

Genome Editing and Engineering

Course No: BT-637



LECTURE-7

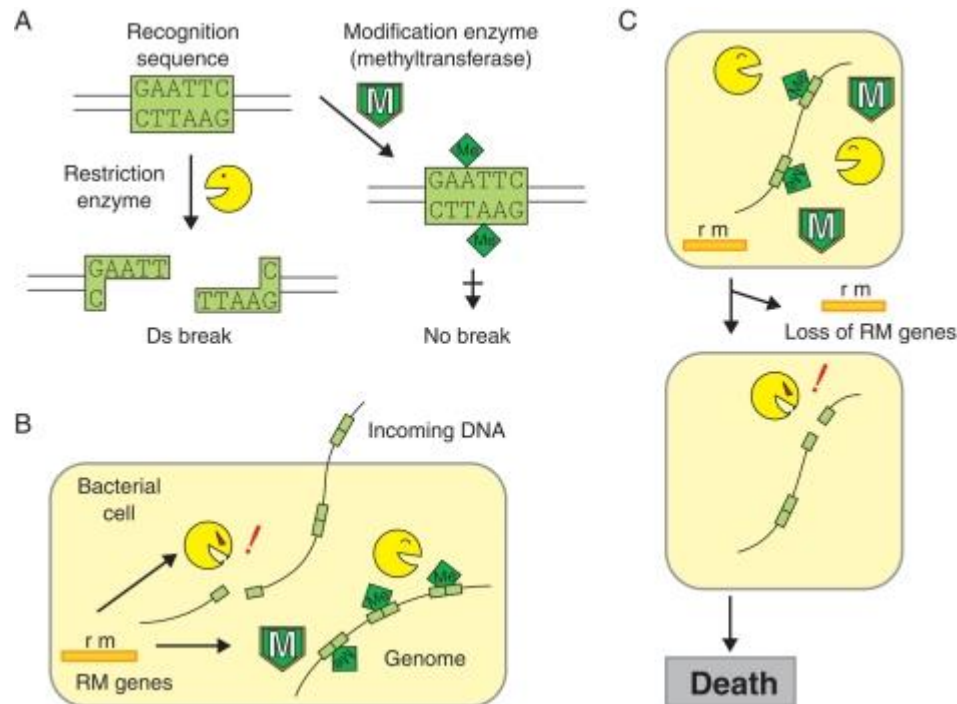
Dr. Kusum K. Singh

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Introduction

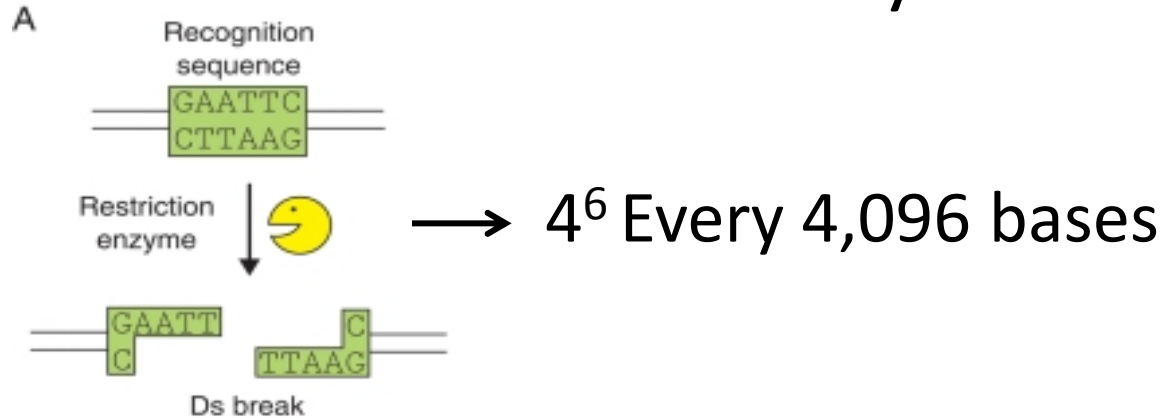
- The Restriction Modification system



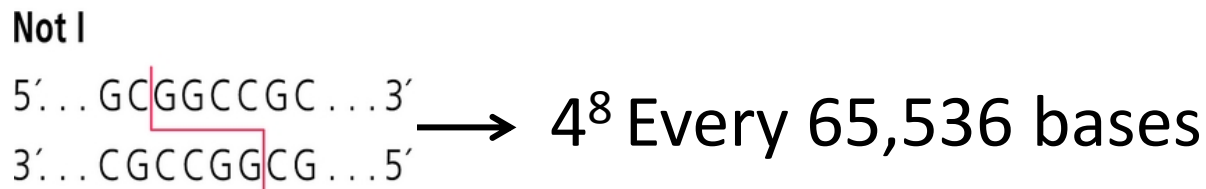
- Not suitable for unique cuts in large DNA

Introduction

- The Restriction Modification system



- Rare-cutter enzyme

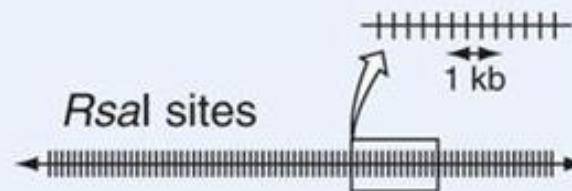


- Not suitable for unique cuts in large DNA

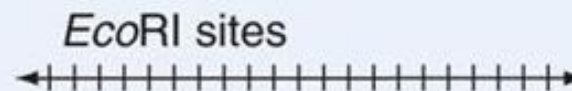
Introduction

Restriction Sites in Human Genomic DNA

1. Four-base



2. Six-base



3. Eight-base



Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 883–887, February 1994
Biochemistry

Chimeric restriction endonuclease

(Flavobacterium okeanoikoites/Escherichia coli/hybrid restriction endonuclease/protein engineering/recognition and cleavage domains)

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Chimeric restriction endonuclease

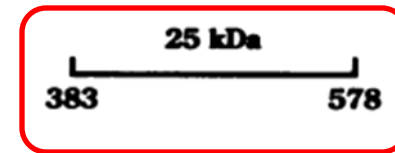
- DNA-binding proteins

Homeodomain motif

Zinc finger motifs

POU domain motifs

λ and lac repressors



Chimeric restriction endonuclease

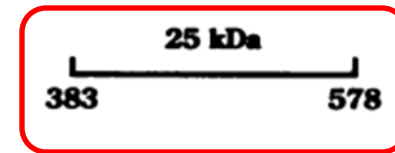
- DNA-binding proteins

Ultrabithorax (Ubx) homeodomain motif

61 codon region

183 bp long

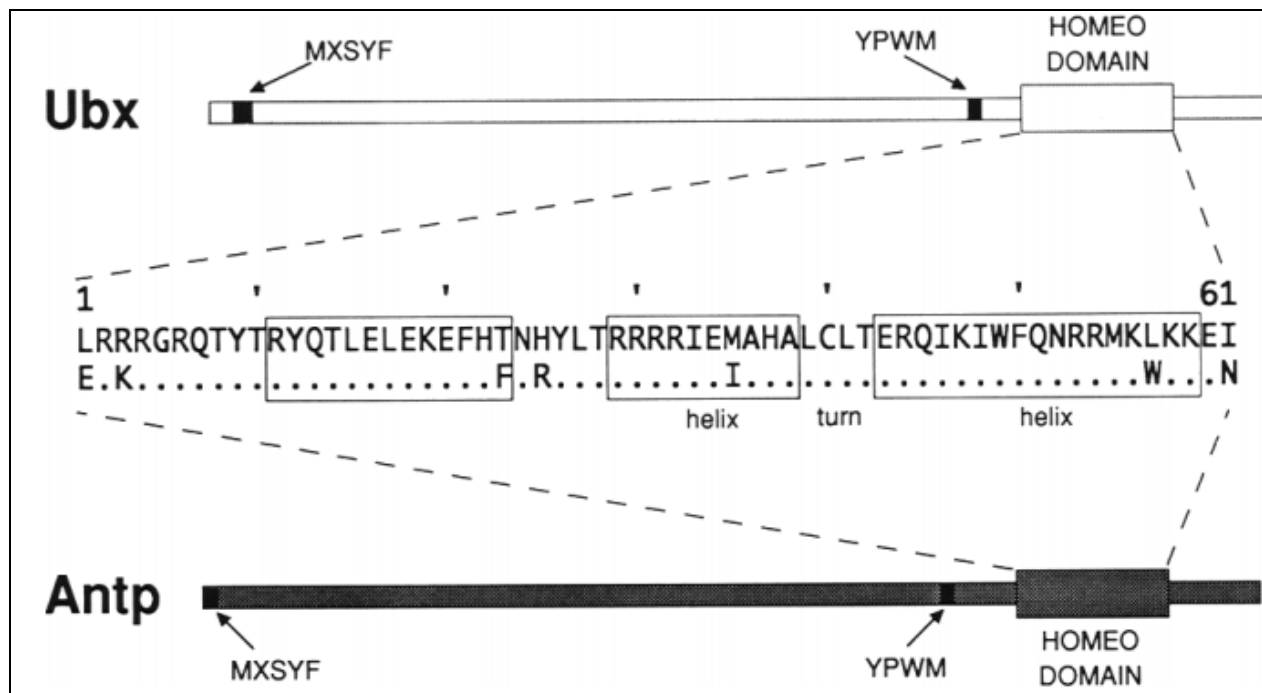
conserved in eukaryotes



Chimeric restriction endonuclease

- DNA-binding proteins

Ultrabithorax (Ubx) homeodomain motif



Chimeric restriction endonuclease

- DNA-binding proteins

Ultrabithorax (Ubx) homeodomain motif

5' TTAAT(G/T)(G/A)CC 3'



Chimeric restriction endonuclease

A

Ubx 5' - primer: 5' - TAC ^{PstI} CTGCAG C GGAGGT TTAAAT ATG CGA AGA CGC GGC CGA - 3'
 Met Arg Arg Arg Gly Arg

3' - primer: 3' - T TAC TTC GAC TTC TTC CTC TAG GTT ^{SpeI} GAT CAGAT - 5'
 Met Lys Leu Lys Lys Glu Ile Gln Leu

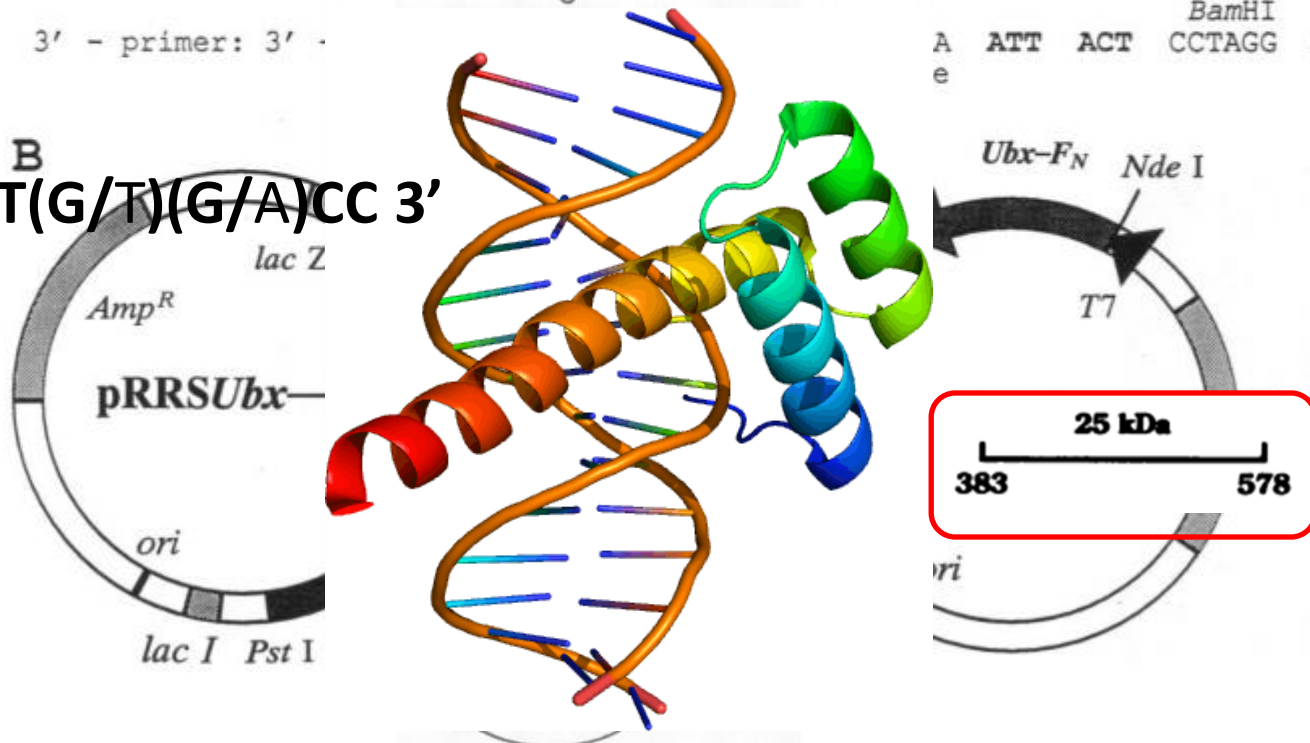
Ubx-FN 5' - primer: 5' - CCA CGG CAT ^{NdeI} ATG CGA AGA CGC GGC CGA - 3'
 Met Arg Arg Arg Gly Arg

3' - primer: 3' -

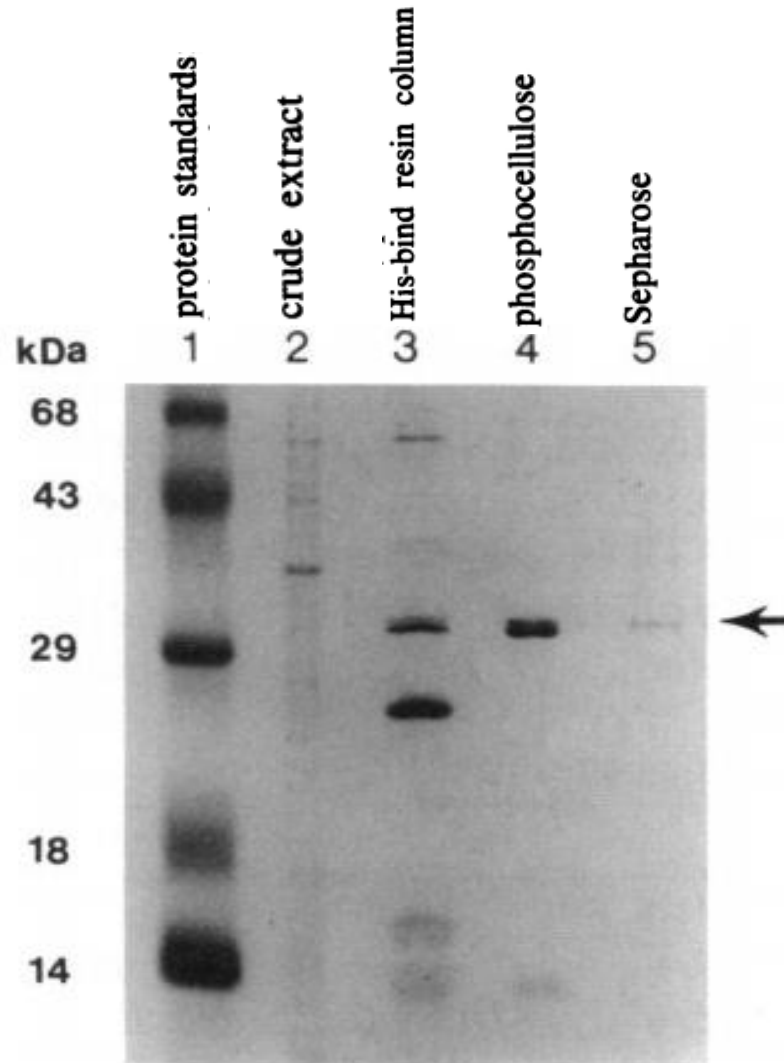
A ^{BamHI} ATT ACT CCTAGG AT - 5'
 e

B

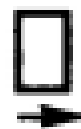
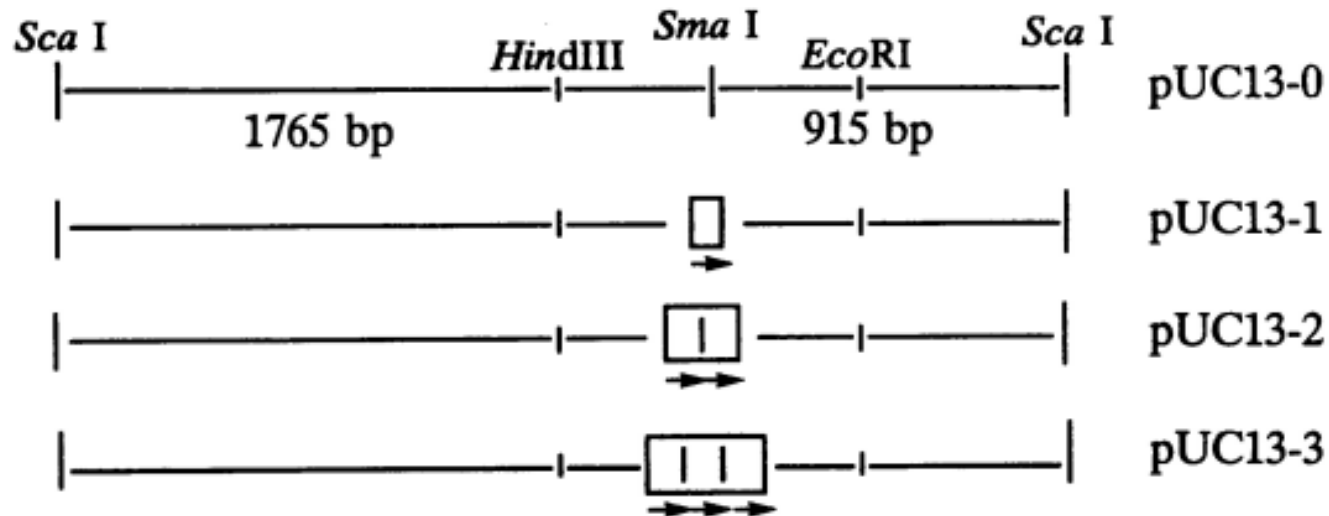
5' TTAAT(G/T)(G/A)CC 3'



Purification of Chimeric Endonuclease



DNA Seq. preference of the Ubx-F_N

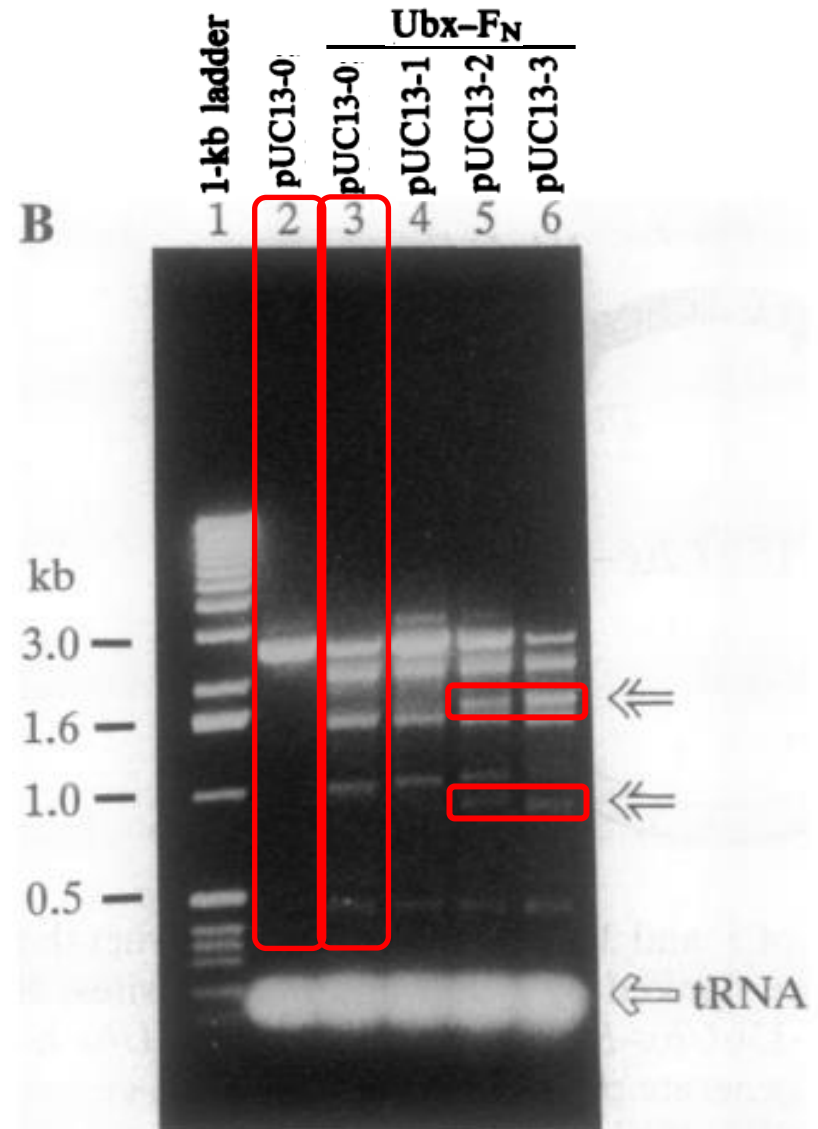
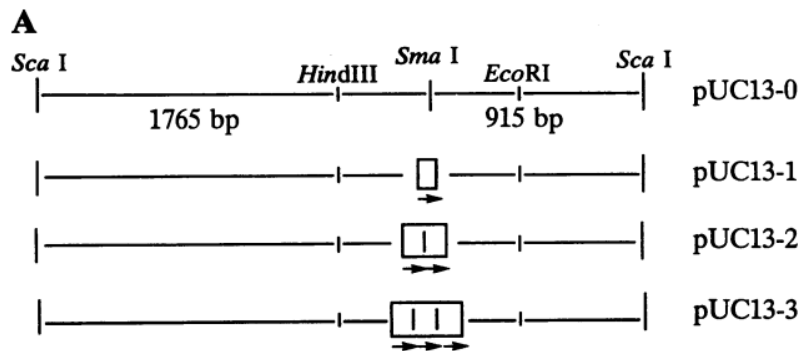


31-base pair DNA fragment

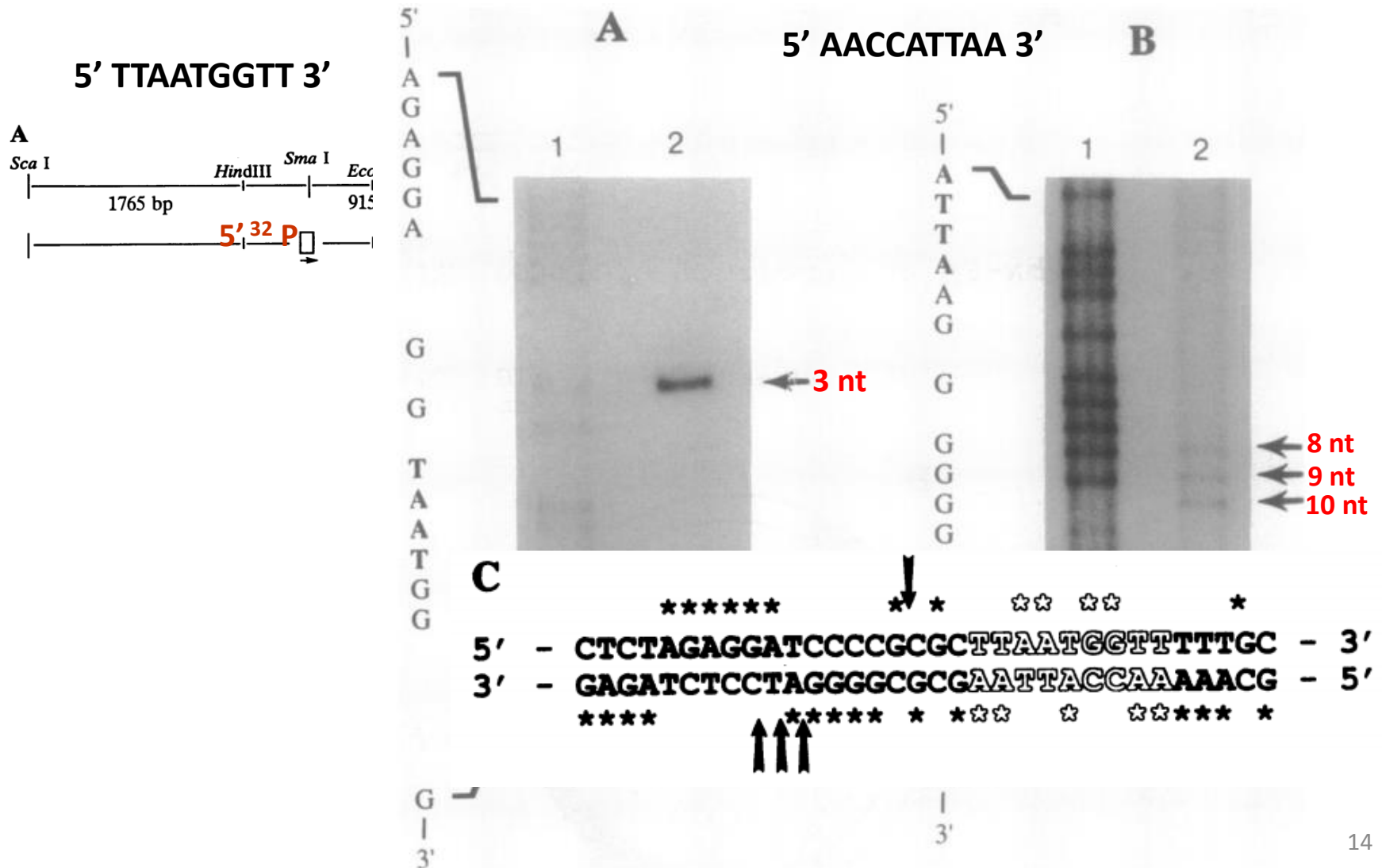
Ubx recognition sequence

5' TTAATGGTT 3'

DNA Seq. preference of the Ubx-F_N



Cleavage distance recognition site



Conclusions of Lecture-7

- Successfully engineered a first chimeric RE
- Hybrid enzyme targets to appropriate site
- Ubx-F_N shows non-specific nucleolytic activity
- Important = “artificial restriction enzymes”

Thank You!