7.02 Fall 2001

RDM

Recombinant DNA methods

Agenda

DAY 5

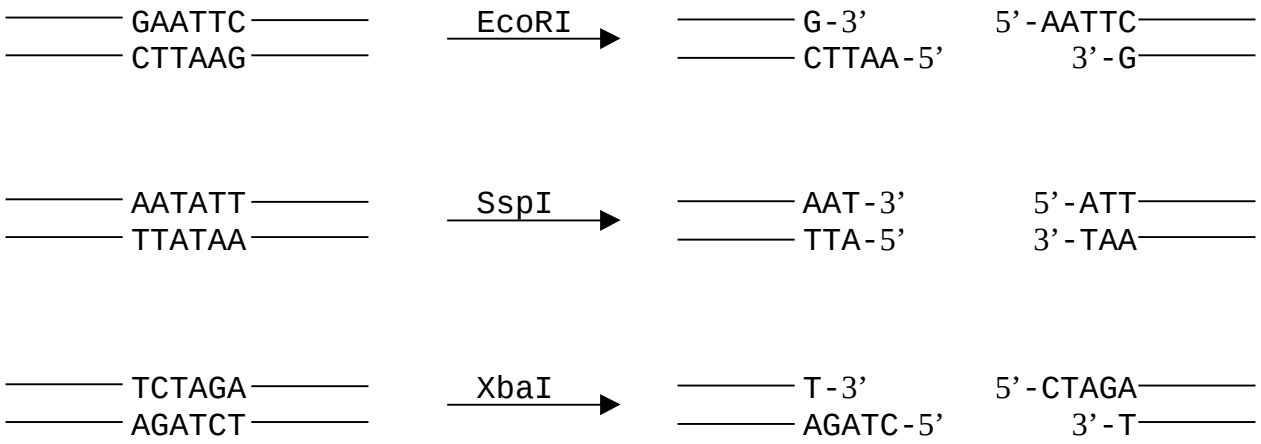
- analytical redigestions of DNA from minipreps
- what enzymes did you pick?
- what could have happened during the ligation/transformation, and how can these events be distinguished by restriction analysis?

Analytical redigestions of DNA from minipreps

• You used minipreps to isolate plasmid DNA from six colonies on your vector+insert plate. Now you want to figure out which of those colonies has the right construct. As negative controls, you also isolated DNA from two colonies on a vector-only plate.
• Restriction digests of these 8 plasmids can reveal which have the insert and which don't. If you pick your enzymes right, you can also rule out a variety of other "weird" events that may have happened during the ligation and transformation.

Which enzymes did you pick?

• The restriction enzymes at our disposal are the following.



• Before arriving at lab today, you should have chosen which of these enzymes to use in your analytical digestions. There are eight possible combinations you could have used:

(1) no enzymes, (2) EcoRI, (3) SspI, (4) XbaI, (5) EcoRI + SspI, (6) EcoRI + XbaI,
(7) SspI + XbaI, (8) all three enzymes

Possible outcomes of the ligation/transformation

• Important note: incompatible sticky ends (such as those generated by EcoRI and XbaI) can sometimes end up getting ligated together. This can lead to the digested vector recircularizing upon itself, even despite all the precautions we have taken. It can also lead to other kinds of odd ligation events.

• In instances of incompatible end ligation, the resulting ligated site is most likely some asymmetric hybrid of the two recognition sites, and it will be resistant to cutting by both of the enzymes that generated those ends.

• Here are some possible outcomes of the ligation and transformation:

(1) Plasmid with one GFP insert that integrated in the correct orientation (this is what we want)
(2) Uncut vector
(3) Recircularized doubly-cut vector
(4) Dimerized doubly-cut vector
(5) Plasmid with two GFP inserts
(6) Plasmid with three GFP inserts
(7) Plasmid with one GFP insert that integrated in the backward orientation

• Draw plasmid maps for each of the seven outcomes listed above, showing all restriction sites for SspI, XbaI, and EcoRI.

• What restriction fragments would you expect in each case, using the enzymes you chose?

• Is there any enzyme combination that can distinguish plasmid (1) from plasmid (6)?