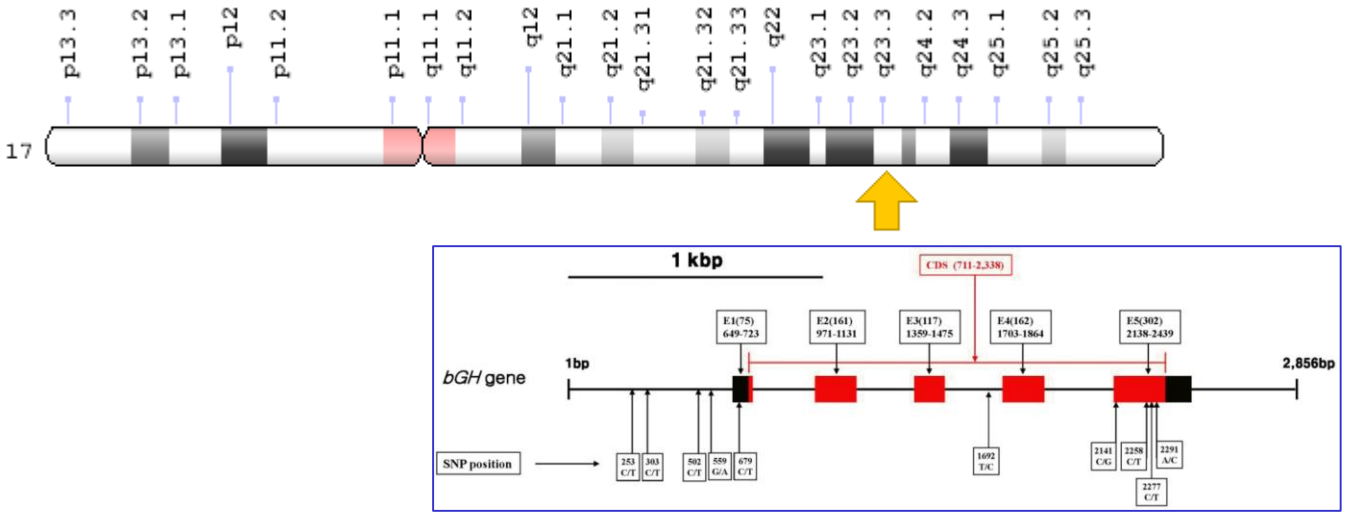
REKOMBİNANT DNA TEKNOLOJİSİ

PROF. DR. KADİR TURAN

Growth hormone (somatotropin or human growth hormone)



Proc. Nat. Acad. Sci. USA
Vol. 69, No. 10, pp. 2904–2909, October 1972

Biochemical Method for Inserting New Genetic Information into DNA of Simian Virus 40: Circular SV40 DNA Molecules Containing Lambda Phage Genes and the Galactose Operon of *Escherichia coli*

(molecular hybrids/DNA joining/viral transformation/genetic transfer)

DAVID A. JACKSON*, ROBERT H. SYMONS†, AND PAUL BERG

Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305

Contributed by Paul Berg, July 31, 1972

ABSTRACT **We have developed methods for covalently joining duplex DNA molecules to one another and have used these techniques to construct circular dimers of SV40 DNA and to insert a DNA segment containing lambda phage genes and the galactose operon of *E. coli* into SV40 DNA. The method involves: (*a*) converting circular SV40 DNA to a linear form, (*b*) adding single-stranded homodeoxypolymeric extensions of defined composition and length to the 3′ ends of one of the DNA strands with the enzyme terminal deoxynucleotidyl transferase (*c*) adding complementary homodeoxypolymeric extensions to the other DNA strand, (*d*) annealing the two DNA molecules to form a circular duplex structure, and (*e*) filling the gaps and sealing nicks in this structure with *E. coli* DNA polymerase and DNA ligase to form a covalently closed-circular DNA molecule.**

MATERIALS AND METHODS

DNA. (*a*) Covalently closed-circular duplex SV40 DNA [SV40(I)] (labeled with [^{3}H]dT, 5×10^4 cpm/μg), free from SV40 linear or oligomeric molecules [but containing 3–5% of nicked double-stranded circles—SV40(II)] was purified from SV40-infected CV-1 cells (Jackson, D., & Berg, P., in preparation). (*b*) Closed-circular duplex λ*dvgal* DNA labeled with [^{3}H]dT (2.5×10^4 cpm/μg), was isolated from an *E. coli* strain containing this DNA as an autonomously replicating plasmid (see ref. 3) by equilibrium sedimentation in CsCl–ethidium bromide gradients (4) after lysis of the cells with detergent. A more detailed characterization of this DNA will be published later. Present information indicates that the

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3240–3244, November 1973

Construction of Biologically Functional Bacterial Plasmids *In Vitro*

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

* Department of Medicine, Stanford University School of Medicine, Stanford, California 94305; and † Department of Microbiology, University of California at San Francisco, San Francisco, Calif. 94122

Communicated by Norman Davidson, July 18, 1973

ABSTRACT **The construction of new plasmid DNA species by *in vitro* joining of restriction endonuclease-generated fragments of separate plasmids is described. Newly constructed plasmids that are inserted into *Escherichia coli* by transformation are shown to be biologically functional replicons that possess genetic properties and nucleotide base sequences from both of the parent DNA molecules. Functional plasmids can be obtained by reassociation of endonuclease-generated fragments of larger replicons, as well as by joining of plasmid DNA molecules of entirely different origins.**

*Eco*RI-generated fragments have been inserted into appropriately-treated *E. coli* by transformation (7) and have been shown to form biologically functional replicons that possess genetic properties and nucleotide base sequences of both parent DNA species.

MATERIALS AND METHODS

E. coli strain W1485 containing the RSF1010 plasmid, which carries resistance to streptomycin and sulfonamide, was obtained from S. Falkow. Other bacterial strains and R

Proc. Nat. Acad. Sci. USA
Vol. 71, No. 5, pp. 1743–1747, May 1974

Replication and Transcription of Eukaryotic DNA in *Escherichia coli*

(restriction/plasmid/transformation/recombination/ribosomal DNA)

JOHN F. MORROW*†‡, STANLEY N. COHEN¶, ANNIE C. Y. CHANG†, HERBERT W. BOYER§, HOWARD M. GOODMAN¶, AND ROBERT B. HELLING§||

Departments of * Biochemistry and † Medicine, Stanford University School of Medicine, Stanford, California 94305; and
Departments of § Microbiology and ¶ Biochemistry and Biophysics, University of California, San Francisco, Calif. 94143

Communicated by Joshua Lederberg, January 4, 1974

ABSTRACT **Fragments of amplified *Xenopus laevis* DNA, coding for 18S and 28S ribosomal RNA and generated by *Eco*RI restriction endonuclease, have been linked *in vitro* to the bacterial plasmid pSC101; and the recombinant molecular species have been introduced into *E. coli* by transformation. These recombinant plasmids, containing both eukaryotic and prokaryotic DNA, replicate stably in *E. coli*. RNA isolated from *E. coli* minicells harboring the plasmids hybridizes to amplified *X. laevis* rDNA.**

by an adaptation of a NaCl-sodium dodecyl sulfate cleared-lysate procedure (12, 13). Transformation of *E. coli* by plasmid DNA (7), isolation of *E. coli* minicells (14), heteroduplex analysis by electron microscopy (15), DNA·RNA hybridization (16, 17), and analysis of fragments generated by *Eco*RI endonuclease by agarose gel electrophoresis (refs. 1, 6, and 18; Helling, Goodman and Boyer, in preparation) have been described elsewhere. Molecular weights of fragments were

DNA ÇALIŞMALARINDA KULLANILAN ENZİMLER

•RESTRİKSİYON ENDONÜKLEAZLARI

(HindIII, EcoRI, BamHI gb. 500'ün üzerinde farklı restriksiyon endonükleazi izole edilmiştir)

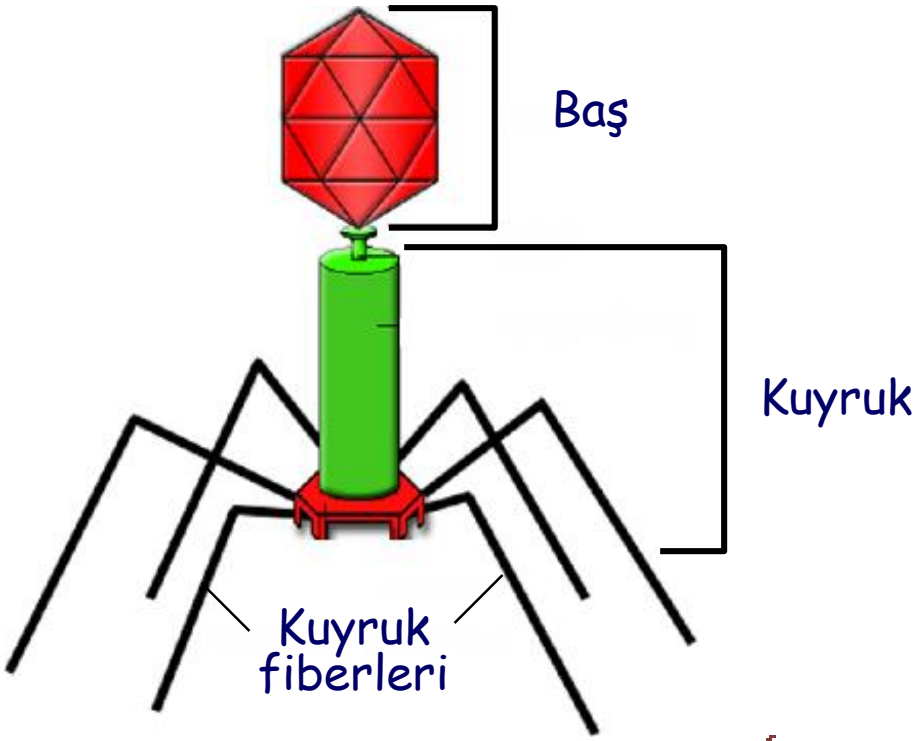
•LİGAZLAR

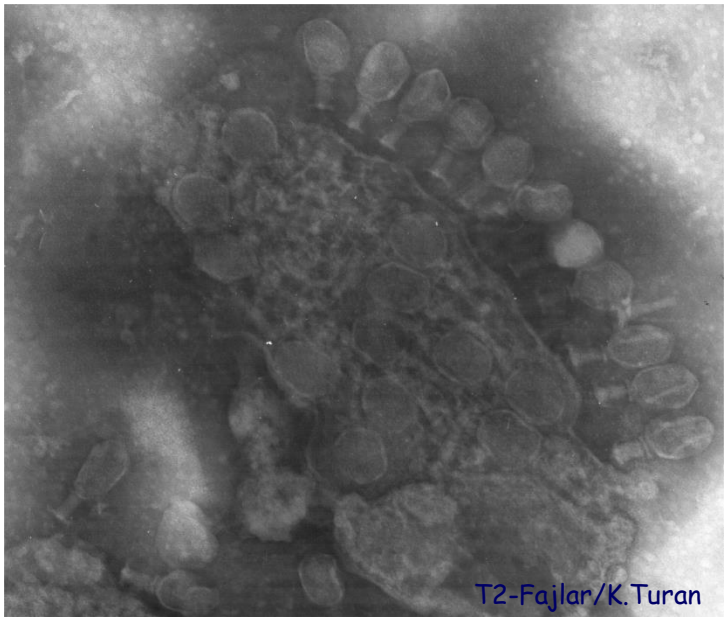
(T4-DNA ligaz enzimi, *E. coli* DNA ligaz enzimi)

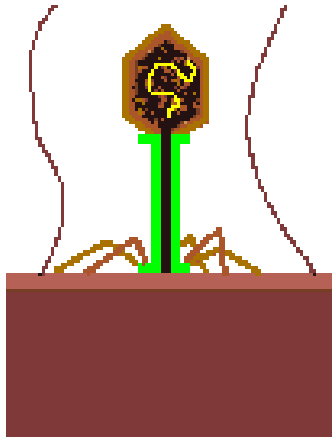
•*DNA POLİMERAZLAR*

(Bakteriyofaj T4 DNA polimerazı, Taq DNA polimeraz, Reverz Transkriptaz gb.)

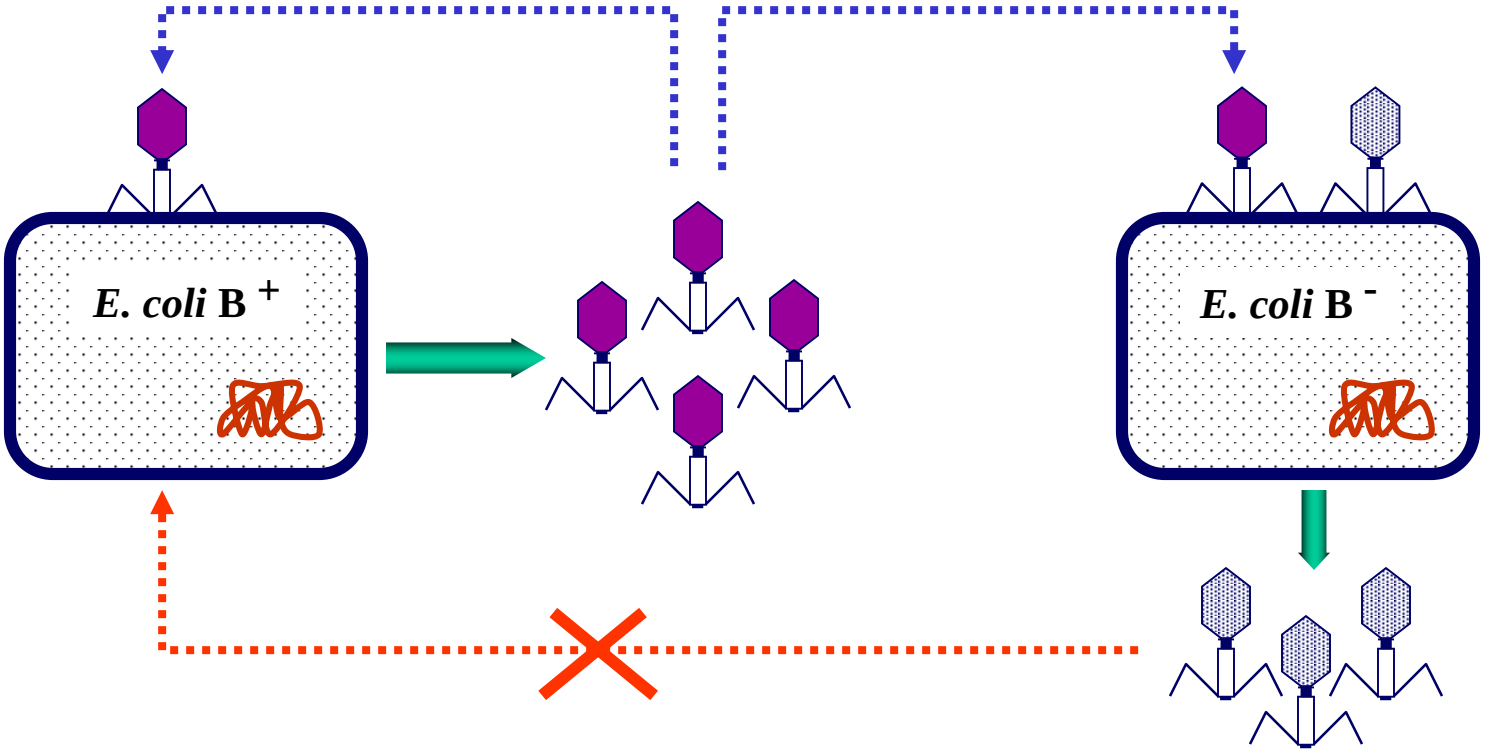
BAKTERİYOFAJLAR (T-fajları)







BAKTERİLERDE RESTRİKSİYON MODİFİKAYON SİSTEMLERİ-I



BAKTERİLERDE RESTRİKSİYON ENZİMLERİ

Tip I Enzimler:

Hedef diziyi tanıma, metilasyon ve kesme işlevi tek bir enzim kompleksinde farklı altbirimler tarafından gerçekleştirilir.Tanıma dizisinden çok uzak noktada kesme yaparlar. Örnek: EcoK enzimi.

Tip II Enzimler:

Metilasyon ve kesme işlevi birbirinden bağımsız enzimler tarafından gerçekleştirilir. Bu enzimler tanıdıkları bölgede kesme yaparlar. DNA tekniklerinde kullanılan restriksiyon endonukleazları bu grup içerisinde yer alırlar.

Tip III Enzimler:

Tip I enzimlerde olduğu gibi metilasyon ve kesme işlevi aynı enzim tarafından gerçekleştirilir. Bu grup enzimlerde hedef diziyi tanıyan altbirim aynı zamanda metilasyon işlevi görür. Tanıma noktasından ya da bu noktanın yakınından kesme yaparlar. Örnek, *E. coli*'de P1 ve P15 plazmitlerinin kodladığı EcoP1 ve EcoP15 enzimleri.

Tip II Restriksiyon Endonükleazları

EcoRI:
Escherichia coli R ırkı

HindII:
Haemophilus influenzae Rd ırkı

Enzyme	Recognition Sequence*	
*Alu*I	A G ↓ C T	→ 4^4=256bp
*Bam*HI	G ↓ G A T C C	
*Bgl*II	A ↓ G A T C T	
*Cla*I	A T ↓ C G A T	
*Eco*RI	G ↓ A A T T C	→ 4^6=4096bp
*Hae*III	G G ↓ C C	
*Hin*dII	G T Py ↓ Pu A C	
*Hin*dIII	A ↓ A G C T T	
*Hpa*II	C ↓ C G G	
*Kpn*I	G G T A C ↓ C	
*Mbo*I	↓ G A T C	
*Pst*I	C T G C A ↓ G	
*Pvu*I	C G A T ↓ C G	
*Sal*I	G ↓ T C G A C	
*Sma*I	C C C ↓ G G G	
*Xma*I	C ↓ C C G G G	
*Not*I	G C ↓ G G C C G C	→ 4^8=65536 bp

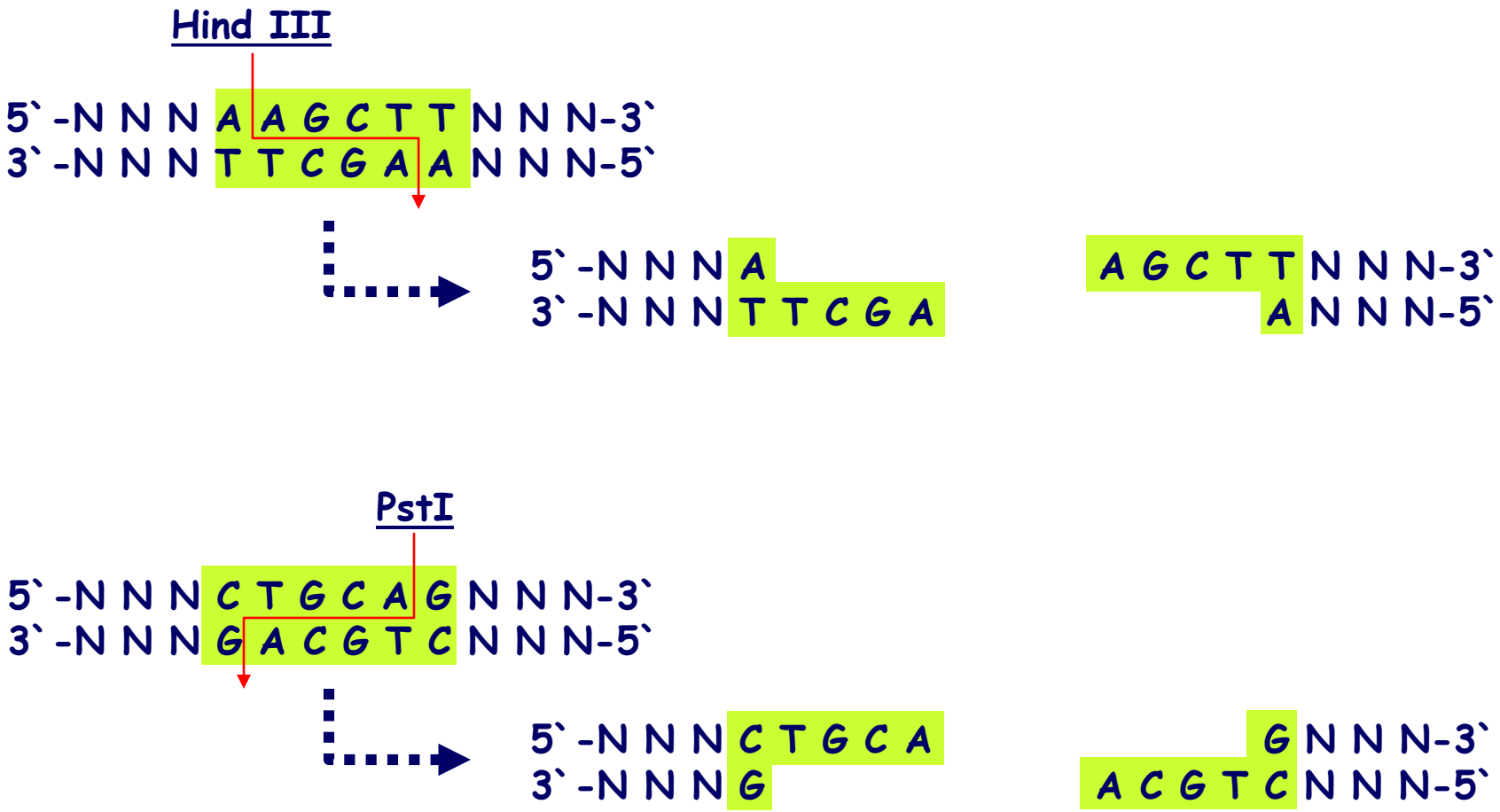
Hedef Dizinin Bulunma Olasılığı: 4^n
n = enzimin tanıdığı dizideki baz çifti sayısı

Tip II Restriksiyon Endonükleazları


Resriksiyon enzimi.swf

Escherichia coli	→	EcoRI
Serratia marcescens	→	SmaI
Providencia stuarti	→	PstI
Haemophilus influenza	→	HindIII

YAPIŞKAN UÇLAR OLUŞTURAN RESTRİKSİYON ENZİMLERİ



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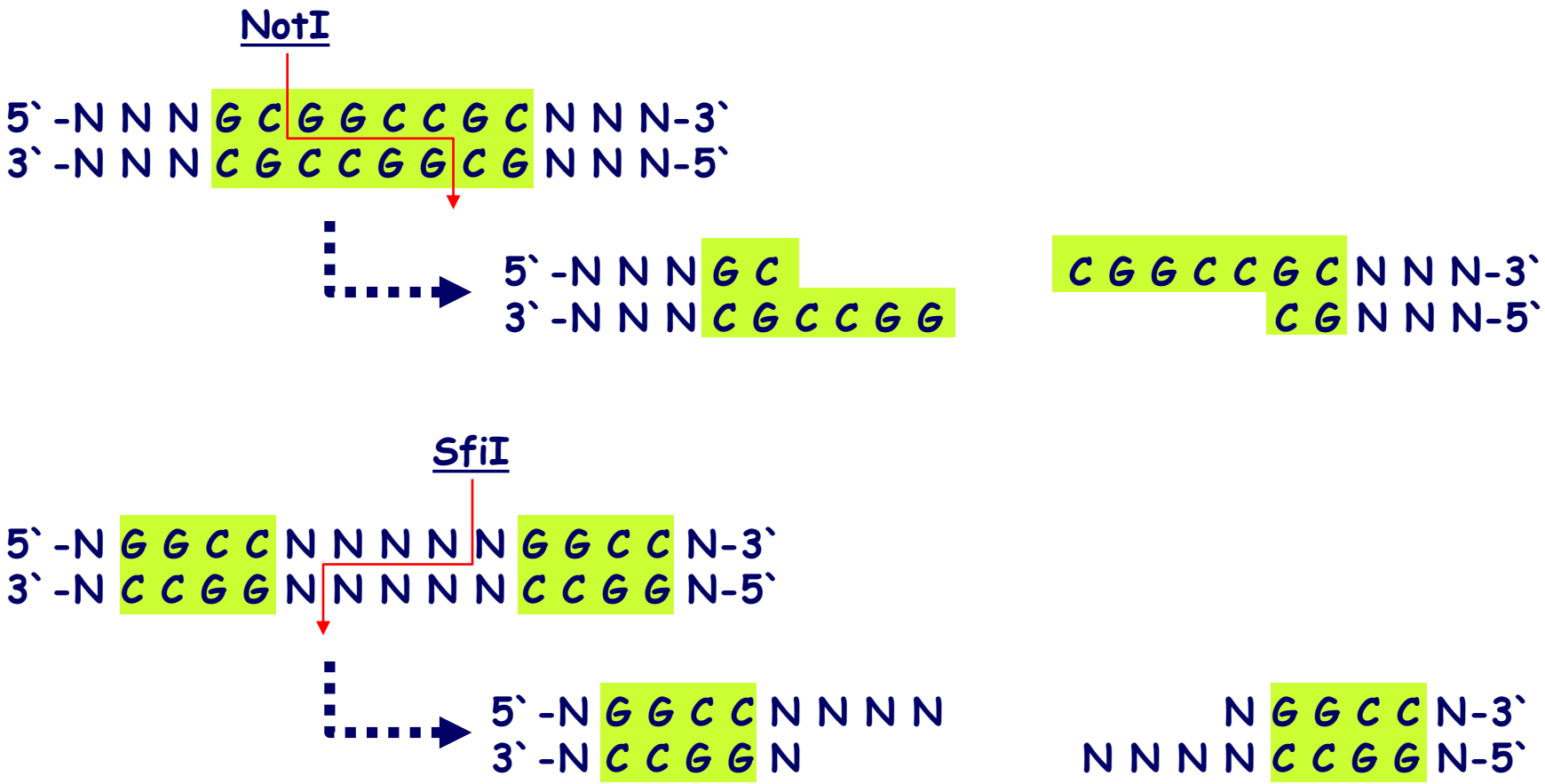
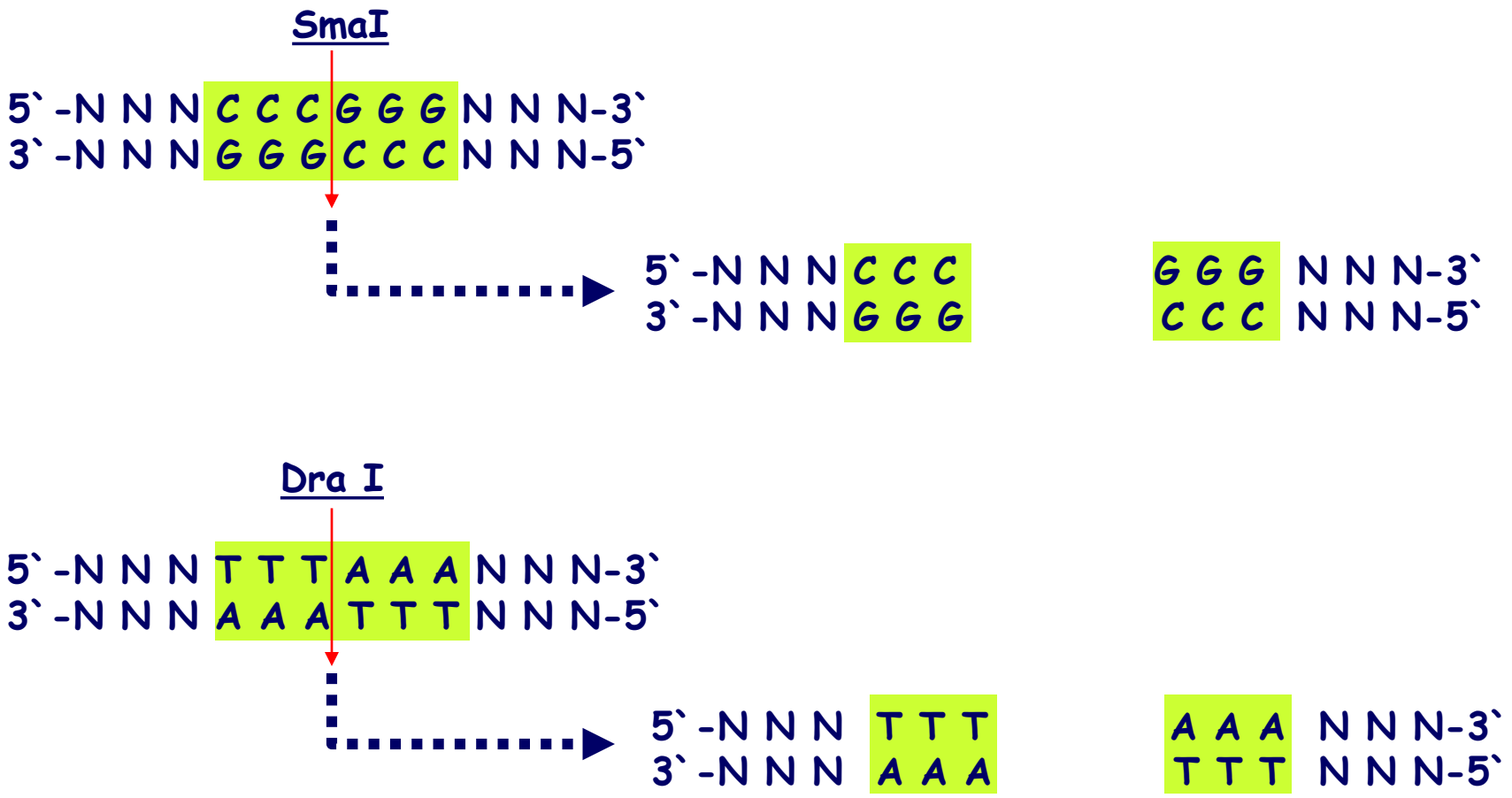


KÜT UÇLAR OLUŞTURAN RESTRİKSİYON ENZİMLERİ



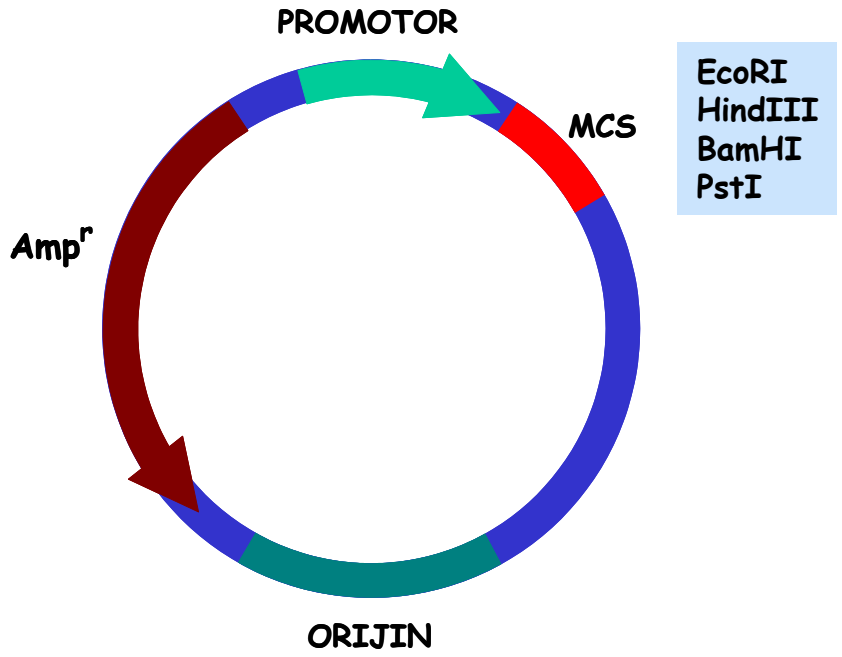
DNA Klonlama Çalışmalarında Kullanılan Vektörler

- Plazmit Vektörler
- Bakteriyofajlar
- Cosmid'ler
- BAC'lar (Bacterial Artificial Chromosomes)
- YAC'lar (Yeast Artificial Chromosomes)

PLAZMİT VEKTÖRLER

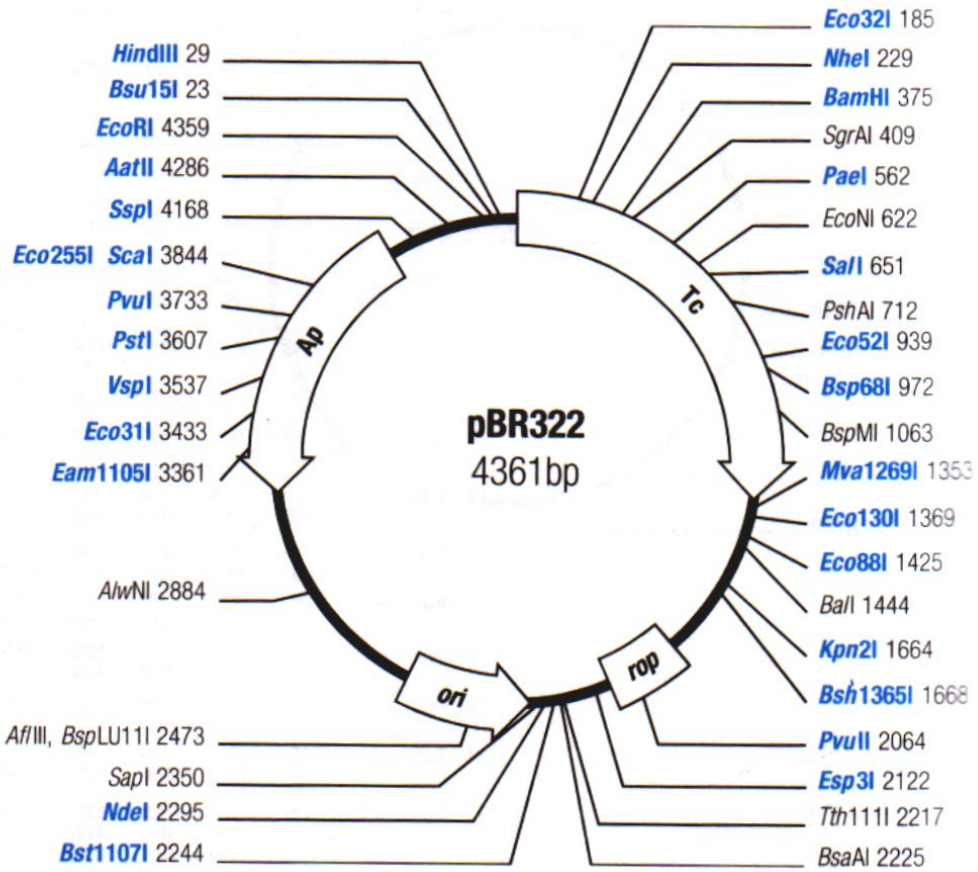
Plazmit Vektörde Bulunması Gereken Bölgeler

- Replikasyon Orijini
  (ColE1, pMB1, pUC)
- İşaret (Marker) geni
  (Amp, Tet, Kan direnç genleri)
- Multiple Cloning Site (MCS)
  Farklı restriksiyon enzimleri için kesme bölgeleri



Plazmit Vektörler

İlk Geliştirilen Yapay Plazmit Vektör (pBR322)

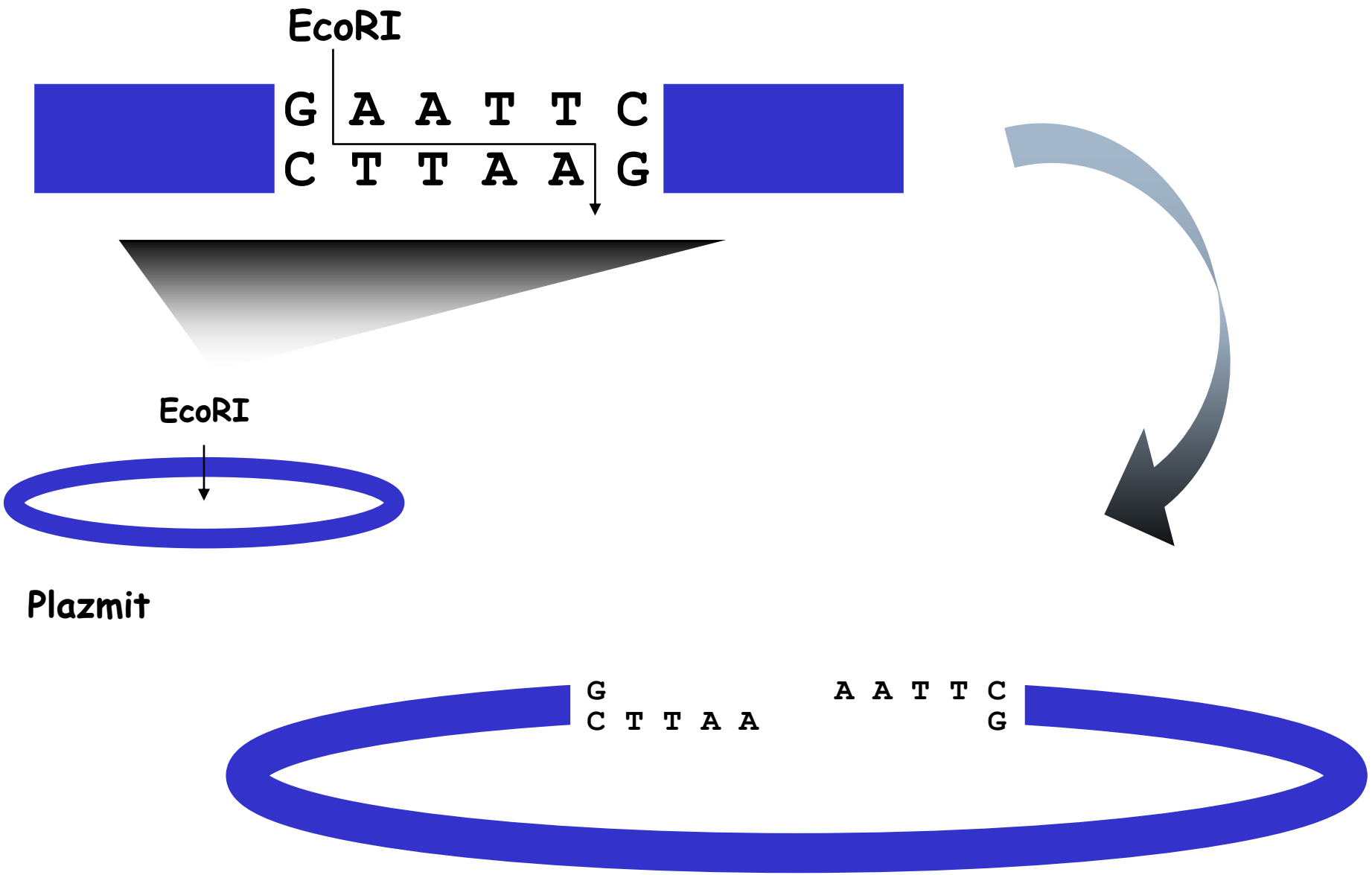


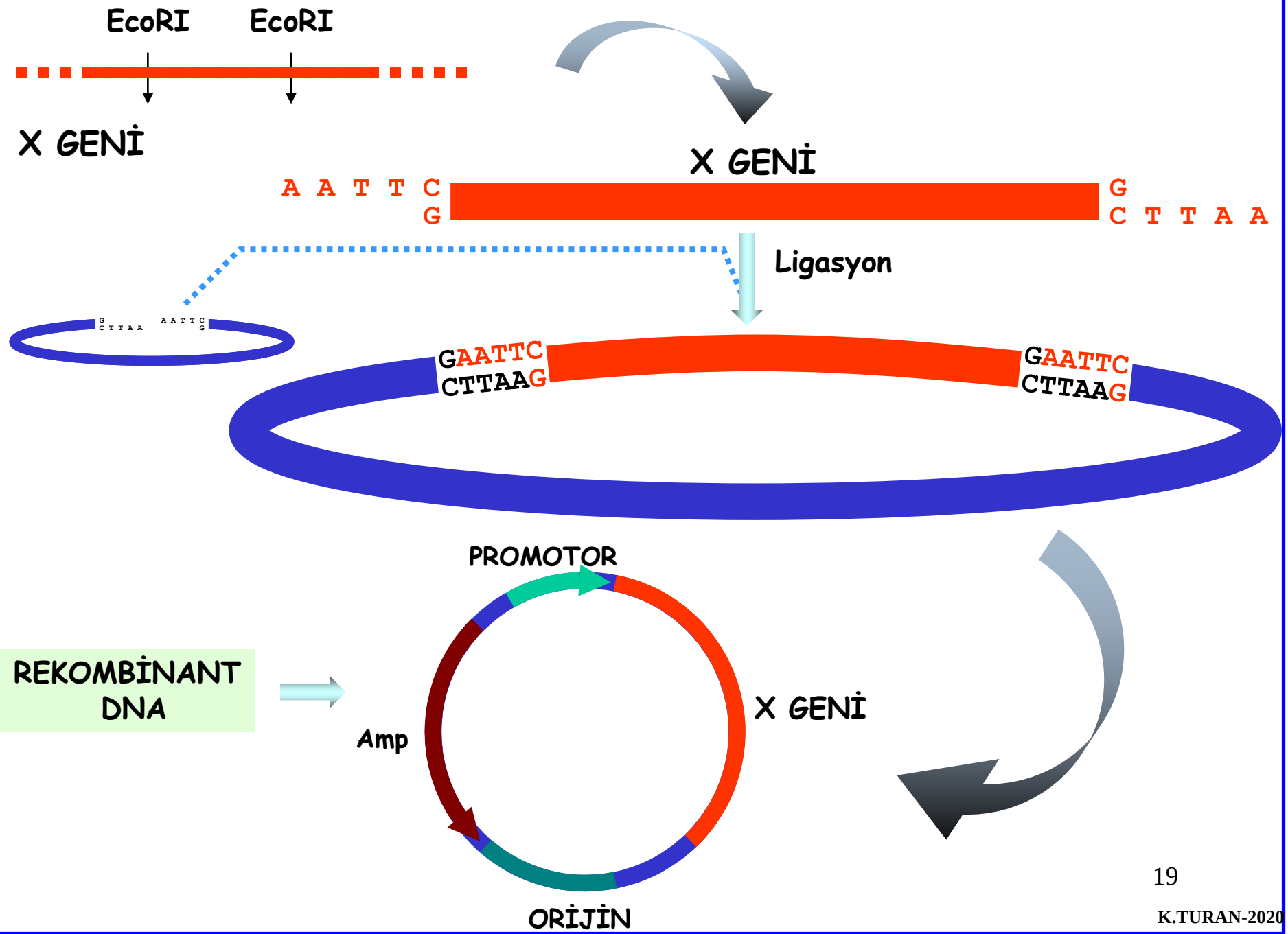
Orijin: pM1

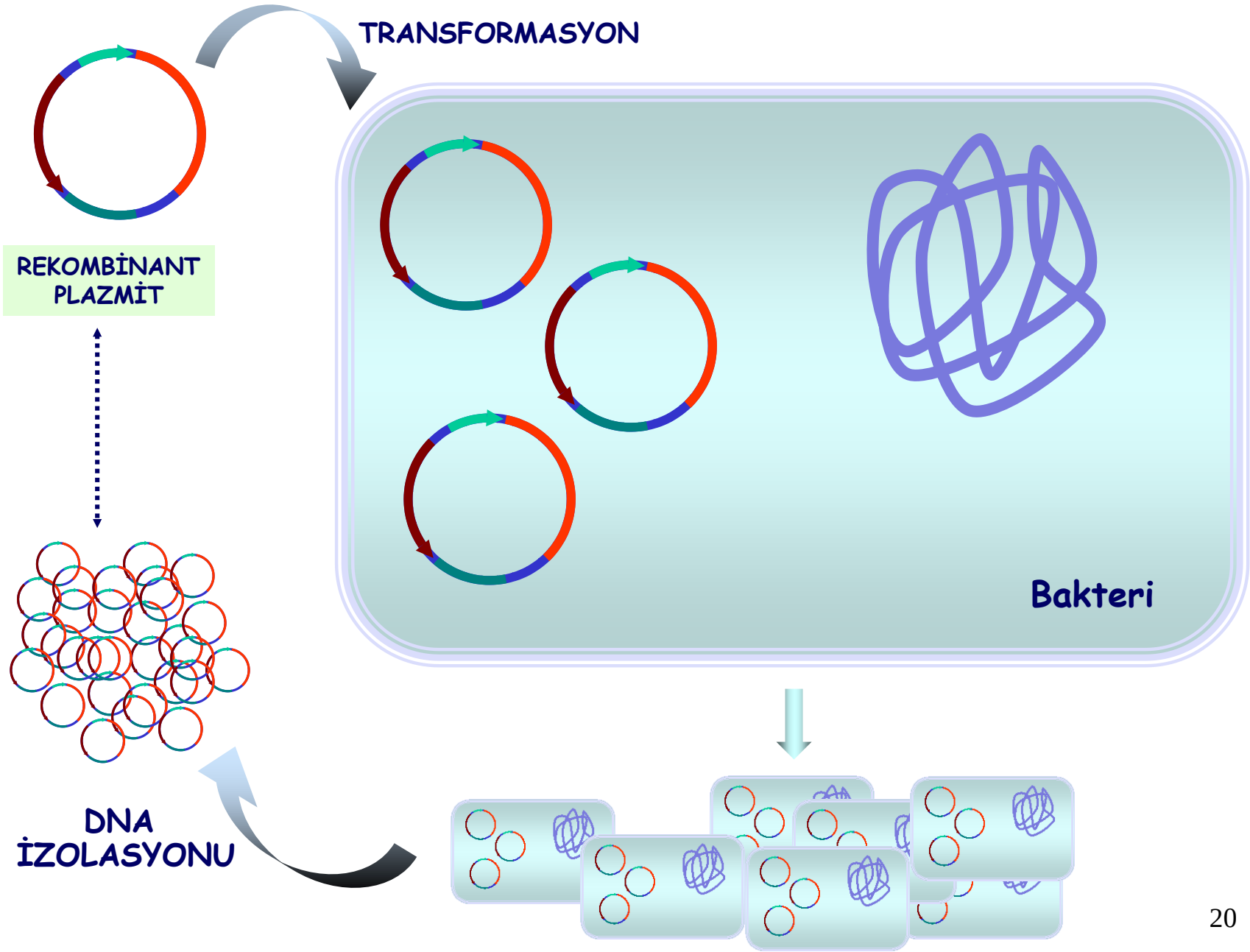
Amp^r:RSF2124

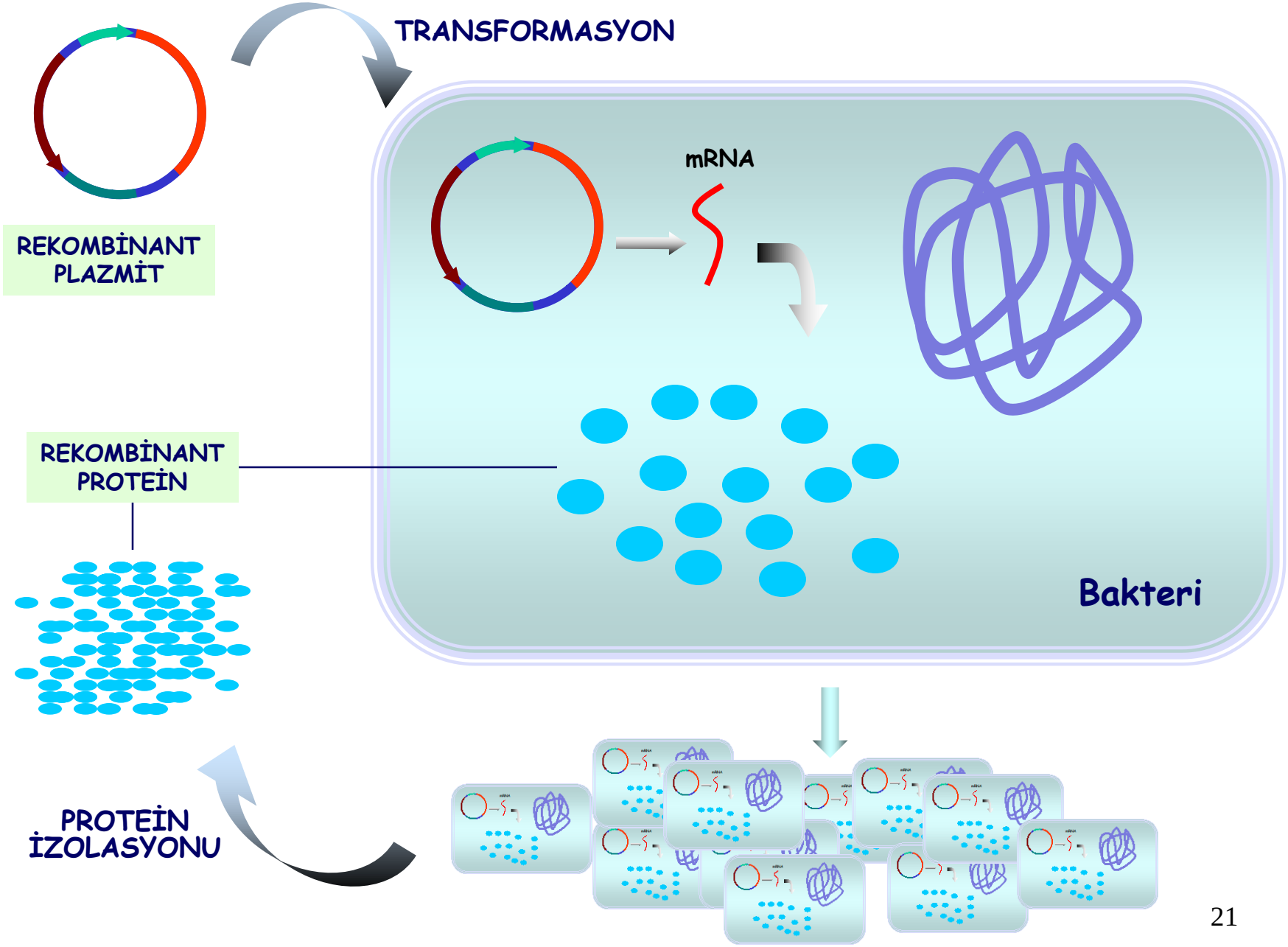
Tc^r: pSC101

REKOMBİNANT DNA ELDESİ



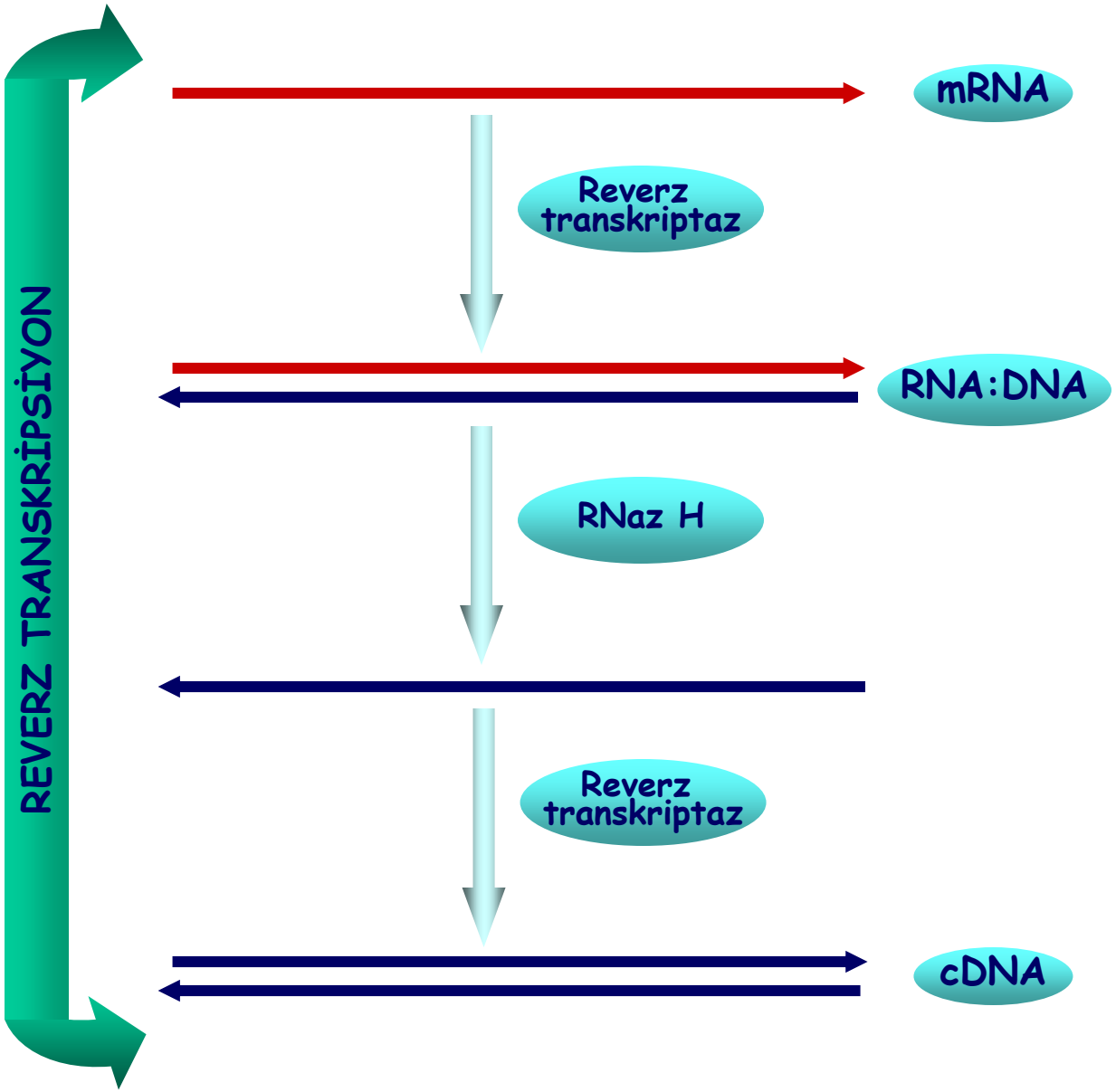

EcoRI
EcoRI
X GENİ
X GENİ
A A T T C
G
G
C T T A A
Ligasyon
GAATTC
CTTAAG
GAATTC
CTTAAG
REKOMBİNANT DNA
PROMOTOR
Amp
X GENİ
ORİJİN


TRANSFORMASYON
REKOMBİNANT PLAZMİT
Bakteri
DNA İZOLASYONU

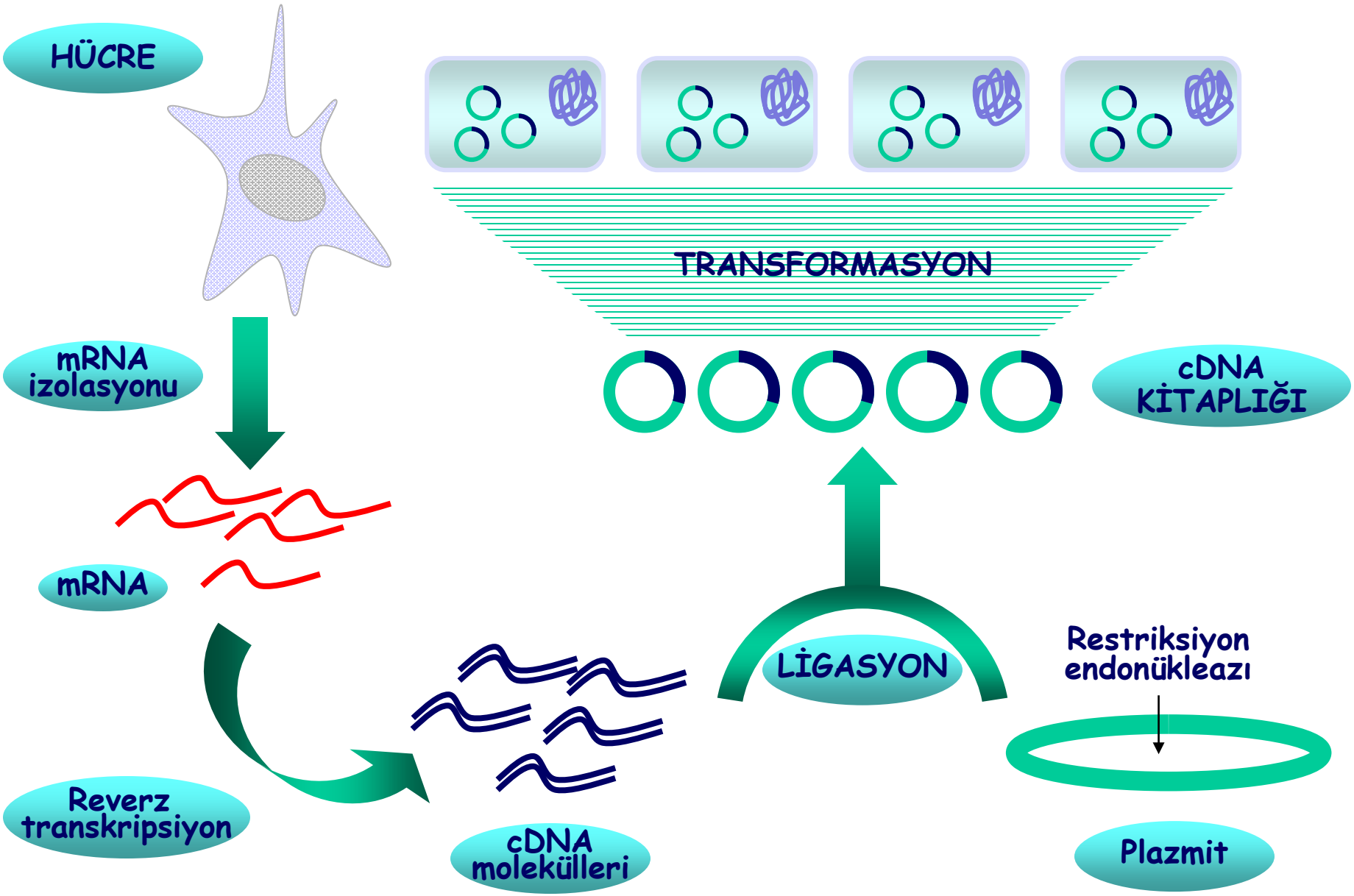

TRANSFORMASYON
REKOMBİNANT PLAZMİT
mRNA
REKOMBİNANT PROTEİN
Bakteri
PROTEİN İZOLASYONU

cDNA ve cDNA KİTAPLIĞI

Cdna.swf



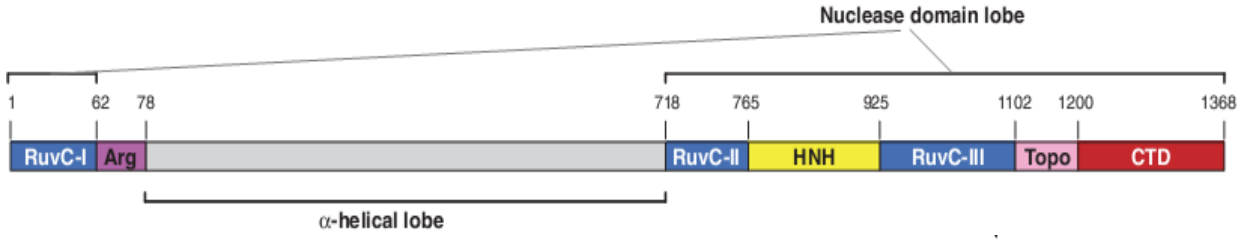
cDNA ve cDNA KİTAPLIĞI

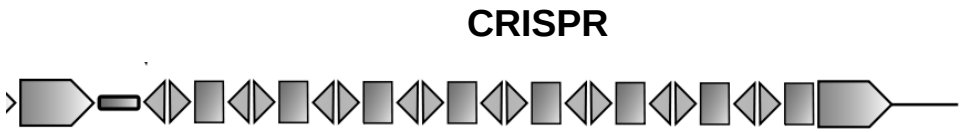


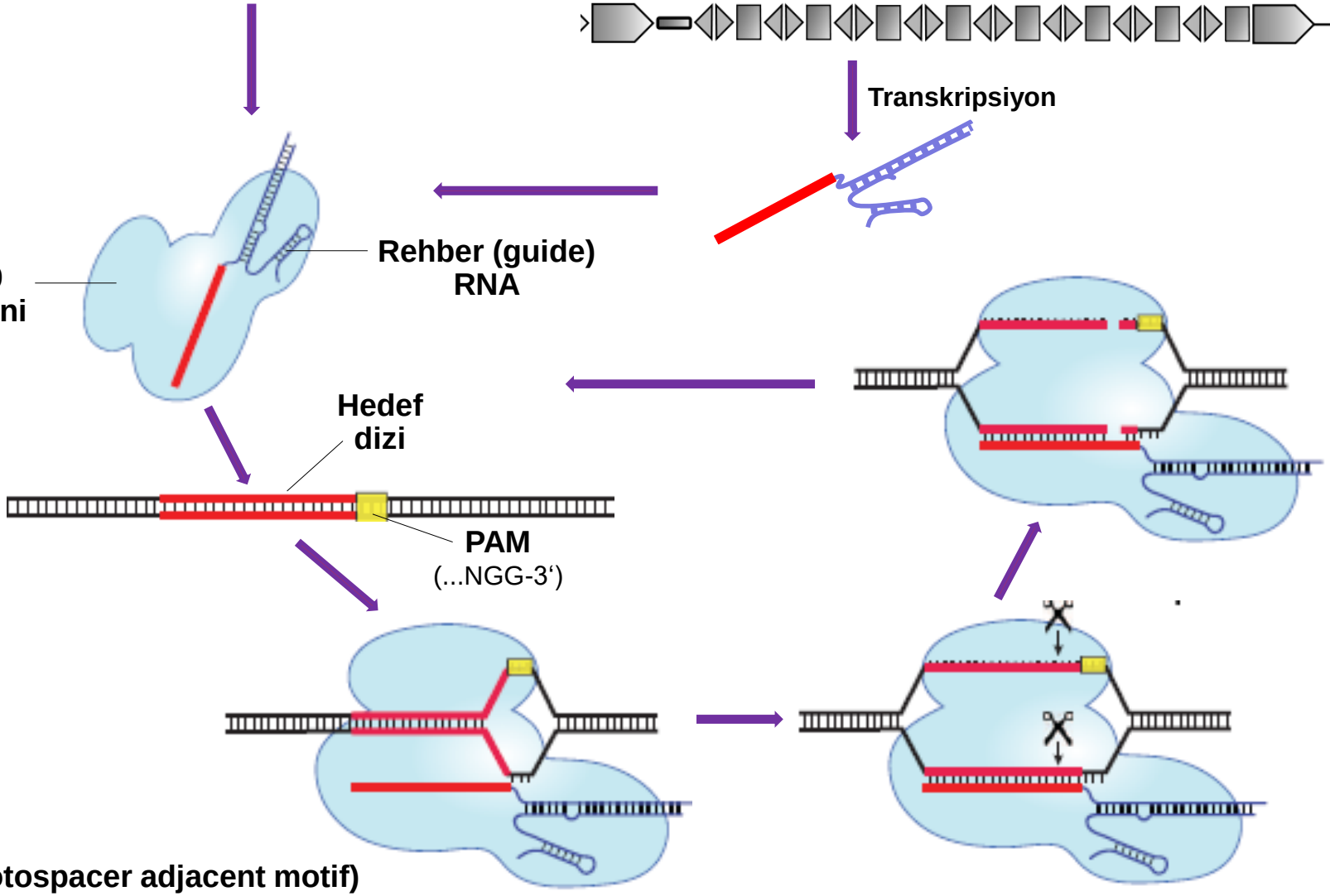
Clustered Regularly-Interspaced Short Palindromic Repeats – Cas9 (CRISPR/Cas9)

(Kümelenmiş Düzenli Aralıklı Kısa Palindromik Tekrarlar)

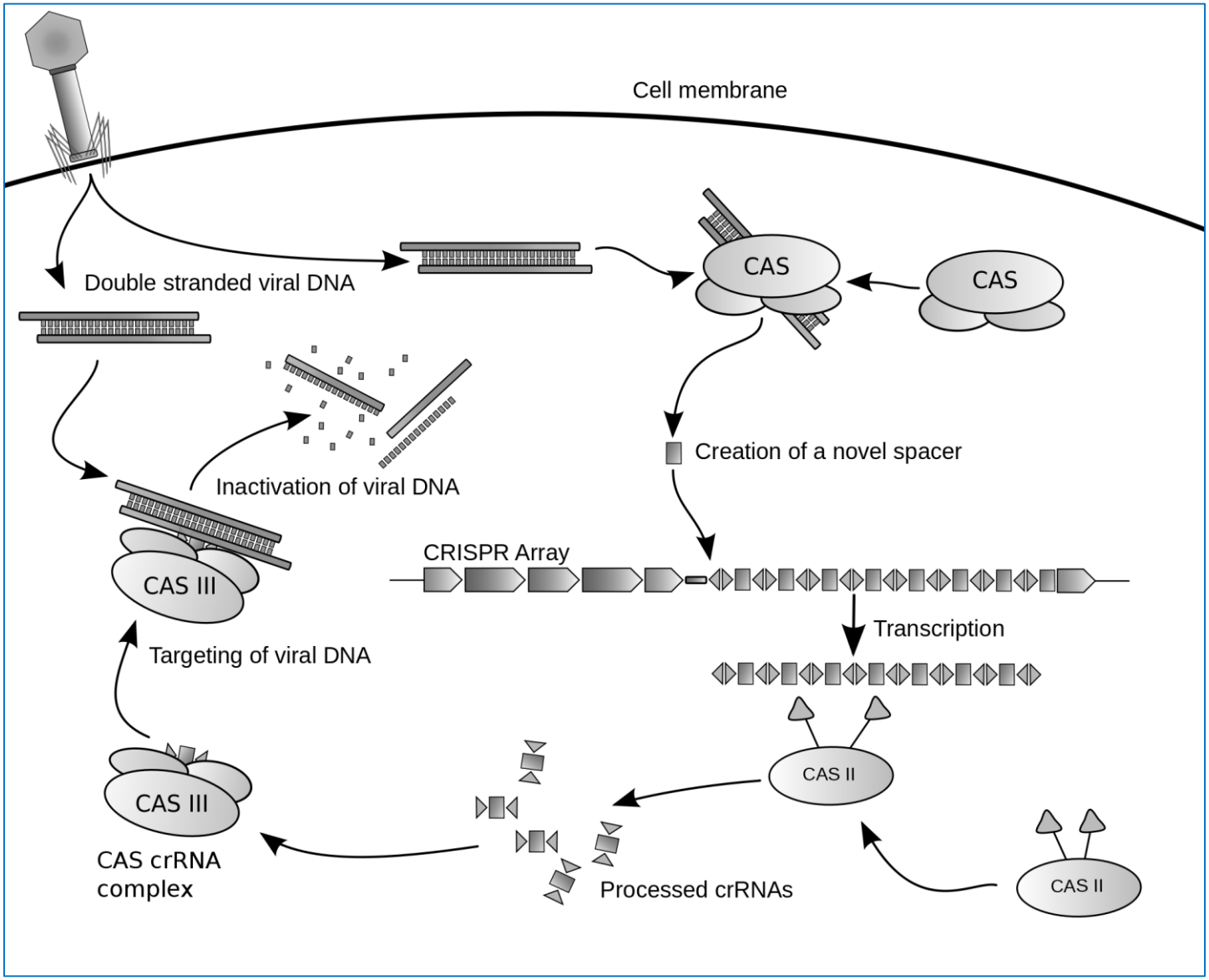
(CRISPR/Cas9)







PAM (protospacer adjacent motif)


Cell membrane
Double stranded viral DNA
CAS
CAS
Creation of a novel spacer
Inactivation of viral DNA
CAS III
CRISPR Array
Transcription
Targeting of viral DNA
CAS II
CAS III
CAS crRNA
complex
Processed crRNAs
CAS II

Clustered Regularly-Interspaced Short Palindromic Repeats : CRISPR

CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

Rodolphe Barrangou,[1] Christophe Fremaux,[2] Hélène Deveau,[3] Melissa Richards,[1] Patrick Boyaval,[2] Sylvain Moineau,[3] Dennis A. Romero,[1] Philippe Horvath[2]*

Clustered regularly interspaced short palindromic repeats (CRISPR) are a distinctive feature of the genomes of most Bacteria and Archaea and are thought to be involved in resistance to bacteriophages. We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences. Removal or addition of particular spacers modified the phage-resistance phenotype of the cell. Thus, CRISPR, together with associated *cas* genes, provided resistance against phages, and resistance specificity is determined by spacer-phage sequence similarity.

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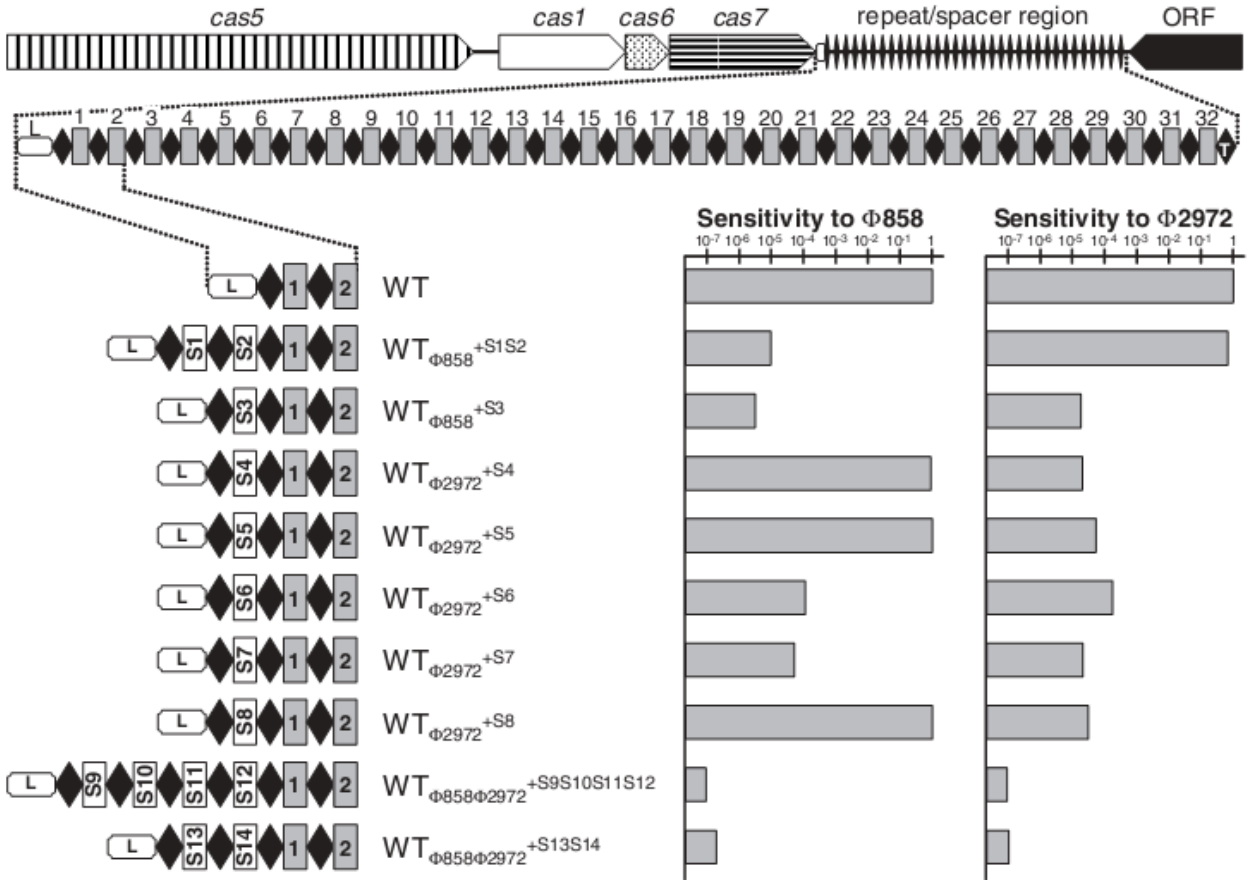
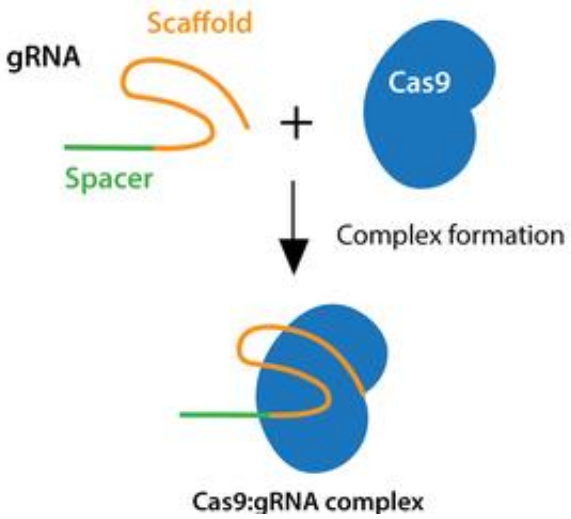
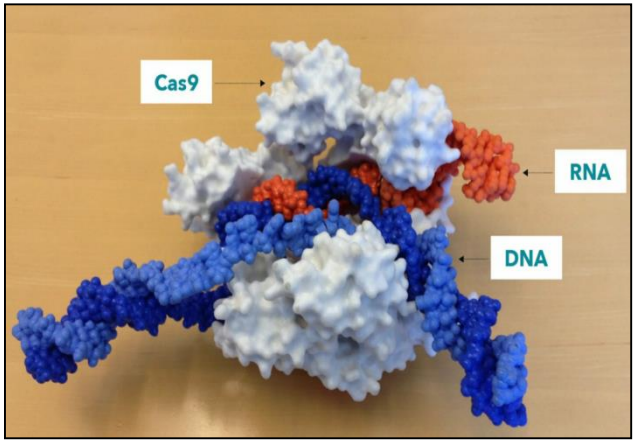
Streptococcus thermophilus

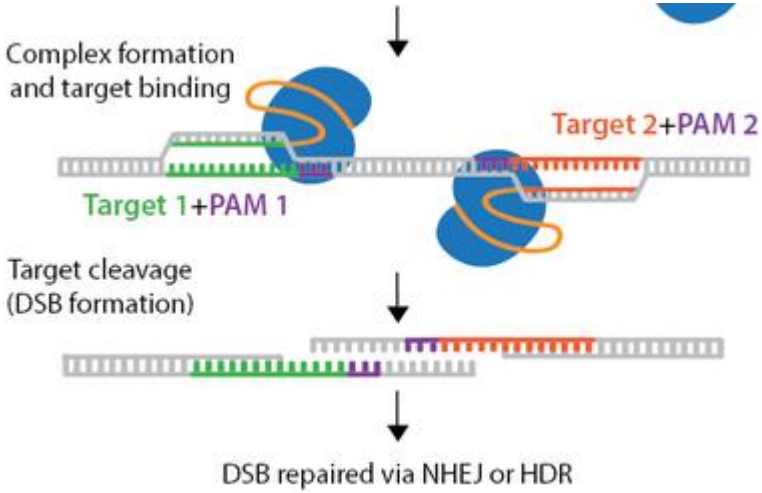


Yoshizumi Ishino-1987

CRISPR/Cas Gene Knockout







SINAVLARDA BAŞARILAR DİLERİM

K.TUTAN