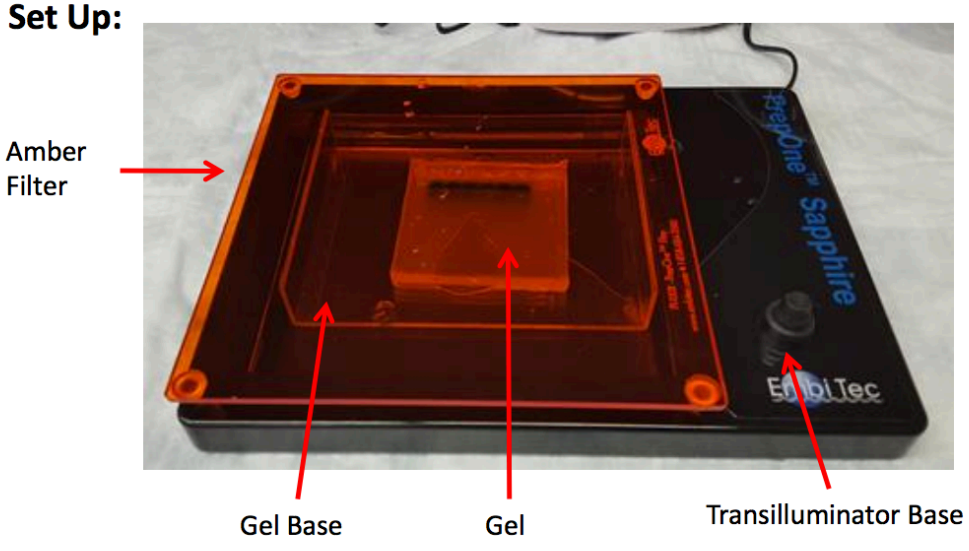
Set Up:


Heat Block
Eppendorf Tubes
Waste Container
Microcentrifuge
ABE
ABE Waste-3l
ABE III
Benchmark
ABE-3
ABE-2
ABE-3
P20 Pipette
Tube Rack
Pink: 2.5B
Yellow: pARA
Blue: pKAN
Green: ddH2O
Tips
P20/P200
Ice Bucket
Clear: RE

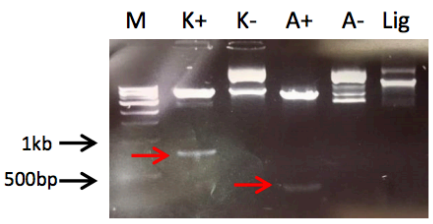
Tube	Fragments and plasmids listed in order of increasing bp size in each tube
K–	*(1) pKAN-R, 5,512 bp* *The plasmid can have all three configurations, and the supercoiled configuration should move the fastest.*
K+	*(1)* pBAD-rfp *fragment, 807 bp (2)* kanR *fragment, 4,705 bp*
A–	*(1) pARA, 4,872 bp* *The plasmid can have all three configurations, and the supercoiled configuration should move the fastest.*
A+	*(1) blank fragment, 377 bp (2)* ampR-ori-araC *fragment, 4,495 bp*
LIG	*(1) double blank plasmid, 754 bp (2)* pBAD-rfp-*blank plasmid, 1,184 bp* *(3) double* pBAD-rfp *plasmid, 1,614 bp (4) pARA, 4,872 bp* *(5)* kanR-*blank plasmid, 5,082 bp (6) pARA-R, 5,302 bp* *(7) pKAN-R, 5,512 bp (8) double* ampR-ori-araC *plasmid, 8,990 bp* *(9)* ampR-ori-araC-kanR *plasmid, 9,200 bp* *(10) double* kanR *plasmid, 9,410 bp*

Visualizing Results: Transilluminator

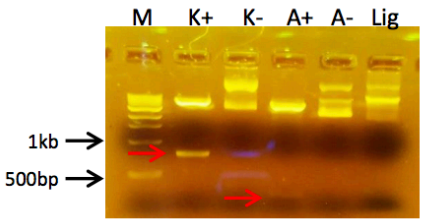
Set Up:



Gel-Doc Faint Band Detector



Transilluminator



Lab 4 Gel Electrophoresis Student Protocol

☐ Check that you have all of the reagents in the first column of table below.

☐ Label 5 new tubes for gel electrophoresis samples (ge K-, ge K+, ge A-, ge A+, ge LIG).

☐ Use P-20 micropipette and new tips for each reagent.
Set up your tubes for gel electrophoresis as indicated in the following table (check off as you go).

☐ Mix reagents by gently pipetting solution up and down, or by flicking the bottom of the closed tube.

Reagents	**ge K- tube**	**ge K+ tube**	**ge A- tube**	**ge A+ tube**	**ge LIG tube**	**M tube**
Distilled Water (dH_20)	4.0 uL	4.0 uL	4.0 uL	4.0 uL	3.0 uL	
Loading Dye (LD)	**2.0 uL**	**2.0 uL**	**2.0 uL**	**2.0 uL**	**2.0 uL**	**2.0 uL**
Nondigested pKAN-R (K-)	4.0 uL					
Digested pKAN-R (K+)		4.0 uL				
Nondigested pARA (A-)			4.0 uL			
Digested pARA (A+)				4.0 uL		
Ligated Plasmid (LIG)					5.0 uL	
DNA Ladder Marker (M)						8.0 uL

☐ Check that you have six tubes with the same total volume of 10 uL.

☐ Spin all six tubes in microcentrifuge for 10 seconds, so all samples are at the bottom of the tubes.

☐ Use a new tip for each sample. Use P-20 micropipette and load 10 uL of each sample in a 0.8 % agarose gel.

☐ Be sure to mark the loading order in your notebook.

☐ For Mini One gel box, run the gel 15-25 minutes.

☐ Take photos with your cell phone every 5 minutes, but **do not leave the light on during the gel run** (as the DNA stain is light sensitive). You should see DNA bands as they move through the gel.

  ☐ If you cannot see the smallest band, move the gel to the blue light transilluminator.

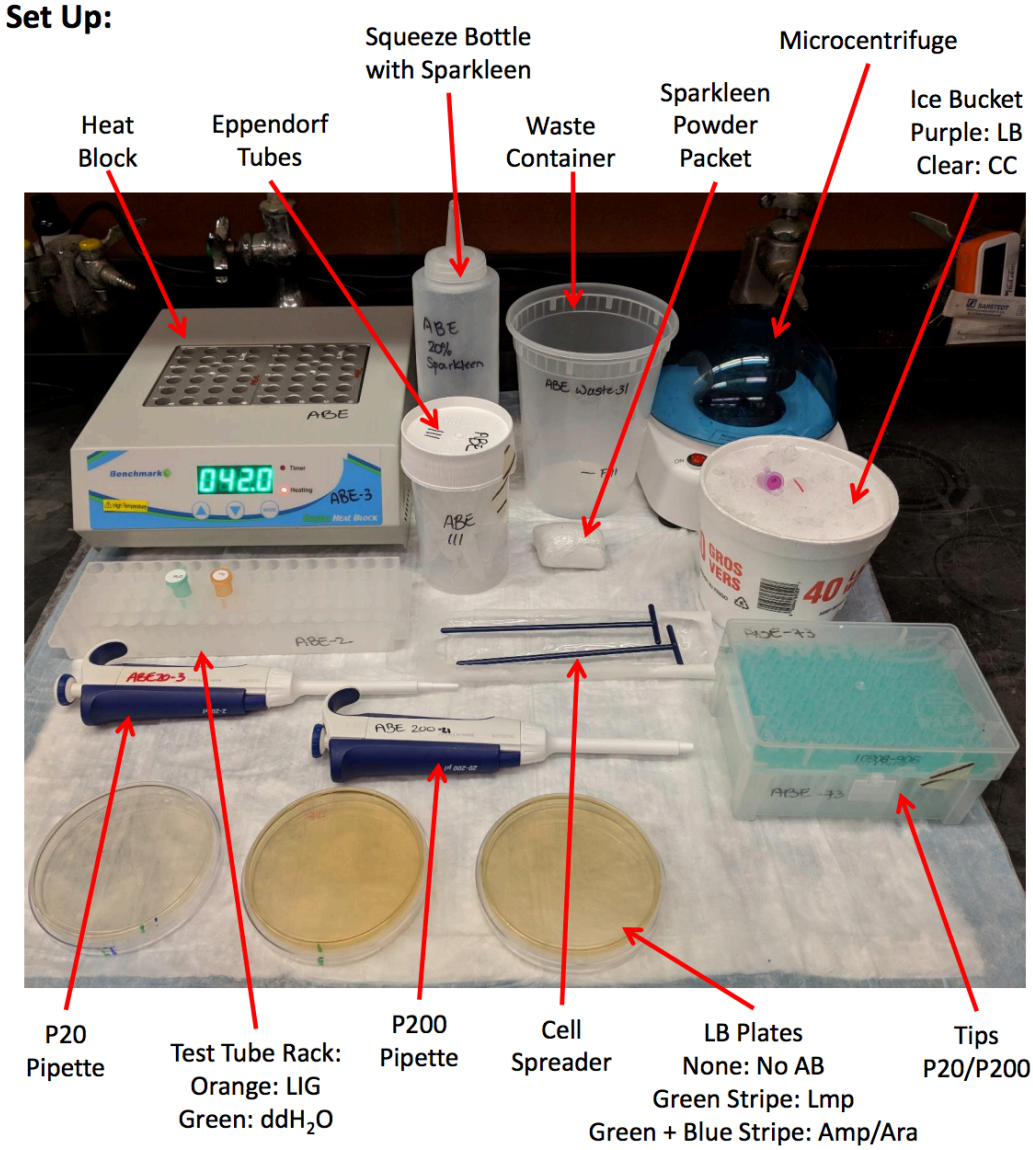
- Note: For other gel box brands, run at 135V for 40 minutes, until dye front is near bottom.
- Transfer gel onto a blue transilluminator, if using Gel Green loading dye.
- Transfer gel onto an uV transilluminator, if using Gel Red loading dye.

TEACHER PREP for Bacterial Transformation (Lab 5/5A/5B)

Proper Storage of Reagents:

STORAGE	REAGENT	NOTE
FREEZER	Frozen Competent cells (tubes) pARA-R DNA : 10 ng/mL for Transformation	Pick up from Distribution Center 1 - 2 days before transformation lab. Leave tubes of cells in isofreeze box. Put entire isofreeze box in freezer. (pARA-R DNA tube that is 70 ng or 80ng/mL is for Restriction Digest Lab, NOT transformation lab)
Refrigerator	Agar Plates of LB (sleeved) Agar Plates of LB/Amp (sleeved) Agar Plates of LB/Amp/Ara (sleeved) LB Broth (tubes)	Store plates upside-down (agar side at the top) in sleeves. If needed, make shorter stacks in new bags. Bring to RT on morning of lab. Bring to RT on morning of transformation

Set Up:



Biowaste station and how to return properly.packing

Lab 5 TEACHER PREPARATION TIMELINE:

1 day ahead (or more):

- Turn on and set incubator to 37°C.
- Label and Set up biowaste beaker for each group, and one biowaste bag for the class.
- Could have students do practice PLATE SPREADING. Use one of the extra LB plates and one spreader to have students practice pipetting water (or red dye) and spreading on top of the agar plate.
- Fullerton Center has already prepared:
    - Aliquot 11 uL pARA-R DNA (10 ng/mL) for each group. Store in freezer.
    - Aliquot sterile LB broth. Store in refrigerator. Have groups share and finish one LB tube, before opening new LB tube.,

Morning of TRANSFORMATION LAB DAY:

- Check incubator temperature. Place stacks of agar plates upside-down into 37C incubator (agar side at the top).

15 minutes before Lab:

- Put crushed ice into Styrofoam cups.
- Thaw frozen competent cells ("Comp Cells") just before lab. Cells can NOT be refrozen for use.
- Aliquot 100 uL Comp Cells per student group and put into crushed ice immediately.

During Lab:

- Set up Practice PLATE SPREADING STATION, if not done the day before.
- Bring LB Broth aliquots out of refrigerator and distribute to each group.
- Distribute 3 agar plates to each group. Have each group double-check that they have 1, 2, 3-striped plates.
- Collect all labeled pARA-R tubes to be returned to distribution center (inside a baggie is fine). Place all unused pARA-R aliquots and original tubes back into freezer.
- Discard used Comp Cell tubes into biowaste bag. If leftover comp cells – you can use for other bacterial experiments, if you have broth or agar plates.

After Lab:

- Have students wipe off their lab work area and pipette with Lysol disinfectant wipes.
- Dump biowaste beakers into class biowaste bag. Wipe outside of beaker with used desk wipe.
- Add disinfectant or 10% bleach into biowaste beakers, Sit for 10 minutes before washing in sink.
- Students take off gloves in the proper way and discard into class biowaste bag. Then wash hands and arms in sink.

1-2 Day After Lab:

- Check agar plates for growth. If many colonies, use "Colony Counter" app on cell phone.
- Take photos on cell phone, and send back to amgenbwoc@gmail.com (or share Dropbox or Google Drive).
- Wrap 2 best agar plates with parafilm, put into baggie, and return to FC. If anything unusual, also wrap and send.
- Stack all USED agar plates into sleeve, tape and mark USED. Can leave outside of biowaste bag, but must be taped.
- Sleeve UNUSED agar plates to return to center. Keep all agar plates in refrigerator until kit return day.

LABORATORY 5: TRANSFORMING BACTERIA WITH THE LIGATION PRODUCTS

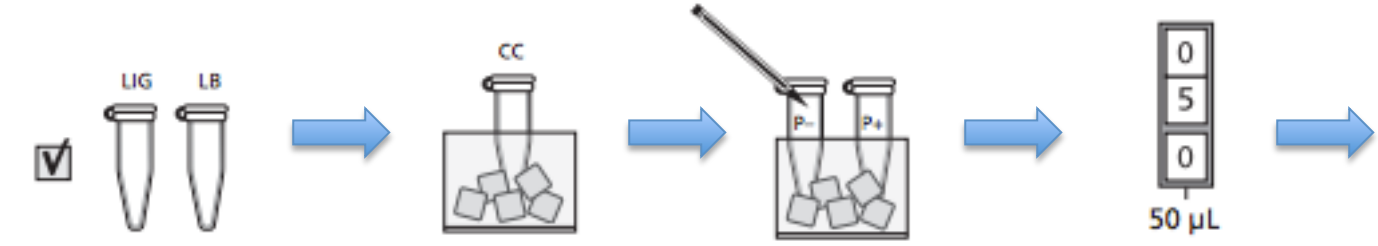
Reference: On-line Student Guide (Complete Sequence) pages 90-97

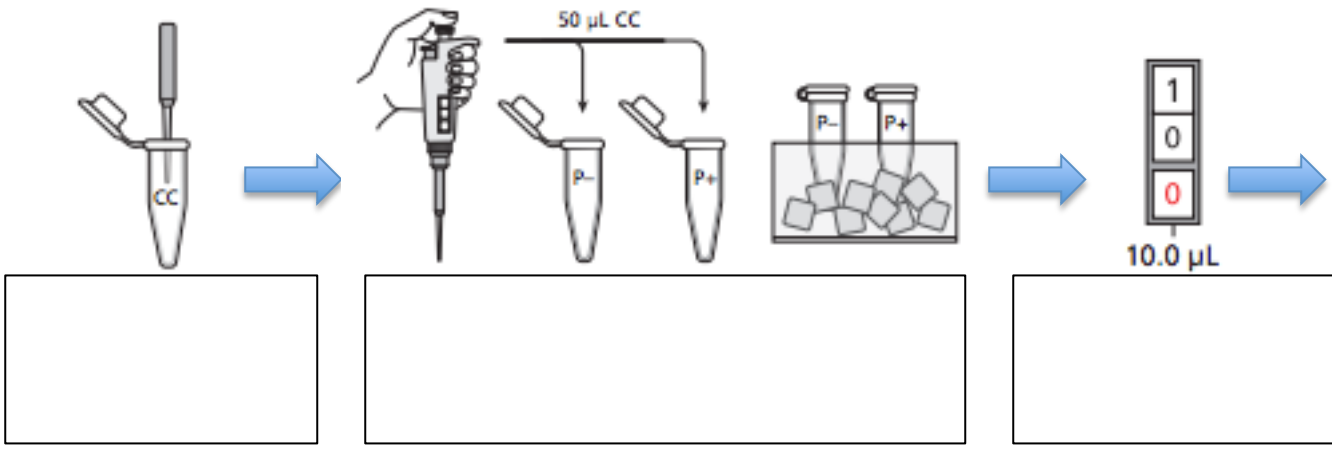
Prelab: Complete BEFORE doing the Lab

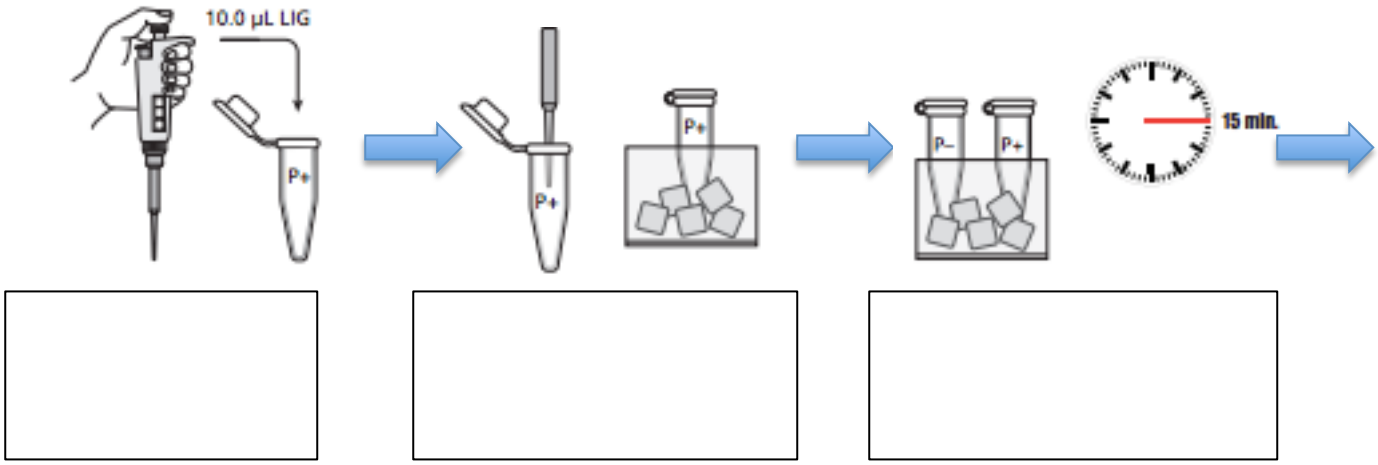
In your own words, describe the **Purpose** of Lab 5:

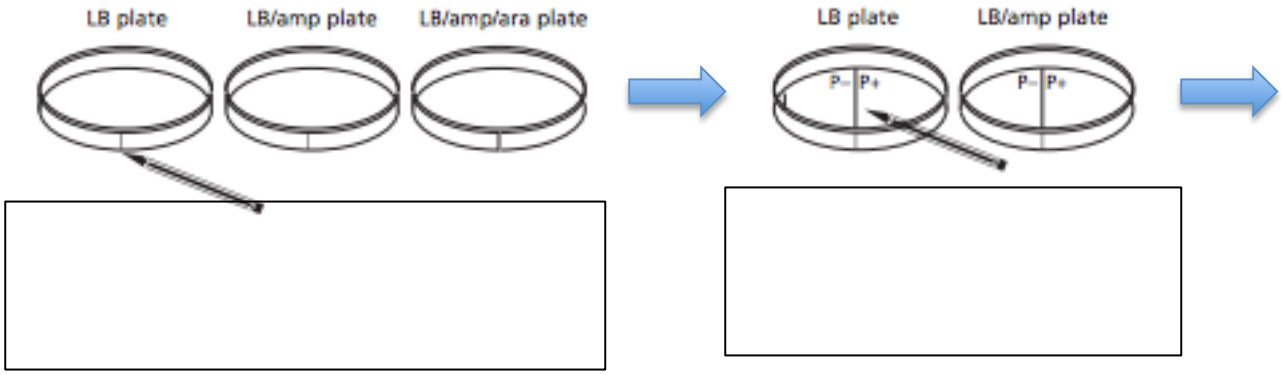
List the **Materials** (Reagents, Equipment, and Supplies) that you will need for Lab 4:

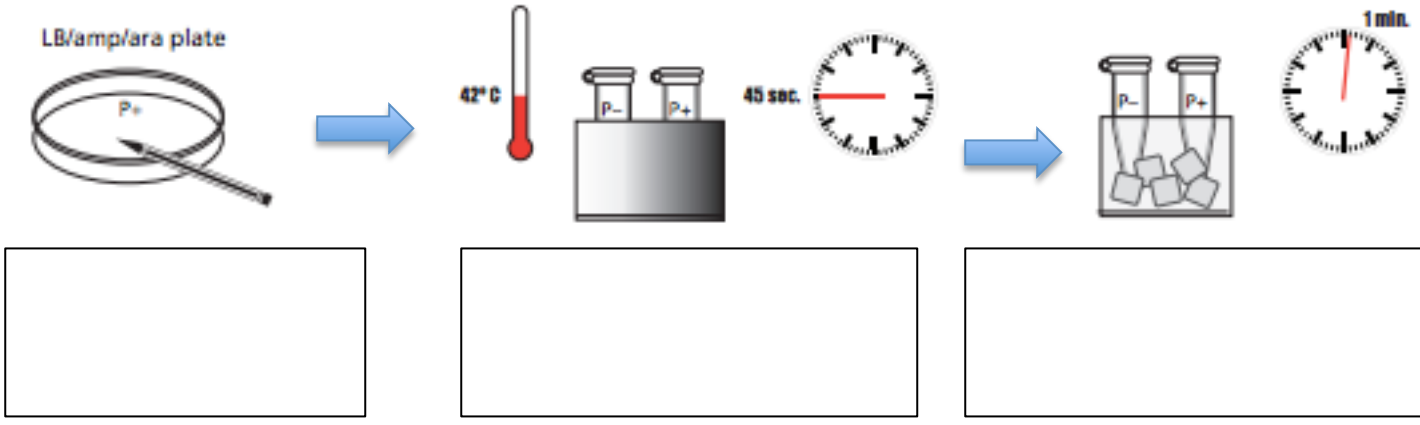
Methods: Finish the flowgram for Lab 4 by labeling each of the diagrams below:

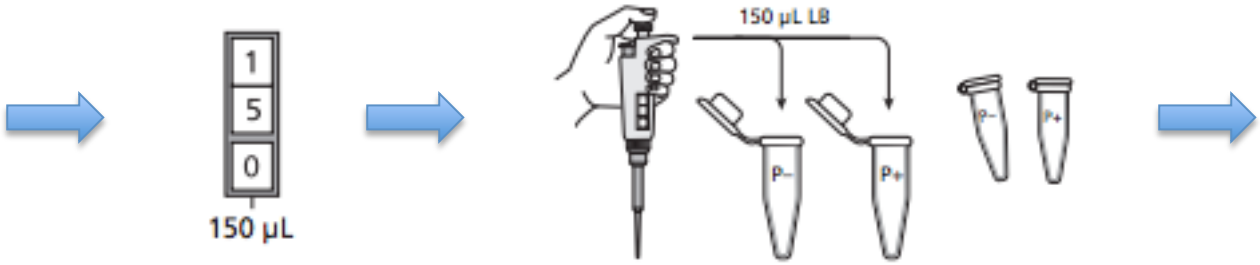


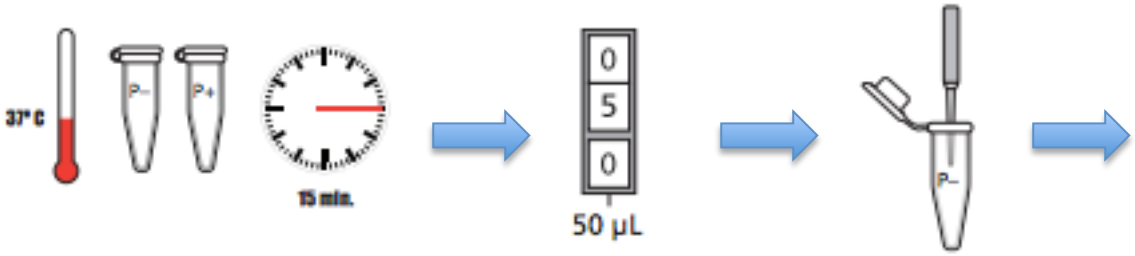

CC
50 µL CC
P–
P+
P–
P+
1
0
0
10.0 µL

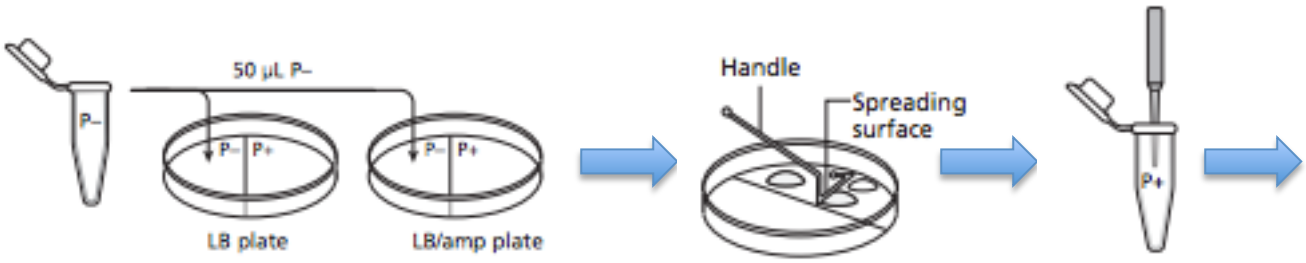

10.0 µL LIG
P+
P+
P+
P–
P+
15 min.

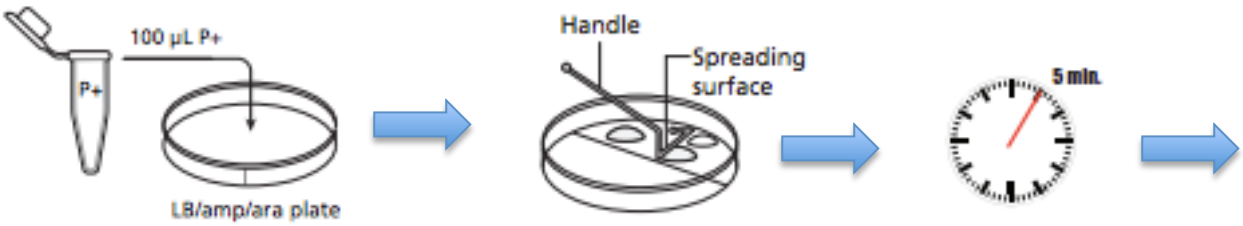

LB plate
LB/amp plate
LB/amp/ara plate
LB plate
LB/amp plate
P–
P+
P–
P+

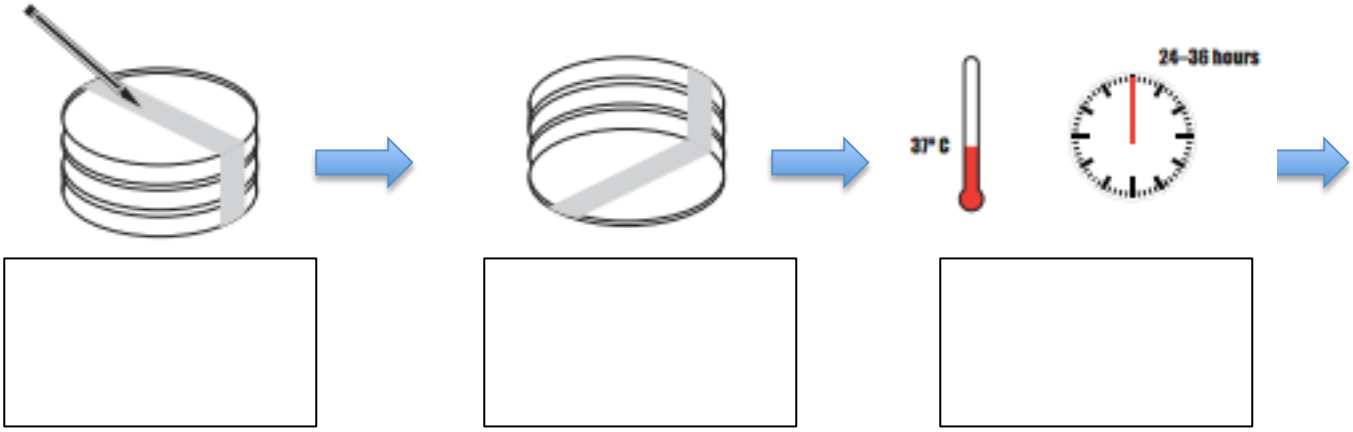

LB/amp/ara plate
P+
42° C
P–
P+
45 sec.
P–
P+
1 min.


1
5
0
150 µL
150 µL LB
P–
P+


37° C
P–
P+
15 min.
0
5
0
50 µL
P–


P–
50 µL P–
P–
P+
P–
P+
LB plate
LB/amp plate
Handle
Spreading surface
P+


P+
100 µL P+
LB/amp/ara plate
Handle
Spreading surface
5 min.


37° C
24–36 hours

BACTERIAL GROWTH PREDICTIONS – Complete this BEFORE doing the lab.

In Table 1: Predict how much bacterial growth you will see on each plate. Mark the plate/plate section with +++ (for high growth), ++ (for medium growth), + (low growth), or - (for no growth):

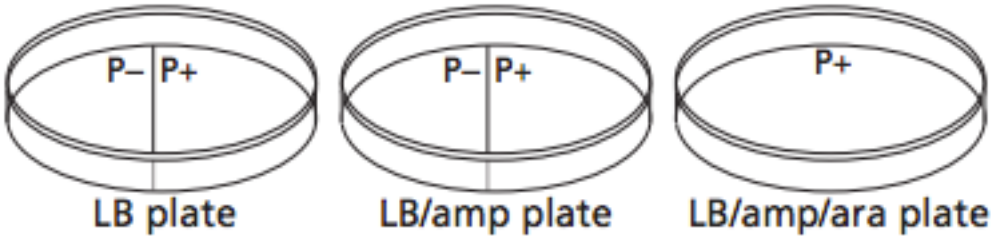


Table 1: P– Control Group (Non-Transformed Bacteria)

Plate Contains:	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)			
Luria Broth ampicillin (LB/amp)			

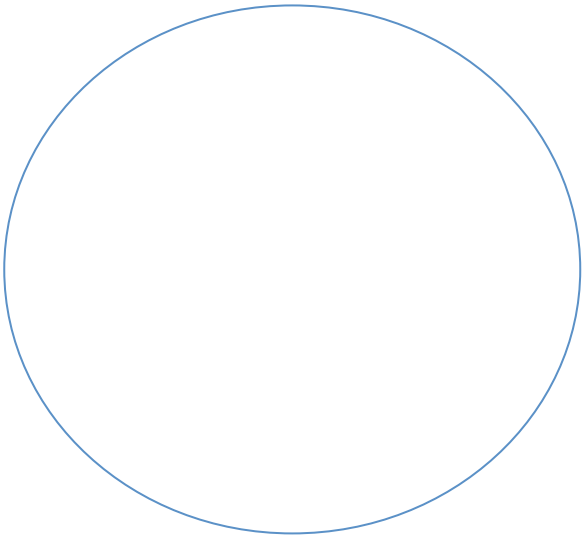
Table 2: P+ Experimental Group (Transformed Bacteria)

Plate Contains:	Predicted Growth	Conclusion If Predicted Growth Occurs	Conclusion If Predicted Growth Does Not Occur
Luria Broth (LB)			
Luria Broth ampicillin (LB/amp)			
Luria Broth ampicillin arabinose (LB/amp/ara)			

After the Lab Analysis Questions:

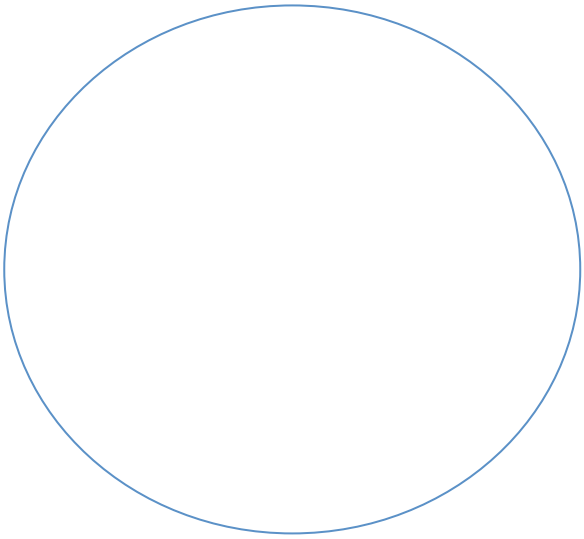
1. Observe your plates after incubation. Make an accurate drawing of each plate (or attach a good quality photograph). The following details should be represented in your drawing:
    a. Growth characteristics – colonies or bacterial lawn
    b. Number of colonies
    c. Color of colonies
    d. Shape of colonies

LB Plate



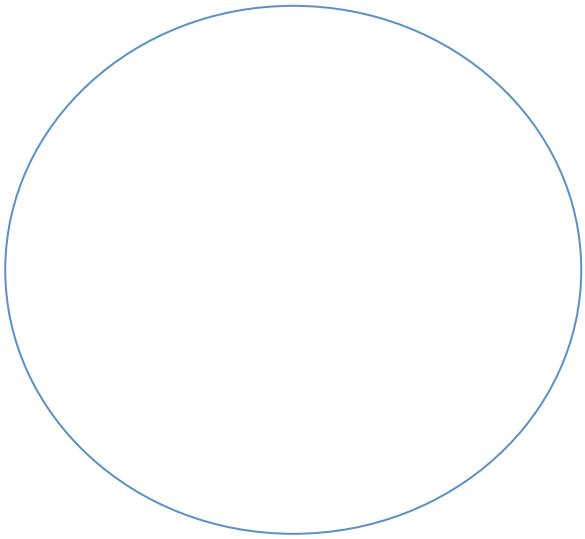
Approximate number of colonies: _____
(if present)

LB/amp Plate



Approximate number of colonies: _____
(if present)

LB/amp/ara Plate



Approximate number of colonies: _____
(if present)

Lab 5 Transformation Analysis

1. Look at the results of your transformation. Do your actual results match your predicted results? If not, what differences do you see, and what are some explanations for these differences? Be thorough in your explanation.

2. How many red colonies were present on your LB/amp/ara plate?

3. Why did the red colonies only appear on the LB/amp/ara plate and not the LB/amp plate?

4. Recombinant plasmids are engineered so that they can replicate in the cell independently of the chromosome replication. Why is it important to have multiple copies of a recombinant plasmid within a cell?

5. How is the information encoded in the rfp gene expressed as a trait? Be sure to use what you have previously learned about gene expression and the relationship between DNA, RNA, protein, and traits.

Lab 5 Bacterial Transformation Student Protocol

☐ **TEACHER PREP:** Thaw the tube of 500 uL frozen competent cells ("Comp Cells") 15 minutes before use. Keep on ice. It is best if you aliquot 100 uL Comp Cells (CC) per student group. Be sure to re-suspend the bacterial cells in the CC tube by **gently pumping** the pipette 2 times before dispensing to multiple tubes..

Cells can NOT be refrozen for another transformation use.
LAB TECHNIQUE NOTES: Wear gloves. Bacterial transformation requires sterile techniques and accurate timing of temperatures. Discard all tips and tubes that touched bacterial cells in the specially marked BIOWASTE containers. Hold all tubes at its rim, to prevent cells from warming up during your pipetting, and return tubes back on ice quickly.

☐ 1. Obtain a Styrofoam cup of crushed ice with the CC (Competent Cells) tube.

☐ 2. Label two clean tubes "P–" and "P+" and put on ice.

☐ 3. Set P-200 micropipette to 50 µL. Get new pipet tip and

☐ 4. Add 50 uL of re-suspended competent cells from the CC tube to the empty chilled P+ tube. Discard tip in the Biowaste cup.

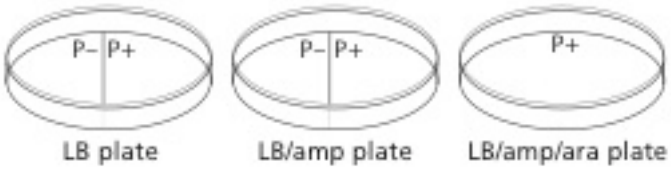
☐ 5. Get new tip. Add 50 uL of competent cells from the CC tube to P– tube. Discard tip in Biowaste.

☐ 6. Set P-20 pipette to 10.0 µL and get new tip. Add your LIG (your ligated plasmid from Lab 3) to the tube labeled "P+". Mix by gently pumping solution two times. Discard tip in Biowaste. Return the P+ tube to ice.

☐ 7. Keep the P– and P+ tubes on ***ice for 10-15 minutes***.

☐ 8. Write small and along the edge; Label your three agar Petri plates on the bottom of each plate (the part that contains the agar) with your group number and class period.

☐ With the plates closed, draw (on the bottom of plate) a line down the middle of the LB plate and the LB/amp plate. Label half of each plate "P–" and the other half "P+." Label the LB/amp/ara plate "P+." The plates will be arranged as follows:



☐ 9. After the incubation on ice, carry the P– and P+ tubes (in the cup of ice) to the ***42°C water bath***. Place the two tubes in the floating microfuge tube rack in the water bath for exactly ***45 seconds.***

☐ 10. After the heat shock, immediately place the tubes back on ice and leave them there for **at least** a minute.

☐ 11. Use P-200 and new tip, add 150 uL of LB broth to the P– tube. Re-suspend gently twice. Discard tip in Biowaste.

☐ 12. Get new tip, add 150 uL of LB broth to the P+ tube. Re-suspend gently twice. Discard tip in Biowaste.

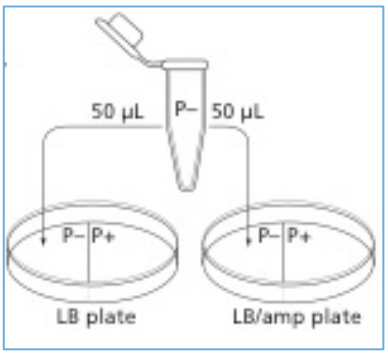
☐ Sit both P+ and P- tubes in microfuge rack at ***room temperature for 15 minutes***.

LAB TECHNIQUE: Use "Clamshell" method to open up agar plates. Keep plates closed as much as possible.
Decide if one partner will pipet the cell solution and the other partners spread cells onto petri dishes.

Lab 5 Bacterial Transformation Student Protocol (page 2)

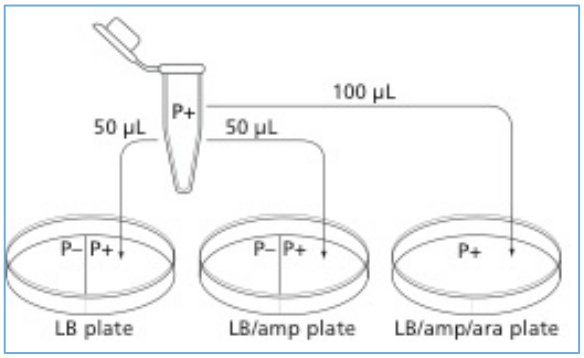
IMPORTANT **LAB TECHNIQUE: You MUST do the spreading of cells in the correct order of LB plate first, then LB/Amp plate second, then LB/Amp/Ara plate last. If you contaminate the tip or spreader, then get a new one.**

☐ 13. Set P-200 to 50 uL. Get new tip. Re-suspend cells twice, then transfer 50 uL cells from the **P–** tube onto the **P-** half of your **LB plate**. Use a sterile cell spreader to gently spread liquid evenly across the entire P– half of the plate.



☐ 14. Transfer 50 uL cells from **P-** tube onto the **P-** half of the **LB/amp plates**. Use same cell spreader to gently spread evenly across the entire P– half of the plate. Discard tip, tube and spreader into Biowaste.

☐ 15. Get new tip. Re-suspend cells gently twice inside the P+ tube. Transfer 50 uL cells from **P+** tube onto the **P+** half of the **LB plate** and then 50 uL cells from **P+** tube onto the **P+** half of **LB/amp plate**.



☐ 16. Use new spreader to gently spread evenly across the entire P+ half of each plate, but do the **LB plate first**, then do the LB/Amp plate.

☐ 17. Transfer *50 uL twice* (for total of 100 uL **P+** cells) onto the **LB/amp/ara plate**. Spread liquid evenly across entire plate, rotating the plate. Discard tip, tube and spreader into Biowaste container.

☐ 18. Allow all three plates to sit right side up for five minutes.

☐ 19. Using two pieces of thin masking tape about 3-4 inches long, tape all three plates together with the 3-stripe agar plate on bottom. Label tape with your group number and class period.

☐ 20. Place the plates in the 37°C incubator upside down to prevent condensation from dripping onto the gels. Note: The 3-stripe agar plate should now be at the top of the stack (as this is what your teacher will need to check tomorrow). Incubate the plates for ***24–36 hours at 37°C***.

☐ 21. Clean up your workspace. Make sure all microfuge tubes, pipette tips, and spreaders that touched cells/broth are in the biowaste bag. If there are unused spreaders, leave them in their bag and give back to teacher. Use disinfectant wipe to wipe off your laminated protocol sheet, tabletop, tube holders, pipette, anything that got contaminated.

☐ 22. Dump your station biowaste cup into the class biowaste autoclave bag. Spray disinfectant into the station biowaste cup. Remove your gloves in the proper manner and discard in biowaste bag.

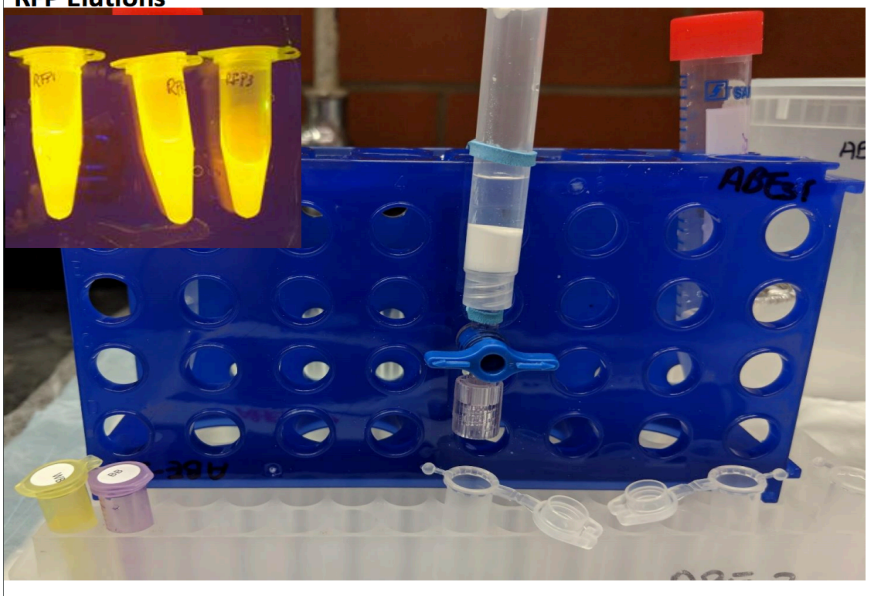
☐ 23. Examine the plates and record detailed observations in your notebook. Take photos and count the number of colonies (note colors) on each plate.

☐ 24. Discard the Petri plates in a sleeve for the biowaste autoclave bag when directed to do so.

Teacher Prep for Lab 6: Overnight Bacterial Culture

Set Up:

RFP Elutions



This is an update for those starting an overnight culture for mFP purification. This update describes what we believe is a more efficient method of producing overnight cultures expressing the mutant fluorescent protein, mFP. **NOTE: Start the culture 3 days before the date of Lab 6.**

Materials/Reagents

- 125 mL Sterile flask containing LB/*amp* broth. Volume will vary but should not exceed 75mL.
- Vented cap for sterile flasks (used for overnight shaking)
- Frozen cells (LMG 194) transformed with pARA-R
- Tube of sterile arabinose (500 mg/mL) final concentration should be 5mg/mL of LB/amp broth
- Shaking incubator

Procedure

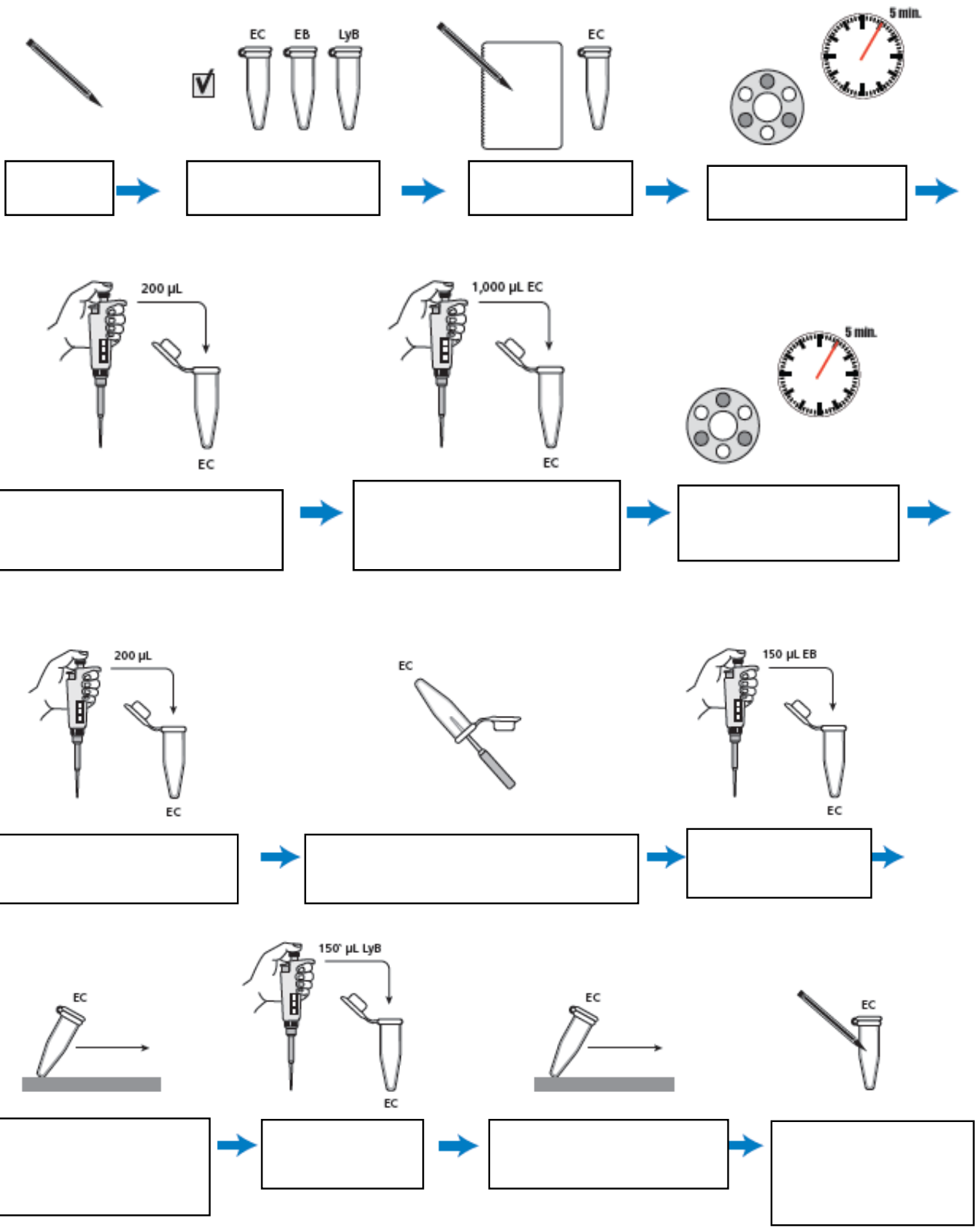
1. Aseptically, add 500 µL of transformed Lab 6 cells into the flask containing LB/amp broth.
2. Secure the vented cap to the flask. This will allow the culture to aerate while the culture is growing. (Be sure the **solid** top of the cap has been removed.)
3. Shake and incubate (300 rpm, 35◦C) the cells according to the directions on the incubator/shaker. Shake for 4-5 hours. Broth should become cloudy or there should be an indication that cells are growing.
4. Following the 4 to 5 hours of shaking, add the appropriate volume of sterile arabinose to the flask so the *final* concentration of arabinose is 5 mg/mL. Continue to shake until the culture becomes a deep pink/red color. This may take up to 72 hours. If the culture is ready early, store in the refrigerator until needed (sometimes the color intensifies).
5. Show (or take photo of) the original culture flask for students to record color.
6. Aliquot 1 mL of culture into microfuge tubes for students. To save time, centrifuge this and give the tube centrifuged tubes to students.
7. Students will discard broth. Dispense another 1 mL of culture liquid on top of pellet. Centrifuge and discard liquid broth.

Rationale

The original protocol has been modified as a result of observing overnight cultures that were not resulting in strong protein expression. It was discovered that the bacteria were somehow restricting the expression of the *rfp* gene after several generations of growth. This drop in expression seems to be an intermittent problem, but negatively impacts mFP purification (Lab 6) when it does occur.

It has been demonstrated that this problem can be circumvented by growing up the culture initially in LB/amp broth before exposing the cells to arabinose. If cells are taken from a red colony directly from an LB/amp/ara plate and used for this overnight culture, the inoculum sometimes contain cells that have managed to repress *rfp* expression. These cells will grow faster than those who are expressing the gene and the overnight culture will contain a mixture of these cells, most of which are not producing mFP. It has also been discovered that if we inoculate LB/amp/ara broth with cells, never exposed to arabinose, but transformed with pARA- R, a portion of these cells will stop expressing rfp at a fairly early point in the growth. This, too, will yield a mix of cells and a poor yield of mFP. By allowing the transformed cells to grow in the absence of arabinose for 4-5 hours before exposing them to arabinose, a greater yield of mFP will result.

Lab 6 Part A: Lysis of Bacterial Culture - FLOW CHART (1 page)



Lab 6 Part A: Lysis of Bacterial Culture Student Protocol

☐ 1. Check your rack for EC (tube of cultured E coli cells), EB (elution buffer), LyB (Lysis buffer).

☐ 2. Examine the EC tube and record its color in your notebook.

☐ 3. Before you can lyse the cells, you will need to separate the cells from the growth *medium.* To do this, spin the EC tube in the microcentrifuge for five minutes (remember to have balanced tube configuration in centrifuge).

☐ 4. Carefully remove the EC tube from the microcentrifuge to avoid disturbing the solid pellet that contains the bacterial cells.

☐ 5. Set P-200 micropipette to 200 µL and use new tip. Carefully remove the supernatant (liquid) without disturbing the cell pellet. (Dispense the supernatant into the Biowaste beaker.)

☐ 6. Bring the EC tube to your teacher, who will add another 1,000 µL (1 mL) of the LB/ amp/ara culture of *E. coli* to your EC tube.

☐ 7. Centrifuge the tube for 5 minutes and remove the liquid into biowaste beaker.
STOP AND THINK: What color is the supernatant? The pellet? What are the contents of each?

☐ 8. Use P200 and use the micropipette to remove as much of the liquid as you can without touching the cell pellet.
LAB TECHNIQUE: Be sure to use a new micropipette tip for each reagent to avoid contamination.

☐ 9. Add 150 µL of EB to the cell pellet in the EC tube.

☐ 10. Close the cap of the EC tube tightly. Drag the tube vigorously across the surface of the microfuge tube rack two times to mix.

☐Examine the EC tube carefully. If there are visible clumps of cells, mix again.

☐ 11. Add 150 µL of LyB to the EC tube. Close EC tube tightly and **flick tube gently with finger, but not too vigorously.**

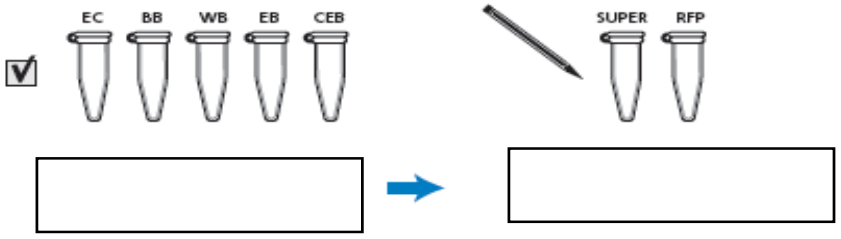
☐ 12. Label the EC tube with your group number and class period and give it to your teacher. Your teacher will incubate the cells at room temperature overnight.

☐ 13. Pour biowaste beaker contents into the biowaste bag. Use disinfecting wipe to wipe outside of beaker to catch any leaks, spray disinfectant or 10% bleach into beaker, sit for at least 10 minutes before washing in sink. Clean lab table with disinfecting wipe. Take gloves off and throw into biowaste bag.

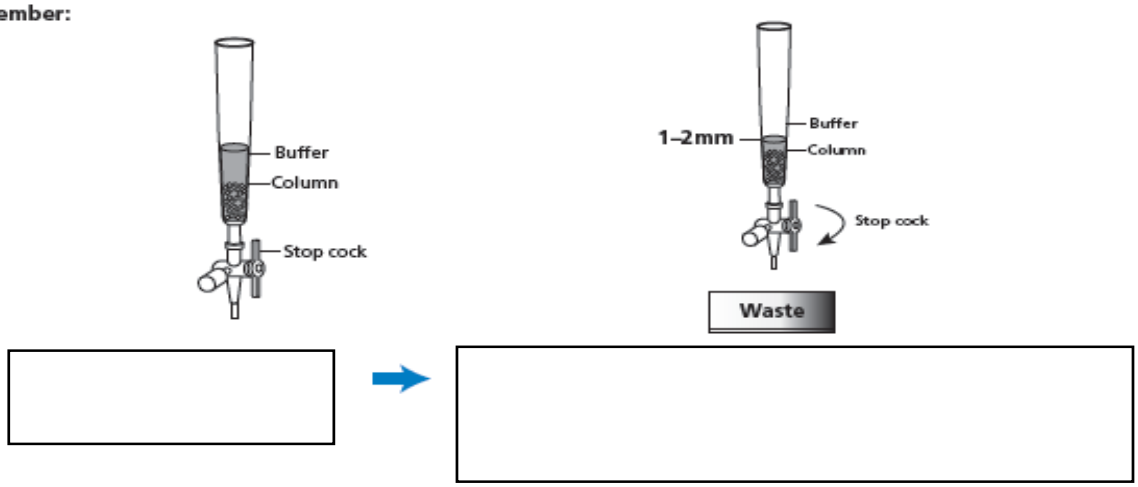
Lab 6 Part B: Column Chromatography - FLOW CHART

3 Students in Assigned Tasks (Page 1 of 2)

One group member:



One group member:



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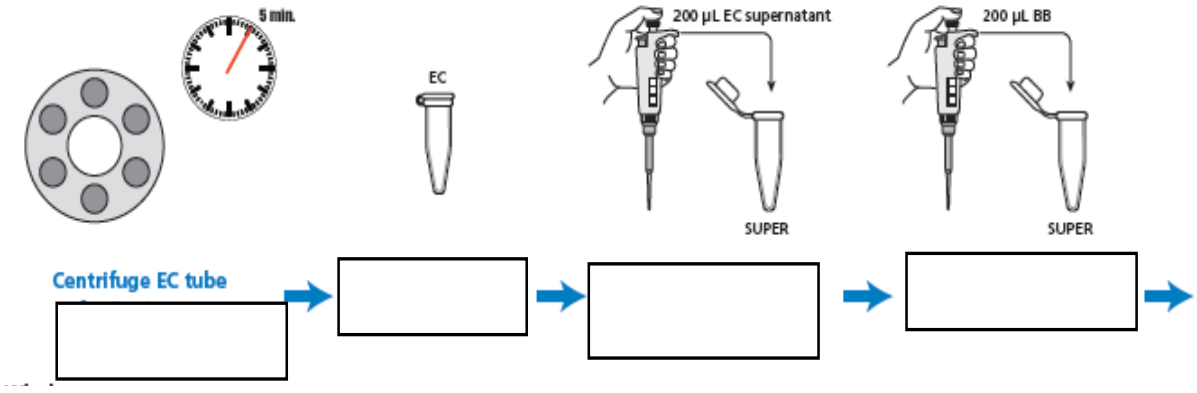
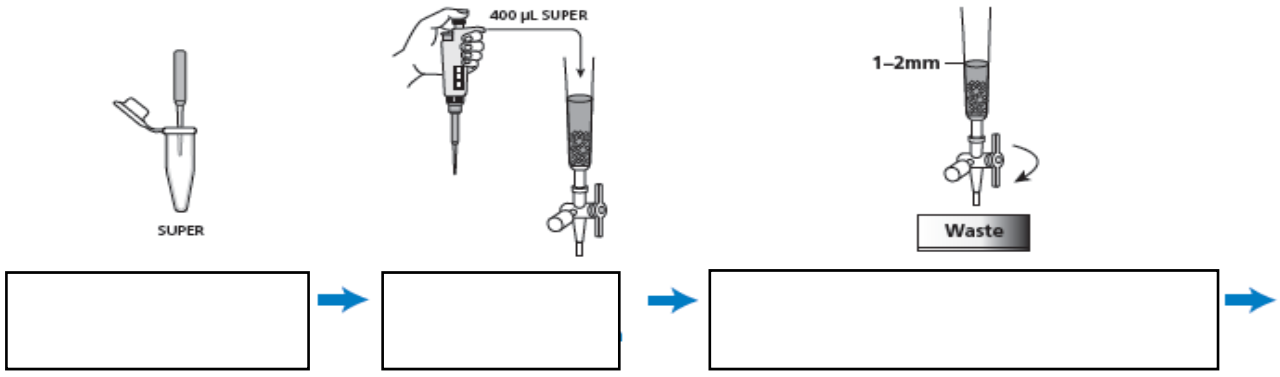
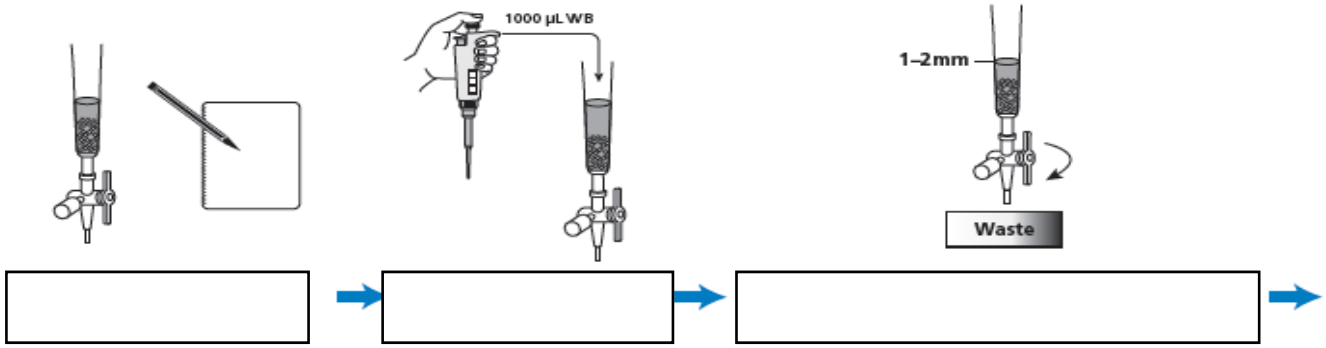
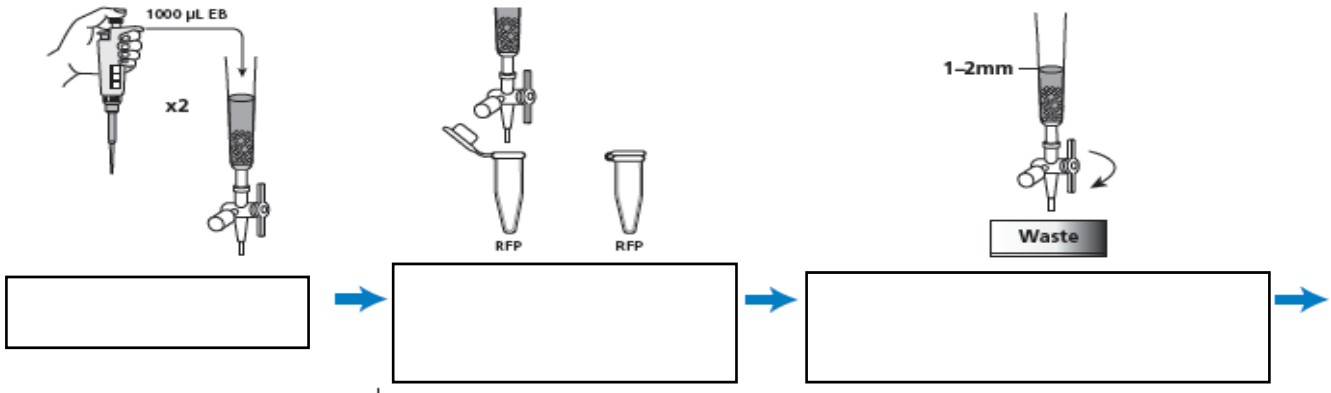


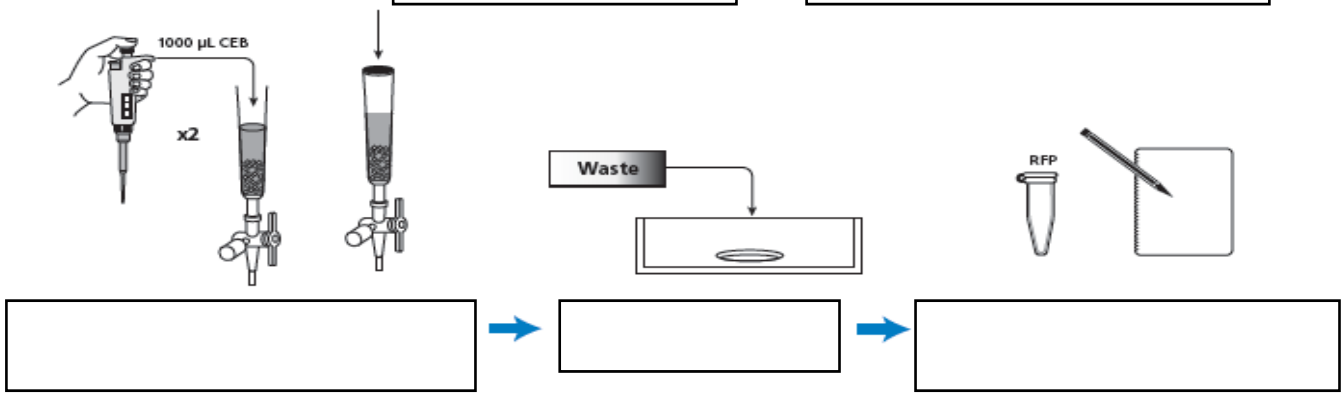
Lab 6 Part B: Column Chromatography - FLOW CHART (Page 2 of 2)

Whole group:









Lab 6B: Chromatography Protocol for 2 students (continue from Part A) – page 1 of 2

Pre-Lab

- ☐ Watch Videos of Hydrophobic Column Chromatography Concept and Lab Tips.
- ☐ Fill out flow chart.
- ☐ Assign tasks. Have one person make sure that your materials are ready (steps 2–3), another person set up the chromatography column (steps 4–5), and another person spin the lysed cells and remove the supernatant (step 6–7).

IMPORTANT LAB TECHNIQUE: Do not allow the column to run dry or disturb the resin bed.

STUDENT ONE	**STUDENT TWO**
Prepare Cell Lysate Supernatant: ☐ Obtain your EC tube (from Part A) ☐ Balance with another team's EC tube to spin the tubes in the high-speed microcentrifuge for five minutes to create a pellet of the cell debris. ☐ Examine the tube. You should see a supernatant and a solid pellet. ☐ **Note colors of the supernatant and pellet.** ☐ Label a new tube "SUPER". ☐ Using the P-1,000 pipette, carefully remove 200 µL of the EC supernatant (without disturbing the cell debris pellet), and transfer the liquid into your clean SUPER tube. If you dislodge the debris pellet, you will have to centrifuge again. ☐ Close the cap of the tube with pellet and discard tube into Biowaste cup/bag. ☐ Using a new tip, add 200 µL of Binding Buffer (BB) to the SUPER tube. ☐ Mix contents by gently pumping the solution in and out OR Closing cap and flicking tube bottom with your finger.	**Check for these reagents:** ☐ Waste cups for liquid and tips ☐ P-1,000 micropipette ☐ Tip box of 1000 uL pipette tips ☐ Binding buffer (BB) ☐ Wash buffer (WB) ☐ Elution buffer (EB) ☐ Column equilibration buffer (CEB) ☐ Label new tube "RFP" **Prepare Chromatography Column** ☐ Obtain the Chromatography column and remove both top and bottom caps (store in safe place). ☐ Clip upright column onto stand. ☐ Add tip to bottom of column. ☐ Place white laminated sheet behind column. ☐ Set the waste cup under the stopcock. ☐ Carefully open the column by turning the stopcock valve, and allow the ethanol storage liquid to drain into the waste cup. ☐ Close the valve once there is about 1–2 mm of liquid left above the resin bed ☐ **Equilibrate the column:** ☐ Add 1 mL Column Equilibration Buffer (CEB) carefully down the side of the column to avoid disturbing the surface of the resin bed. ☐ Open valve to drain the liquid from the column. ☐ Add another 1 mL CEB onto the column. ☐ Close the valve when 1-2 mm liquid is left above the resin bed.

WORK TOGETHER

☐ **Add the Supernatant into the Column:**

- ☐ After the Column Equilibration buffer has drained from the column, carefully pipet 400 µL of the SUPER tube (supernatant and Binding Buffer mixture) down the side of the chromatography column.
- ☐ Open the valve and drain into the waste cup, until 1–2 mm of liquid is left above the resin bed.
- ☐ Examine the column and locate the red fluorescent protein.
  Is it spread throughout the resin bed, or does it appear to be restricted to a single band?
  Record your observations in your notebook.

☐ **Add Wash Buffer to the Column:**

- ☐ Using a new tip, add 1,000 µL (1 mL) of WB gently down the side of the chromatography column.
- ☐ Open the valve and drain into the waste cup, until 1–2 mm of liquid is left above the resin bed.
- ☐ Examine the column and locate the red fluorescent protein.
  Has the location of the red fluorescent protein changed in the resin bed?

☐ **Add Elution Buffer to the Column:**

- ☐ Using a new tip, add 1 mL of EB down the side of the chromatography column. Add 1 mL EB again.
- ☐ Place a white laminated sheet behind the column.
- ☐ Add a tip to the bottom of the column (if desired). This gives you more time to determine the eluate color and to collect only the pinkest drops.
- ☐ Open the valve and drain into the waste cup until you see the RFP (pink liquid) **almost ready** to fall off the column. Close the valve.
- ☐ Get new microfuge tube ready to collect the concentrated pink liquid (RFP) droplets.
- ☐ Open the valve and drain/collect only the RFP (pinkest liquid drops) into the microfuge tube.
- ☐ Close the valve, cap and label the microfuge tube when done.
- ☐ Set the waste cup back under column. Drain the rest of the clear eluate to drain into the waste cup.

☐ **Add Ethanol Storage Liquid to the Column (if you are the last class to use the columns):**

- ☐ Add 1 mL 20% Ethanol to the chromatography column to prepare it for the next school.

☐ **OR Add Equilibration Buffer to the Column** (if another class will use the columns at your school):

- ☐ Add 2 mL Column Equilibration buffer to the column.

☐ **Clean Up the Column:**

- ☐ Remove the bottom pipet tip (if used) and give to teacher.
- ☐ Attach both the top cap and bottom cap of the column tightly.
- ☐ Tear down the stand and clips for storage.
- ☐ Place the capped column upright in the storage container.
- ☐ Pour the contents of the waste cup down the sink drain and rinse.
- ☐ Be sure all of the buffer bottles are closed tightly.

☐ **Compare your RFP tube** with class (place all tubes on white paper, blue light or uV transilluminator).

- ☐ Take a photo of all tubes together. Is there a difference in intensity of color from sample to sample?
- ☐ Record your observations in your notebook. Troubleshoot and discuss any steps that you may have performed incorrectly.

Lab 6 Part B: Column Chromatography Starting with Supernatant

Assign tasks. Have STUDENT ONE set up the chromatography column and STUDENT TWO prepare the supernatant.

IMPORTANT LAB TECHNIQUE: Do not allow the column to run dry or disturb the resin bed.

STUDENT ONE

☐ 1. **Set up your chromatography column** on the stand with the clip as directed by your teacher. Be careful not to dislodge the stopcock attached to the lower portion of the tube.

☐ 2. **Prepare the column:**

- ☐ Set the liquid waste cup under the stopcock.
- ☐ Carefully open the column by turning the stopcock valve, and allow the ethanol storage liquid to drain into the waste cup.
- ☐ Close the valve once there is about 1–2 mm of liquid left above the resin bed, so that the liquid is not draining from the column into the waste cup.

☐ 3. **Equilibrate the column:**

- ☐ Add 2 mL Column Equilibration Buffer (CEB) carefully down the side of the column to avoid disturbing the surface of resin bed.
- ☐ Open the stockcock valve to drain the liquid from the column.
- ☐ Close the valve when 1-2 mm liquid is left above the resin bed.

STUDENT TWO

☐ 1. **Check for all of the reagents listed.**

- Binding buffer (BB)
- Wash buffer (WB)
- Elution buffer (EB)
- Column equilibration buffer (CEB)

☐ 2. **Check for these supplies:**

- Tube of 200 uL Supernatant "SUPER"
- two clean 1.5-mL microfuge tubes
- Waste cup(s) for liquid and tips
- White laminated sheet
- P-1,000 pipette and box of 1000 uL tips
- OR disposable transfer pipets

☐ 3. **Prepare the SUPERNATANT:**

- Add 200 µL of BB to the SUPER tube. Mix by gently pumping twice.

WORK TOGETHER

☐ 4. **Add the Supernatant into the Column:**

- ☐ After the Column Equilibration buffer has drained from the column, carefully pipet 400 µL of the SUPER tube (supernatant and Binding Buffer mixture) down the side of the chromatography column.
- ☐ Open the valve and drain into the waste cup, until 1–2 mm of liquid is left above the resin bed.
- ☐ Examine the column and locate the red fluorescent protein. Is it spread throughout the resin bed, or does it appear to be restricted to a single band? Record your observations in your notebook.

☐ 5. **Add Wash Buffer to the Column:**

- ☐ Using a new tip, add 1,000 µL (1 mL) of WB gently down the side of the chromatography column.
- ☐ Open the valve and drain into the waste cup, until 1–2 mm of liquid is left above the resin bed.
- ☐ Examine the column and locate the red fluorescent protein. Has the location of the red fluorescent protein changed in the resin bed?

☐ 6. **Add Elution Buffer to the Column**:

- ☐ Using a new tip, add 1,000 µL of EB twice (2 mL total), gently, down the side of the chromatography column.
- ☐ Place a white laminated sheet (or paper behind the column. Add a tip to the bottom of the column (if desired).
- ☐ Open the valve and drain into the waste cup until you see the RFP (pink liquid) almost ready to fall off the column. Close the valve.
- ☐ Get 1-2 new microfuge tube(s) ready to collect the concentrated pink liquid (RFP) droplets.
- ☐ Open the valve and drain the RFP into the tube. Close the valve, cap and label the tube when done.
- ☐ Set the waste cup back under the stopcock. Drain the rest of the eluate to drain into the waste cup.

☐ 7**. Add Ethanol Storage Liquid to the Column:**

- ☐ Add 1 mL 20% Ethanol to the chromatography column to prepare it for the next school.

☐ 8. **Clean Up the Column:**

- ☐ Remove the bottom pipet tip (if used), cap both the top and bottom of the column tightly.
- ☐ Tear down the stand and clips for storage. Stand the capped column upright in the storage container.
- ☐ Pour the contents of the waste cup down the sink drain. Be sure all of the buffer bottles are closed tightly.

☐ 9. **Compare your RFP tube** with class (on white paper, blue light or uV transilluminator).
Is there a difference in intensity of color from sample to sample? Record your observations in your notebook.

Lab 6 Part B: Chromatography from Supernatant