Working With Data from *Principles of Life* by Hillis

Recombinant DNA

Introduction

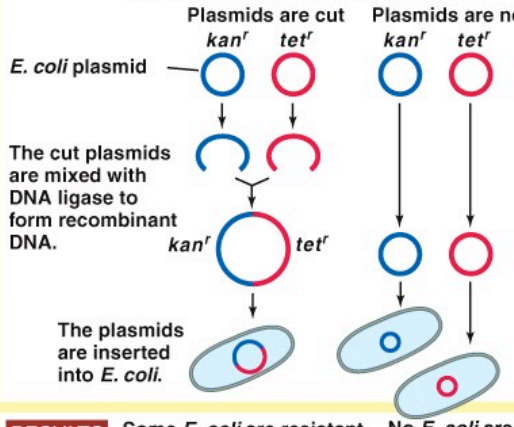
In 1973, Stanley Cohen and Hebert Boyer pioneered the field of recombinant DNA technology when they demonstrated that biologically functional recombinant bacterial plasmids can be constructed in the laboratory. Specifically, the scientists used restriction enzymes to cut two *E. coli* plasmids containing a resistance gene for either kanamycin or tetracycline. The plasmids were joined together using the enzyme DNA ligase, and the resulting plasmid was transformed back into *E. coli*. When the bacteria were plated onto media containing both kanamycin and tetracycline, approximated 1 in 10,000 cells grew, indicating that they were resistant to both antibiotics. To demonstrate that this result represented a genetic transformation and was not the result of a spontaneous mutation that arose during the experiment, cells that carried individual plasmids for either kanamycin or tetracycline were also plated on media containing both antibiotics. Approximately 1 in 10^6 cells grew on these control plates, and represented bacteria that acquired antibiotic resistance to the second antibiotic only as a result of spontaneous mutation. These important controls confirmed that the *E. coli* that grew on the experimental plates represented transformed cells that had taken up the biologically functional recombinant DNA plasmid.

INVESTIGATION

HYPOTHESIS

Biologically functional recombinant chromosomes can be made in the laboratory.

METHOD *E. coli* plasmids carrying a gene for resistance to either the antibiotic kanamycin (*kan*^{r}) or tetracycline (*tet*^{r}) are cut with a restriction enzyme.



RESULTS Some *E. coli* are resistant to both antibiotics. No *E. coli* are doubly resistant.

CONCLUSION

Two DNA fragments with different genes can be joined to make a recombinant DNA molecule, and the resulting DNA is functional.

ANALYZE THE DATA

Two plasmids were used in this study: pSC101 had a gene for resistance to tetracycline and pSC102 had a gene for resistance to kanamycin. Equal quantities of the plasmids—either intact, cut with *Eco*RI, or cut with *Eco*RI and then sealed with DNA ligase—were mixed and incubated with antibiotic-sensitive *E. coli*. The *E. coli* were then grown on various combinations of the antibiotics. Here are the results:

DNA treatment	Number of resistant colonies: Tetracycline only	Kanamycin only	Both antibiotics
None	200,000	100,000	200
*Eco*RI cut	10,000	1,100	70
*Eco*RI, then ligase	12,000	1,300	570

A. Did treatment with *Eco*RI affect the transformation efficiency? Explain.
B. Did treatment with DNA ligase affect the transformation efficiency of each cut plasmid? Which quantitative data support your answer?
C. How did doubly antibiotic-resistant bacteria arise in the "none" treatment? (Hint: see Concept 9.3.)
D. Did the *Eco*RI followed by ligase treatment increase the appearance of doubly antibiotic-resistant bacteria? What data support your answer?

Original Paper

Cohen, S. N., A. C. Y. Chang, H. W. Boyer, and R. B. Helling. 1973. Construction of biologically functional bacterial plasmids *in vitro*. *Proceedings of the National Academy of Sciences* 70: 3240–3244. http://www.pnas.org/cgi/reprint/70/11/3240

Links

(For additional links on this topic, refer to the Chapter 13 Investigation Links.)

Working With Data from *Principles of Life* by Hillis
University of Arizona: Biology Learning Center: Creating Recombinant DNA
http://www.biology.arizona.edu/molecular_bio/problem_sets/Recombinant_DNA_Technology/recombinant_dna.html

Access Excellence: Inserting a DNA Sample into a Plasmid
http://www.accessexcellence.org/RC/VL/GG/inserting.html

Access Excellence: Plasmid Insertion
http://www.accessexcellence.org/RC/VL/GG/plasmid.html

DNA Interactive: Manipulation (To view animation, choose "Techniques," and then click on the links to "Cutting & pasting" and "Transferring & storing")
http://www.dnai.org/b/index.html

Massachusetts Institute of Technology: Inventor of the Week: Cloning of Genetically Engineered Molecules
http://web.mit.edu/invent/iow/boyercohen.html

Analyze the Data

Question 1 (from textbook Figure 13.4)
Two plasmids were used in this study: pSC101 had a gene for resistance to tetracycline and pSC102 had a gene for resistance to kanamycin. Equal quantities of the plasmids,which were either intact, cut with *Eco*Rl, or cut with *Eco*Rl and then sealed with DNA ligase—were mixed and incubated with antibiotic-sensitive *E. coli*. The *E. coli* were then grown on various combinations of the antibiotics. Here are the results:

	Number of resistant colonies		
DNA treatment	Tetracycline only	Kanamycin only	Both antibiotics
None	200,000	100,000	200
*Eco*RI cut	10,000	1,100	70
*Eco*RI, then ligase	12,000	1,300	570

A. Did treatment with *Eco*Rl affect the transformation efficiency? Explain.

B. Did treatment with DNA ligase affect the transformation efficiency of each cut plasmid? Which quantitative data support your answer?

C. How did doubly antibiotic-resistant bacteria arise in the "none" treatment? (Hint: see Concept 9.3.)

D. Did the *Eco*Rl followed by ligase treatment increase the appearance of doubly antibiotic-resistant bacteria? What data support your answer?

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Question 2

In another part of the study, some pSC101 was cut with the restriction enzyme, *Eco*RI but not sealed up with DNA ligase. Cut or intact pSC101 were presented to *E. coli* cells sensitive to antibiotics. The results are shown in Table 1 (from original paper).

TABLE 1. *Transformation by covalently closed circular and EcoRI-treated plasmid DNA*

Plasmid DNA species	Transformants per μg DNA		
	Tetracycline	Kanamycin (neomycin)	Chloram-phenicol
pSC101 covalently closed circle	3×10^3	—	—
*Eco*RI-treated	2.8×10^1	—	—

What can you conclude from this experiment?