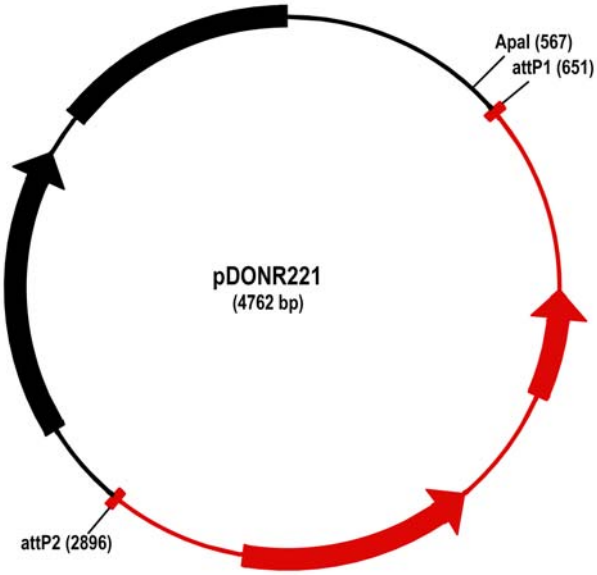
(Lab day 1 continued)

Identifying positive colony my restriction digestion:

The following is a map of the donor vector pNONR/221:



When you did BP recombination, the red part of the vector was replaced with your PCR product. All you PCR product have an *Apa* I site that was incorporated in the reverse primer. All the coding sequence you amplified did not contain any *additional* Apa I site. So, if you have a positive clone and you use *Apa* I to digest the mini-prep DNA, you should get a DNA fragment that is the size of PCR product plus (651-567 =) 84 base pair.

The following is a protocol for Apa I digest of the mini-prep DNA:

1. Label four microcentrifuge tubes with the corresponding name of the plasmids or label four microcentrifuge tubes 1, 2, 3, and 4 respectively and make a note of which colony is what.
2. You are going to set up four reactions with the difference being the mini-prep DNA. So, are can set up a reaction mixture first and aliquot it to the four tubes and then add you mini-prep DNA to individual tubes.

   You are going to set up 20 µl reactions, and you need:

	For each reaction	For 4 reactions
10 times concentrated Buffer 4 (from New England Bio-labs)	2 µl	10 µl
BSA (100X)	0.2 µl	1 µl
RNase:	0.1 µl	0.5 µl
Apa I	0.1 µl	0.5 µl
H_2O	13.6 µl	68 µl

Mini-prep DNA	4 µl	

Note: you are making an excess amount of the mixture just to make sure you will have enough to aliquot. When you are experienced with the procedure, you do not really need to do that.

3. Incubate the reactions at 37 °C for one hour.

If this lab is in the evening, which is shorter then the morning session, after the incubation is done, the TAs will store you samples at -20°C for you till next lab. Otherwise, you are going to finish all five steps in one lab session.

(Lab day 2)

4. In the next lab session, make a 1% agarose gel. Load you samples along with two of the TA's samples. You will have a total of six samples.
5. Run the gel and take a photo of the gel using the UV box as you did in you PCR lab.