

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.

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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), dH₂O, 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, 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
dH ₂ O	Distilled water	1000 uL	2uL

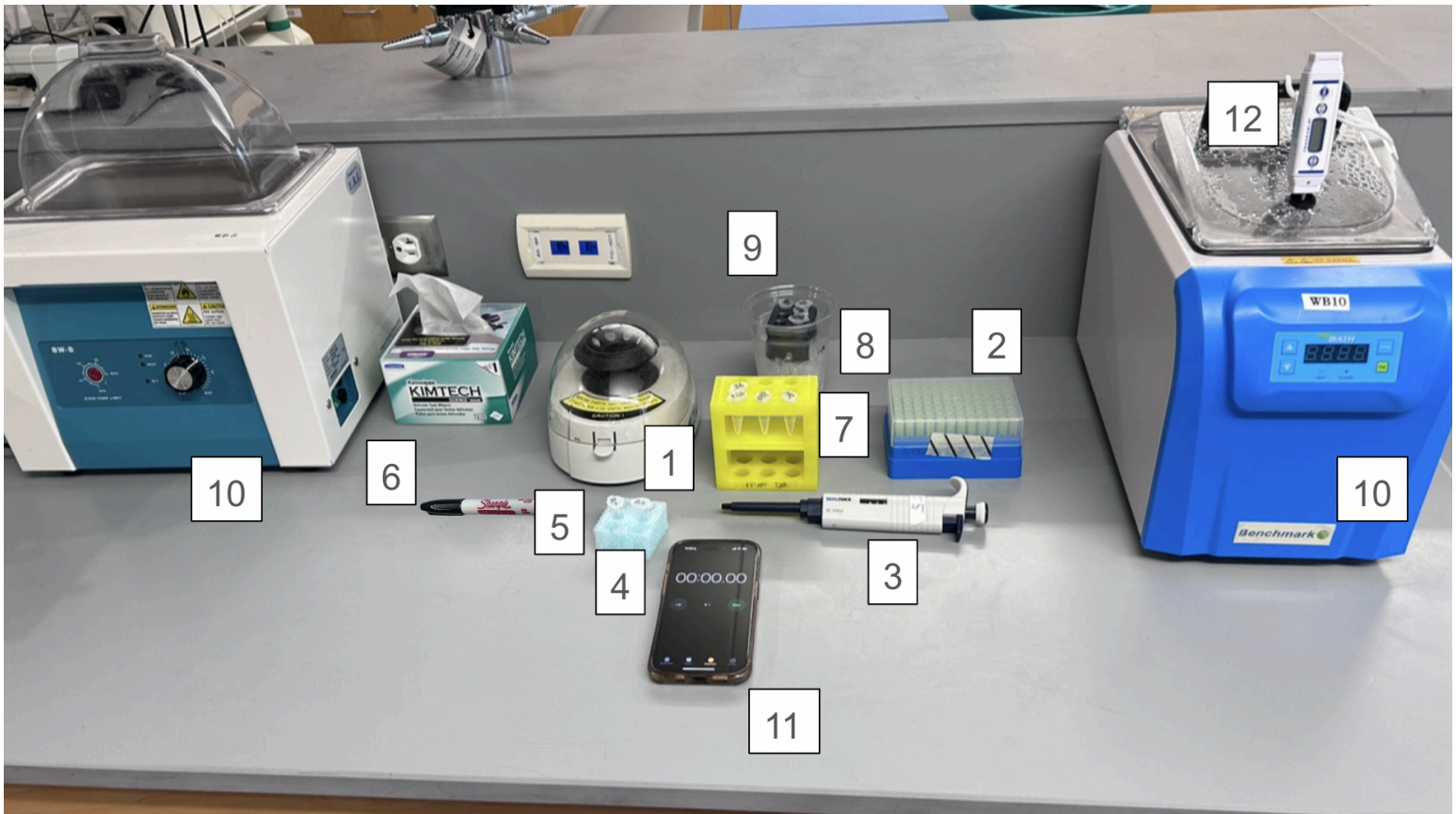


Restriction Digest

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**, **2A (RP)**, **RE**, **dH₂O** (should be aliquoted by teacher)
8. Microfuge tubes that must be on ice containing either of the following: **BamHI**, **HindIII**, **pARA 2A** & **2.5x restriction buffer**
9. Small gray cool rack
10. Water bath (set to 37°C for this experiment) (both types that may be provided in kits are being displayed)
11. Timer (**WE DO NOT PROVIDE!**)
12. Thermometer

Laboratory 2A Flowchart

