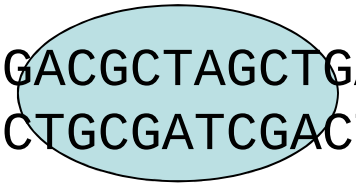
Restriction Endonucleases

Restriction Enzymes

- Enzymatic Activity
- Biological Role
- Diversity
- Recognition Sequence
- Digestion Conditions
- Typical Reaction
- Double Digest
- Class Project
- Computer Analysis

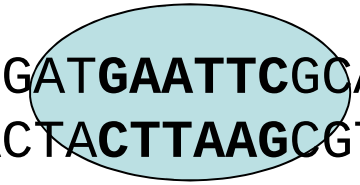
Enzyme Activity

Scanning



GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

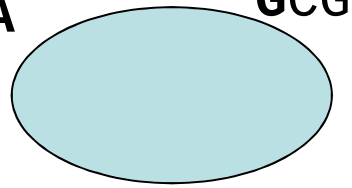
Recognition Sequence



GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Cleavage

GGACGCTAGCTGAT**G**
CCTGCGATCGACTA**CTTAA**



AATTCGCATCGGATCCGAATCCGCTCTTTCAA
GCGTAGCCTAGGCTTAGGCGAGAAAGTT

Biological Role of RE

- Restriction Modification System -restriction enzymes are paired with methylases.

- Methylases are enzymes that add methyl groups to specific nucleotides within the recognition sequence. The methylation prevents recognition by the restriction enzyme.

- Therefore, the restriction enzyme within a cell doesn’t destroy its own DNA. However the restriction enzyme can destroy foreign DNA which enters the cell such as bacteriophage.

Diversity of Enzymes

EcoRI	*Esherichia coli* R	G/AATTC
BamHI	*Baccilu amyloliquefaciens* H	G/GATCC
HindIII	*Haemophilus influenzae* Rd	A/AGCCT
PstI	*Providencia stuartii*	CTGCA/G
PmeI	*Psuedomonas mendocina*	GTTT/AAAC

Recognition Sequences

EcoRI	G/AATTC
BamHI	G/GATCC
HindIII	A/AGCCT
PstI	CTGCA/G
PmeI	GTTT/AAAC
HincII	GTY/RAC
FunII	G/AATTC

Features
Palindromic
Length
    4 cutters, 6 cutters etc
Site of cleavage
    Sticky ends
        3’ overhang
        5’ overhang
        blunt end
    Compatibility
Multiple Recognition sequences
Isoschisomers
Type II vs Type III RE

Digestion Conditions

- XbaI
  - Buffer 2: (10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.9 at 25°C.
  - 100 µg/ml BSA
  - Incubate at 37°
  - 1 Unit digest 1 µg DNA in 1 hour
  - Heat inactivate 65° for 20min

Typical RE Reaction

20 µl reaction.

10 µl DNA (~1 µg total)
7 µl water
2 µl 10X reaction buffer
1 µl RE 10units/µl

Incubate 1 hour at appropriate temperature

Note:

1. 10 fold excess enzyme ensures complete digestion.
2. Enzyme should never exceed 1/10th of reaction.
3. BSA is often recommended because it

Double Digest

Enzyme		Supplied NEBuffer	% in B1	% in B2	% in B3	% in B4
SacI		NEBuffer 1 + BSA	100	50	10	100
SacII		NEBuffer 4	25	75	10	100
SalI		NEBuffer 3 + BSA	0	0	100	0
SapI		NEBuffer 4	75	50	0	100
Sau3AI		NEBuffer 1 + BSA	100	50	10	100
Sau96I		NEBuffer 4	50	100	100	100
SbfI		NEBuffer 4	75	50	0	100

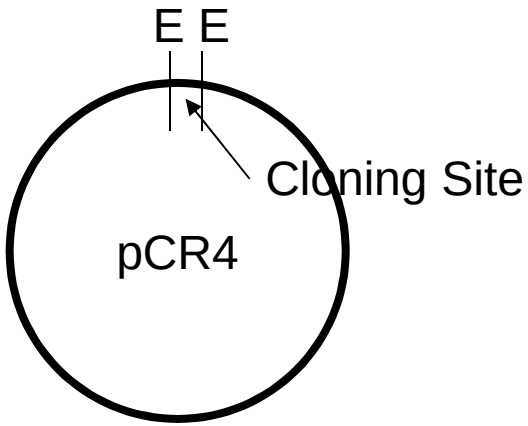
Double Digest Option

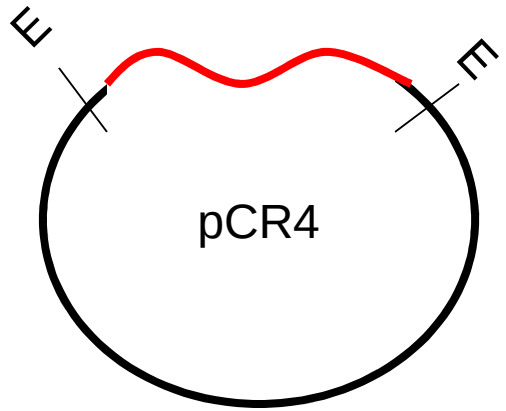
1. Mix the enzymes in the same compatible buffers
2. Conduct sequential digest

Caution: some enzymes display star activity in certain buffers which causes them to digest the DNA at sites other than the standard recognition site.

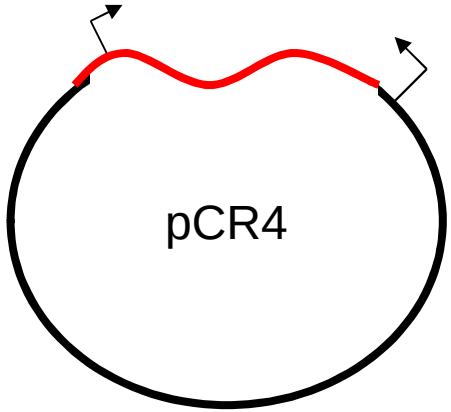
Class Project

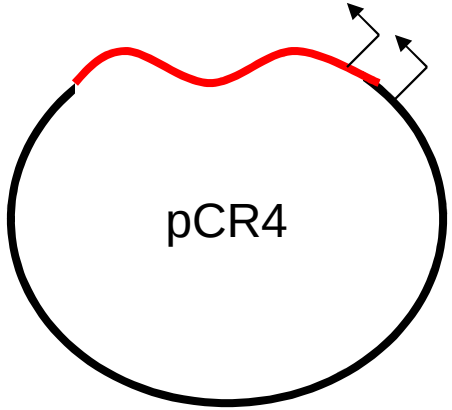
1. Digest with EcoRI to release cloned insert





2. Orientation of Insert





Class Project

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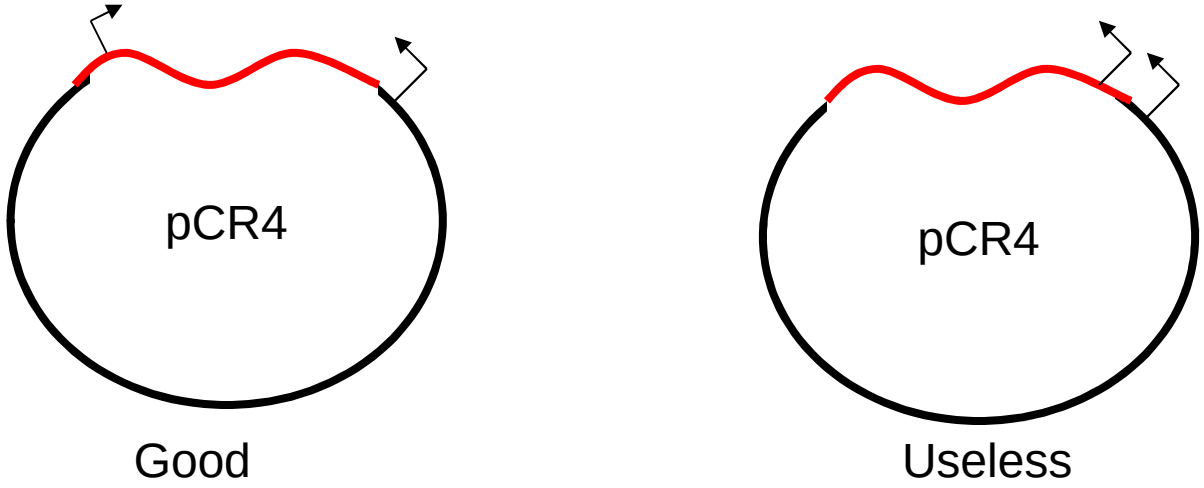

1. Identify internal restriction sites
   - Best internal sites will match one of the external sites

2. Digest with enzymes to cut internal site and external site – analyze by electrophoresis

Computer Analysis

- Use TAGC program in Biology workbench to identify restriction sites within amplified region.

- Analyze full PCR product, including primer sequences.

- In initial analysis identify all restriction sites.
- In second analysis, search for these useful sites.

In pCR4 Vector cloning region

EcoRI, PmeI, PstI, SpeI, NotI

Other available restriction enzymes

BamHI, HindIII, SphI, XbaI