RDM Day 3 Interpretation Questions and Answers

1. On Day 4, you see the following number of colonies on each of your transformation plates. (These plates are labeled as the transformations are labeled on page 18 of your RDM manual.)

Plate #	# of colonies
1	77
2	22
3	8
4	~700
5	212
6	7

Interpret these results by including the answers to the questions: Do you think that you were successful in the goal of this experiment? Did you have any contamination? Did your CIP treatment from Day 2 work completely? Explain your answers.

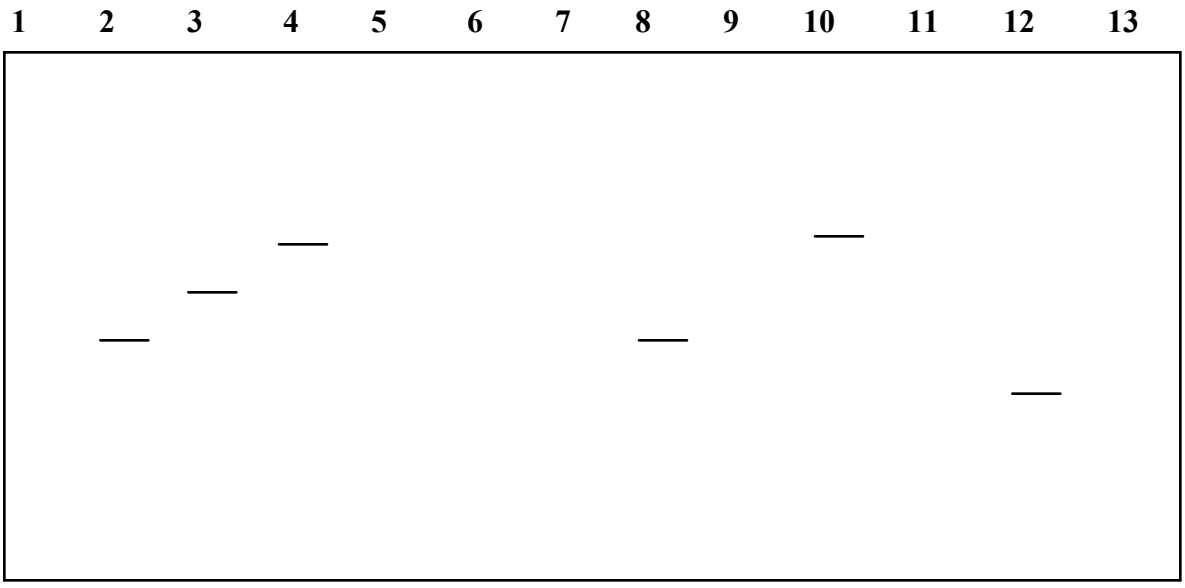
You did seem to be successful in the goal of the experiment, because you got more colonies on the vector + insert plate (#1) than you did on the vector only plate (#2). However, not every step of your experiment worked completely perfectly. For instance, your CIP treatment was incomplete, given that there are a fair number of colonies on your vector alone plate (#2). In addition, you did have a little bit of contamination, as you did get 7 or 8 colonies on the two plates that should have given no colonies – plate #6 (ligation buffer) and plate #3 (insert alone).

2. You have a mutant strain of cells that contains the *lacZ* gene in the 7.02 mini-Tn10 inserted into one of three genes: *mrk*, *nat*, or *irm*. You also have wild-type cells of the same strain with no *lacZ* insertion. Your UROP Professor asks you to design an experiment to identify which gene received the insertion. You design very similar sets of primers to those you used in 7.02 on RDM Day 3. You run the following PCR reactions and load a gel with the following lanes:

Lane	What's loaded in that lane
1	1 kb ladder
2	PCR rxn on control DNA with *mrk* For and Rev primers
3	PCR rxn on control DNA with *nat* For and Rev primers
4	PCR rxn on control DNA with *irm* For and Rev primers
5	PCR rxn on control DNA with *mrk* For and *lacZ* Rev primers
6	PCR rxn on control DNA with *nat* For and *lacZ* Rev primers
7	PCR rxn on control DNA with *irm* For and *lacZ* Rev primers
8	PCR rxn on mutant DNA with *mrk* For and Rev primers
9	PCR rxn on mutant DNA with *nat* For and Rev primers
10	PCR rxn on mutant DNA with *irm* For and Rev primers
11	PCR rxn on mutant DNA with *mrk* For and *lacZ* Rev primers
12	PCR rxn on mutant DNA with *nat* For and *lacZ* Rev primers
13	PCR rxn on mutant DNA with *irm* For and *lacZ* Rev primers

RDM Day 3 Interpretation Questions and Answers (continued)

You correctly determine that *lacZ* inserted into *nat*. Draw what your gel looked like, showing any bands that you saw in each lane. (You saw a band of 1.2kb in lane 2, a band of 1.8 kb in lane 3, and a band of 2.7 kb in lane 4.) Please label each band with its correct approximate size.



Lane 2 = 1.2 kb
Lane 3 = 1.8 kb
Lane 4 = 2.7 kb
Lane 8 = 1.2 kb
Lane 10 = 2.7 kb
Lane 12 = a band some length between about 200 bp and 2 kb