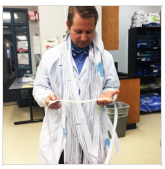

Oct 02, 2020

Lab 5--Cloning a PCR Fragment into a Plasmid (Paper Activity)

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1 Works for me dx.doi.org/10.17504/protocols.io.bmytk7wn

Harley King Workspace USG Fall 2020 BSCI:414

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ABSTRACT

This activity includes a pdf containing instructions for creating a paper plasmid and PCR fragment (gene of interest). I remember doing this with undergraduates with another professor but I couldn't find a similiar activity online. So I recreated it.

This paper activity helps students with:
1) scanning and finding restriction sites
2) contemplating sticky end vs blunt digestion
3) visualizing cloning concepts using tactile materials

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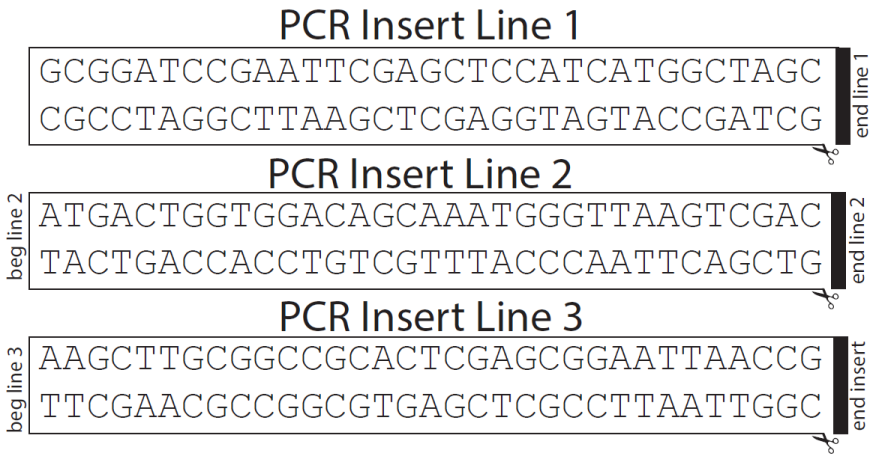
Download and Print Activity 5m

1 Download and print the " F20_Lab5_cloning_activity.pdf

Create PCR Insert 10m

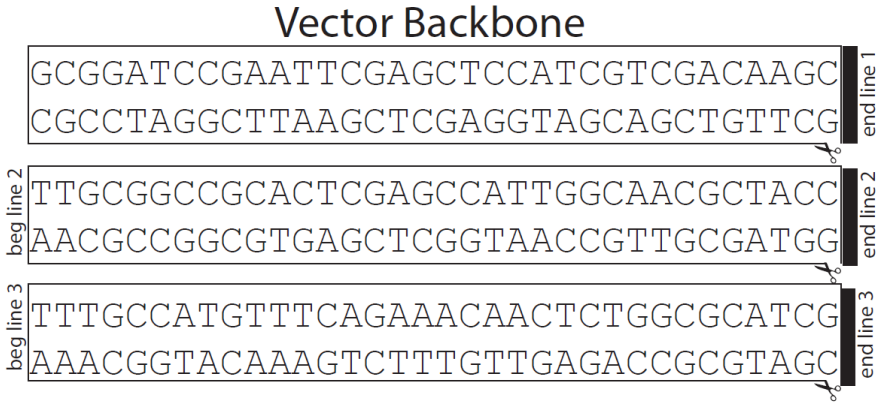
2
1. Use scissors to cut out the three lines of “PCR Insert."
2. Overlap the lines and secure them with tape.
3. Keep the PCR Insert in a line. Do not loop.



Connect PCR Insert Lines with tape.

Create the Plasmid 10m

3
1. Use scissors to cut out the seven lines of “Vector Backbone."
2. Overlap the lines and secure them with tape. Clear off your bench, this is about 6-ft long!
3. Connect the beginning of line 1 to the end of line 7 to form a loop. This represents a circularized vector or plasmid.



Connect Vector Backbone Lines with tape.

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Flushly cut the left side of line.

Use tape when bases line up.

Annotate PCR Insert 15m

4 1. Beginning on the left side of the PCR Insert, scan for a start codon. Underline it.
2. Continue scanning for a stop codon. Box it.
3. Use a marker or highlighter to color the bases between the start and stop codon.

5 Translate the codons into amino acids. Write the one-letter-abbreviation underneath the coding strand.

6 1. Scan upstream and downstream of the PCR Insert for palindromic sequences representing endonuclease target sequences e.g. GAATTC, *EcoRI*. Outline the target sequences and write the name of the endonuclease that recognizes the sequence.
2. Identify the cleavage sites within the sequence.

Find Restriction Sequences in the Plasmid 10m

7 Beginning anywhere in the vector backbone loop, scan for palindromic sites representing endonuclease cleavage sites. Outline the cleavage sites and write the name of the endonuclease that recognizes the site.

Digest and Ligate 15m

8 1. Pick a 5' cleavage site in the PCR Insert. Using scissors, cut the double stranded DNA the same way the endonuclease would cleave.
2. Repeat with a 3' cleavage site in the PCR Insert.

9 1. Locate the same 5' PCR Insert cleavage site in the "Vector Backbone" and cut.
2. Locate the 3' PCR Insert cleavage site in the "Vector Backbone". Cut.

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10 Tape the digested PCR Insert into the digested “Vector Backbone”. Verify that the overlaps are complimentary

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