Amgen Biotech Experience
Aliquoting Guide

We have provided sufficient reagents for the number of students you indicated on the Materials Request form, including a bit extra for pipetting errors. All solutions should be aliquoted into the provided 1.5 mL microcentrifuge tubes, unless otherwise indicated.

Laboratory 1. An Introduction to Microvolumetrics and Pipetting

Label tube	Contents of tube	Aliquot	Actually used
S1	Solution 1 - blue & purple dye	12µL	10µL
S2	Solution 2 - blue, purple & yellow dye	12µL	10µL
S3	Solution 3 - purple dye	12µL	10µL

Laboratory 2. Restriction Analysis of pARA and pKAN-R

Label tube	Contents of tube	Aliquot	Actually used
A	pARA (80ng/µL)	10µL	8µL
K	pKAN-R (80ng/µL)	10µL	8µL
RE	*Bam*H I and *Hind* III	5µL	4µL
2.5xB	2.5x restriction buffer	20µL	16µL
*dH_2O	Distilled water	1000µL	4µL

*dH_2O is used for several labs.

Laboratory 2a. pARA-R Restriction Digest: An Introduction to Plasmids and Restriction Enzymes

Label tube	Contents of tube	Aliquot	Actually used
RP-2a	pARA-R (70ng/µL)	10µL	8µL
RE	*Bam*H I and *Hind* III	3µL	2µL
2.5xB	2.5x restriction buffer	10µL	8µL
*dH_2O	Distilled water	1000µL	2µL

Laboratory 3. Ligation of pARA and pKAN-R Restriction Fragments

Label tube	Contents of tube	Aliquot	Actually used
5xB	5x ligation buffer	4µL	3µL
**LIG	T4 DNA ligase	2µL	2µL
*dH_2O	Distilled water	1000µL	2µL

**NOTE: Students work directly in the LIG tube, do not aliquot any extra.

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Laboratory 4. Confirmation of Restriction and Ligation

Label tube	Contents of tube	Aliquot	Actually used
M	1 kb ladder	10µL	10µL
LD	Loading dye	12µL	10µL
*dH_2O	Distilled water	1000µL	19µL

Reminder: The ladder already contains loading dye. Also, Solution 2 (Lab 1) is the same as loading dye.

Laboratory 4a. Confirmation of pARA-R Restriction Digest

Label tube	Contents of tube	Aliquot	Actually used
M	1 kb ladder	10µL	10µL
LD	Loading dye	6µL	4µL

Reminder: The ladder already contains loading dye. Also, Solution 2 (Lab 1) is the same as loading dye.

Laboratory 5. Transformation of *E. coli* with Recombinant Plasmid

Label tube	Contents of tube	Aliquot	Actually used
LB	Luria Broth	325µL	300µL
CC***	Competent Cells	100µL	100µL

Students will use the "Ligase" tube that was prepared in Lab 3; it contains 10 µL of their recombinant plasmid and you will *not* have to label or aliquot these.
***We recommend having students bring their empty tubes on ice to you to receive the competent cells directly into their working tubes (50µL each to P- and P+) instead of aliquoting them before class. The cells perform best if they are used immediately after they are thawed.

Laboratory 5a. Transformation of *E. coli* with pARA-R

Label tube	Contents of tube	Aliquot	Actually used
RP-5a	pARA-R (10ng/µL)	12µL	10µL
LB	Luria Broth	325µL	300µL
CC***	Competent Cells	100µL	100µL

***We recommend having students bring their empty tubes on ice to you to receive the competent cells directly into their working tubes (50µL each to P- and P+) instead of aliquoting them before class. The cells perform best if they are used immediately after they are thawed.

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PCR Laboratory

NOTE: You must mix the primers with the MasterMix ***no more than three weeks before*** the PCR laboratory is going to be performed. Thaw the MasterMix and primers, and use as soon as they have thawed. Briefly vortex and flash spin both tubes before mixing. Once MasterMix and primers have been combined, vortex and flash spin before aliquoting. Aliquot on wet ice and immediately refreeze.

You will mix a ratio of 10μL of primers to 12.5uL of MasterMix. You will need to calculate how much to mix using the following formulae:
1. Total Quantity Needed = 120μL x # lab groups
2. Primer Needed = [Total Quantity Needed/22.5μL] x 10μL
3. MasterMix Needed = [Total Quantity Needed/22.5 μL] x 12.5 μL

For example, if I have 40 lab groups I would do the following:
1. Total Quantity Needed = 120μL x 40 = 4800μL
2. Primer Needed = [4800μL/22.5μL] x 10μL = 213.33 x 10μL = 2,133.3μL primer
3. MasterMix Needed = [4800μL/22.5μL] x 12.5μL = 213.33 x 12.5μL = 2,666.67μL MasterMix

We provide enough extra primer and MasterMix for you to aliquot the amounts shown below.

Part A: Performing PCR

Label tube	Contents of tube	Aliquot	Actually used
PCR	MasterMix with Primers (see note above)	120µL	92µL
+	pARA-R plasmid	3µL	2µL
-	pARA plasmid	3µL	2µL

NOTE: The DNA Ladder used here is 100bp DNA Ladder. This is NOT the same DNA Ladder used in Laboratory 4/4a.

Part B: Separate the PCR Products Using Gel Electrophoresis

Label tube	Contents of tube	Aliquot	Actually used
M	DNA ladder (100bp)	12µL	10µL

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Laboratory 6. Purifying the Fluorescent Protein

See teacher instructions for Lab 6 on methods for growing the culture of transformed cells. We recommend doing this step with the commercially ligated/purified plasmid used in the short series (pARA-R). The sterile broth provided has been pre-calculated so there is enough for each group to get the 2mL required.

NOTE: You must grow the culture at least 24 hours to get good expression of the rfp before you can begin Lab 6.

Day 1. Lysing of cells from the overnight mFP expression.

Label tube	Contents of tube	Aliquot	Actually used
EC	overnight *E.* coli culture	1mL****	2mL
LyB	Lysis buffer	160µL	150µL
EB	Elution buffer	160µL	150µL

****You will need to do this twice into the same tube, once before and once following centrifugation after the supernatant has been discarded.

Day 2. Purification of RFP using column chromatography

Size of tube	Label tube	Contents of tube	Aliquot	Actually used
15 mL	BB	Binding buffer (4M $(NH_4)_2SO_4$)	250µL	200µL
15 mL	CEB	Column equilibration buffer (2M $(NH_4)_2SO_4$)	3.5mL	3mL
15 mL	WB	Wash buffer (1.3M $(NH_4)_2SO_4$)	1.5mL	1mL
15 mL	EB	Elution buffer (10mM TE)	2.5mL	2mL

Be certain that the last group of students flush the columns with 2 mL of equilibration buffer. Allow the equilibration buffer to drain but leave about 0.5cm of it above the resin bed. Be certain that all tubes are capped, that the extensions have been removed and small yellow caps replaced, and check to see that the stop cocks are in the off position before storing for the next school or class. Always store the columns UPRIGHT. Return the columns to the plastic, lidded box.