LAB 2 RESOURCES

ATTENTION TEACHERS:

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

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

LAB 2A KIT ITEMS	LABELS	VOLUMES to aliquot
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**	3µL-4µL per group (**note this is for RE, both BAM HI and HIND III combined)**
HindIII enzyme*	**HindIII**	3µL-4µL per group (**note this is for RE, both BAM HI and HID III combined)**
2.5x Restriction buffer	**2.5X Rest**	12 µL per group

Notes: *Mix BamHI and HindIII (1:1 ratio) and label as RE ie 24 µL of Bam HI and 24µLof HindIII then aliquot 4 µL into a microcentrifuge tube to each group. Each group will only use 2 µL.

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.

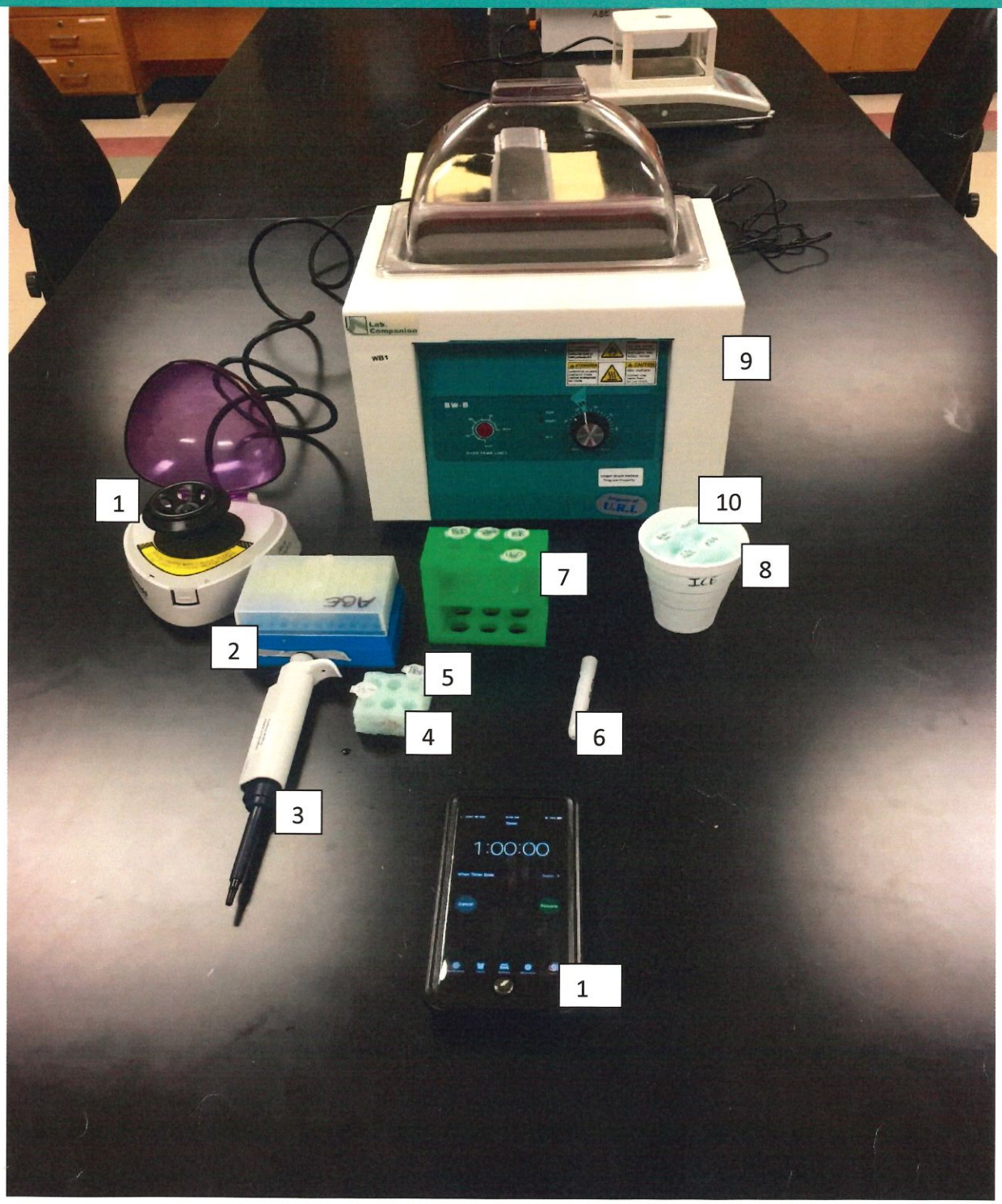
Label Tube	Contents	Aliquot	Actually Use
2A (RP)	pARA	10 uL	4 uL
RE	BamHI and HindIII	3-4uL	2 uL
2.5xB	2.5x Restriction buffer	12 uL	10 uL
dH20	Distilled water	1000 uL	2uL

Restriction Digest

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

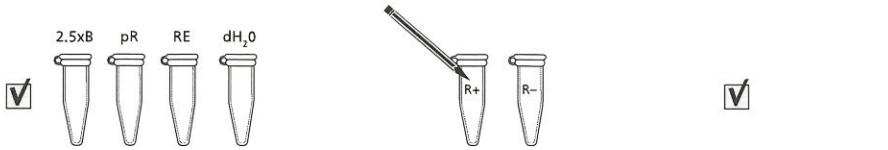
- Calibrate water bath to 37℃ the day before the lab to ensure temperature is correct for the restriction digest of student samples (60 minutes).
- Do not leave the digest in the water for over 2 hours, as BamHI will begin to cut DNA randomly.
- Store digested samples at -20℃ until you are ready to run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

Lab 2A

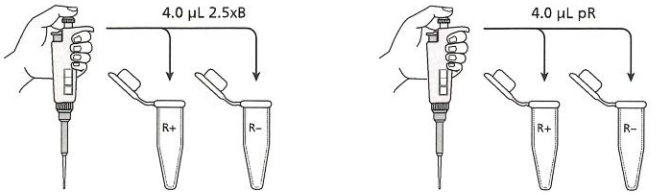


1. Mini-centrifuge
2. P20-200 pipette tips
3. P-20 pipette
4. Green microfuge tube float holding tubes labeled **R+** and **R-**
5. Microfuge tubes
6. Sharpie marker
7. Microfuge tube rack holder holding tubes labeled **2.5xB**, **pR**, **RE**, **dH_2O (** should be aliquoted by **teacher**)
8. Cup to 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 buff**
11. Timer **(WE DO NOT PROVIDE!)**

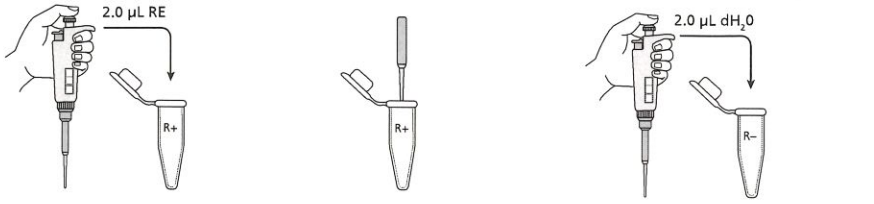
Laboratory 2A Flowchart



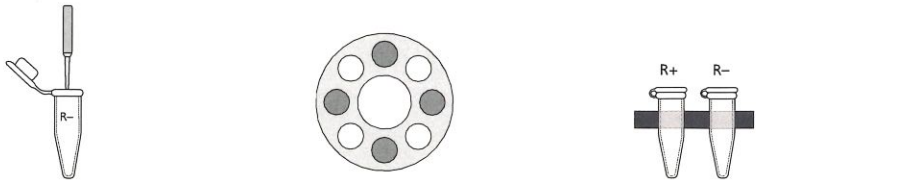
Check reagents (2.5xB, pR, RE, dH_2O) → Label 2 tubes R+ and R– → Review Table 2A.2 →



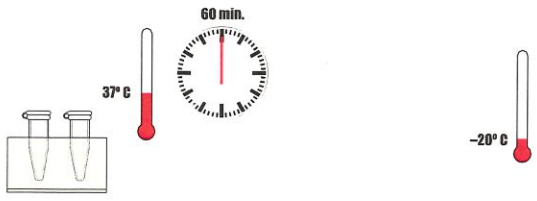
Pipette 4.0 µL 2.5xB into R+ and R– tubes → Pipette 4.0 µL pR into R+ and R– tubes →



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