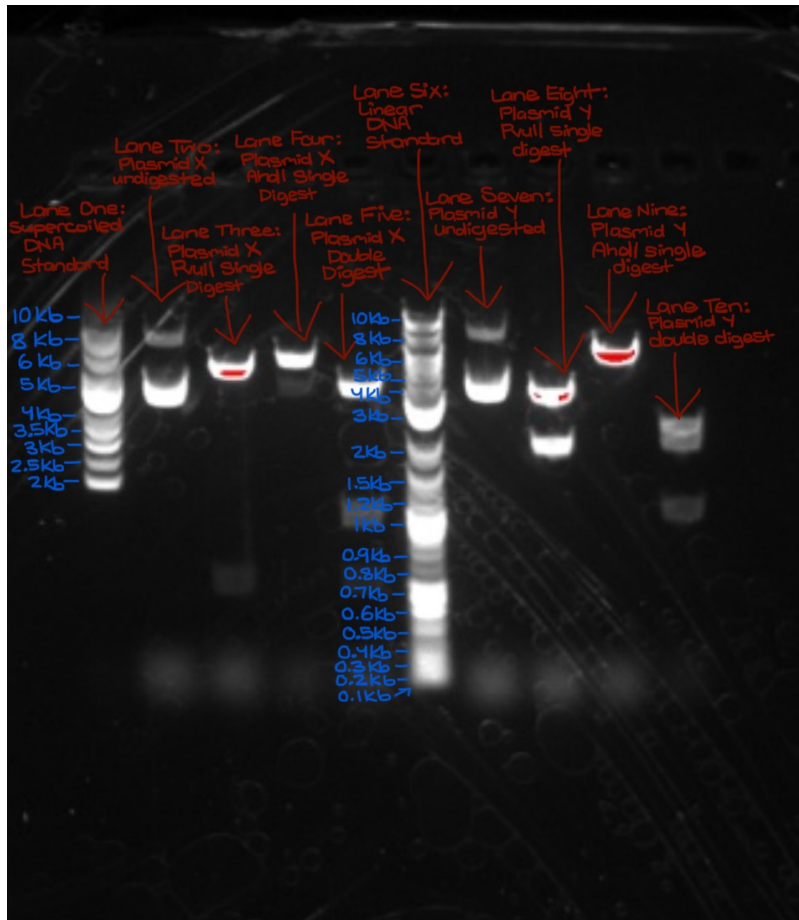


## Restriction Enzyme Digestion Worksheet

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### Q1. Restriction digest gel

Include a well-labeled image of your agarose gel. Remember to label all your lanes, and the molecular weights of the DNA standards clearly. **(4 pts)**



### Q2. Estimation of undigested plasmid size

Looking at your labeled gel in Question 1, estimate (without calculating) the size of your undigested plasmids. Briefly explain how you could make this estimation. (Hint: use the supercoiled DNA standard to help you.) **(2 pts)**

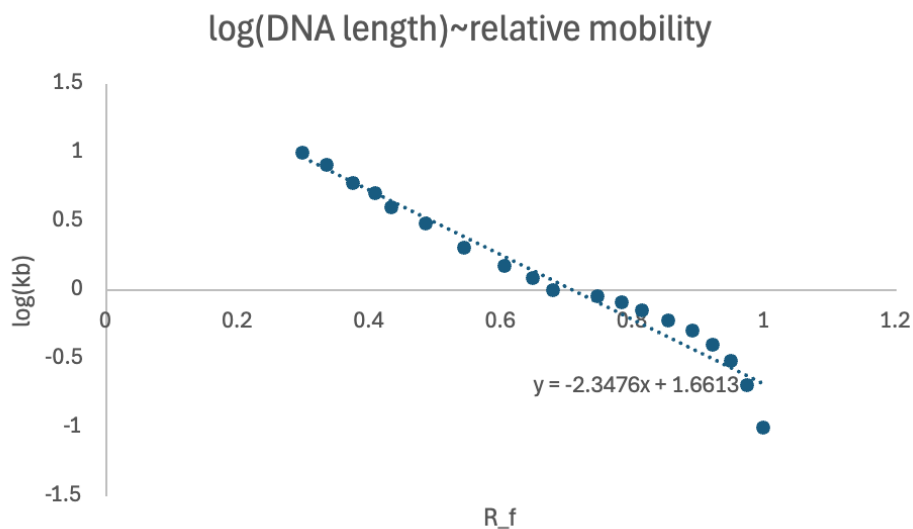
I estimated that both undigested DNA plasmid X and Y are 5 kilobases. I was able to make this estimation by examining their migration patterns relative to the supercoiled DNA standard. More specifically, I lined up the point that each undigested DNA plasmid had migrated to the corresponding point on the ladder to ultimately reach 5 kilobases. This is because the undigested DNA plasmids migrated quickly, meaning that they are in the supercoiled rather than the linear form. Thus, I used the supercoiled ladder and not the linear ladder.

Q3. Graphs of undiluted enzyme (generated in EXCEL)

Generate a standard curve by plotting the log(DNA length) as a function of the relative mobility. Include a picture of your standard curve. You should provide the graph with your axes appropriately labeled with the correct units and display the equation of line. (5 pts)

**Relative mobility  $R_f$**  is defined as:

$$R_f = \frac{\text{Distance migrated by band}}{\text{Distance migrated by dye front or end of the gel}}$$



Q4. Lengths of the DNA fragments in Plasmid X and Y

From the standard curve, calculate the lengths of all the DNA fragments generated for “Plasmid X” and “Plasmid Y” restriction digestion conditions present in each lane. Clearly summarize your results for each single and double digested sample. Show a sample calculation. (4 pts)

Sample calculation: Plasmid X undigested

$$\begin{aligned}\log(\text{kb}) &= -2.3475(R_f) + 1.6613 \\ \log(\text{kb}) &= -2.3476(1.12/3.33) + 1.6613 \\ \log(\text{kb}) &= 0.336 \cdot -2.3476 + 1.6613 \\ \log(\text{kb}) &= 0.8725064 \\ 10^{0.8725064} &= \text{kb} = 7.44\end{aligned}$$

lane	rep		mig_dist	Rf	log(kb)	kb
2	1		1	0.719424	-0.02762	0.938381
2	2		0.94	0.676259	0.073714	1.184989
3	1		1.17	0.466135	0.567	3.689779
3	2		2.43	0.968127	-0.61148	0.244638
4	1		1.06	0.815385	-0.2529	0.558603
4	2		1.25	0.961538	-0.59601	0.253508
5	1		1.29	0.617225	0.212303	1.630433
5	2		2.03	0.971292	-0.6189	0.240489
7	1		0.97	0.702899	0.011175	1.026066
7	2		1.3	0.942029	-0.55021	0.281704
8	1		1.36	0.809524	-0.23914	0.576583
8	2		1.65	0.982143	-0.64438	0.226789
9	1		1.11	0.991071	-0.66534	0.216103
10	1		1.52	0.716981	-0.02188	0.950857
10	2		1.61	0.759434	-0.12155	0.75588
10	3		2.05	0.966981	-0.60878	0.246159

#### Q5. Identity of Plasmid X and Y

Based on your results and referring to the plasmid maps in the lab manual, identify Plasmid X and Plasmid Y. Determine which one corresponds to pGEM 3 and which corresponds to pGEM 4. **(2 pts)**

Plasmid X corresponds to pGEM3, and Plasmid Y corresponds to pGEM 4. pGEM3 has a Pvu II site at 1.92 kb and pGEM4 has a Pvu II site at 0.55 kb. Lane eight (Plasmid Y single digest Pvu II) has a mark at 0.577 kb.