

# Genome Editing and Engineering

Course No: BT-637



## LECTURE-3

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# Introduction

- FokI (Flavobacterium okeanokoites)
- Functional domains of FokI

# A brief Recap

- FokI (Flavobacterium okeanokoites)
- Type II S cleaves d.s DNA
- Recognizes nonpalindromic, pentanucleotide
- Cleaves 9/13 nucleotide downstream of recognition site GGATG
- Since 10 bp /turn
- Enzyme probably interacts with one face
- Cleaves at next helical turn
- Two separate domains



*Proc. Natl. Acad. Sci. USA*  
Vol. 89, pp. 4275–4279, May 1992  
Biochemistry

# Functional domains in *Fok* I restriction endonuclease

(*Flavobacterium okeanokoites*/*Escherichia coli*/methyltransferase/restriction endonuclease/recognition and cleavage domains)

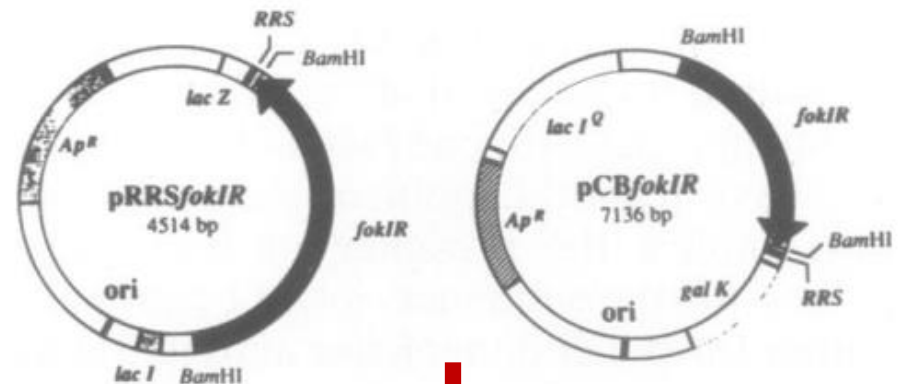
LIN LI, LOUISA P. WU, AND SRINIVASAN CHANDRASEGARAN\*

Division of Environmental Chemistry and Biology, Department of Environmental Health Sciences, The Johns Hopkins University School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205-2179

*Communicated by Hamilton O. Smith, January 15, 1992 (received for review November 25, 1991)*

# Cloning of FokI

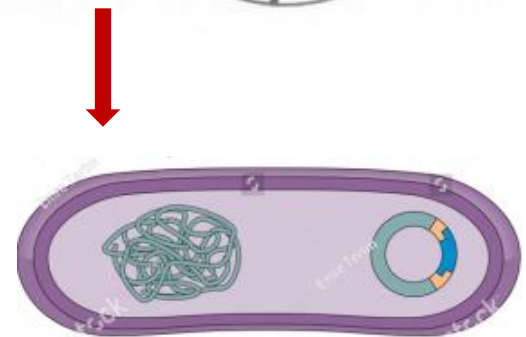
- To study domains of FokI enzymes
- Good quantity of purified proteins
- Overproducer clone



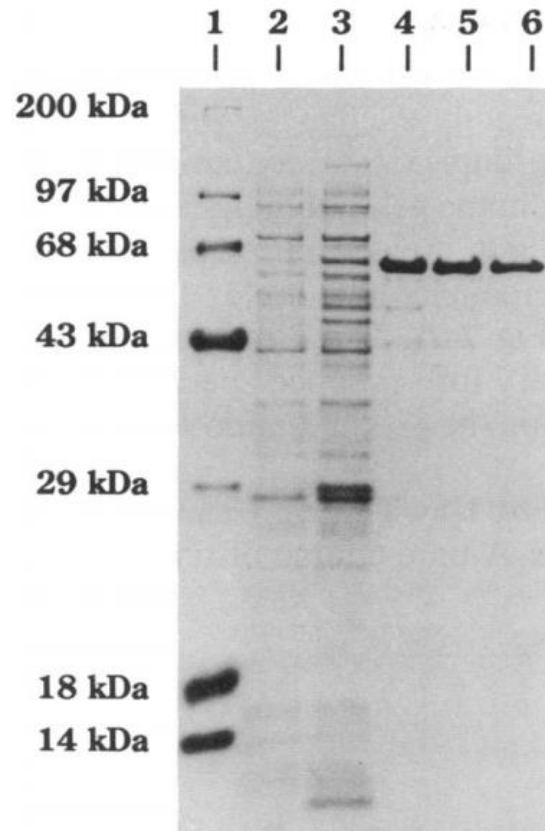
## FokIR

5' primer: 5' - TA BamHI RBS 7-bp spacer ATG GTT TCT AAA ATA AGA ACT - 3'  
Met Val Ser Lys Ile Arg Thr

3' primer: 3' - 24-bp complementary strand BamHI  
TTA TTG CCG CTC TAT TTG AAA ATT ACT CCTAGG AT - 5'  
Asn Asn Gly Glu Ile Asn Phe

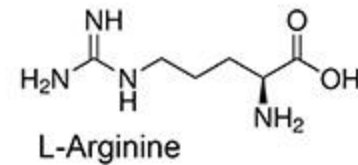
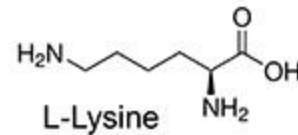
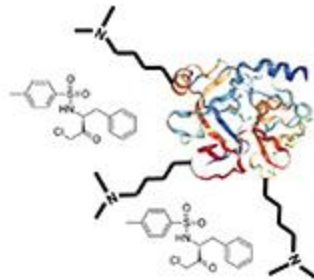


# Purified FokI from clone

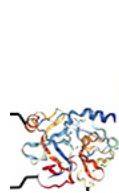


**FIG. 2.** SDS/PAGE profiles at each step in the purification of *Fok* I endonuclease. Lanes: 1, protein standards; 2, crude extract from uninduced cells; 3, crude extract from cells induced with 1 mM isopropyl  $\beta$ -D-thiogalactoside; 4, phosphocellulose pool; 5, 50–70% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fractionation pool; and 6, DEAE pool.

# Trypsin cleavage study of FokI

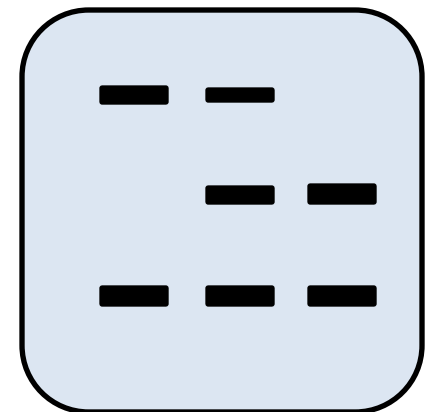


## Time course experiment



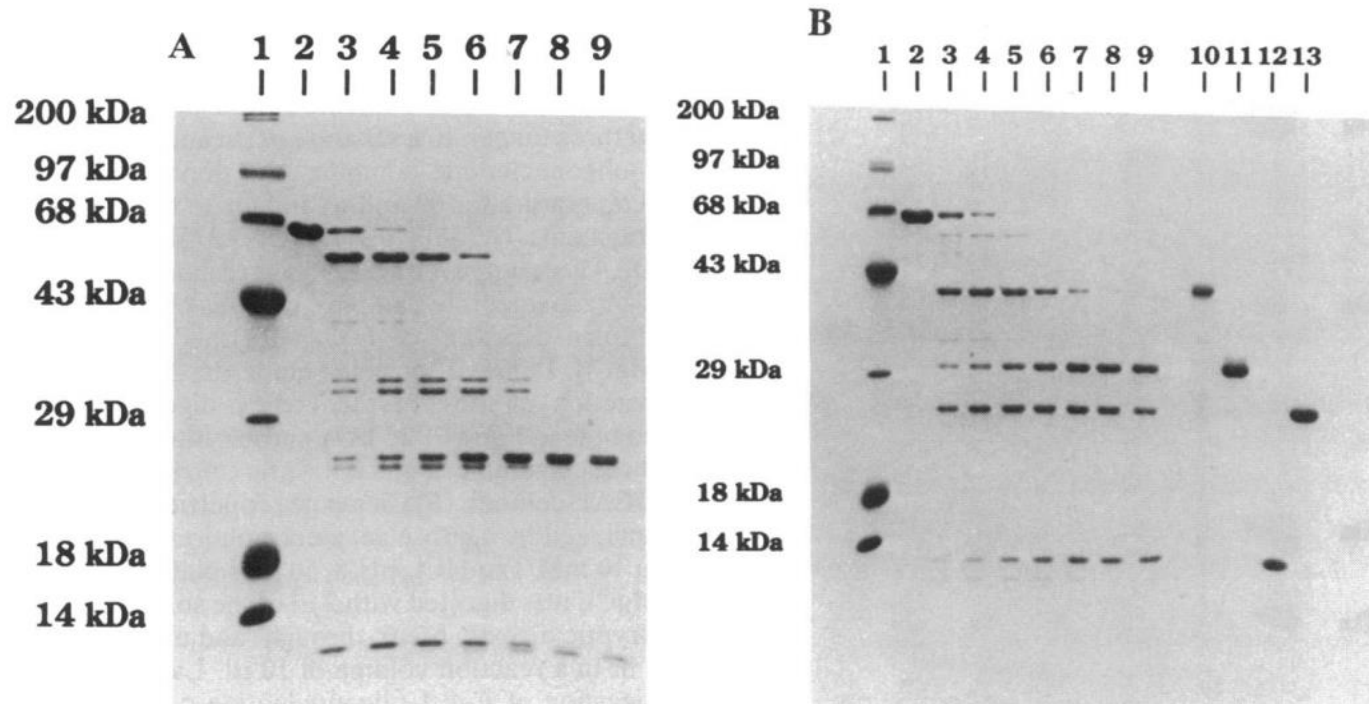
R.T. (1 hr)

2.5 min  
5.0 min  
10.0 min  
20.0 min  
40.0 min  
80.0 min  
160.0 min



SDS-PAGE

# Trypsin cleavage study of FokI



Lanes (A and B): 1, protein standards;

2, *Fok I* endonuclease;

3, 2.5 min (of trypsin digestion);

4, 5.0 min;

5, 10 min;

6, 20 min;

7, 40 min;

8, 80 min;

9, 160 min

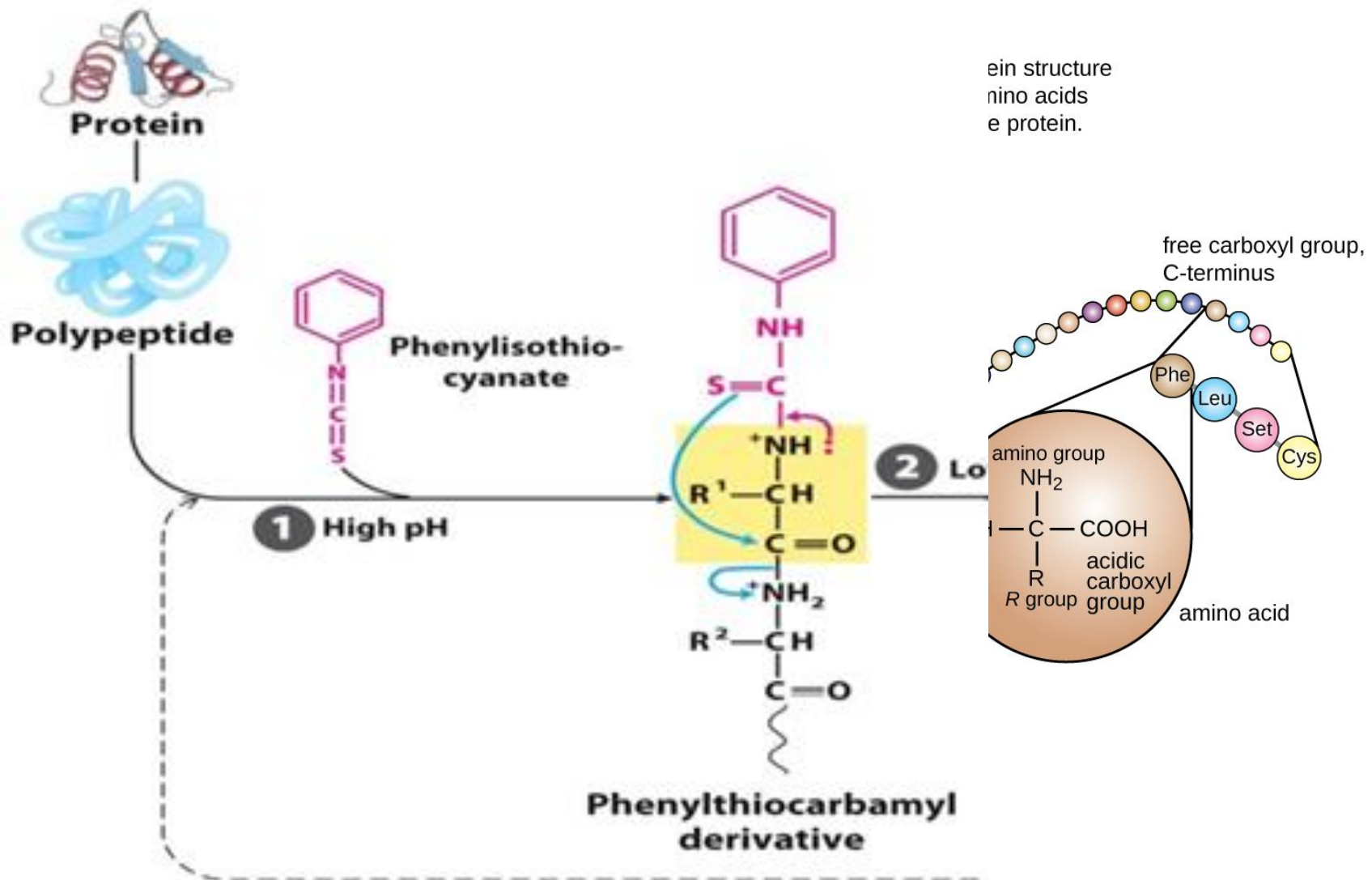
HPLC-purified tryptic fragments of 41 kDa (lane 10)

30 kDa (lane 11)

11 kDa (lane 12)

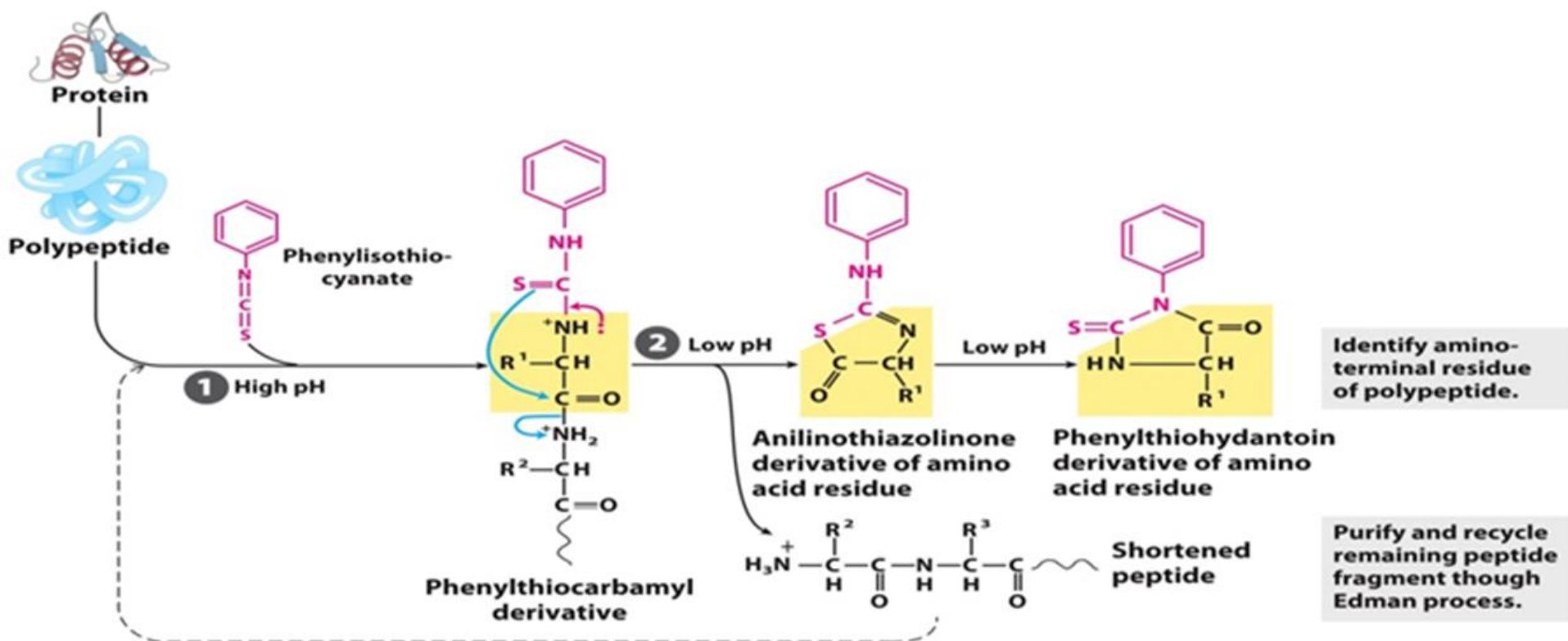
25 kDa (lane 13)





ein structure  
nino acids  
e protein.

**Figure 3-27**  
Lehninger Principles of Biochemistry, Sixth Edition  
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**Figure 3-27**  
 Lehninger Principles of Biochemistry, Sixth Edition  
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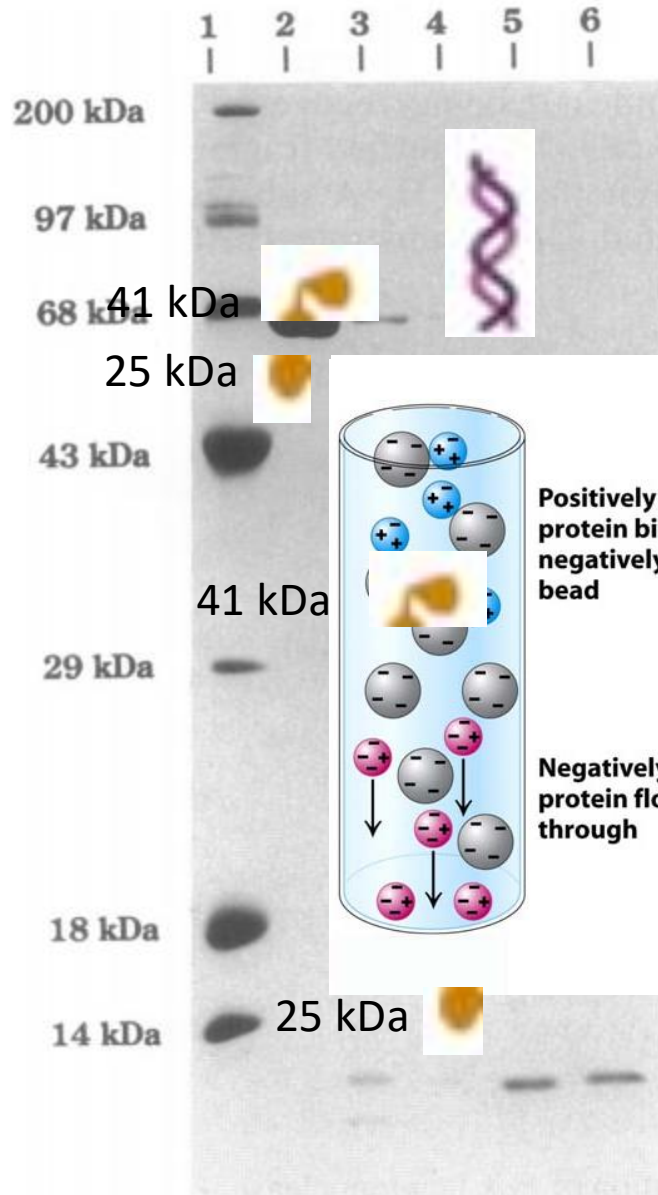
# Amino terminal sequencing

Table 1. Amino-terminal sequences of *Fok* I fragments from trypsin digestion

Frag- ment	Amino-terminus sequence	DNA substrate
8 kDa	VSKIRTFG*VQNPGKFENLKRVVQVFDRNS	—
58 kDa	SEAPCDAIHQ	—
25 kDa	QLVKSELEEK	+
41 kDa	VSKIRTFGWV	+
30 kDa	VSKIRTFGWV	+
11 kDa	FTRVPKRKY	+

\*, Unidentified amino acid.

# DNA binding properties of the fragments



1, protein standards;

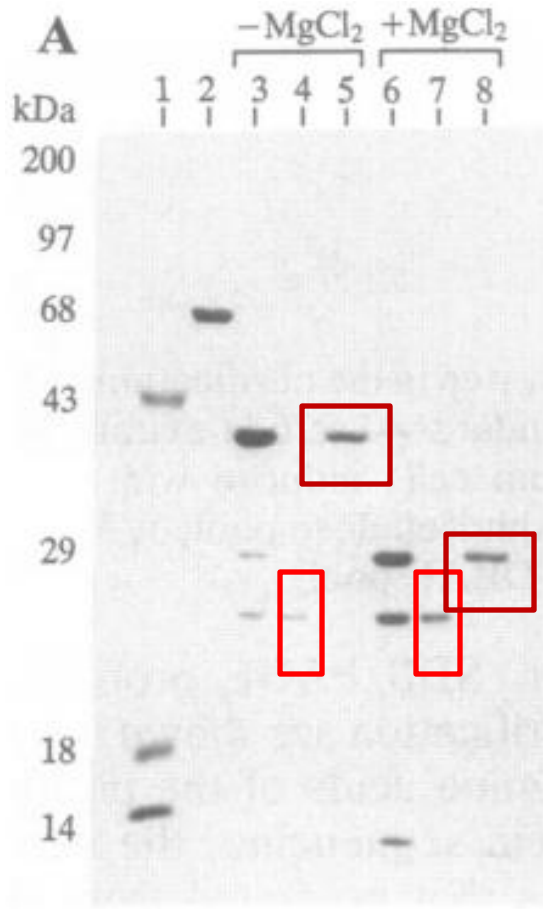
2, *Fok* I endonuclease;

3, 10-min trypsin digestion mixture of *Fok* I-oligonucleotide complex;  
 tryptic fragments that bound to the oligo(dT)-cellulose column;  
 160-min trypsin digestion mixture of *Fok* I-oligonucleotide complex;  
 tryptic fragments that bound to the oligo(dT)-cellulose column.

**41 and 30 kDa bind DNA**

**25 kDa is not retained**

# Cleavage properties of the fragments



1, protein standards 2, *Fok* I endonuclease

3–5 trypsin digestion of *Fok* I–oligonucleotide complex without MgCl<sub>2</sub>

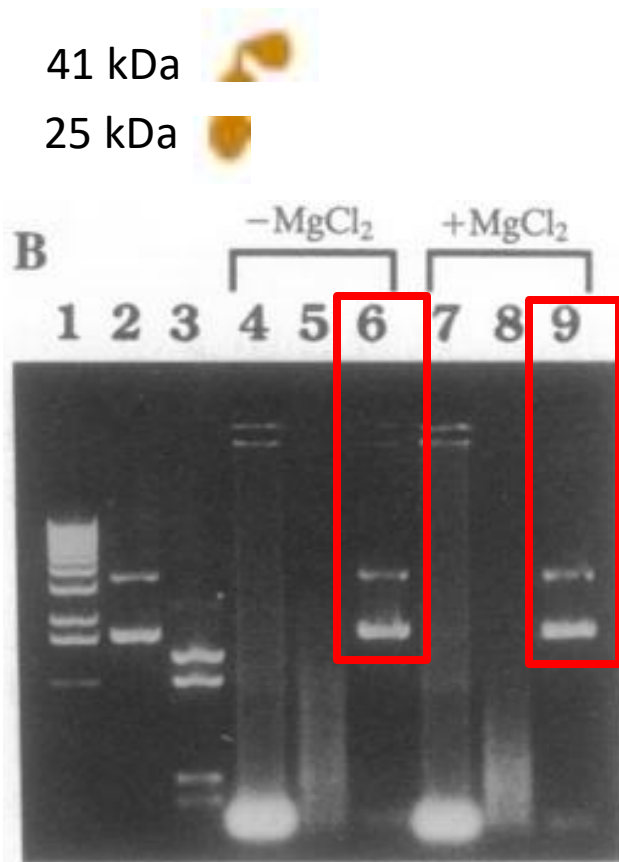
6–8 trypsin digestion of *Fok* I–oligonucleotide complex with MgCl<sub>2</sub>

4 and 7, 25-kDa carboxyl-terminal fragment in the break-through volume

5 and 8, tryptic fragments of *Fok* I that bound to the DEAE column.

- 41 and 30 kDa bind to DEAE-Sephadex column
- 25 kDa do not bind to DEAE column

# Cleavage properties of the fragments



1, 1-kilobase (kb) ladder

2, pTZ19R;

3, pTZ19R digested with *Fok* I endonuclease

4-6 digestion of *Fok* I-oligonucleotide complex without MgCl<sub>2</sub>

7-9 digestion of *Fok* I-oligonucleotide complex with 10 mM MgCl<sub>2</sub>

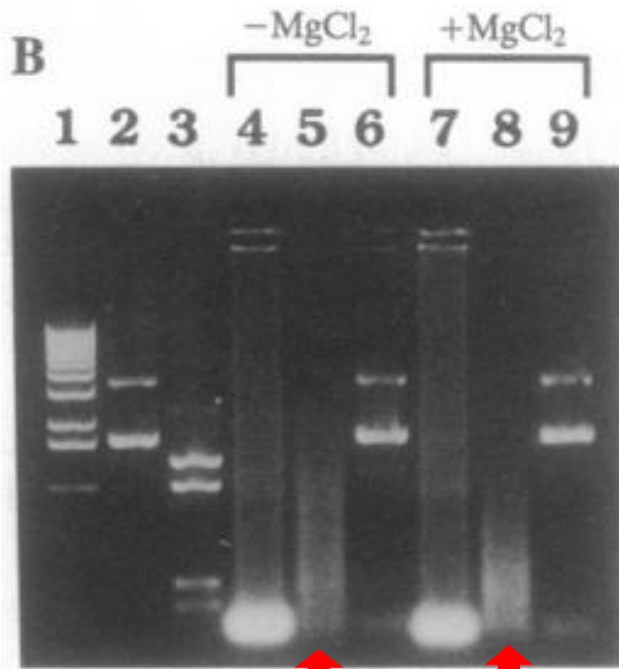
4 and 7 tryptic digests of *Fok* I-oligonucleotide complex

6 and 9 tryptic fragments of *Fok* I that bound to the DEAE column.

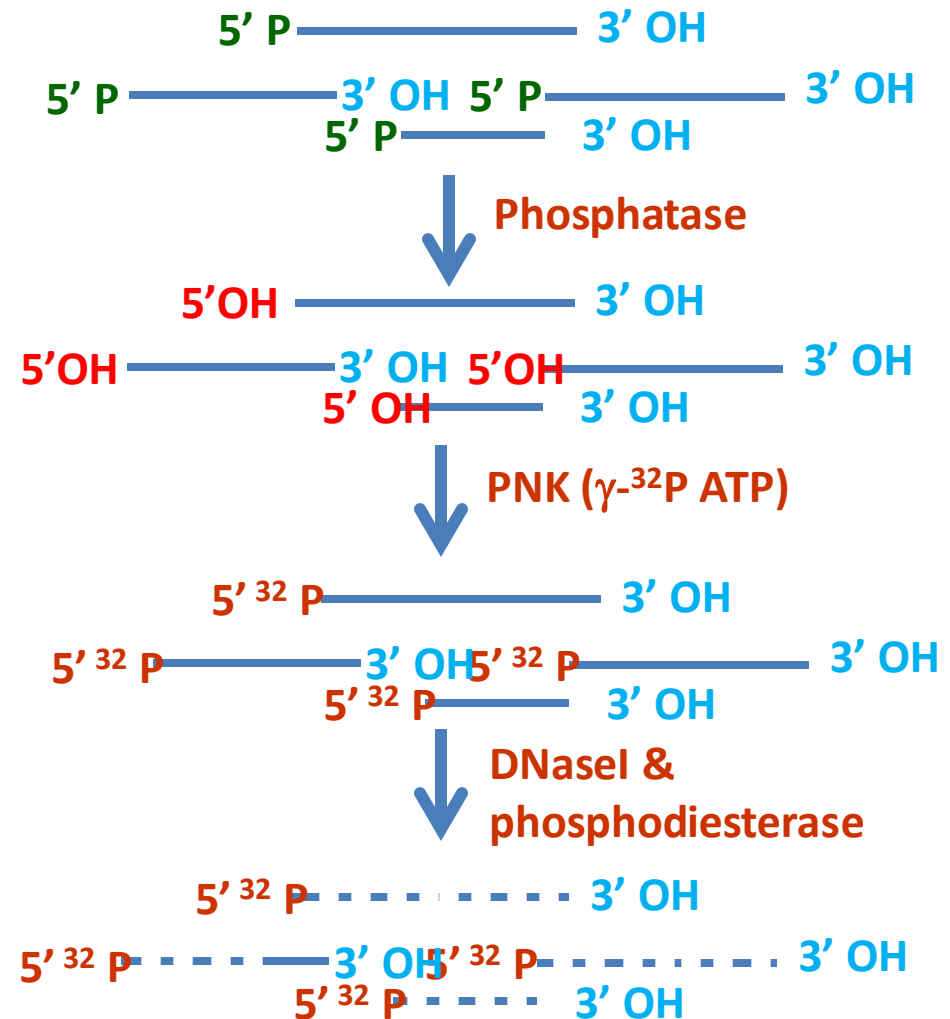
5 and 8, 25-kDa carboxyl-terminal fragment in the break-through volume.

- 25 kDa cleaves DNA in small pieces
- Nonspecific in cleavage

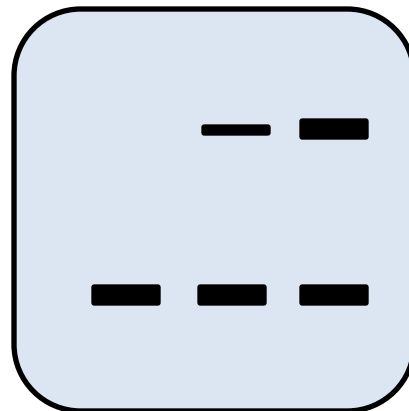
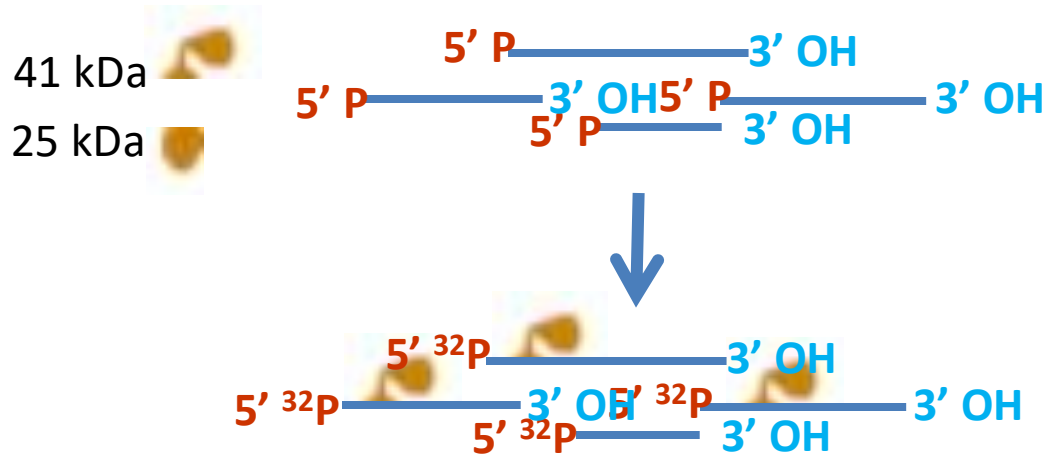
# Cleavage properties of the fragments



- 25 kDa cleaves preferentially
- 5' G > A >> T ~ C
- 25 kDa = cleavage domain



# Gel mobility shift assay



Nondenaturing-PAA

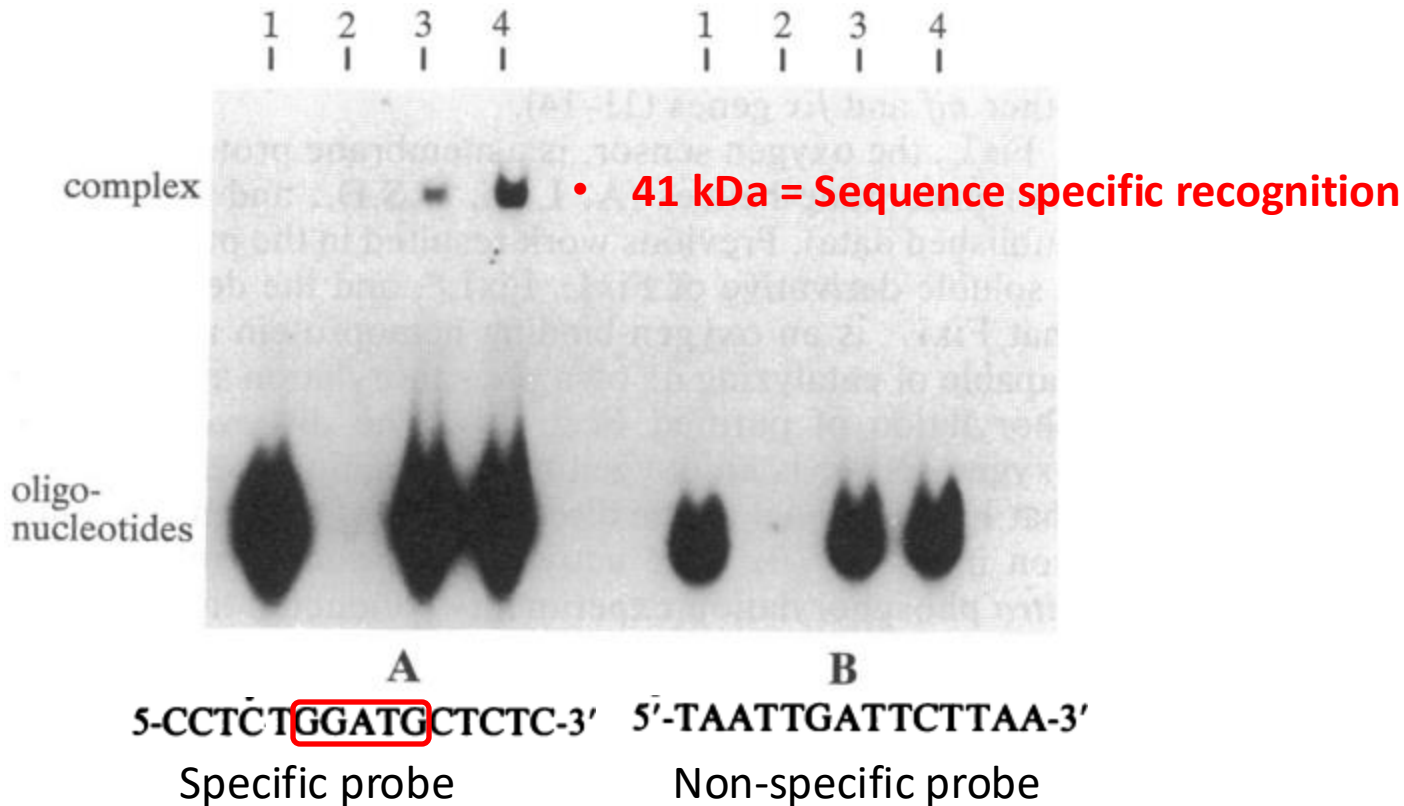


# Gel mobility shift assay

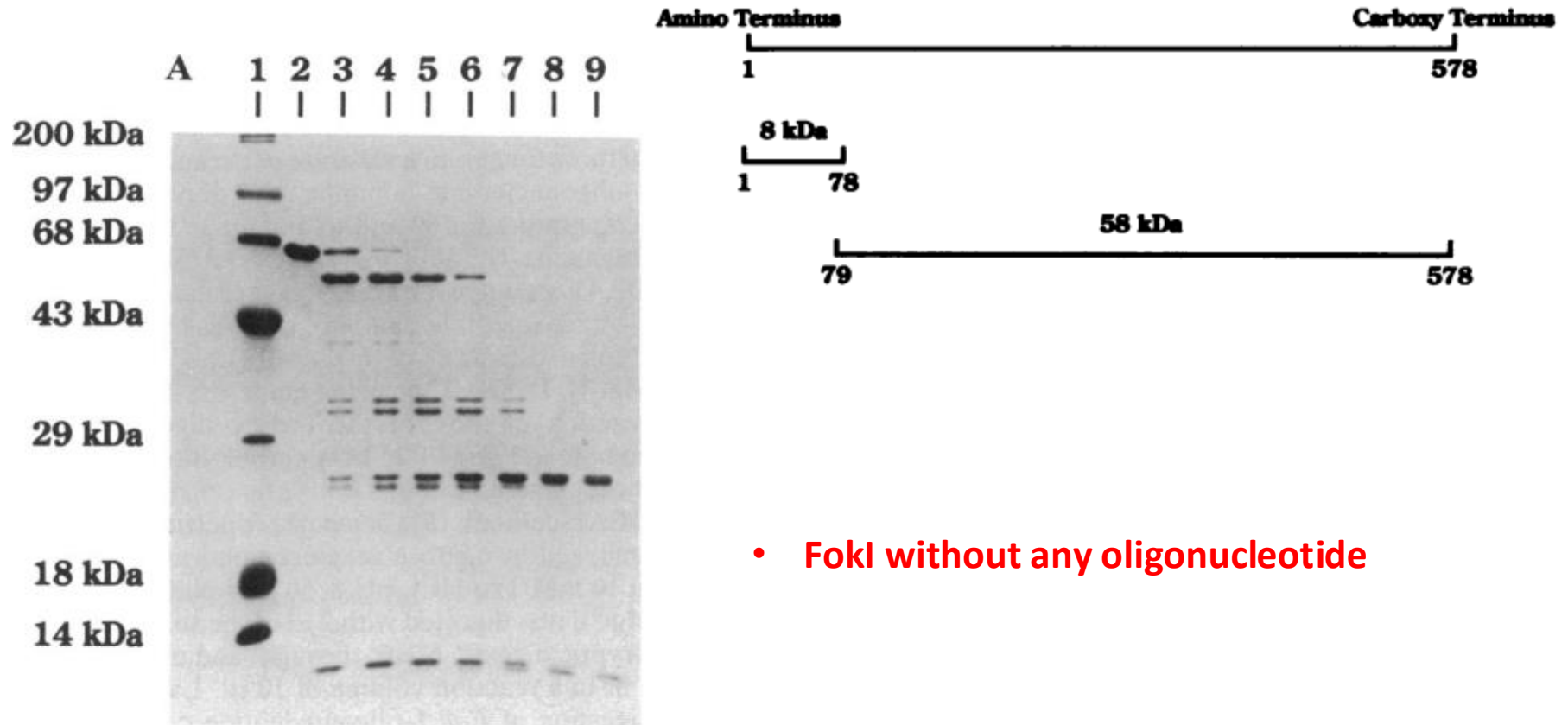
1, specific oligonucleotide duplex

2, 41-kDa amino-terminal fragment–oligonucleotide complex

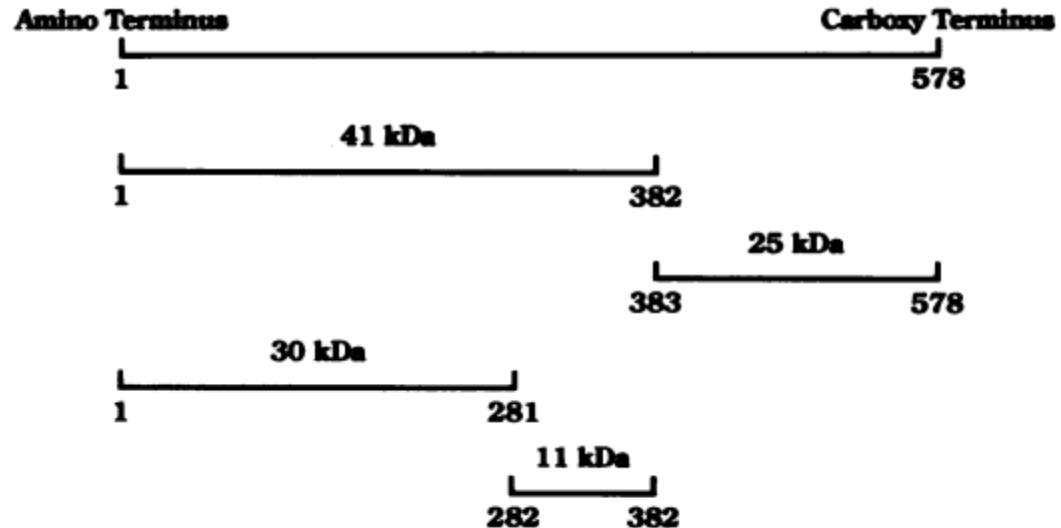
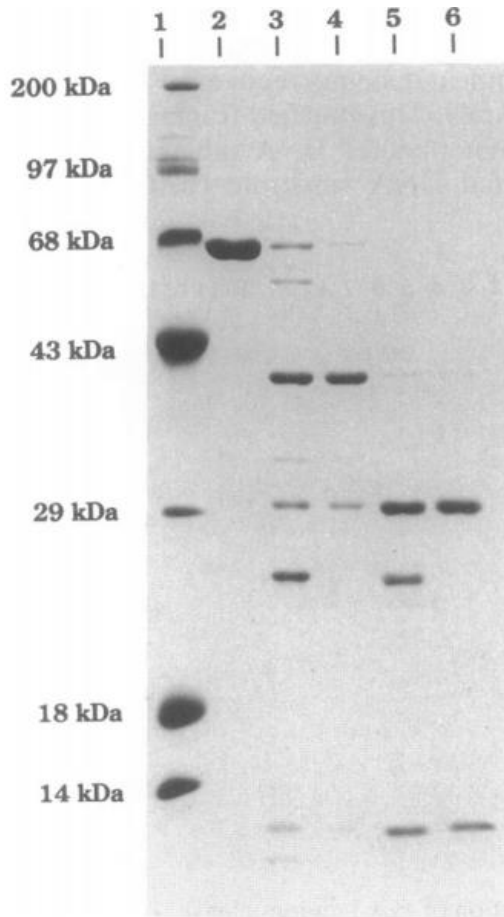
3 and 4, specific probe incubated with complex for 30 and 120



# Functional domains of FokI



# Functional domains of FokI



- Two domains:
  - a) Seq. specific recognition (N-terminus)
  - b) Endonuclease activity (C-terminus)

# Future Outlook

- **Mutational analysis of the domains**
- **Modular structure**
- **Feasibility to construct chimeric endonucleases**

# Conclusions of Lecture-3

- The two domains of FokI
- Recognition domain (amino-terminal)
- Cleavage domain (carboxy-terminal)
- Formation of “the complex” via Gel mobility shift assay

**Thank You!**