DAY	SEM		8:00–8:55 AM	9:00 – 9:55 AM	10:00 – 10:55 AM	11:00 – 11:55 AM	12:00 – 12:55 PM	1:00–1:55 PM	2:00 – 2:55 PM	3:00 – 3:55 PM	4:00 – 4:55 PM	5:00 – 5:55 PM
MON	BTech	III		BT202	BT203	MA201	BT204					HS200
		V		BT 311					BT303	BT302	BT304	BT301
		VII		BT612		BT645/ BT623/ BT610/ BT651	BT641		BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621	
	MTech (BT)	I		BT612	BT501	BT610/ BT651	BT641		BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621	
	MTech (BE)	I		BT612	BT501	BT645/ BT610/ BT651	BT641		BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621	
		III				BT623						
	PhD			BT612		BT645/ BT623/ BT610/ BT651	BT641		BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621	
TUE	BTech	III	BT205	BT201	BT202	BT203	BT204					HS200
		V		BT 311					BT302			~~BT301M~~
		VII		BT401M	BT612		BT641					BT604/ BT605/ BT615/ BT621
	MTech (BT)	I		BT503	BT612	BT501	BT641					BT604/ BT605/ BT615/ BT621
	MTech (BE)	I		BT521	BT612	BT501	BT641					BT604/ BT605/ BT615/ BT621
		III										
	PhD				BT612		BT641					BT604/ BT605/ BT615/ BT621
WED	BTech	III	MA201	BT205	BT201	BT202	~~BT201M~~					HS200
		V			BT302			HS1xx		BT301	~~BT301M~~	BT304
		VII			BT401M	BT612	HS2xx					BT618/ BT620/ BT634/ BT636
	MTech (BT)	I			BT503	BT612			BT 510			BT618/ BT620/ BT634/ BT636
	MTech (BE)	I			BT521	BT612			BT 530			BT618/ BT620/ BT634/ BT636
		III										
	PhD					BT612						BT618/ BT620/ BT634/ BT636

THU	BTech	III		MA201	BT205	BT201	~~BT201M~~					HS200
		V		BT 312				HS1xx	BT301	~~BT301M~~	BT303	BT304
		VII		BT645/ BT623/ BT610/ BT651		BT401M	HS2xx					BT637/ BT640/ BT643/ BT644
	MTech (BT)	I		BT610/ BT651		BT503			BT 510			BT637/ BT640/ BT643/ BT644
	MTech (BE)	I		BT645/ BT610/ BT651		BT521			BT 530			BT637/ BT640/ BT643/ BT644
		III		BT623								
	PhD			BT645/ BT623/ BT610/ BT651								BT637/ BT640/ BT643/ BT644
FRI	BTech	III		BT203	MA201	BT204	~~BT201M~~					
		V		BT 312				HS1xx		BT303		
		VII			BT645/ BT623/ BT610/ BT651	BT641	HS2xx			BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621
	MTech (BT)	I		BT501	BT610/ BT651	BT641				BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621
	MTech (BE)	I		BT501	BT645/ BT610/ BT651	BT641				BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621
		III			BT623							
	PhD				BT645/ BT623/ BT610/ BT651	BT641				BT618/ BT620/ BT634/ BT636	BT637/ BT640/ BT643/ BT644	BT604/ BT605/ BT615/ BT621

Genome Editing and Engineering

Course No: BT-637

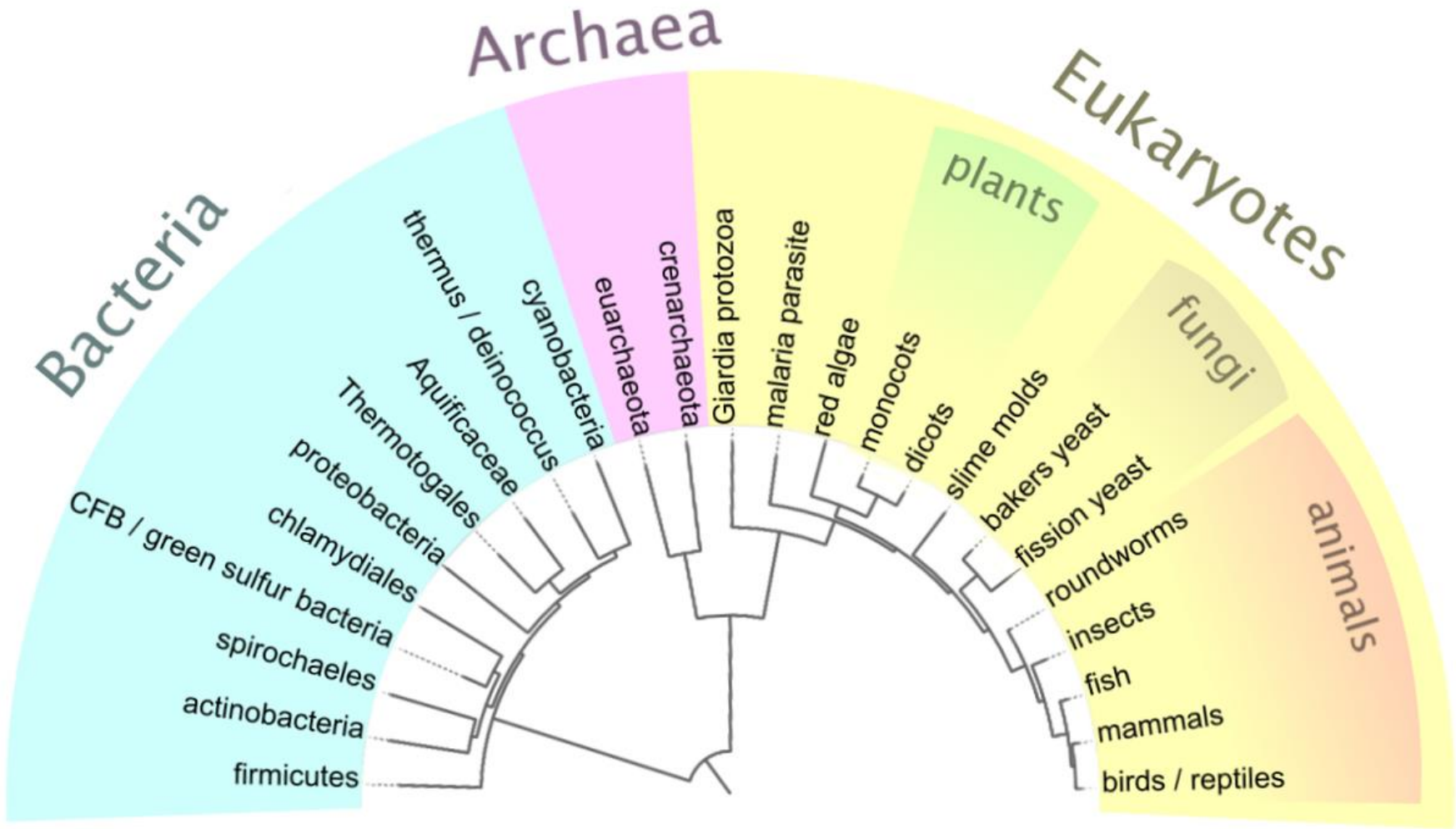
LECTURE-1

Dr. Kusum K. Singh

Department of Biosciences and Bioengineering

Indian Institute of Technology Guwahati

Introduction



- **All lives are connected by DNA (A,T,G,C)**

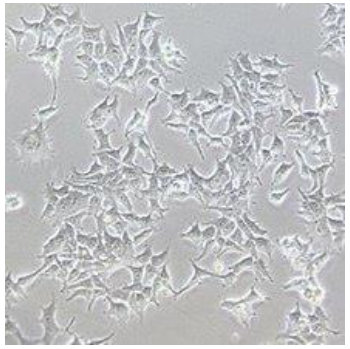
Code of life

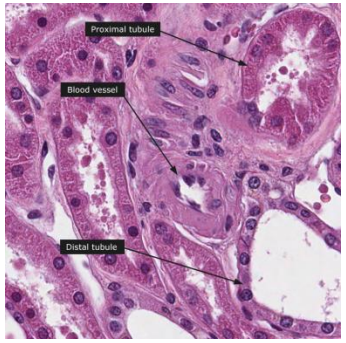
- The code when strung together

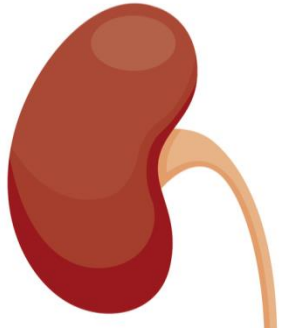
Total number of genes

- The code when strung together

	Chimp	Human	Mouse	Rat	Dog	Primate	Rodent	Ingroup	MRCA	Total # Unique
Total # of Families	9,693	10,349	11,410	9,969	9,663					15,389
Total # of Genes	20,947	22,763	24,502	22,557	18,213					

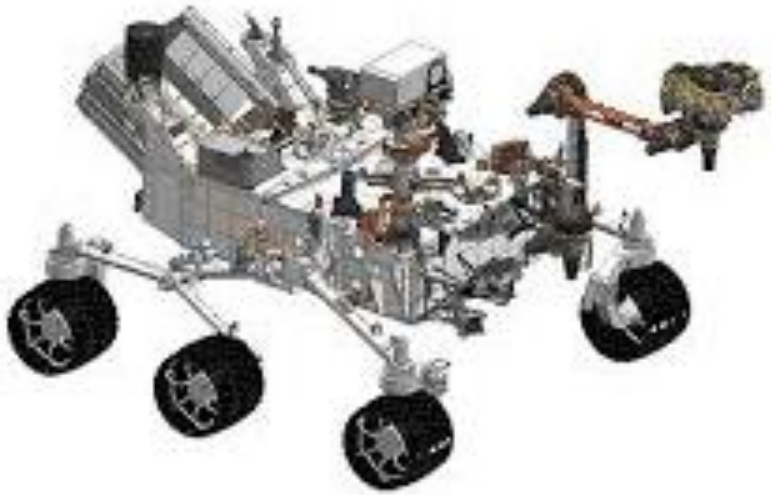




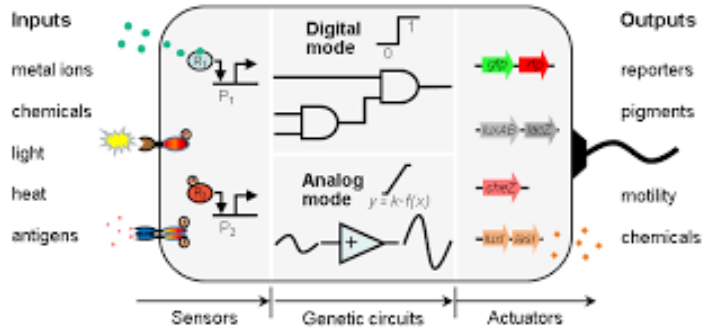


Tools to manipulate

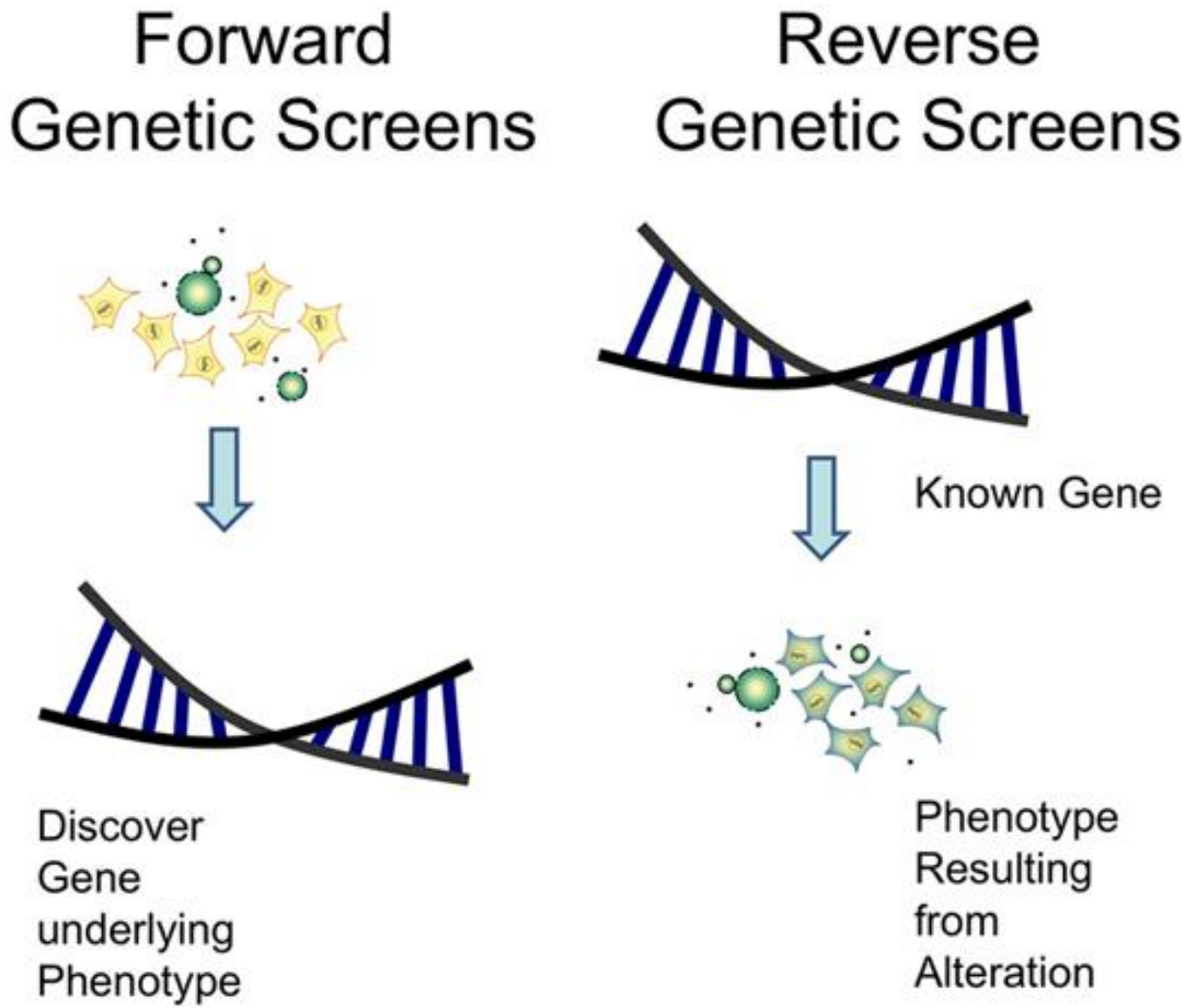
- Engineers



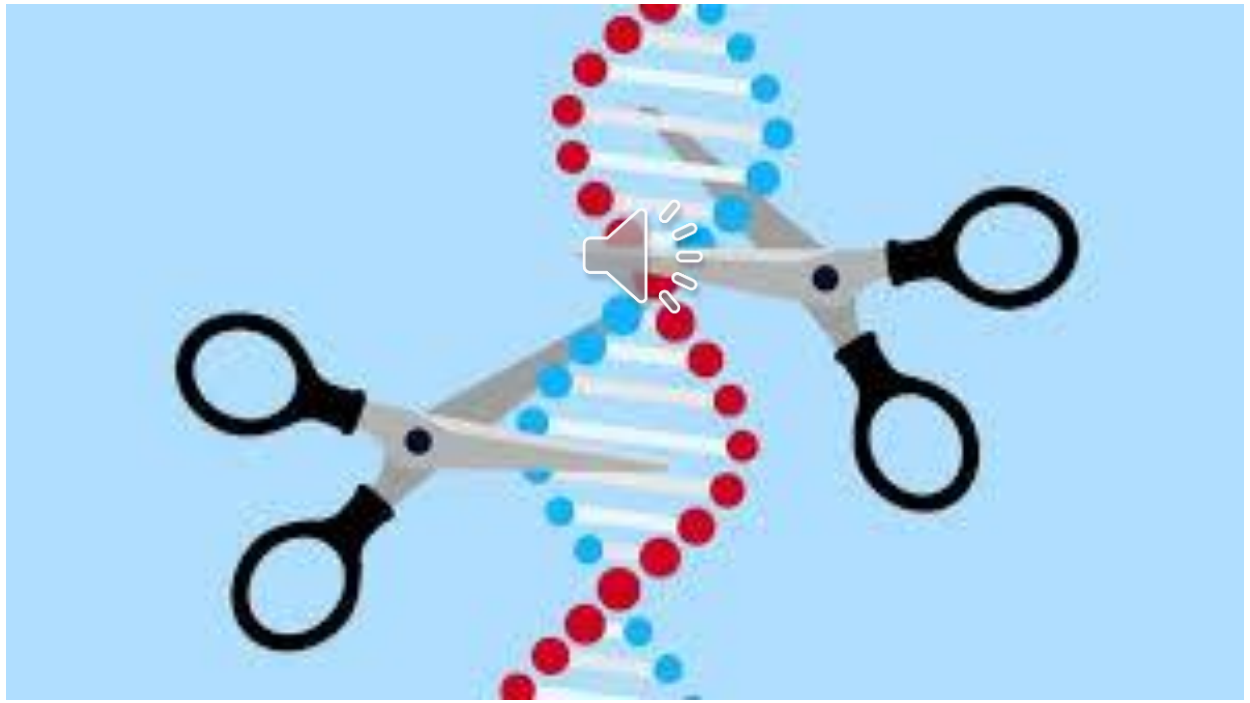
- Biologist



Approaches to study

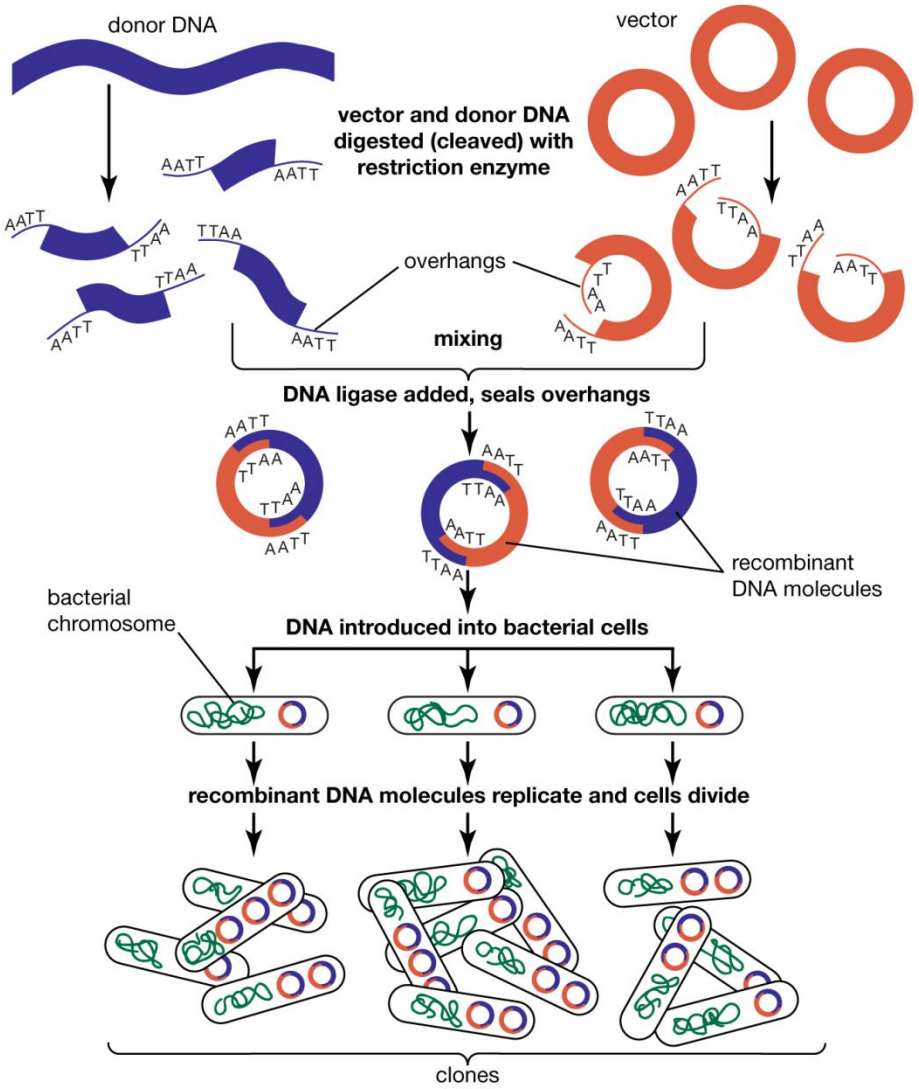


Tools to manipulate DNA



- Meganucleases, ZFNs, TALENs, and CRISPR/CAS

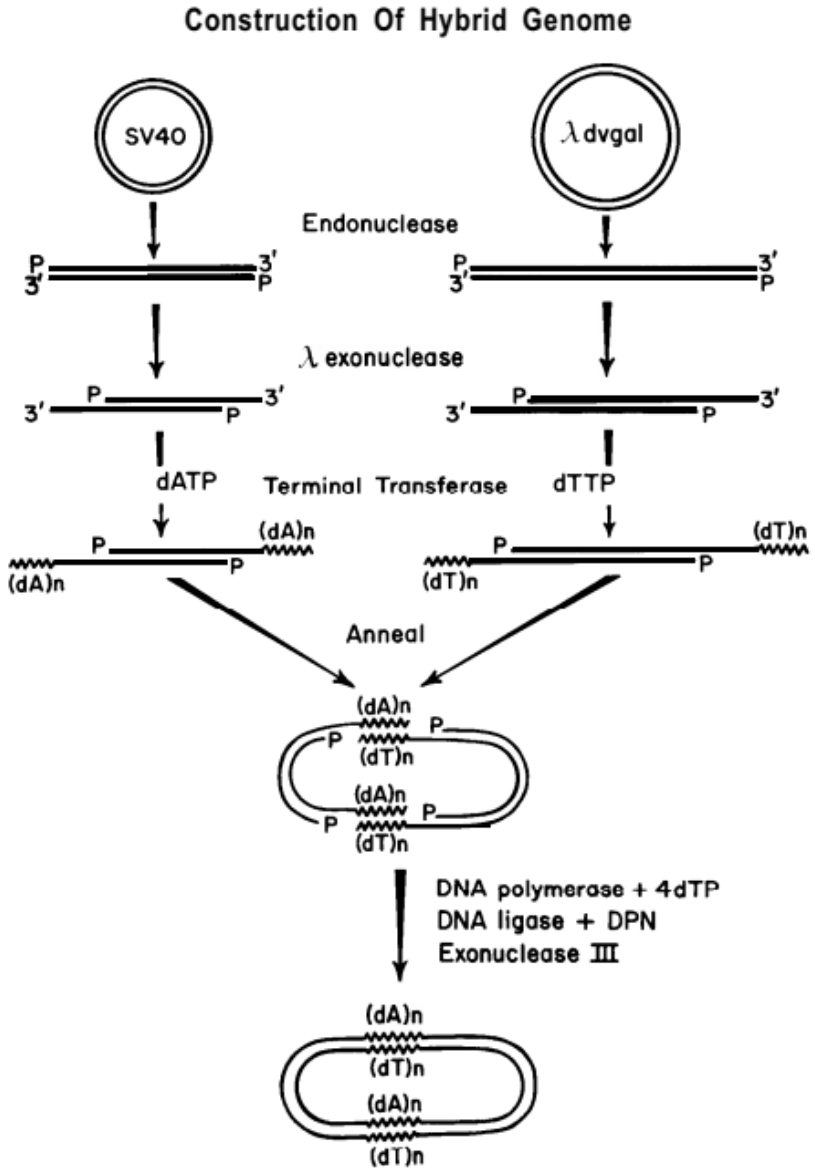
First tool – Recombinant DNA



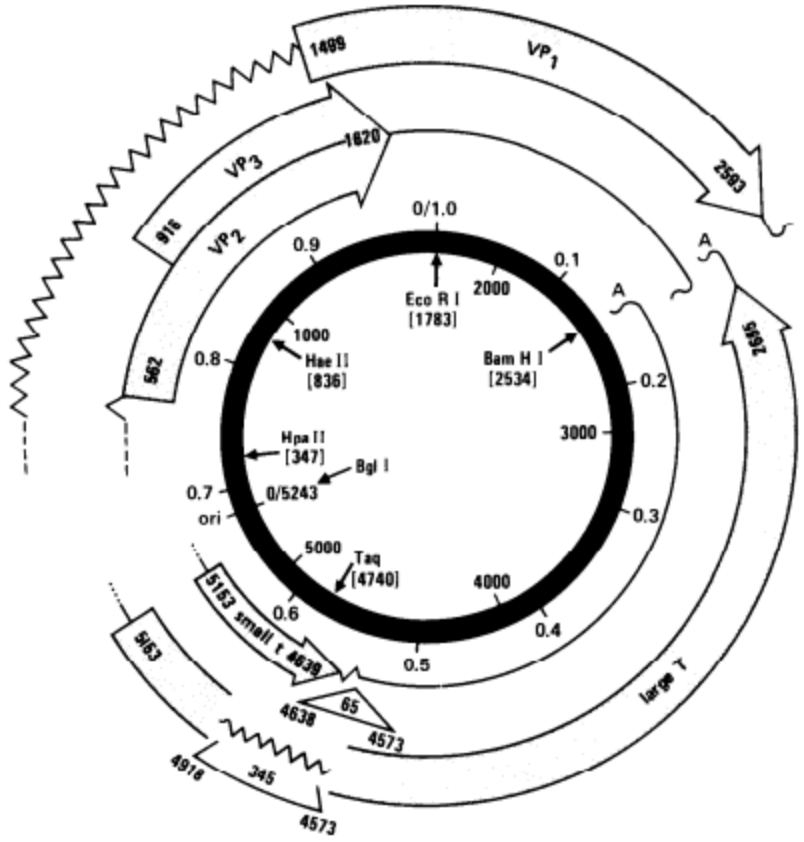
Recombinant DNA (1972)

- E.coli gene of galactose operon was inserted into SV40 (λ phage)

Recombinant DNA (1972)



Recombinant DNA (1972)



β-globin XGPRT DHFR n

Restriction Enzymes

Nobel Price in Physiology or Medicine (1978)

Werner Arber

Daniel Nathans

Hamilton O. Smith

1973). The beauty of restriction/modification systems was captured in a piece written by 10-year-old Syl-
via Arber in 1978, the year that her father Werner Arber, together with Hamilton Smith and Dan Nathans
was awarded the Nobel prize:

> When I come to the laboratory of my father, I usually see some plates lying on the tables. These plates contain colonies of bacteria. These colonies remind me of a city with many inhabitants. In each bacterium there is a king. He is very long, but skinny. The king has many servants. These are thick and short, almost like balls. My father calls the king DNA, and the servants enzymes. The king is like a book, in which everything is noted on the work to be done by the servants. For us human beings these instructions of the king are a mystery.
>
> My father has discovered a servant who serves as a pair of scissors. If a foreign king invades a bacterium, this servant can cut him in small fragments, but he does not do any harm to his own king. Clever people use the servant with the scissors to find out the secrets of the kings.
>
> To do so, they collect many servants with scissors and put them onto a king, so that the king is cut into pieces. With the resulting little pieces it is much easier to investigate the secrets. For this reason my father received the Nobel Prize for the discovery of the servant with the scissors.

published in a News and Views article in *Nature Structural Biology* [Conforti 2000]).

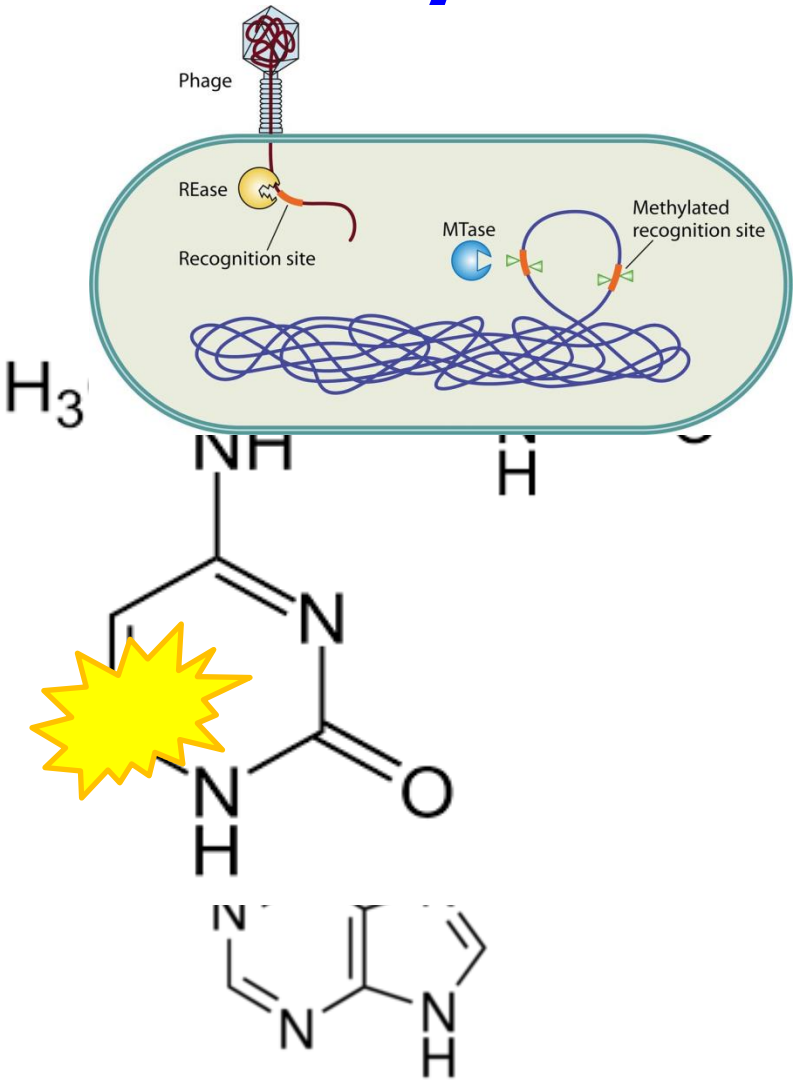
Classification Restriction Enzymes

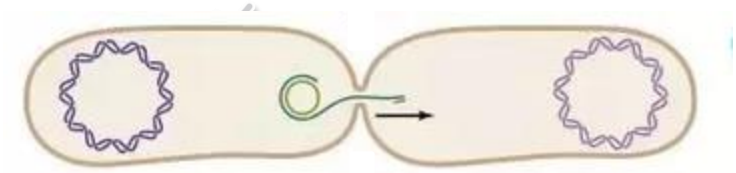
- Domain Structure
- Cofactor requirements
- Length and symmetry of recognition sequences
- Position of cleavage
- Mode of action

- Most important belong to class II

Classification Restriction Enzymes

- Defense Mechanism
- Cognate methylase enzymes
- Cytosine to 4-m-cytosine/5-m-cytc
- Adenine to 6-m-adenine





Classification Restriction Enzymes

least 1 kb away (for review)

TABLE 1. Summary: Major classes of restriction enzymes

Type of enzyme	Structure	Cofactor in vitro	DNA recognition sequence	Cleavage site
I	Complex, with three different subunits (endonuclease [hsdR], methyltransfersase [hsdM], recognition [hsdS])	SAM, ATP	Asymmetric and complex	>1,000 bp from recognition site. The best-studied class I enzyme (LlaG1) cleaves at two asymmetric recognition sites in a head-to-head inverted repeat. The enzyme. The helicase domain of the enzyme catalyzes one-dimensional stepwise translocation of dsDNA between the recognition sites (Smith et al. 2009a,b). For review, see Bourniquel and Bickle (2002).

Classification Restriction Enzymes

Classification Restriction Enzymes

II	Homodimers. Endonuclease and methylase activities are on different molecules	Mg^{2+}	Dyad symmetry	At recognition site

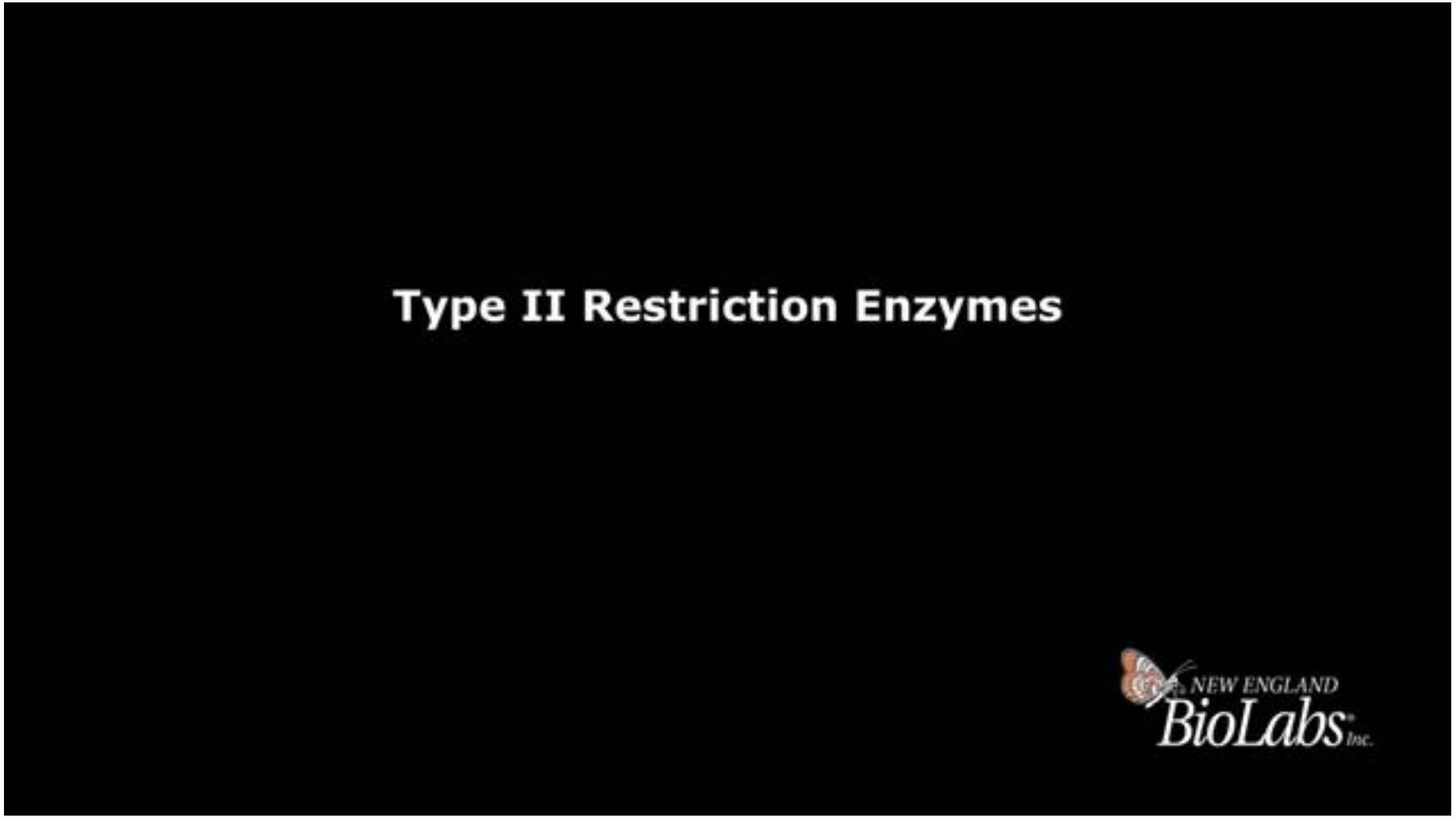
Bam HI

5′. . . G▼G A T C C . . . 3′
3′. . . C C T A G▲G . . . 5′

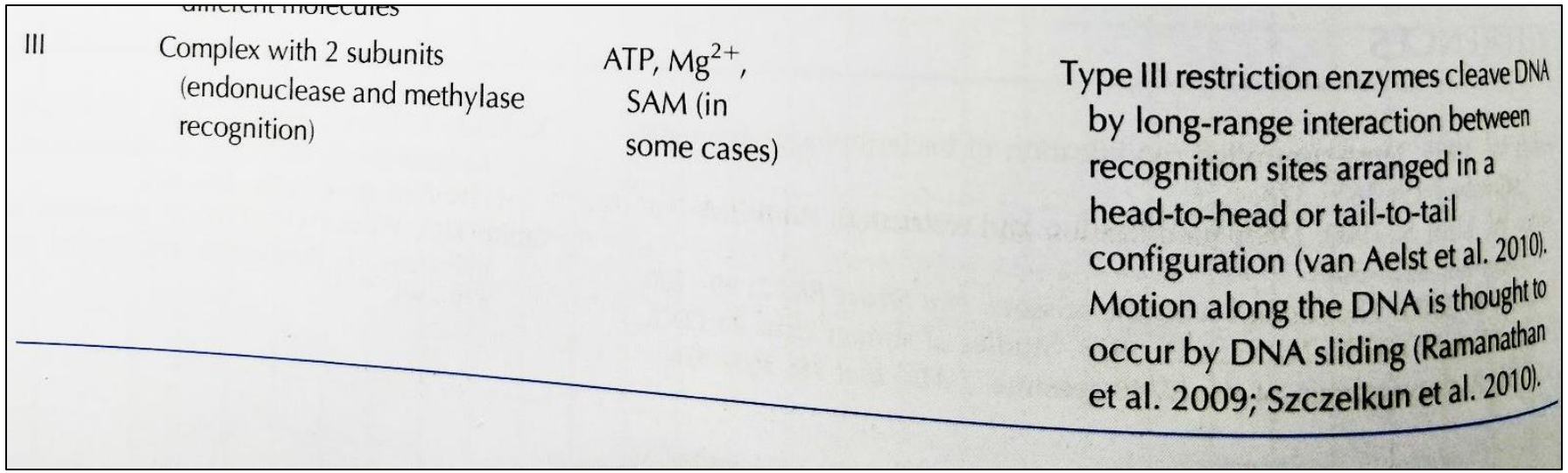
BglII

5′. . . A▼G A T C T . . . 3′
3′. . . T C T A G▲A . . . 5′

Classification Restriction Enzymes



Classification Restriction Enzymes



III	Complex with 2 subunits (endonuclease and methylase recognition)	ATP, Mg^{2+}, SAM (in some cases)	Type III restriction enzymes cleave DNA by long-range interaction between recognition sites arranged in a head-to-head or tail-to-tail configuration (van Aelst et al. 2010). Motion along the DNA is thought to occur by DNA sliding (Ramanathan et al. 2009; Szczelkun et al. 2010).

Classification Restriction Enzymes

- Brief historical start of genome editing.
- Discovery and classification of R.E.
- Perspective of 10 year old girl on R.E.
- Framework for future lectures.

- Restriction Enzymes
- Type II Restriction Enzymes: subtypes
- Type II P
- Type II S (Shifted
- Type II C
- Type II T

Gene, 16 (1981) 73–78
Elsevier/North-Holland Biomedical Press

New restriction endonucleases from *Flavobacterium okeanokoites* (*Fok*I) and *Micrococcus luteus* (*Mlu*I)

(Class II enzymes; DNA sequencing; $\begin{matrix} GGATGN_9{}^{\downarrow} \\ CCTACN_{13}{}_{\uparrow} \end{matrix}$; $A^{\downarrow}CGCGT$)

Hiroyuki Sugisaki and Susumu Kanazawa

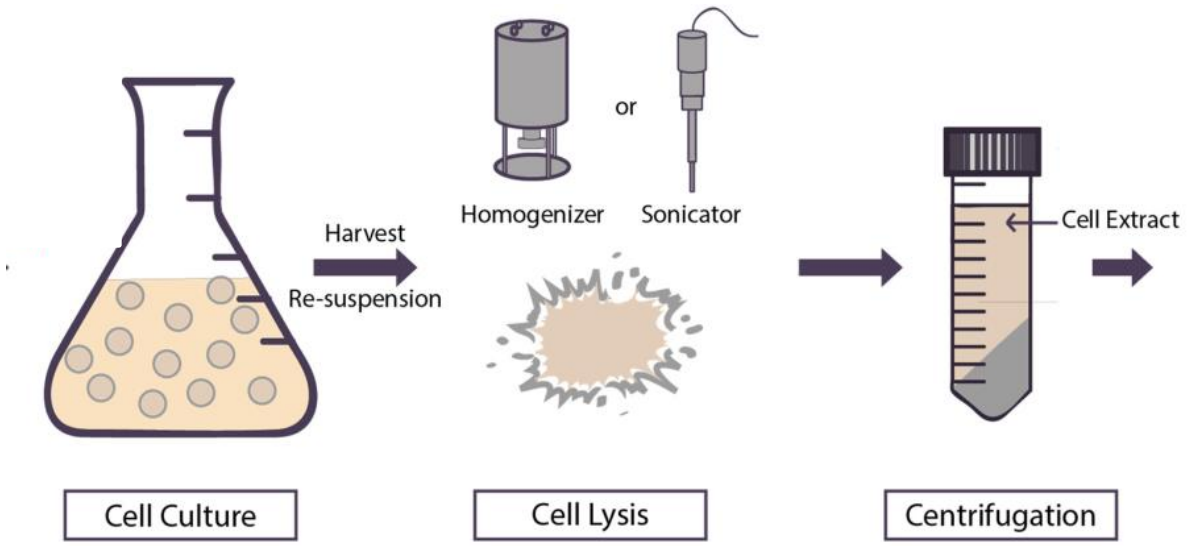
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611 (Japan)

Purification of novel enzyme

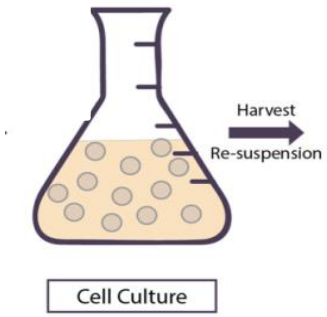
Flavobacterium okeanokoites IFO12536

1 liter culture was about 4 g (wet weight).

20 g of frozen cells



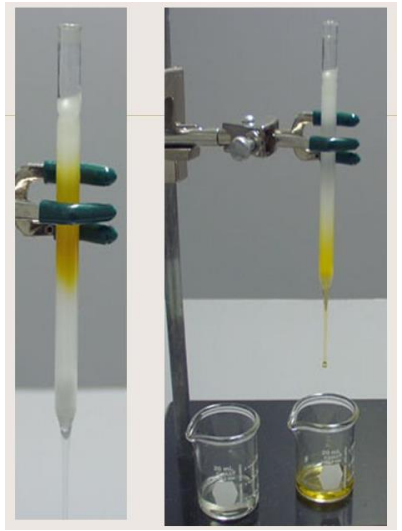
Purification of novel enzyme



(a)

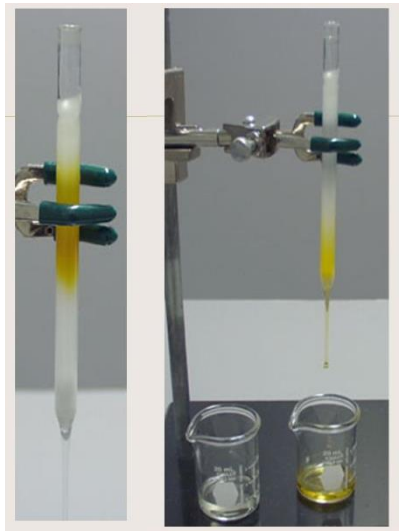
DNA-modifying enzymes prepared using P11 chromatography

Source of enzyme	Enzyme name
Escherichia coli RY13	*Eco*RI
Haemophilus influenzae Rd	*Hin*dII, *Hin*dIII
Providencia stuartii	*Pst*I
Serratia marcescens	*Sma*I
Bacillus amyloliquefaciens H	*Bam*HI
Bacillus globigii	*Bgl*I, *Bgl*II
Klebsiella pneumoniae	*Kpn*I
Arthrobacter luteus	*Alu*I
Streptomyces albus G	*Sal*GI
Streptomyces achromogenes	*Sac*I, *Sac*II
Proteus vulgaris	*Pvu*I, *Pvu*II
Bacillus caldolyticus	*Bcl*I
Haemophilus aegyptius	*Hae*II, *Hae*III
Haemophilus haemolyticus	*Hha*II
Haemophilus parainfluenzae	*Hpa*I, *Hpa*II
Staphylococcus aureus 3A	*Sau*3A
Streptomyces phaechromogenes	*Sph*I
Thermus aquaticus	*Taq*I
Xanthomas badrii	*Xba*I
Xanthomonas holicola	*Xho*I, *Xho*II
Escherichia coli carrying cloned T_4 DNA ligase gene	T_4 DNA ligase
Escherichia coli carrying cloned DNA polymerase gene	DNA polymerase I

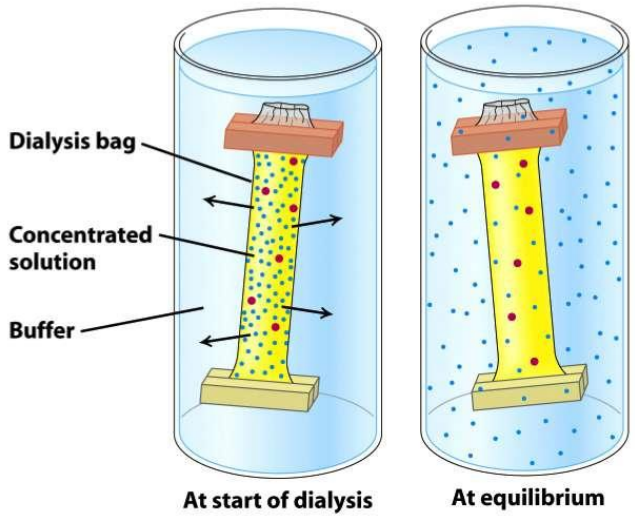


Resin Type	Cation Exchanger
Net charge of molecule of interest	+
Charge of resin	−
Running conditions	0.5–1.5 pH units below the pI of the molecule of interest

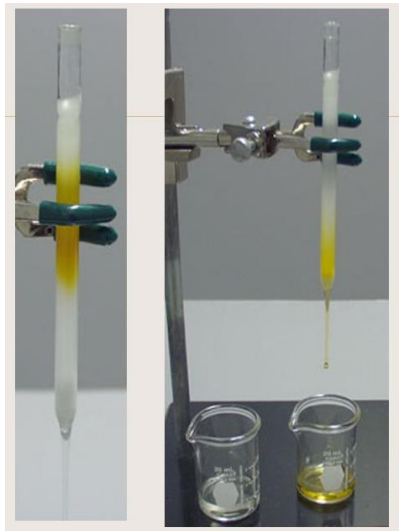
Purification of novel enzyme



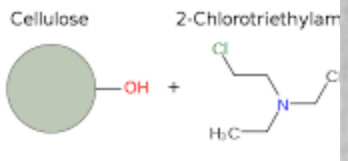
P-11 column (cation-exchanger)

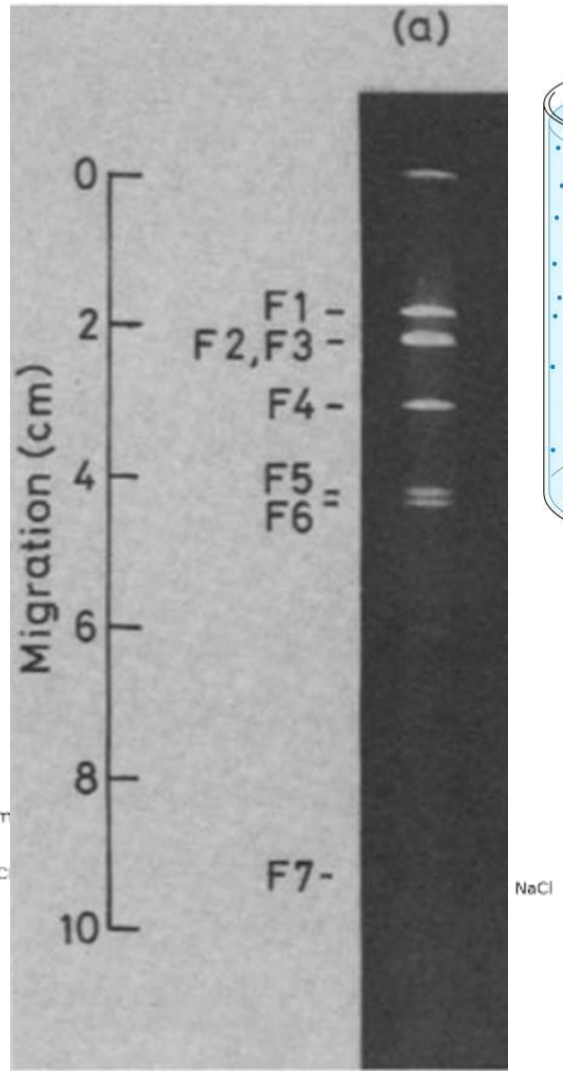


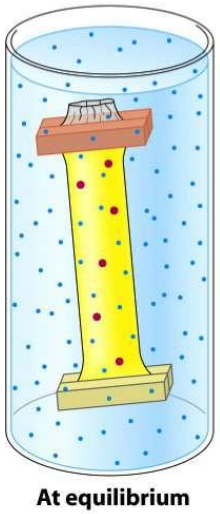
Purification of novel enzyme



DE-52 column
(anion- exchanger)







Resin Type	Anion Exchanger
Net charge of molecule of interest	−
Charge of resin	+
Running conditions	0.5–1.5 pH units above the pI of the molecule of interest

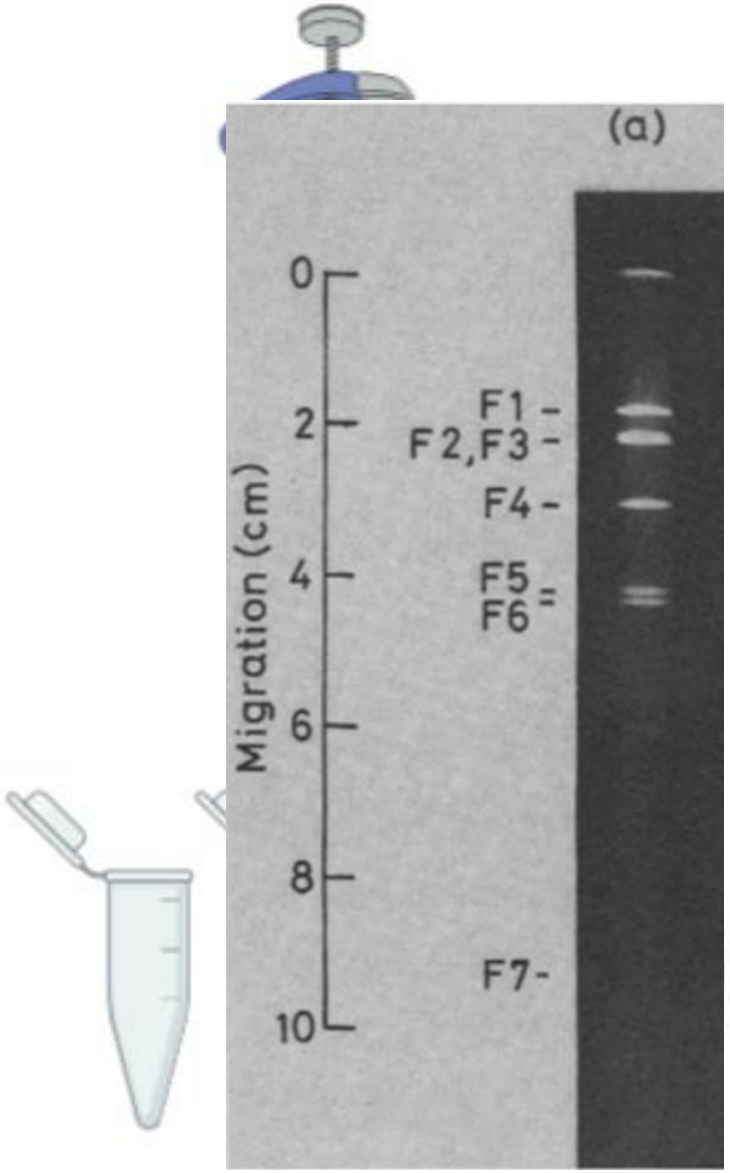
Endonuclease activity of purified novel enzy

TABLE I

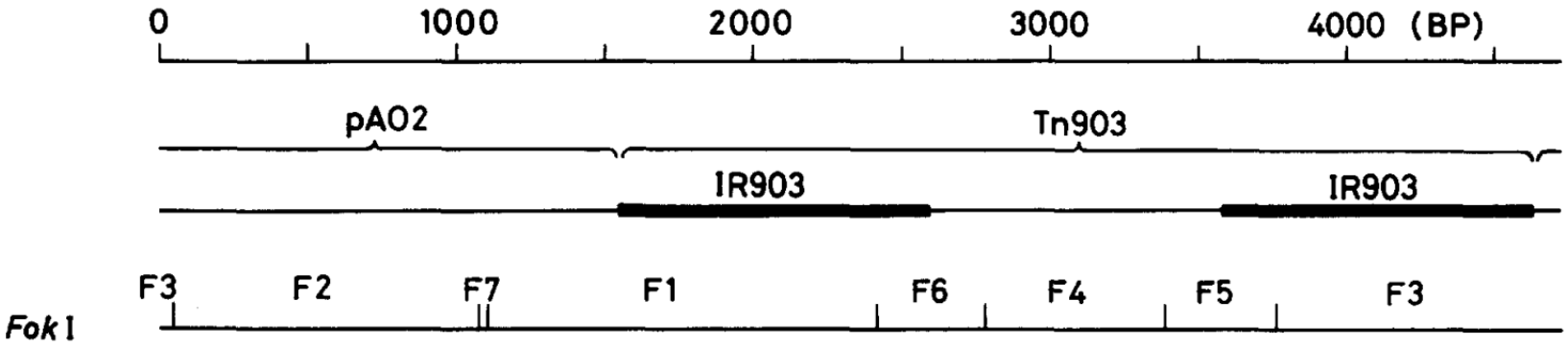
Approximate KCl concentrations elu
Whatman P-11 and DE-52 columns a

Enzymes	P-11 column (M)	DE-5 colu (M)
*Fok*I	0.30–0.34	0.08

One Unit = 1µg of pBR322 DNA



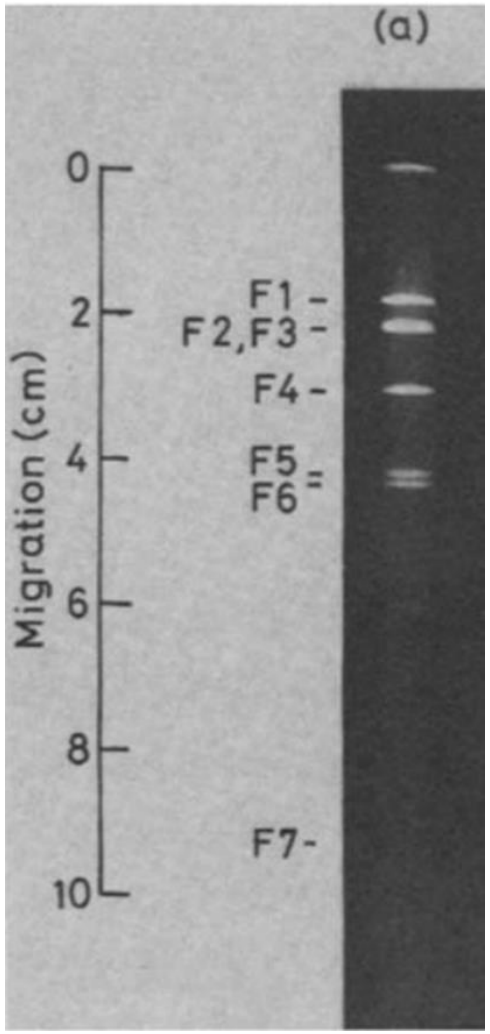
Physical map of pAO43

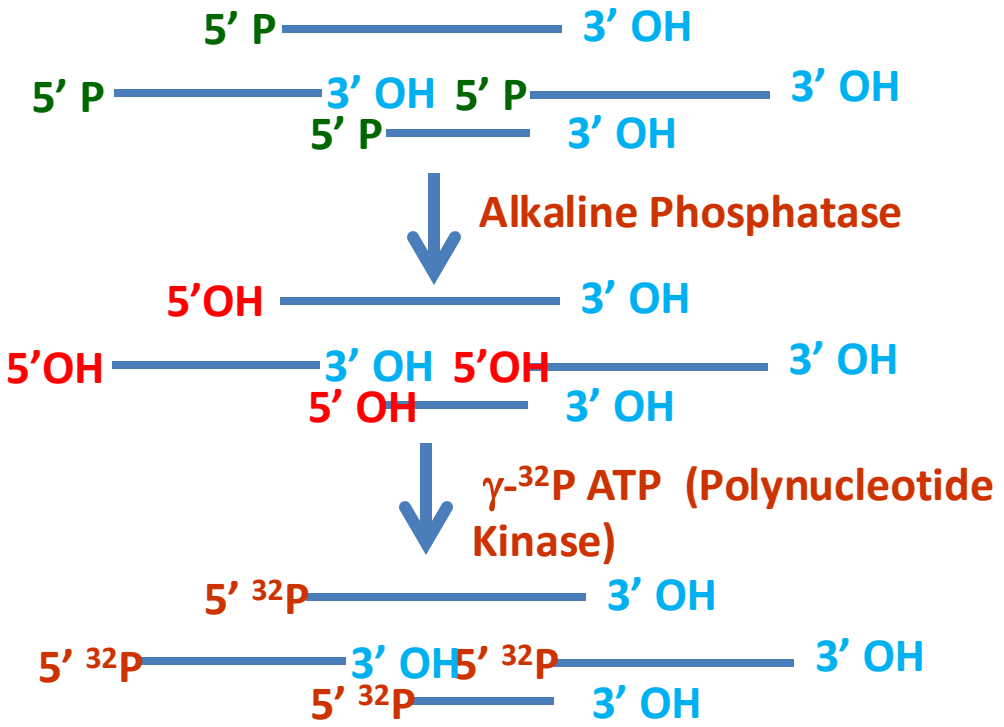


No unique sequence present at the 5’ termini of the fragments

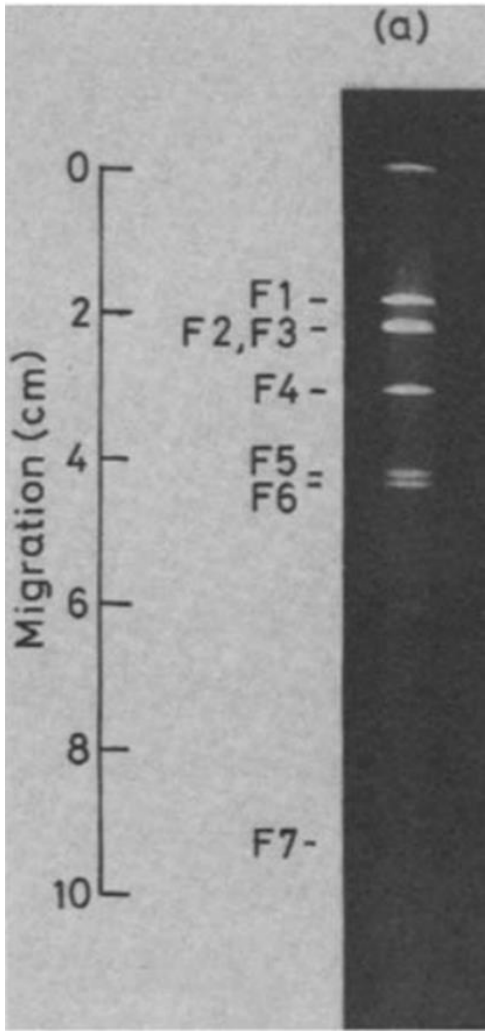
5’ terminal sequencing

Cleavage site specificity of FokI





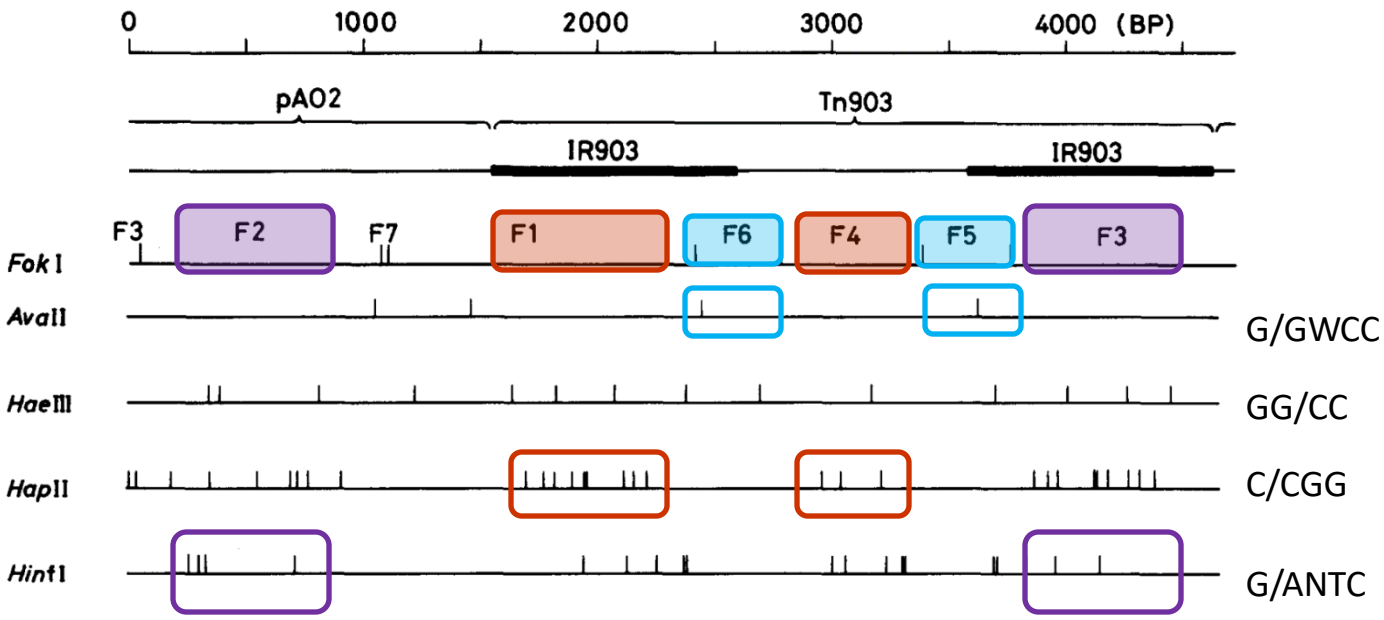
Cleavage site specificity of FokI



5' P F1 and F4 (**HapII**) **C/CGG**

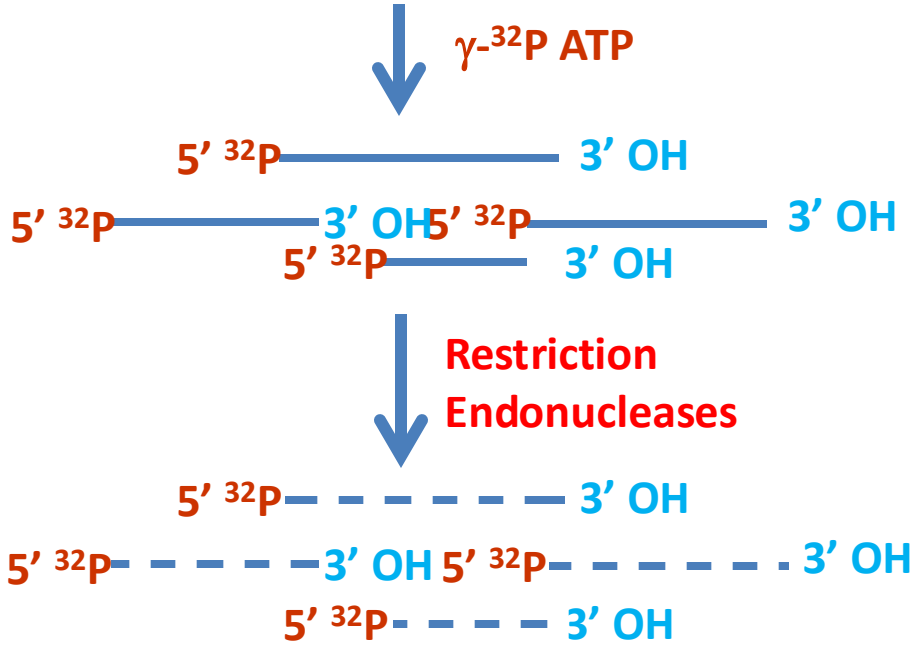
5' P F2 and F3 (**HinfI**) **G/ANTC**

5' P F5 and F6 (**AvaII**) **G/GWCC**

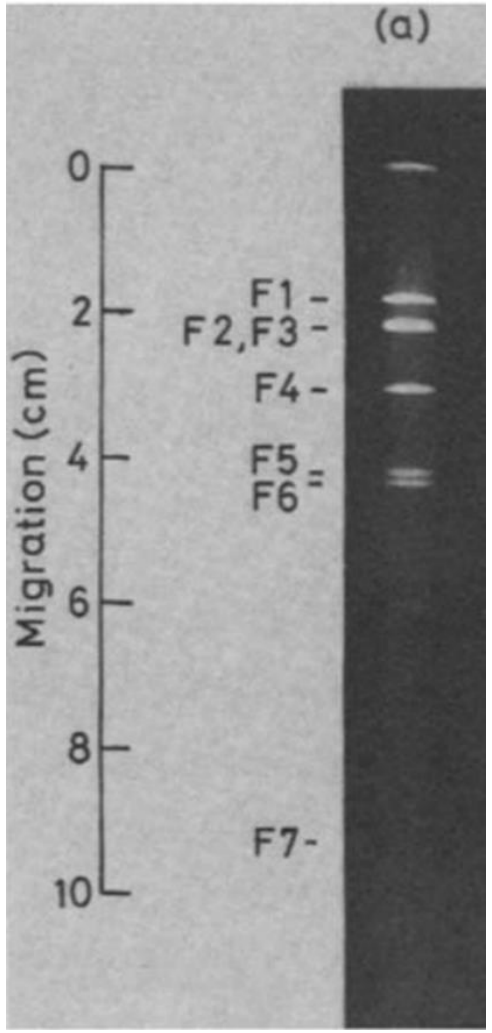


5' terminal sequences of the sub-fragments

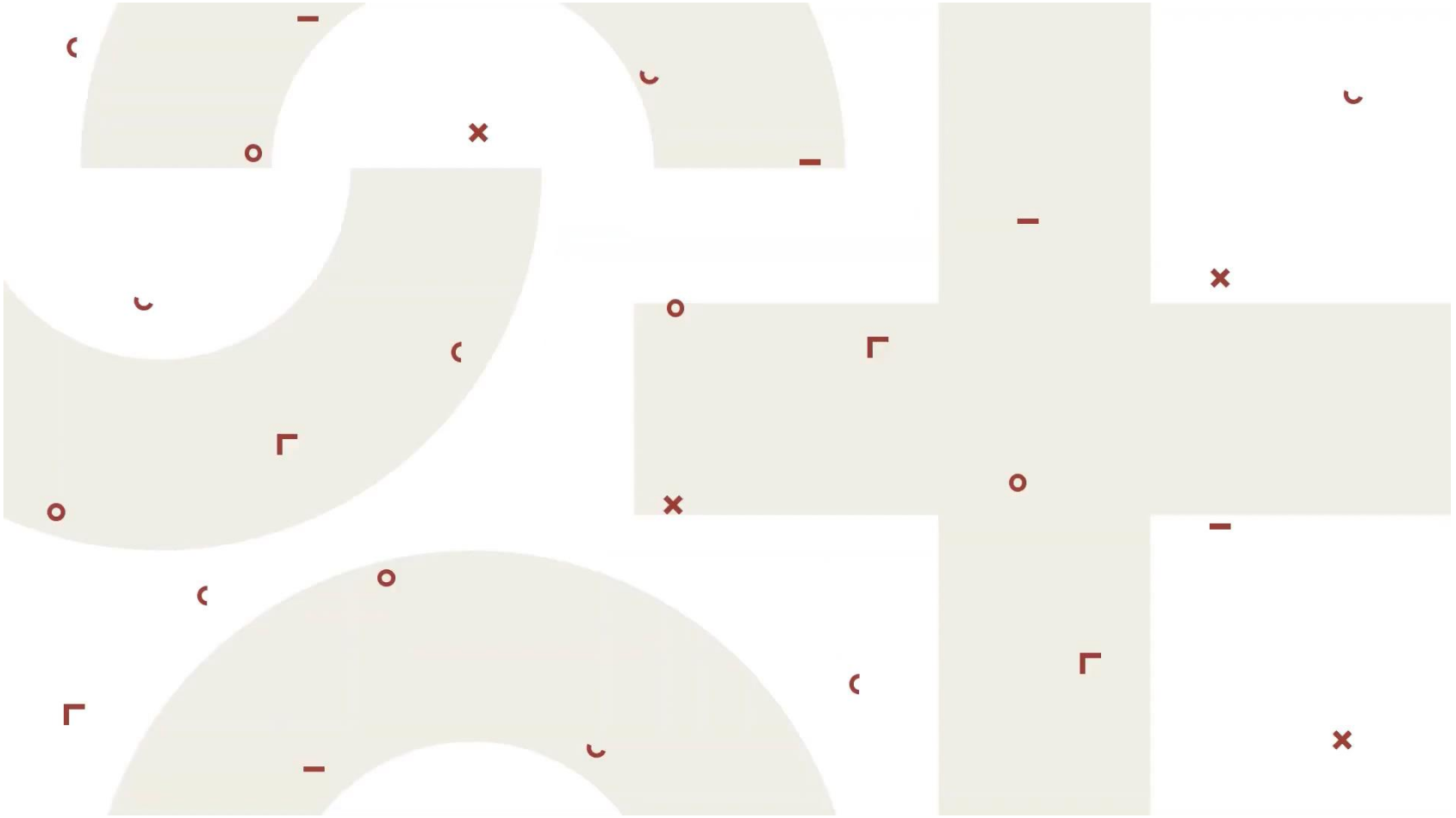
Maxam Gilbert Sequencing on fragments of pAO43



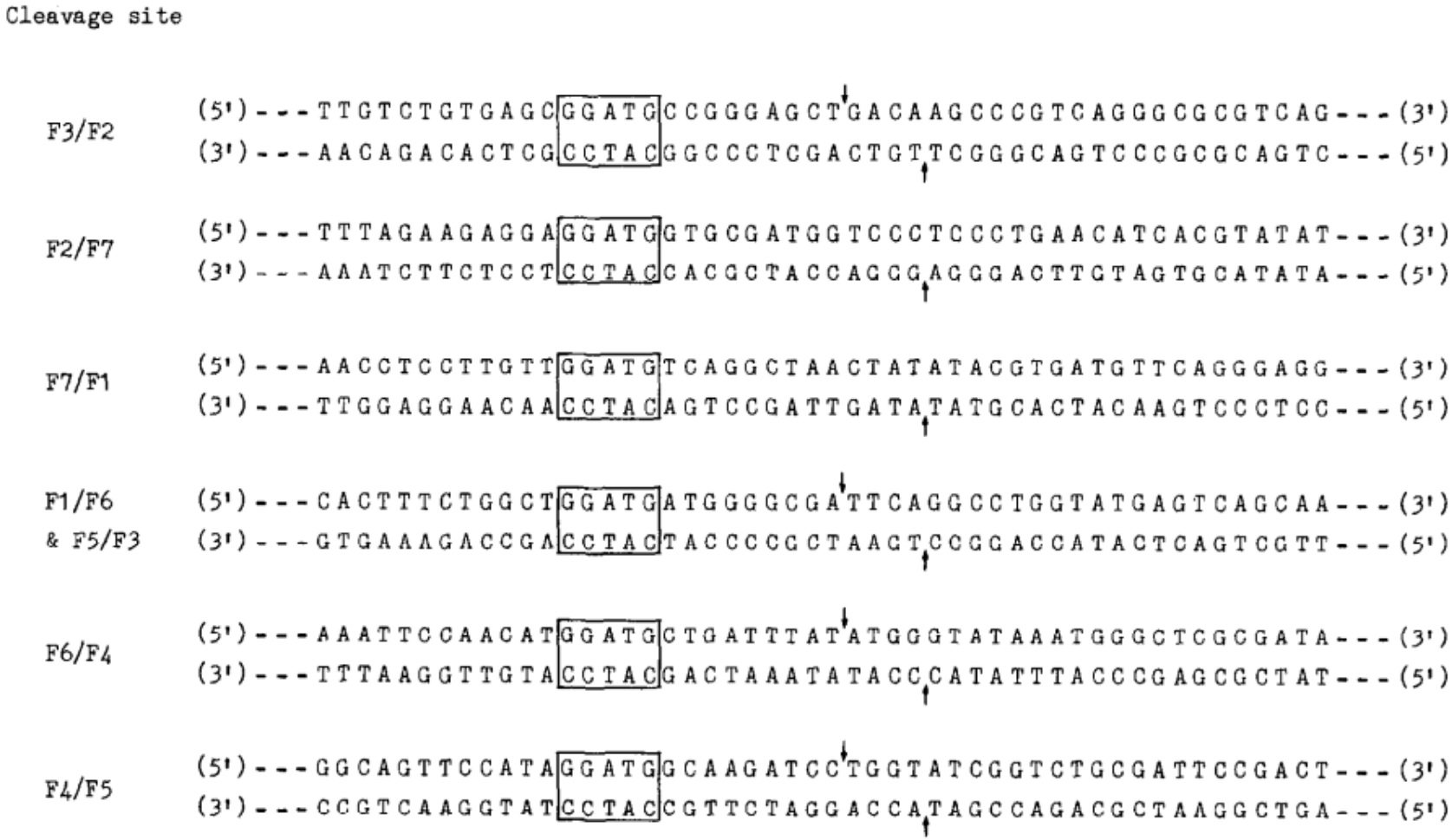
5' terminal sequences of the sub-fragments



Maxam Gilbert Sequencing on fragments of pAO43

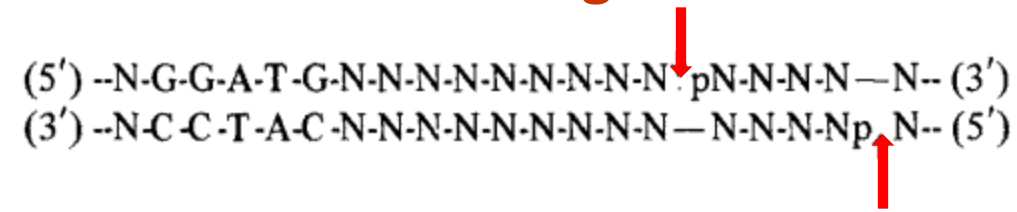


Cleavage site specificity of FokI

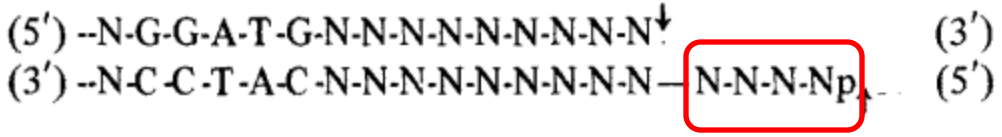


Analysis of terminal sequencing

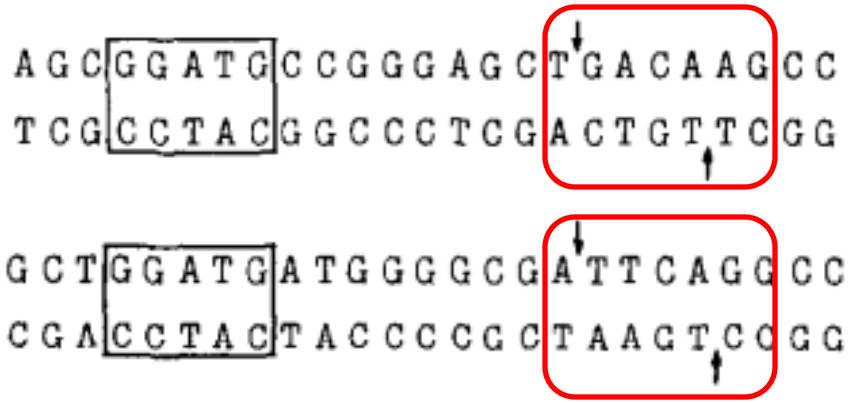
- FokI introduces double stranded cleavage



- Protruding 5’ends of four nucleotides



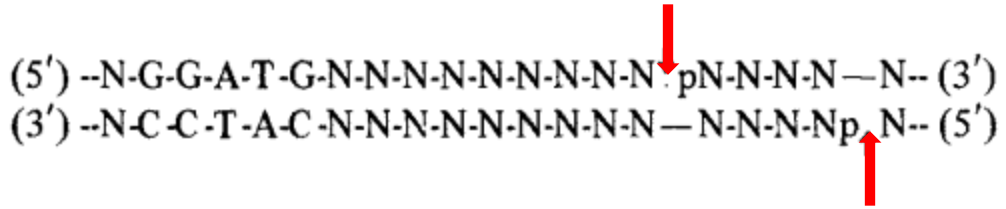
- There is no similarity in the sequences at the cleavage site



Analysis of terminal sequencing

- A Penta nucleotide seq. (GGATG) occurs at nt position 9 and 13 from the cleavage site

- GGATG is the recognition site of FokI (pBR322)
- Mode of cleavage :



Conclusions of Lecture

- Type II S (R.E).
- Fok I purification P-11 and DEAE.
- Terminal 5’ sequencing: Maxam-Gilbert sequencing.
- Recognition sequence of FokI “GGATG”.

Thank You!