
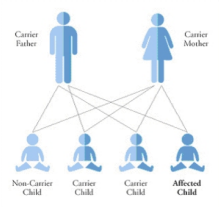


DNA Engineering


20.109(F14)
M1D2 Lecture
09.11.14
Natalie Kuldell



What's so important about rDNA?



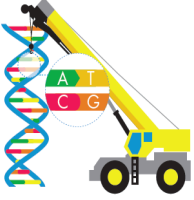
Genetics



Molecular Genetics

What's so important about rDNA?

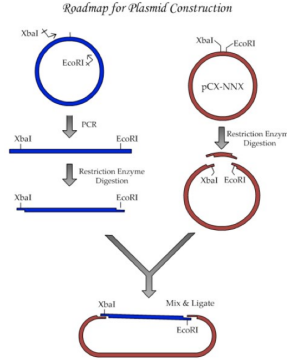
- Protein overproduction
- Metabolic engineering & compounds
- DNA and RNA research
- change genotype of an organism
- correct genetic defect



Molecular Genetics

Mod 1 DNA Engineering

Roadmap for Plasmid Construction




Introduction to Restriction Enzymes

- What are they?
- Where do they come from?
- What do they do there?
- How do cells protect themselves from themselves?

- From knowing to doing
- Advanced Practice

What are Restriction Enzymes: Molecular Visualization

- <http://youtu.be/aA5fyWjh5S0>



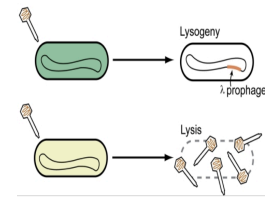
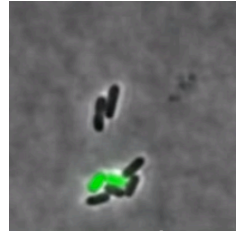
Where do restriction enzymes come from?

<i>Thermus aquaticus</i>	TaqI	3'... CCG G... 5' 5'... TCG A... 3' 3'... AGC T... 5'
<i>Haemophilus haemolyticus</i>	HhaI	5'... GCG C... 3' 3'... CGG G... 5'
<i>Desulfovibrio desulfuricans</i>	DdeI	5'... C T N A G... 3' 3'... G A N T C... 5'
<i>Moraxella bovis</i>	MboII	5'... G A A G A (N) ₈ ... 3' 3'... C T T C T (N) ₈ ... 5'
<i>Escherichia coli</i>	EcoRV	5'... G A T A T C... 3' 3'... C T A T A G... 5'
	EcoRI	5'... G A A T T C... 3' 3'... C T T A A G... 5'
<i>Providencia stuarti</i>	PstI	5'... C T G C A G... 3' 3'... G A C G T C... 5'
	MstII	5'... C C T N A G G... 3'

Watson, Myers, Caudy, Witkowski "rDNA: genes and genomes"

Restriction Enzymes: what is their natural biological function?

- <http://youtu.be/sLkZ9FPHJGM>



Proc Natl Acad Sci U S A. Dec 30, 2008; 105(52): 20705–20710.

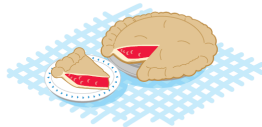
How cells protect themselves from their own restriction enzymes?

Introduction to Restriction Enzymes

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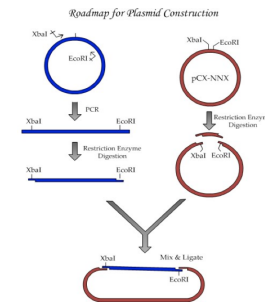
Restriction Enzymes: from knowing to doing

- In Practice
 - Recognition seq lengths
 - Types of cutters
 - Shared recog sequences
 - Shared overhangs
 - Buffer conditions
 - Storing and diluting
 - Specificity
 - Lack of activity
- PI for an hour
 - Anticipating pitfalls
 - Setting up helpful controls

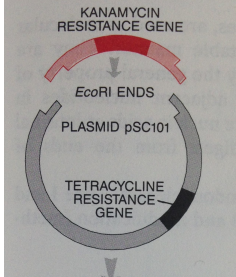


Restriction Enzymes: from knowing to doing

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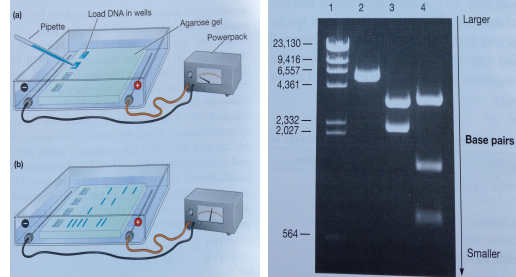


PI for an hour....



- Kanamycin resistance gene is ~1000 bp and comes from a plasmid you know very little about...
- pSC101 is ~9200 bp and is named after Stanley Cohen (early DNA engineer & Lasker prize winner)

Bird's eye view of gel electrophoresis

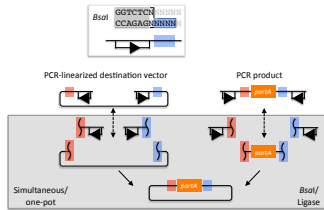


Watson, Myers, Caudy, Witkowski "rDNA: genes and genomes"

Alternative Strategy



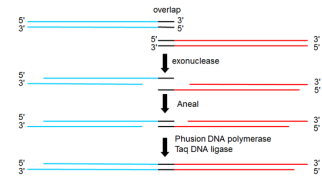
- Golden Gate Assembly



Alternative Strategy



- Gibson Assembly



Summary

