LAB 2 RESOURCES

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

LAB 2A KIT ITEMS	LABELS	VOLUME
P-20 micropipette		--
P-20 pipette tips		--
Minicentrifuge		--
Water bath set to 37°C and thermometer		--
FREEZER BOX ITEMS		
pARA-R (2a concentration)	**pARA-R 2a**	10uL per group
BamH1 enzyme	**BamHI**	5uL per group
HindIII enzyme	**HindIII**	5uL per group
2.5x Restriction buffer	**2.5X Rest**	10uL per group

Notes: Mix BamHI and HindIII (1:1 ratio) and label as RE

Lab2A and 4A:
NEW Pre-stain gel Method: Spin the SYBR safe DNA gel stain tube and then mix with your pipette before aliquoting it into melted agarose solution. Pipet 15µL of the SYBR safe stain (from the vial) into 150mL of melted agarose just before pouring your gel. Gently, swirl the melted agarose to mix the SYBR safe. Please put return all unused stock of SYBR safe (amber tube). We will need the stock tube for the next kit cycle.
Make sure to always keep the SYBR safe away from light.

Post Stain Method: We have also included the SYBR safe stain (in the bottle) for post staining if you prefer to keep using this staining method.

Diluting 20x SB Buffer to 1x SB buffer---- Mix 9mLs of 20x SB Buffer with 171 mLs of deionized water
You can find this in the ABE Teacher Guide on page OV-30

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

Lab 2A

Kit Materials:

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

Aliquoting:

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

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

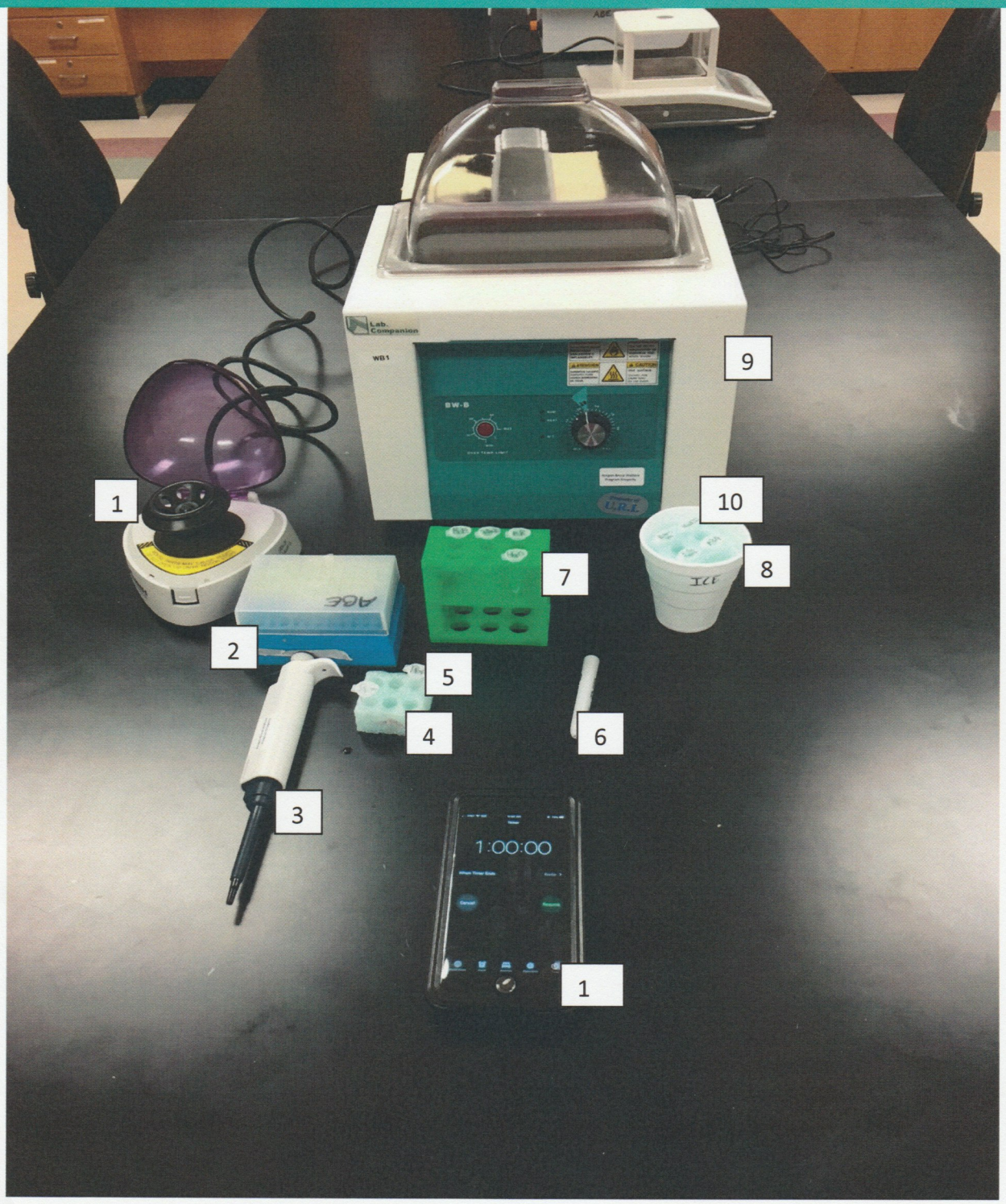
Label Tube	Contents	Aliquot	Actually Use
2A	pARA	10 uL	8 uL
RE	BamHI and HindIII	5 uL	4 uL
2.5xB	2.5x Restriction buffer	10 uL	8 uL
dH20	Distilled water	1000 uL	4 uL

Restriction Digest

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

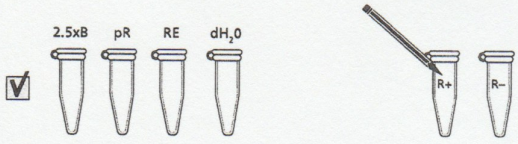
- Calibrate water bath to 37˚C the day before the lab to ensure temperature is correct for the restriction digest of student samples (60 minutes).
- Do not leave the digest in the water for over 2 hours, as BamHI will begin to cut DNA randomly.
- Store digested samples at -20˚C until you are ready to run the ligation protocol (Lab 3).
- Please empty out and wipe down the water baths before returning.

Lab 2A



1. Mini-centrifuge
2. P20-200 pipette tips
3. P-20 pipette
4. Green microfuge tube float holding tubes labeled **R+** and **R-**
5. Microfuge tubes
6. Sharpie marker
7. Microfuge tube rack holder holding tubes labeled **2.5xB**, **pR**, **RE**, **dH_2O (** should be aliquoted by **teacher**)
8. Cup to hold ice
9. Water bath (set to 37°C for this specific experiment)
10. Microfuge tubes that **must** be on ice containing either of the following: **BamH1, HindIII, pARA 2A & 2.5x restriction buffer**
11. Timer **(WE DO NOT PROVIDE!)**

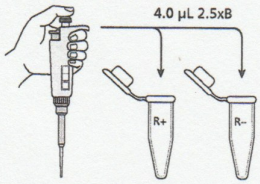
Laboratory 2A Flowchart



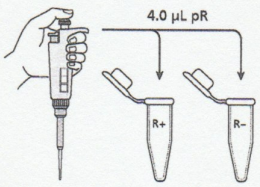
Check reagents (2.5xB, pR, RE, dH_2O)

Label 2 tubes R+ and R–

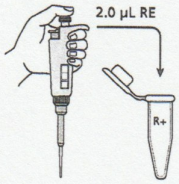
Review Table 2A.2



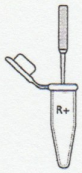
Pipette 4.0 µL 2.5xB into R+ and R– tubes



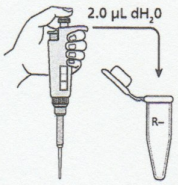
Pipette 4.0 µL pR into R+ and R– tubes



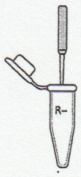
Pipette 2.0 µL RE into R+ tube



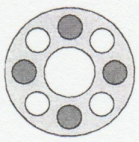
Use pipette to mix reagents in the R+ tube



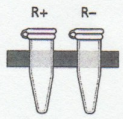
Pipette 2.0 µL dH_2O into R– tube



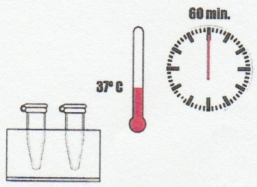
Use pipette to mix reagents in the R– tube



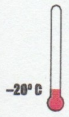
Centrifuge the R+ and R– tubes



Place tubes in floating microfuge tube rack



Teacher will: Place R+ and R– tubes into 37°C water bath and incubate for 60 minutes



Teacher will: Place the R+ and R– tubes in the freezer at –20°C