Discussion Leader Activity: Recombinant DNA Technology
Constructing and Deconstructing Plasmids

Author/Creator: Ryan Schutte (3rd year graduate student)  
Created for: Bio 93

Activity Type: Small group activity and presentations

Time Needed in Discussion: 45-50 minutes

Purpose
• To provide students an opportunity to work with plasmids, restriction enzymes, and gel electrophoresis
• To allow students to design their own experiment which draws on key concepts presented in the Recombinant DNA Technology lecture

Abstract

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-class prep</td>
<td>30 minutes</td>
</tr>
<tr>
<td>Teacher passes out materials</td>
<td></td>
</tr>
<tr>
<td>Students work on individual plasmid/gel to figure out group</td>
<td>10-15 minutes</td>
</tr>
<tr>
<td>Students work in groups on project/presentation</td>
<td>20-25 minutes</td>
</tr>
<tr>
<td>Presentations</td>
<td>10-15 minutes</td>
</tr>
</tbody>
</table>

Supplies
• 6 Plasmids and corresponding gel worksheets for use in assigning groups
• Poster paper with pens

Pre-class prep
• Make plasmids and gels to handout at start of discussion (see attached sample)
• Prepare two instruction sheets

In Class
1. (10-15) Have each student work individually on their plasmid/gel. Tell students that those who have a plasmid should sketch what their gel would look like if they were to add restriction enzymes and do gel electrophoresis. Those with gels need to draw their plasmid based on the gel you gave them. Make your way around the room checking to see if any student is having a difficult time. After around 5+ minutes have the students start talking to neighbors trying to find their matching plasmid and gel. Have students break into groups based on which gel/plasmid they received.

2. (20-25 minutes) Hand out an instruction sheet to each group. Half the groups should get Sheet A and the other half Sheet B. Set A groups will be designing a plasmid that will allow them to meet certain criteria then should explain how to transform bacteria. Set B groups will work on identifying transformed or non transformed cells and then how to verify their
results with gel electrophoresis. Students should make a poster detailing key concepts using
diagrams or linear flow charts.

Set A: Provide students with a putative gene map showing a gene (insulin) flanked by
restriction sites. Tell students to describe how to transform bacteria to make insulin.

Concepts Set A students should demonstrate:
A. Using PCR to amplify DNA of interest
B. Designing plasmid suitable for bacterial transformation - has at least 1 restriction site in
common with gene, one selectable marker to separate transformed and non transformed
bacteria, and another to separate bacteria transformed with a plasmid containing the
insulin gene from those without the gene.
C. Brief explanation of their experiment up to and including transformation of bacteria.

Set B: Provide students with plasmid containing the lacZ and ampicillin resistance gene as well
as the same plasmid but with the insulin gene inserted into the lacZ gene.

Concepts Set B students should demonstrate:
A. How to separate transformed and non-transformed bacteria.
B. How to separate bacteria transformed with a plasmid containing the insulin gene and
those transformed without the gene. How to verify their results with gel electrophoresis -
what the lanes would look like.

3. (10-15 minutes) Have students give brief presentation of their posters. Ask for volunteers,
but have at least one from each group present and ask the other groups if there is anything
they had that was not discussed. Have a few questions ready to promote discussion, i.e., if a
group draws a gel, but does not label poles, ask them where the + and - ends are and where
small or large fragments would be found.

Things to Ask or Emphasize
• At each step, think of information from the earlier weeks of lecture that you can elicit
from the students and remind them that they can’t forget what they learned earlier.
• Ask students what would happen if two fragments were exactly the same size - would they
show as one or two bands?
• Emphasize the polarity of the gel, how the fragments differentially segregate based on
size, how they can increase the resolving ability of their gel, and the general theory behind
electrophoresis.
• Emphasize characteristics good plasmids have. Have students discuss importance of
selective markers (ampicillin or neomycin resistance and lacZ gene). How to identify
restriction enzyme cut sites. Also make sure students understand hydrogen bonds hold the
bases together while phosphodiester bonds comprise the backbone and that DNA ligase
fuses plasmid sticky ends by reforming phosphodiester bonds.

Comments
• Be prepared for students to have problems with the group pairing plasmids. These
plasmids and gels should be very easy and straightforward. If students have an extremely
difficult time, you could instead have students work together in groups to solve 3 plasmids
and 3 gels. From experience, having students infer fragment size is a bad idea. All
fragments should have a clear size. This activity is mostly to get them to randomly assort themselves and bring some additional interactivity.

- Have students draw plasmids and gels and include necessary information, such as restriction sites, selective markers, poles, and how to select for transformants.

- This is mostly a way to re-expose the students to lecture material and get them to link the use of plasmids and gels to other concepts from this difficult lecture.

Sample matched gel and plasmid:

Set A Instructions

Design and draw a plasmid that:

1) Has two restriction enzyme cut sites: but only one shared with your gene of interest (like above)
Would allow you to distinguish between transformed and non transformed bacteria after transform a laboratory strain of *E.Coli* (bacteria).

How does this work? What will you need?
How would you tell these colonies apart?

Would allow you to distinguish between bacteria transformed with plasmids containing your gene of interest and those that do not.

How does this work? What will you need?
How would you tell these colonies apart?

Set B Instructions

For the plasmid above:

1) How would you distinguish between transformed and non transformed bacteria.  What would you need to do so?  What would these colonies look like?

2) How would you distinguish between bacteria transformed with and without your gene of interest?  What would you need to do so?  What would these colonies look like?

3) How could you test if you isolated the correct transformants?  Draw this process on your poster paper.