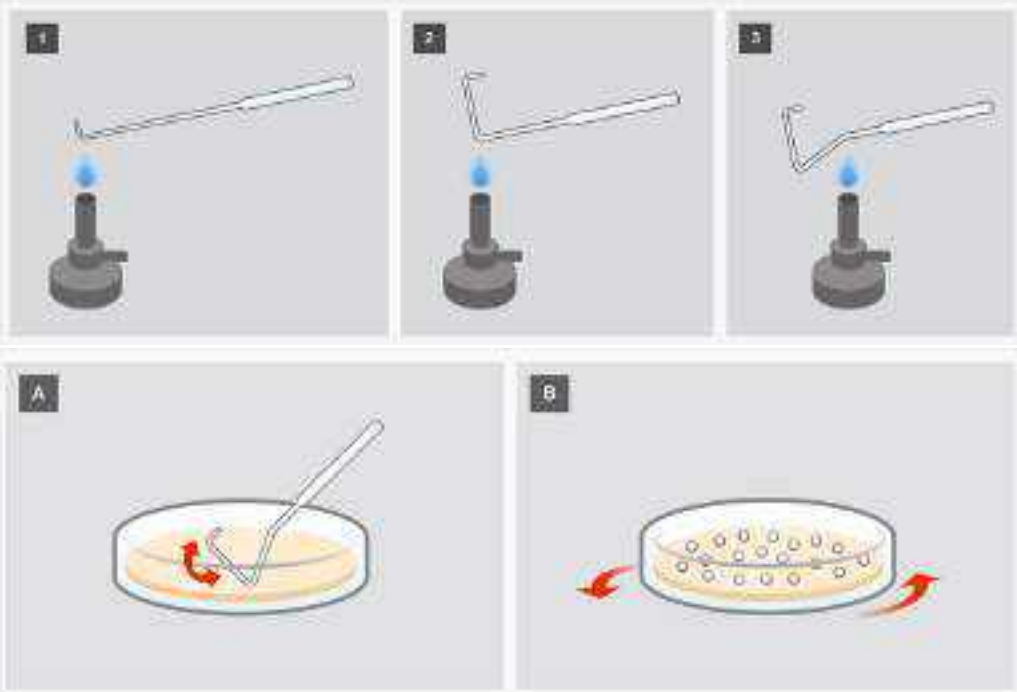
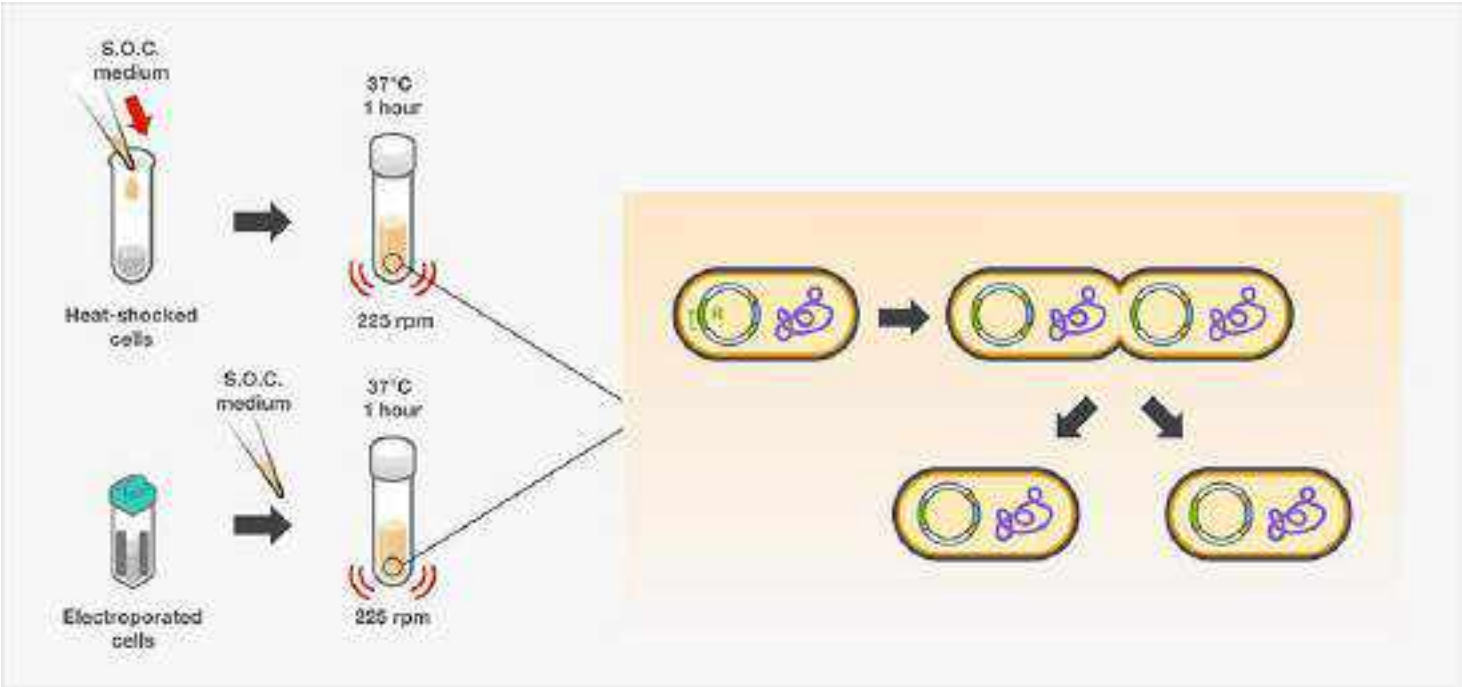


E. coli transformation



On Plate Color selection

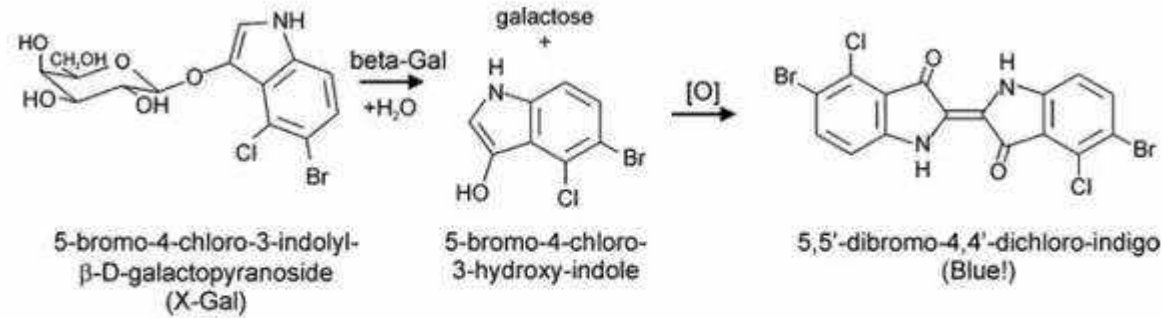
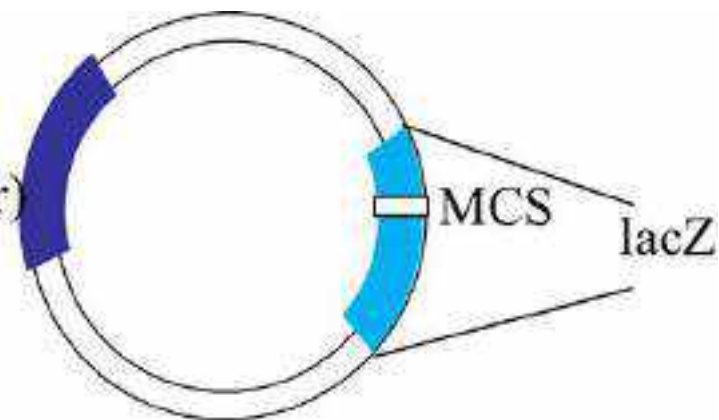
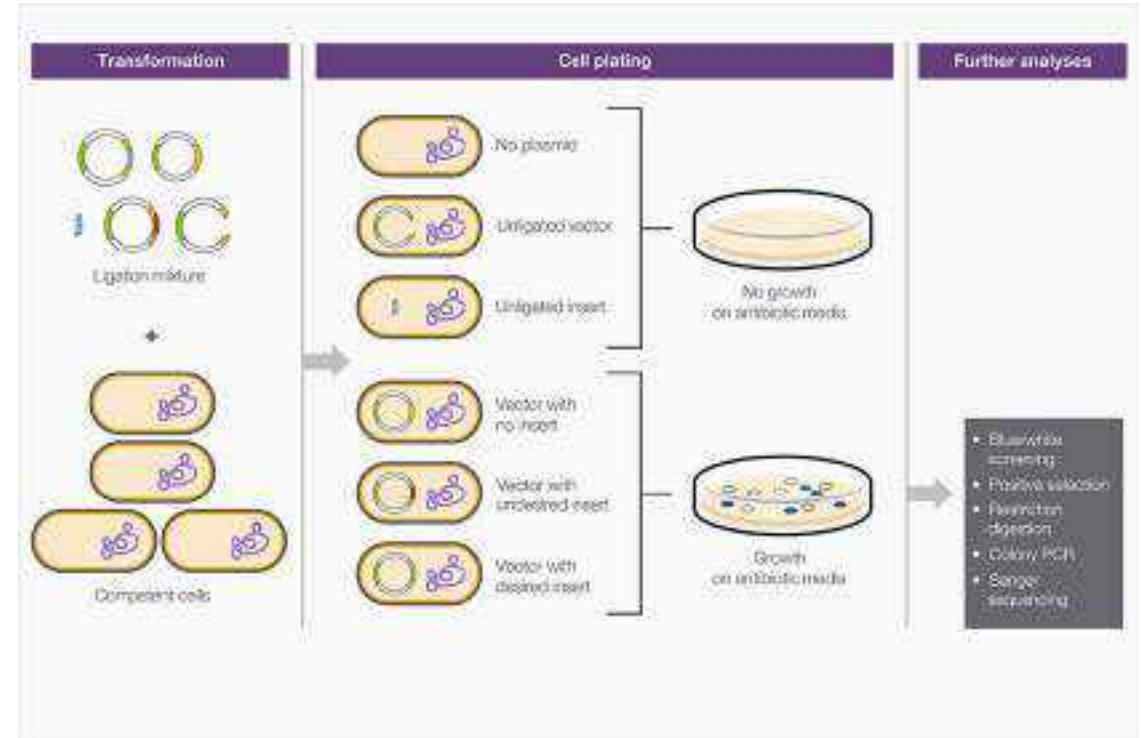
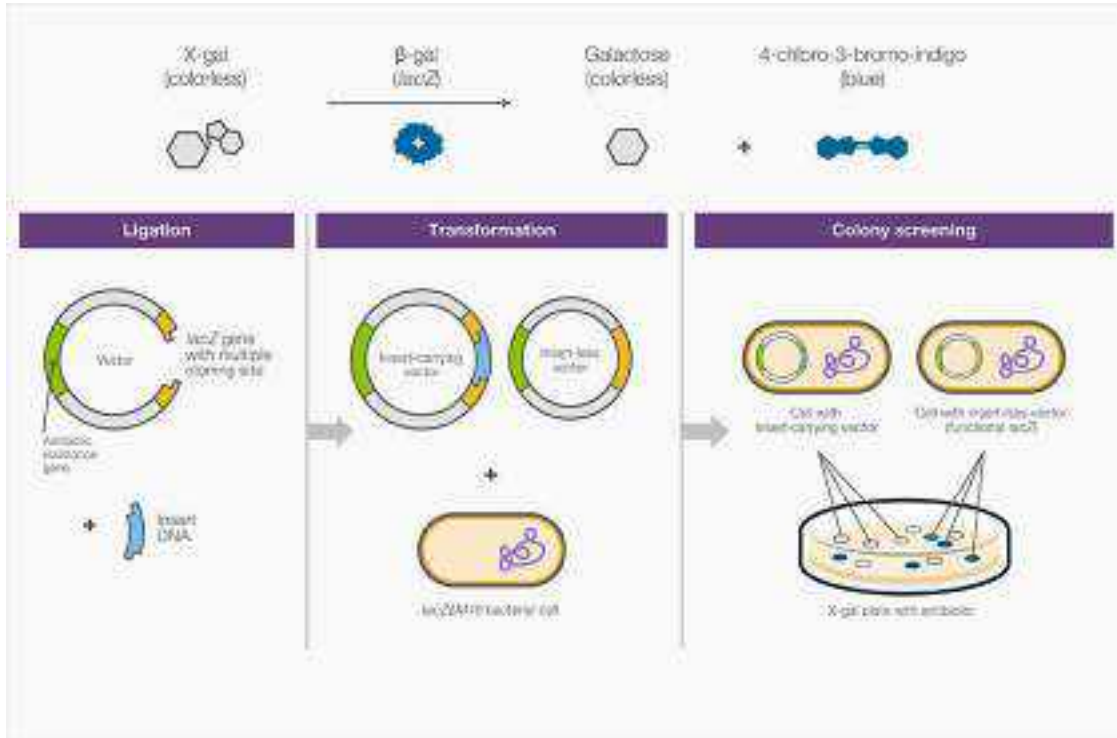
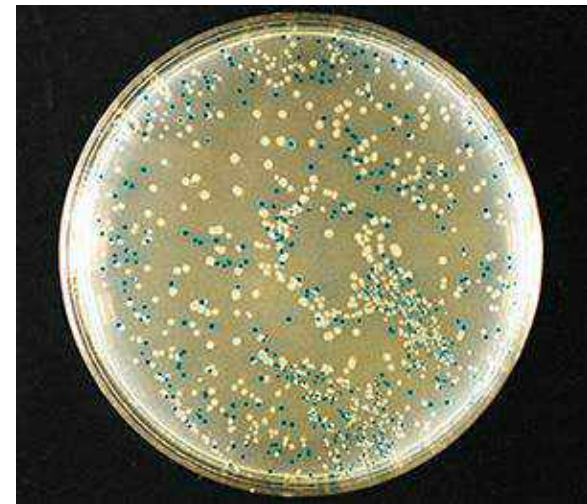
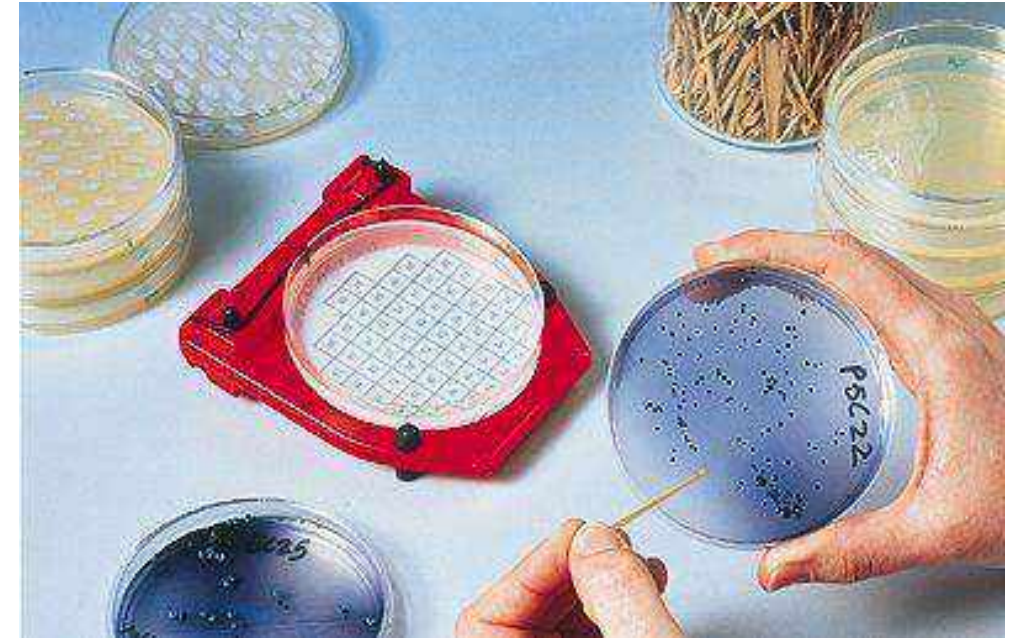
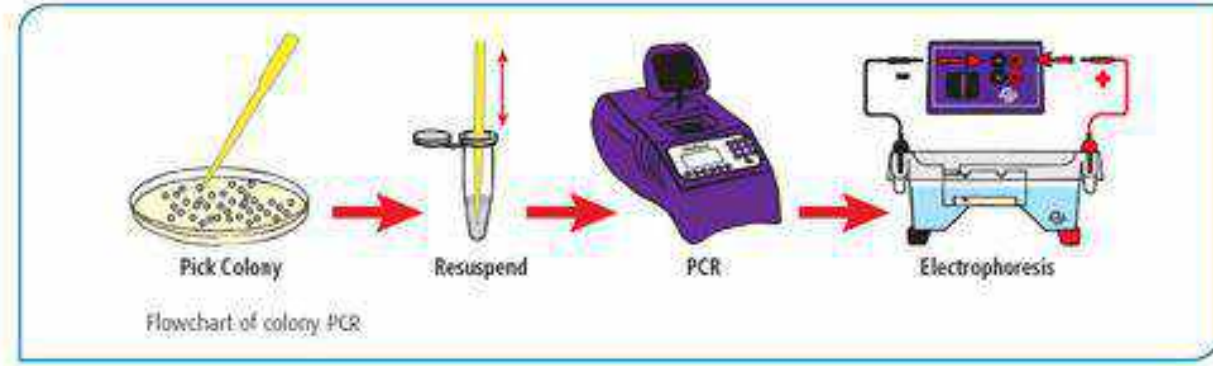
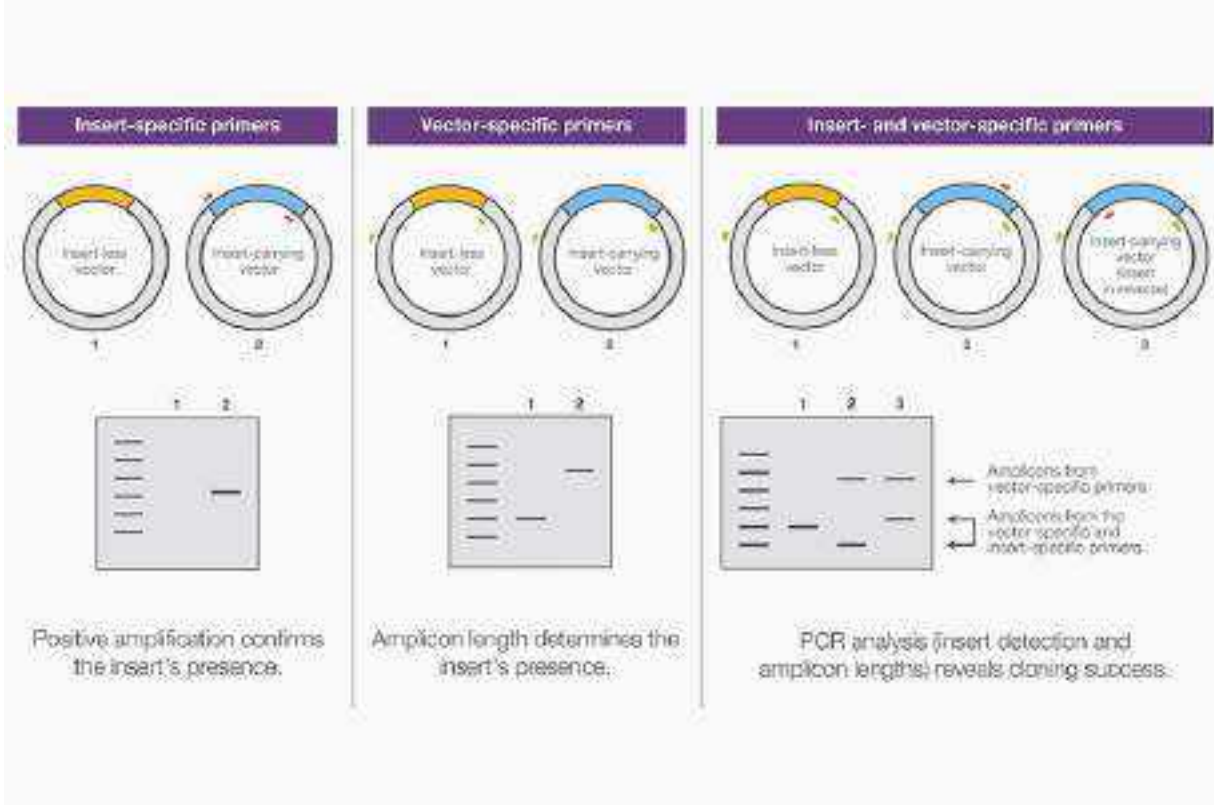


Fig. 1

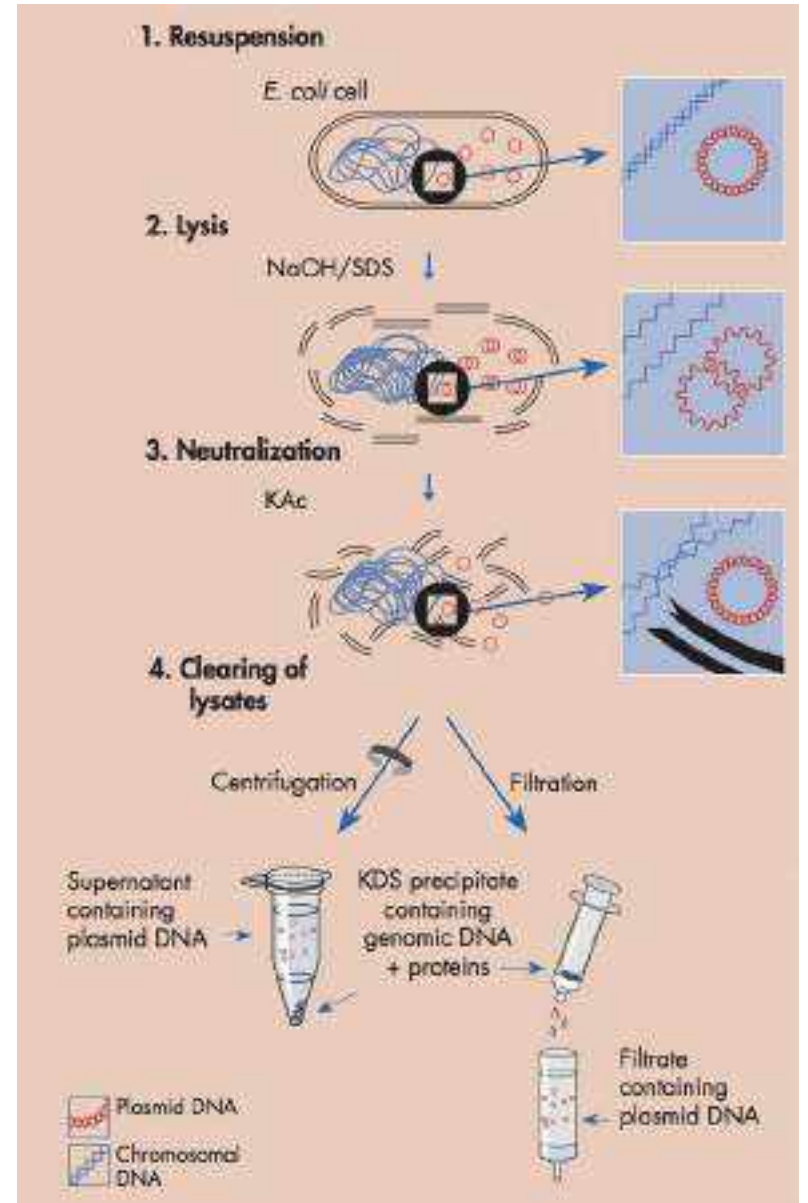
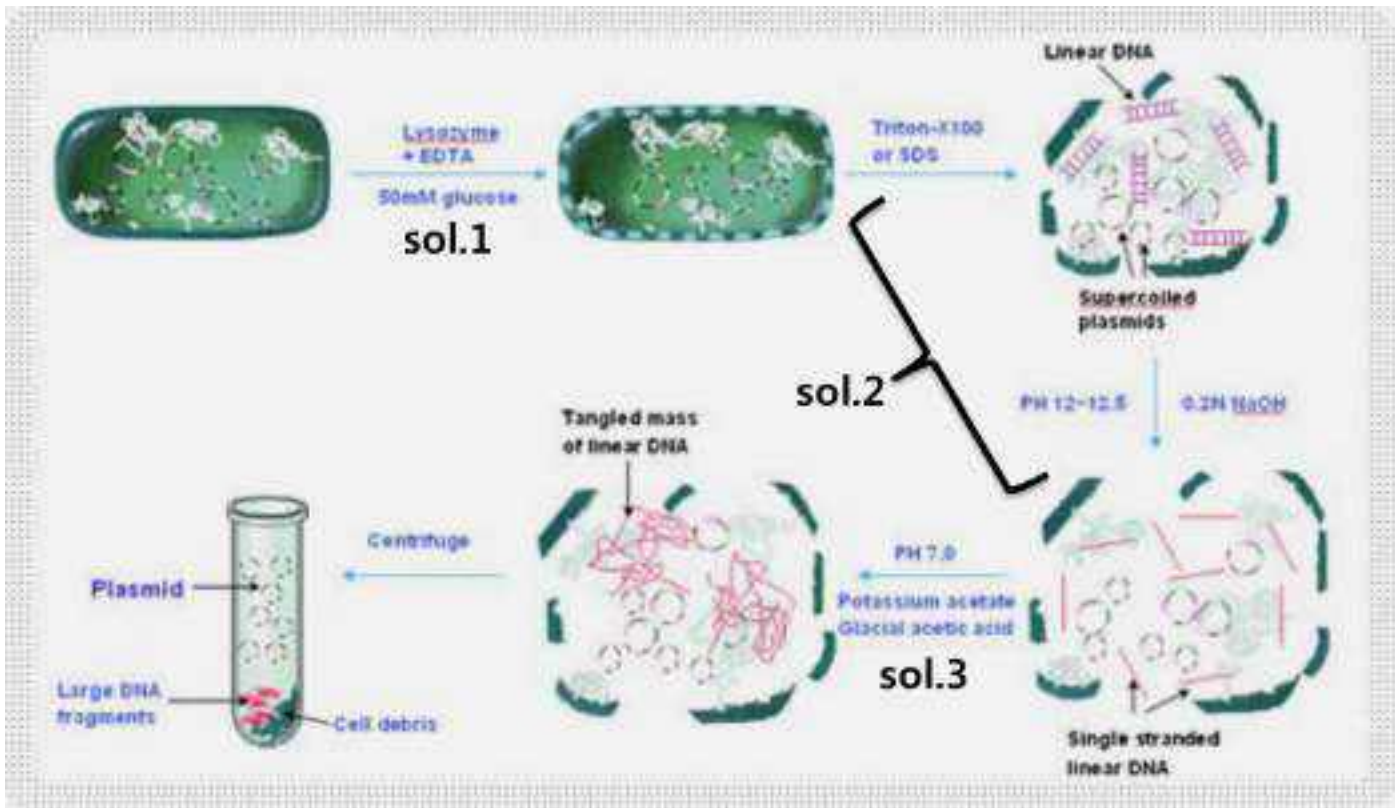
Structure of X-gal and its hydrolysis products



Insert confirmation by colony PCR



Plasmid Isolation from E.coli (Alkaline cell lysis)





Kontruksi Pada Binary Vector



Ti (Tumor inducing) Plasmid

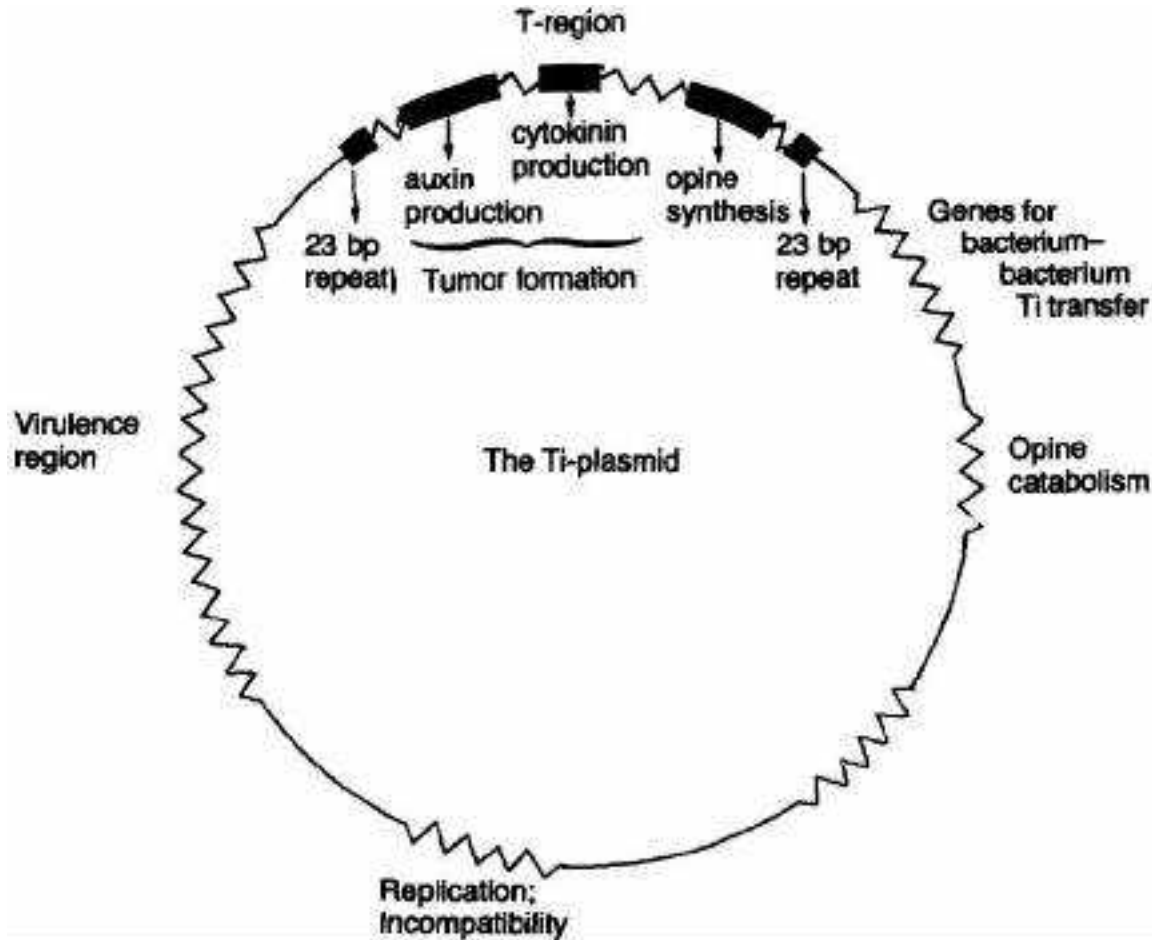
Structure of Ti Plasmid

Virulence region, encode:

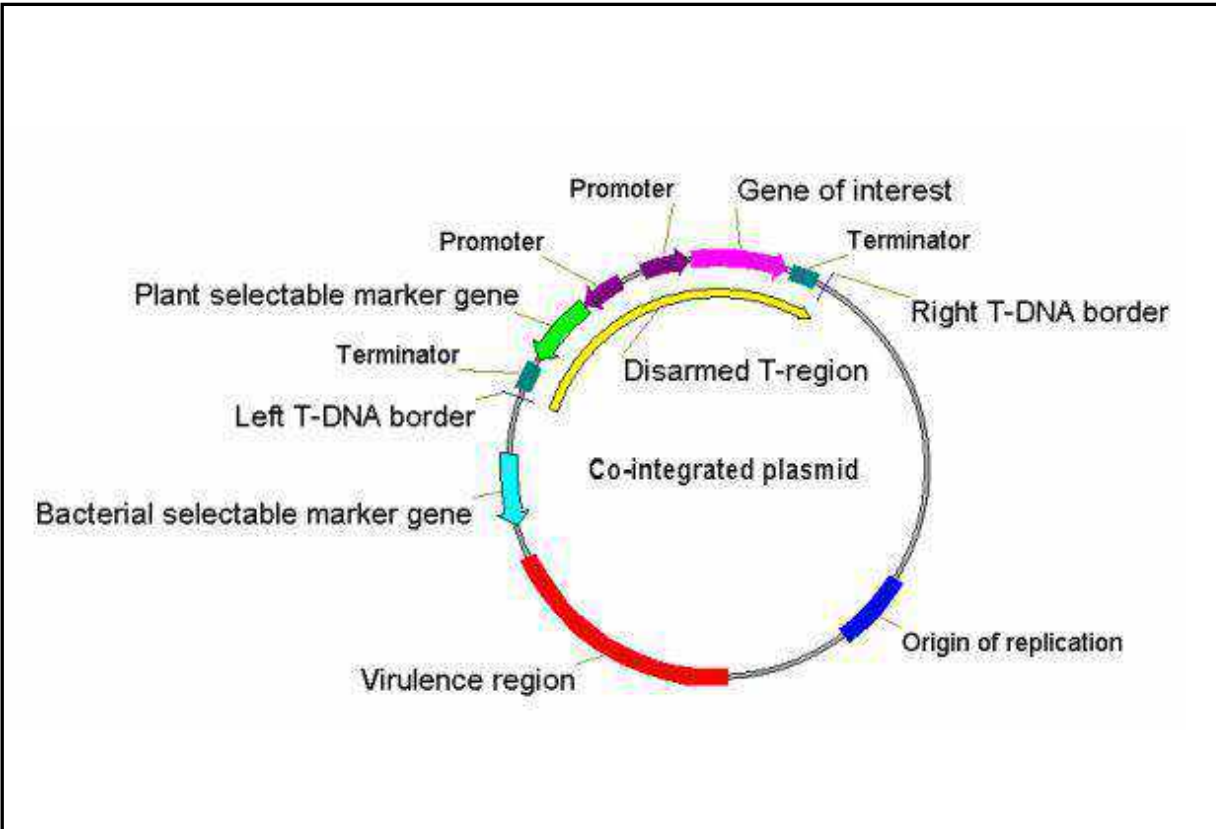
- Opine catabolism
- Gene for bacterium-bacterium Ti transfer
- T-DNA region (Transfer DNA)

Virulence (*vir*) genes:

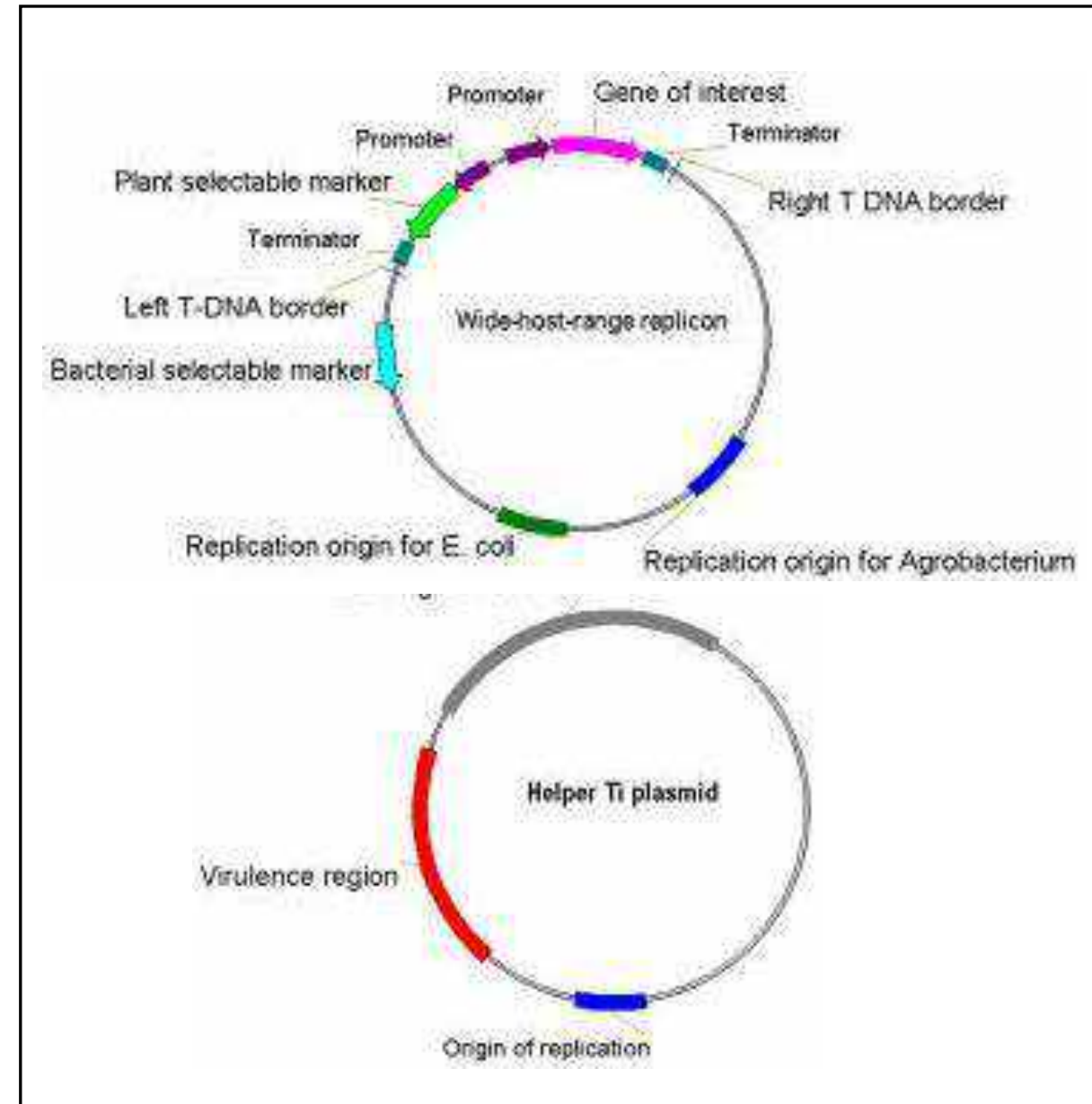
- virA* : a receptor which reacts to the presence of phenolic compounds such as acetosyringone, syringaldehyde or acetovanillone which leak out of damaged plant tissues
- virB* : a pore/pilus-like structure protein.
- virC* : protein binds the overdrive sequence.
- virD1* and *virD2*: endonucleases which target the direct repeat borders of the T-DNA segment; *virD4* is the coupling protein.
- virE* : binds to T-strand protecting it from nuclease attack
- virG* : signaling protein that activates *vir*-gene expression after binding to a consensus sequence, once it has been phosphorylated by *virA*.



Binary vs Co-integrated Plasmid

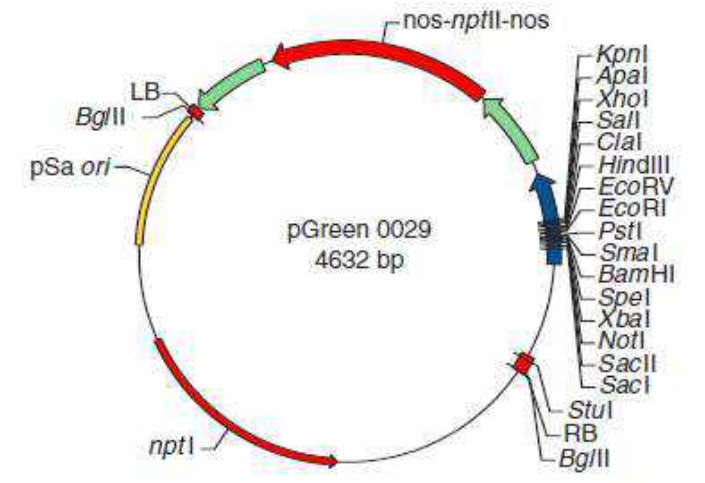
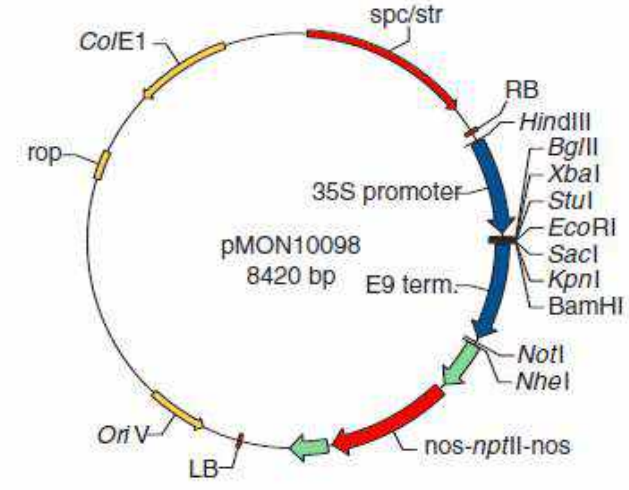
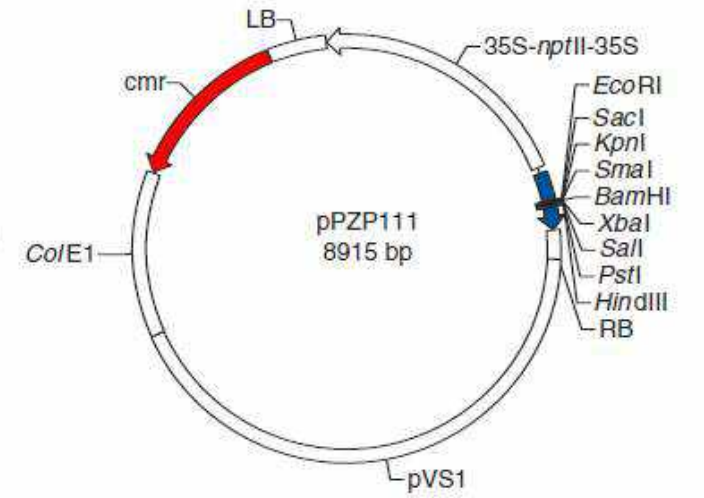
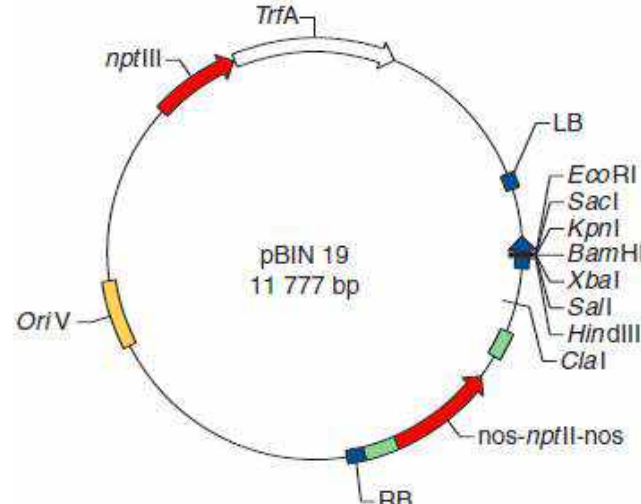
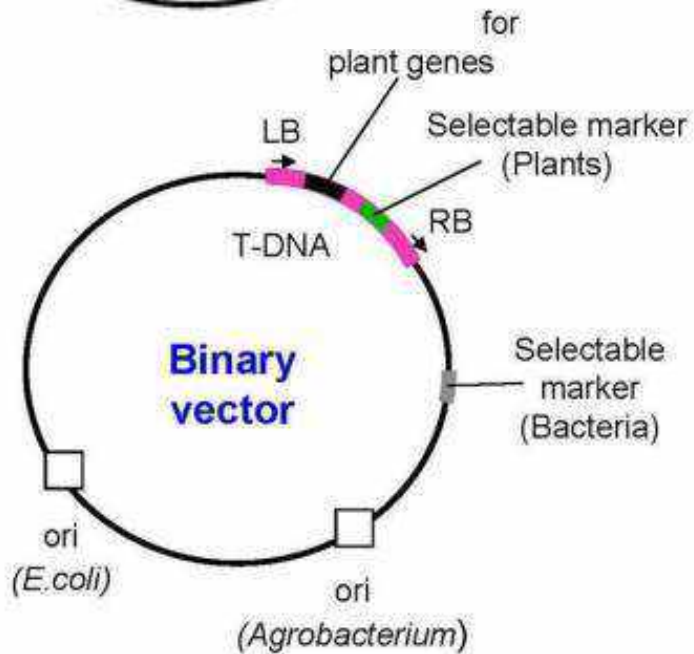
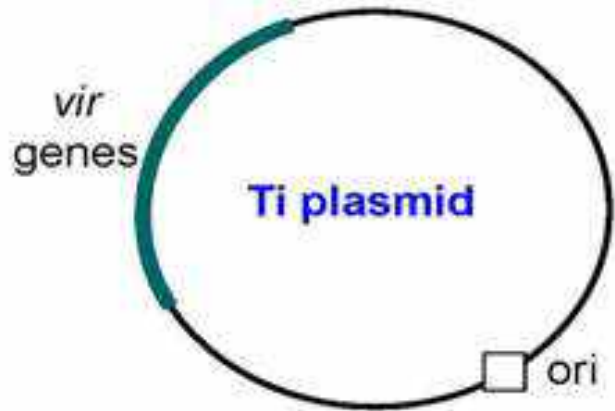


Co-integrated Vector



Binary Vector

Binary Ti Plasmid



Trends in Plant Science

Table 1. A selection of binary Ti vectors published in the peer-reviewed literature^a

Vector ^b	Size (kb)	Unique restriction sites in T-DNA	LacZ	Bacterial selection on	Selectable marker at	Replication origin		Mobilization	Ref.
						<i>Agrobacterium</i>	<i>E. coli</i>		
pBIN19	11 777	9	Yes	Kanamycin	RB	pRK2	pRK2	Yes	14
pC22	17 500	2	No	Ampicillin, streptomycin and spectinomycin	RB	pRi	ColE1	Yes	21
pGA482	13 200	7	No	Tetracycline	RB	pRK2	ColE1	Yes	31
pPCV001	9200	6	No	Ampicillin	RB	pRK2	ColE1	Yes	6
pCGN1547	14 440	5	Yes	Gentamicin	LB	pRi	ColE1	Yes	32
pJJ1881	25 700	4	No	Tetracycline	LB	pRK2	pRK2	Yes	24
pPZP111	8909	9	Yes	Chloramphenicol	LB	pVS1	ColE1	Yes	7
pGreen0029	4632	18	Yes	Kanamycin	LB	pSa	pUC	No	5

^aIn the cases of families of binary vectors only those which confer kanamycin resistance on transgenic plants are described. Sizes are based on restriction enzyme digestion patterns or on nucleotide sequence.

^bSee Box 1 for e-mail addresses for obtaining most of the plasmids listed.

Abbreviations: LacZ, β galactosidase α subunit gene for α -lac complementation; LB, left border; RB, right border. Mobilization refers to the ability of the plasmid to be transferred from *E. coli* to *Agrobacterium* by conjugation.

Table 2. Disarmed *Agrobacterium tumefaciens* strains defined by the *Agrobacterium* chromosomal background and the Ti plasmid they harbour^a

<i>Agrobacterium</i> strain ^a	Chromosomal		Ti plasmid	Opine ^b	Ref.
	Background	Marker gene ^c	Marker gene ^c		
LBA4404	TiAch5	rif	pAL4404	spec and strep	3
GV2260	C58	rif	pGV2260 (pTiB6S3ΔT-DNA)	carb	32
C58C1	C58	–	Cured	–	33
GV3100	C58	–	Cured	–	34
A136	C58	rif and nal	Cured	–	35
GV3101	C58	rif	Cured	–	34
GV3850	C58	rif	pGV3850 (pTiC58Δonc. genes)	carb	36
GV3101::pMP90	C58	rif	pMP90 (pTiC58ΔT-DNA)	gent	6
GV3101::pMP90RK	C58	rif	pMP90RK (pTiC58ΔT-DNA)	gent and kan	6
EHA101	C58	rif	pEHA101 (pTiBo542ΔT-DNA)	kan	37
EHA105	C58	rif	pEHA105 (pTiBo542ΔT-DNA)	–	Succinamopine 38
AGL-1	C58, RecA	rif, carb	pTiBo542ΔT-DNA	–	Succinamopine 39

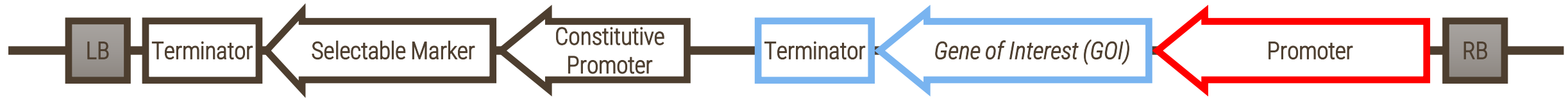
^aSee Box 1 for e-mail addresses for obtaining most of the strains listed.

^bGrouped according to the opine catabolism of the original progenitor wild-type strain and/or non-disarmed parental Ti plasmid. This generally accepted classification of *Agrobacterium* strains does not necessarily imply that their disarmed counterparts still make opines.

^cAntibiotic resistance gene used to select for that strain of *Agrobacterium* or Ti plasmid.

Abbreviations: rif, rifampicin resistance; gent, gentamicin resistance; nal, nalidixic acid resistance; kan, kanamycin resistance gene for bacteria (*nptI* or *nptIII*); carb, carbenicillin and ampicillin resistance; spec and strep, spectinomycin and streptomycin resistance; –, no marker gene present.

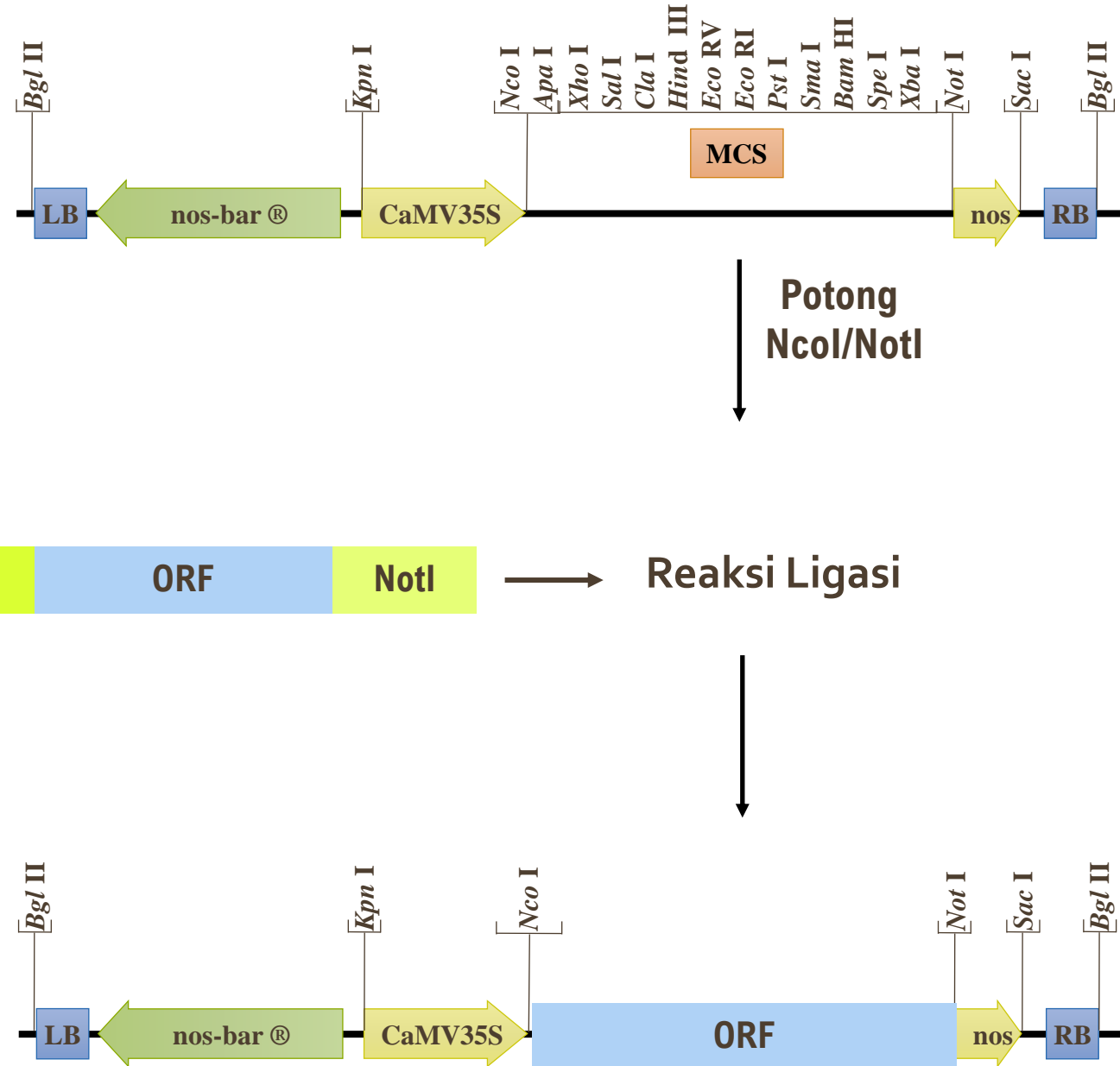
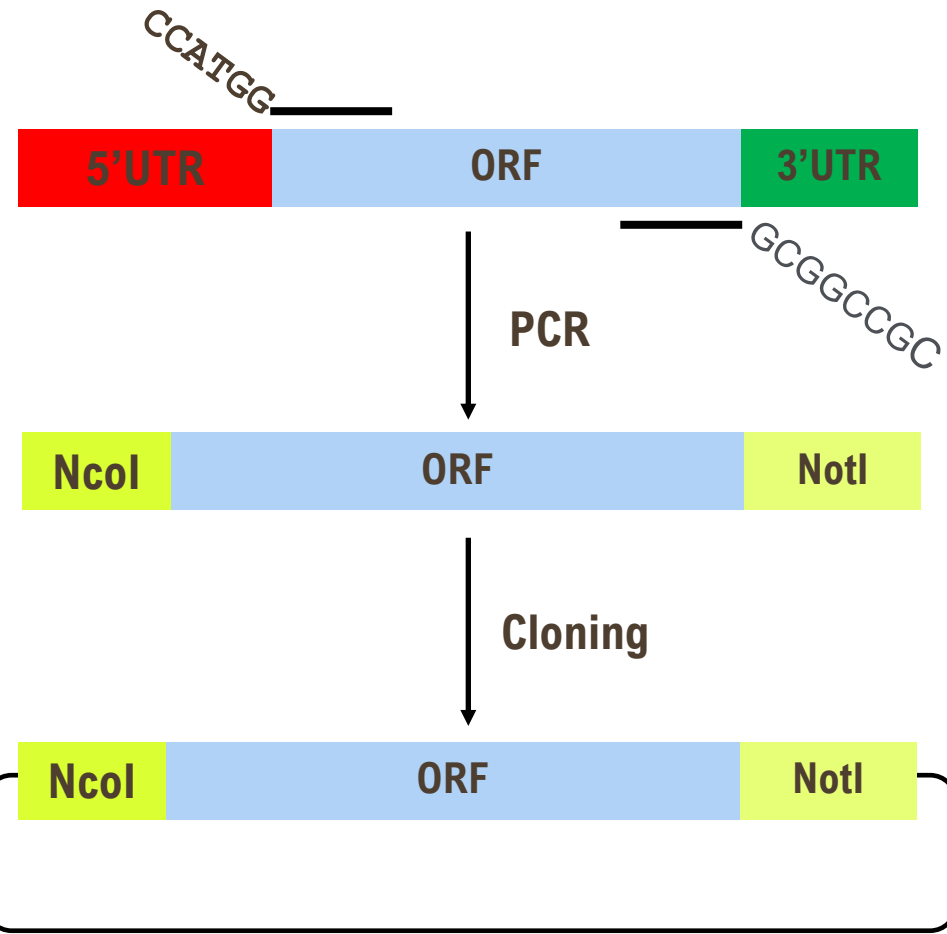
Binary Ti Plasmid : T-DNA structure



NO	Selectable Marker Genes	Chemical Agent
1	BASTA (bar) gene	Phosphinothricin (Basta atau Glyphosat)
2	Hygromycin B phosphotransferase (<i>hygB</i>)	Hygromycin B
3	kanamycin resistance gene	Acetamide

NO	Promoter	Contoh
1	Constitutive promoters	Opine promoters, CaMV 35S promoter, Plant ubiquitin promoter (Ubi), Rice actin 1 promoter (Act-1)
2	Inducible promoters	Temperature-regulated promoters, Light-regulated promoters, Stress-regulated promoters
3	Tissue-specific promoters	Root promoters, Fruit promoters, Seed promoters

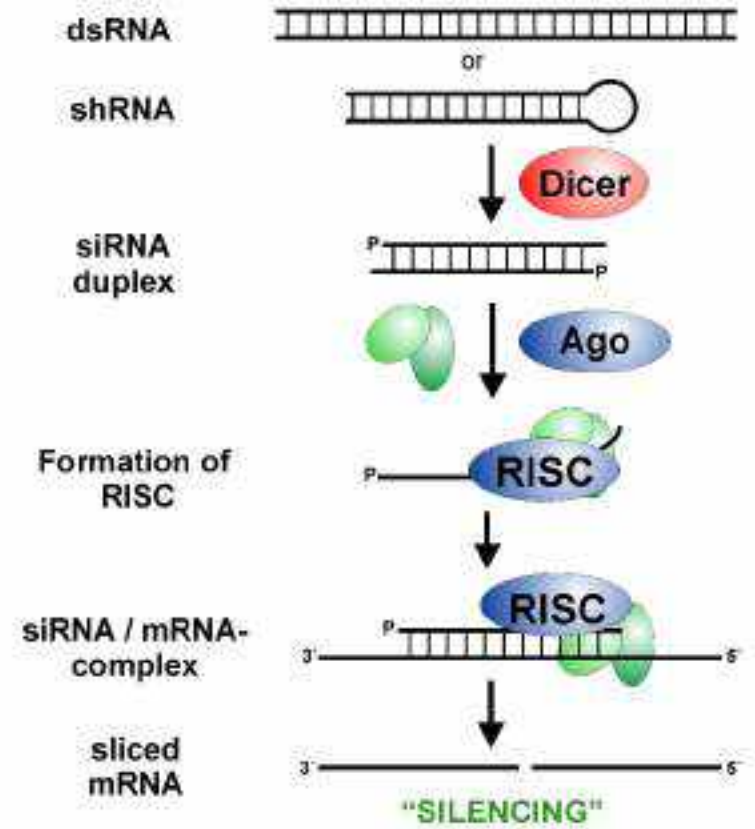
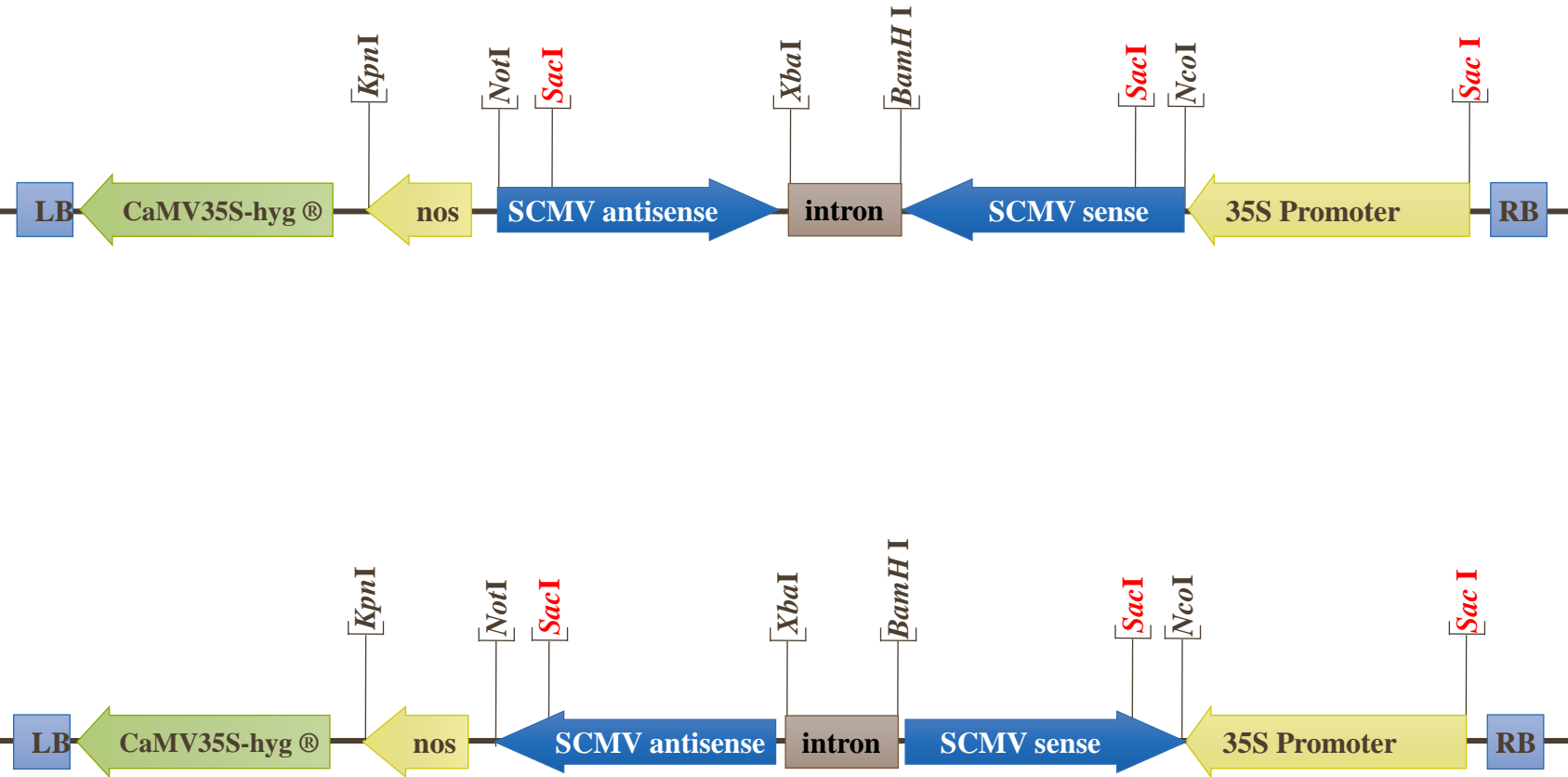
Inseri Gen pada vektor





RNAi construct

RNAi construct

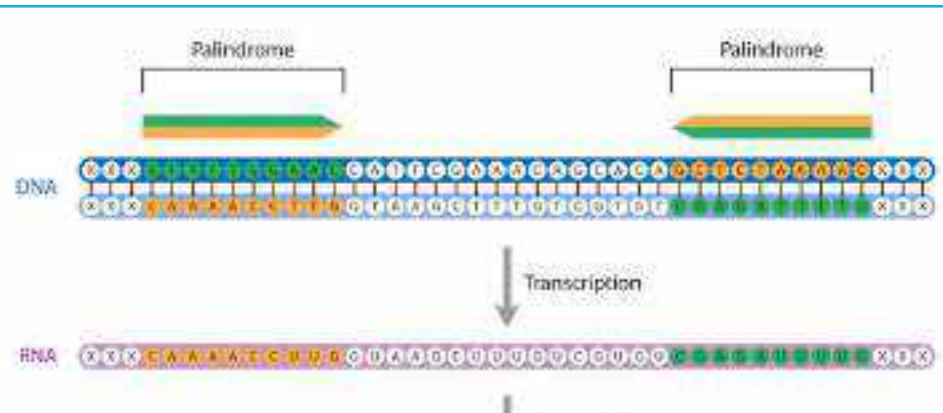




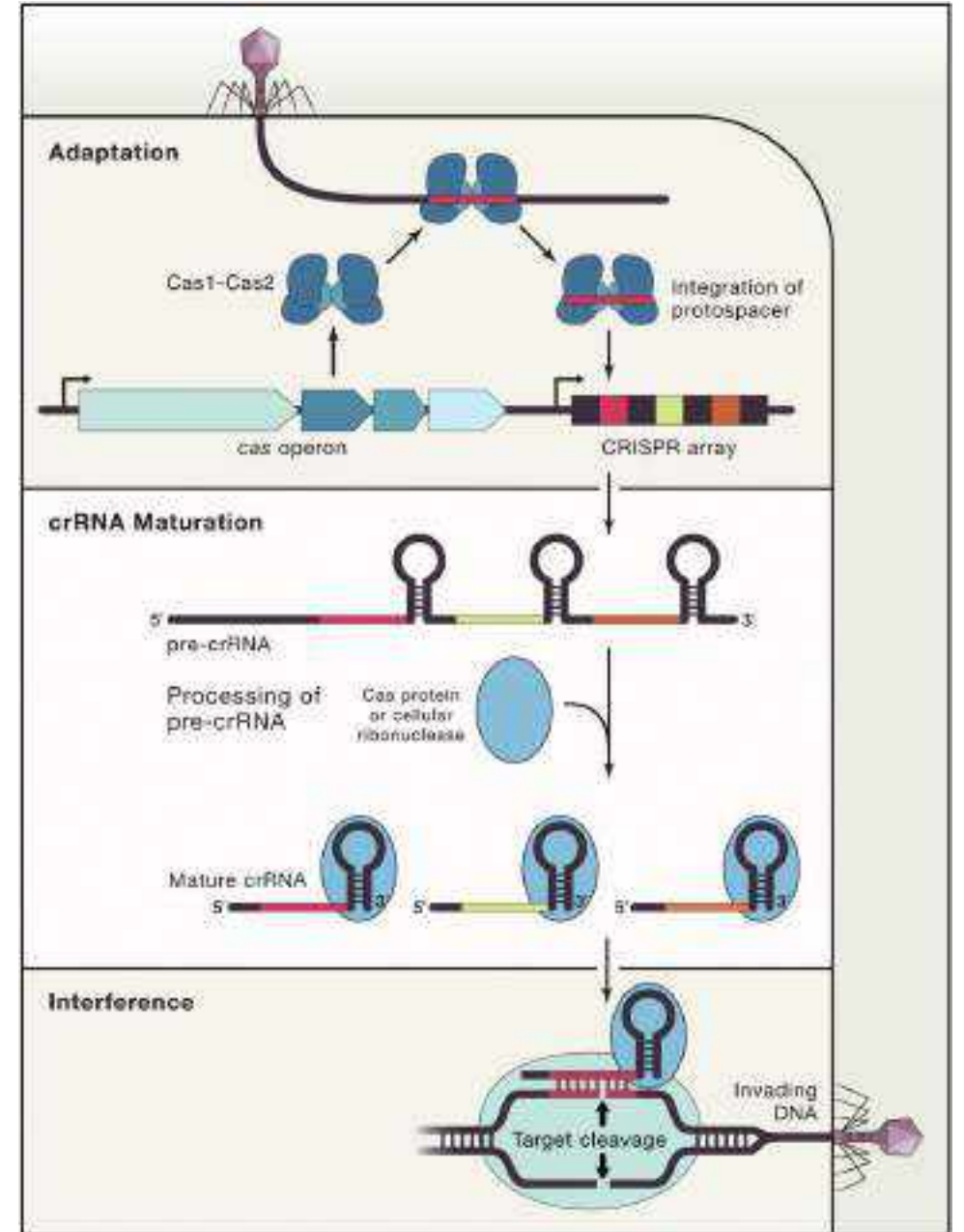
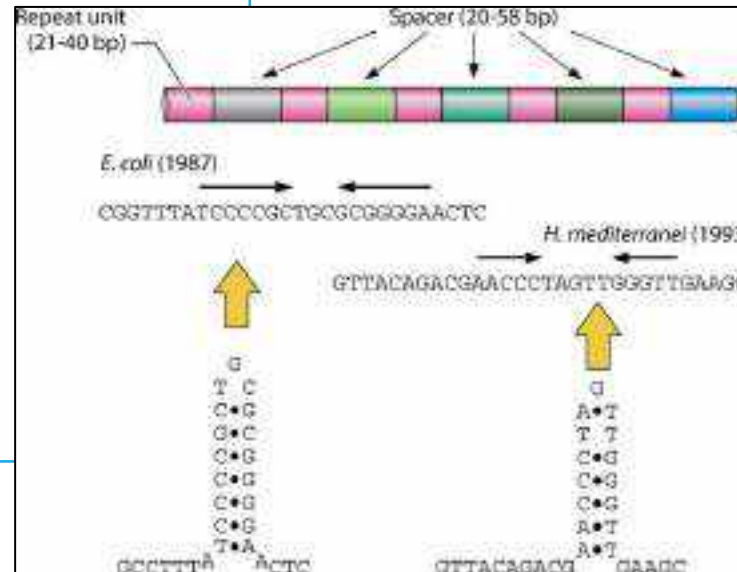
CRISPR – Cas9

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) - Cas Immunity in Prokaryotes

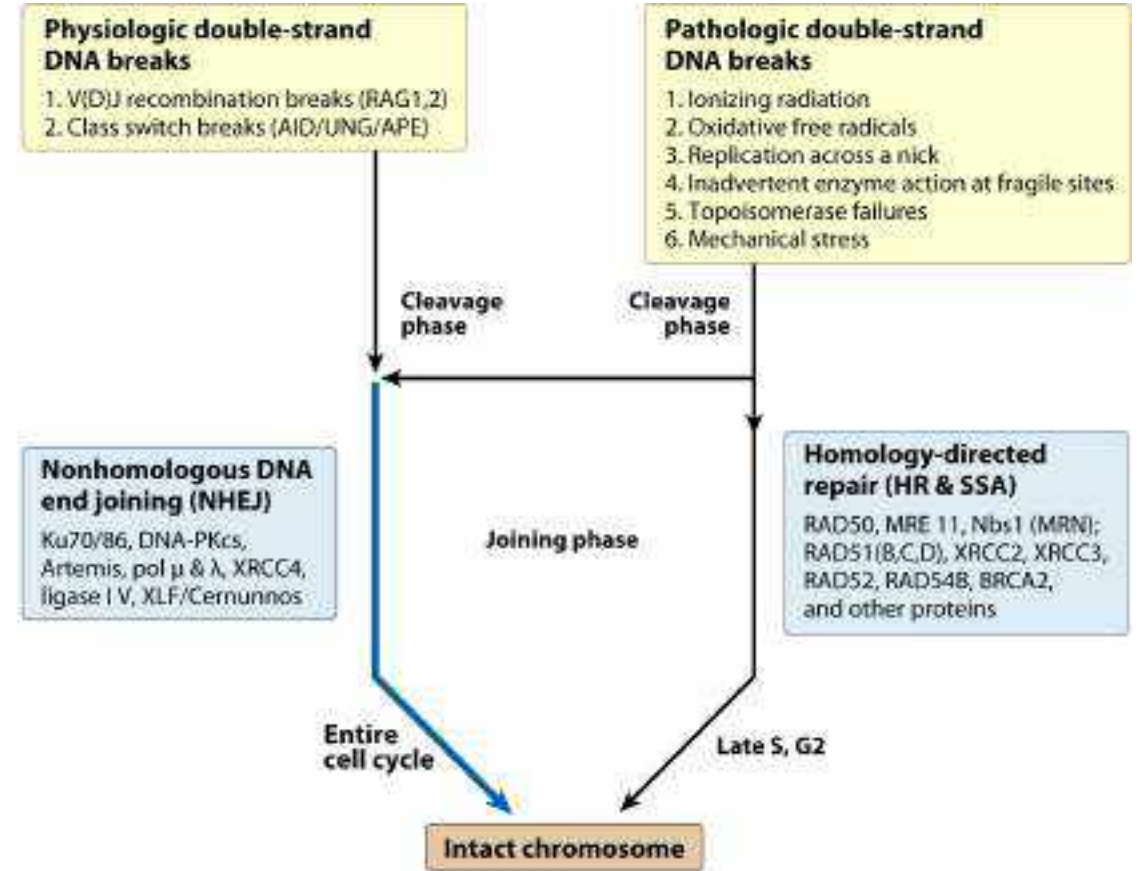
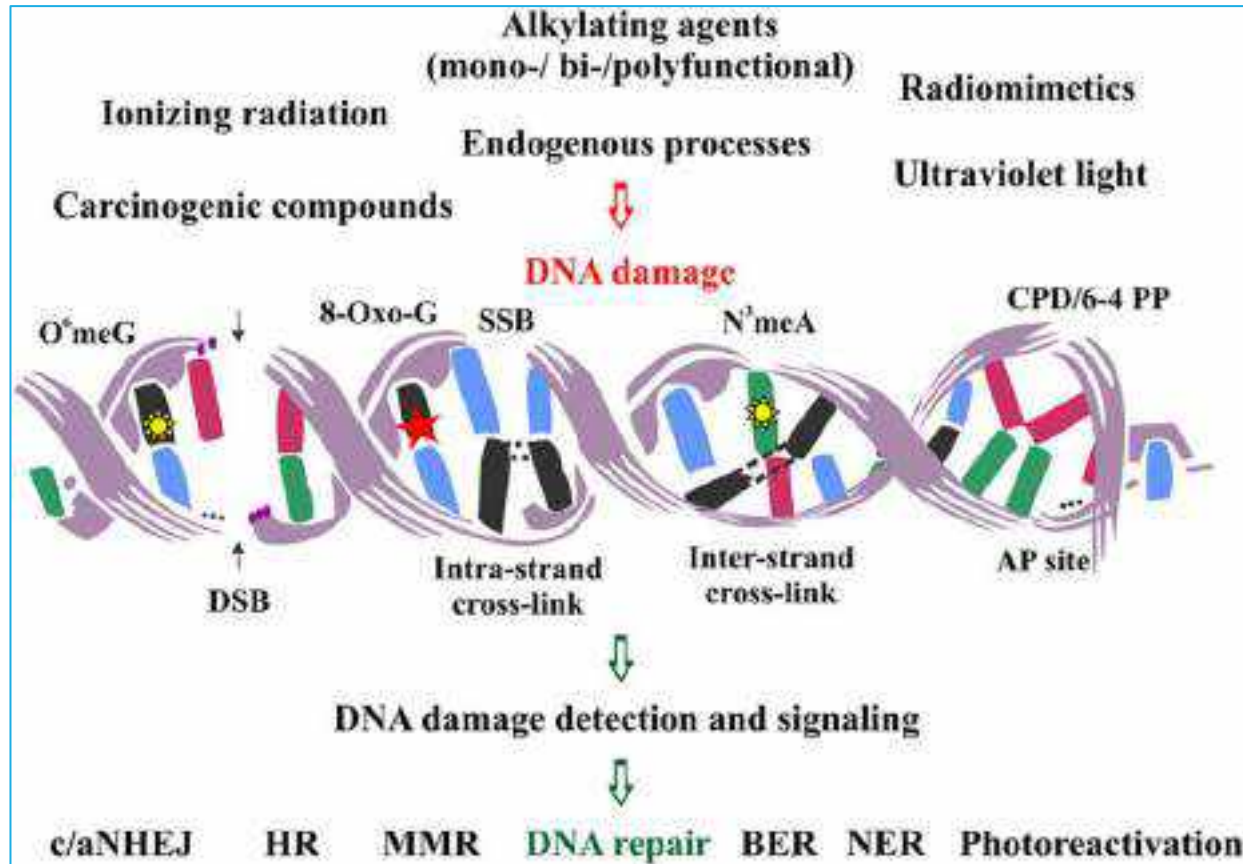
Palindromic



Repeat



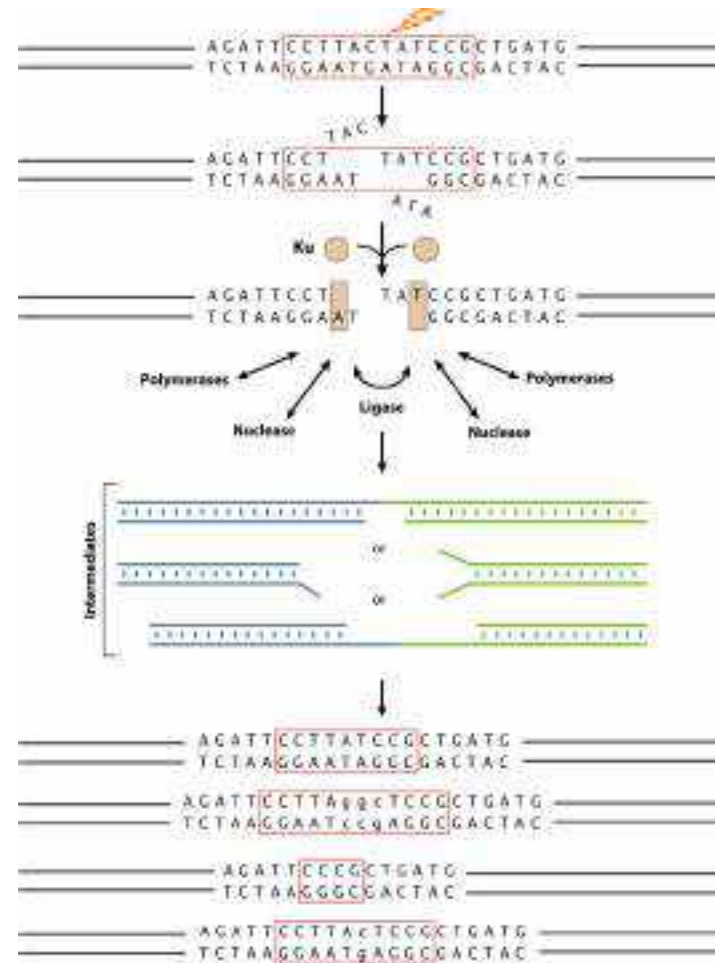
Double-strand DNA Break (DSB) Repair By The Nonhomologous DNA End-joining (NHEJ) and Homology-directed repair (HDR) Pathway



Lieber MR. 2010. Annu. Rev. Biochem. 79:181-211

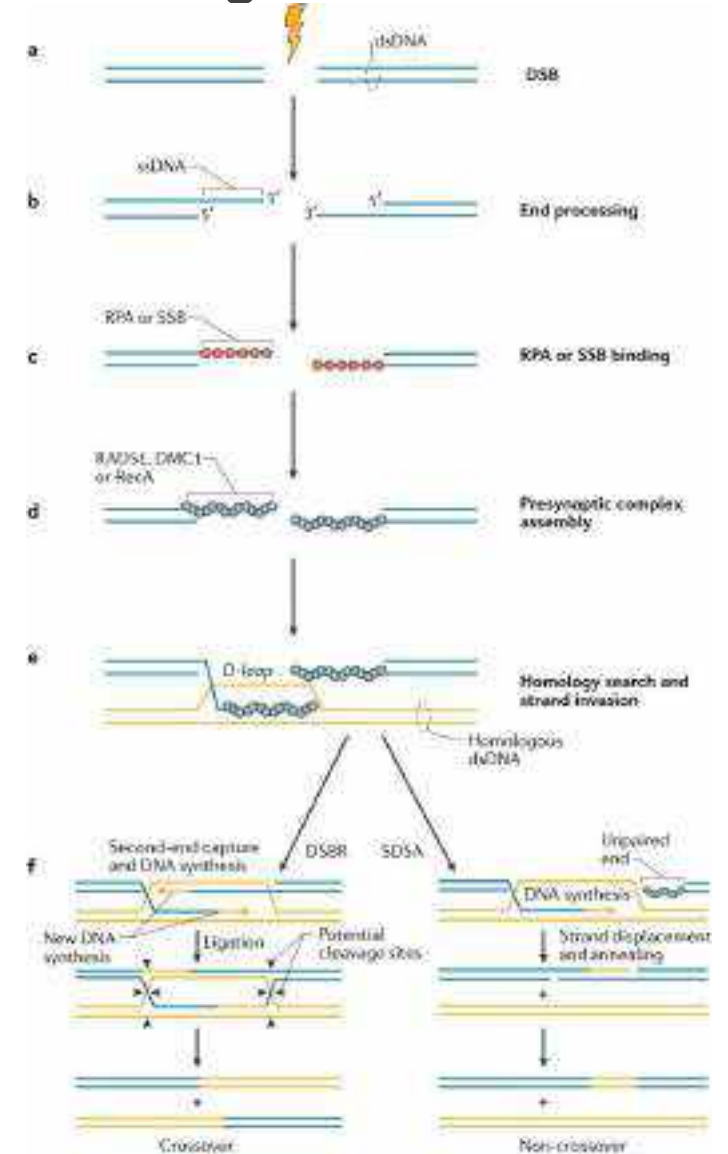
DOUBLE-STRAND DNA BREAK (DSB) REPAIR

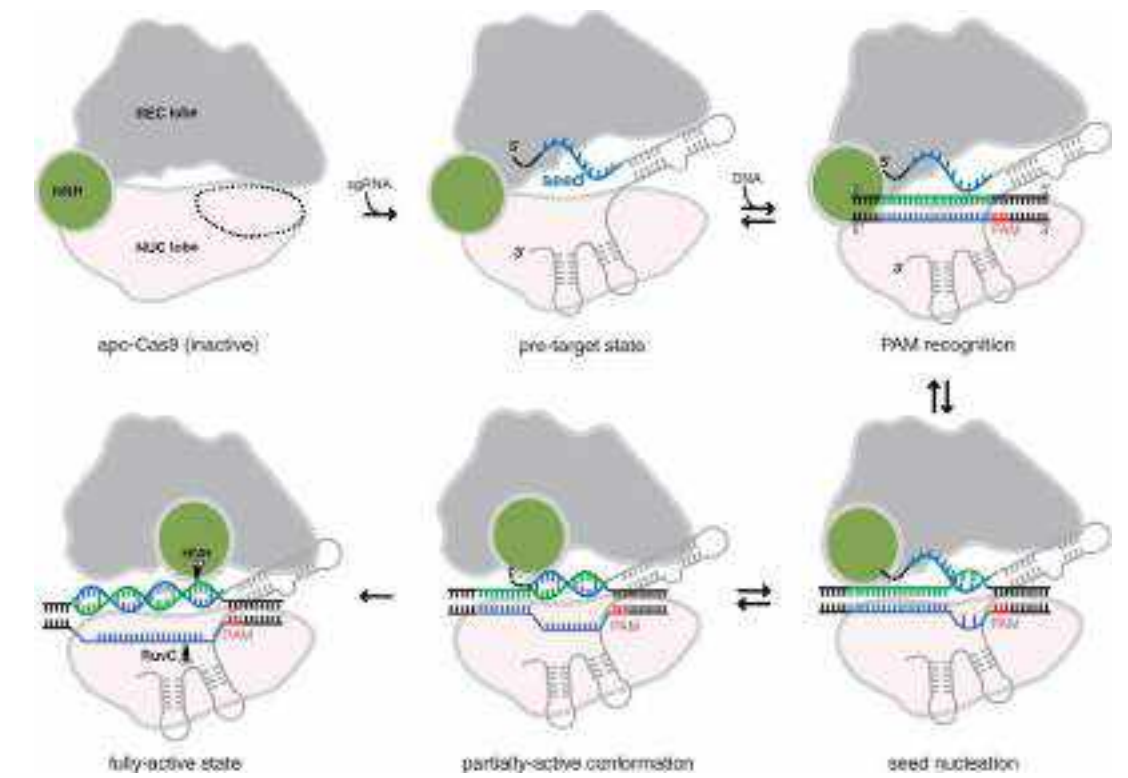
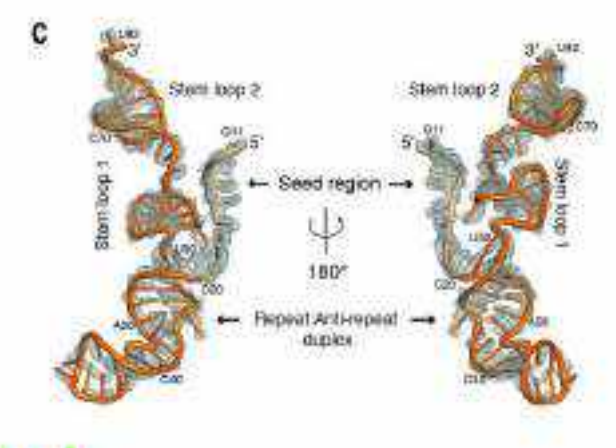
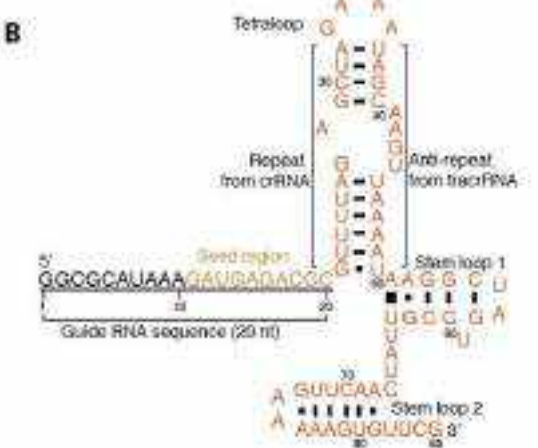
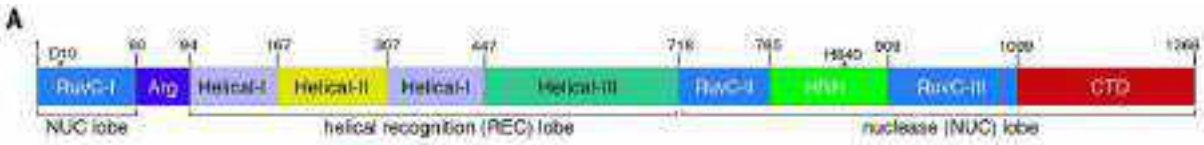
Nonhomologous DNA End-joining (NHEJ) Pathway



Lieber MR, 2010.
Annu. Rev. Biochem. 79:181–211

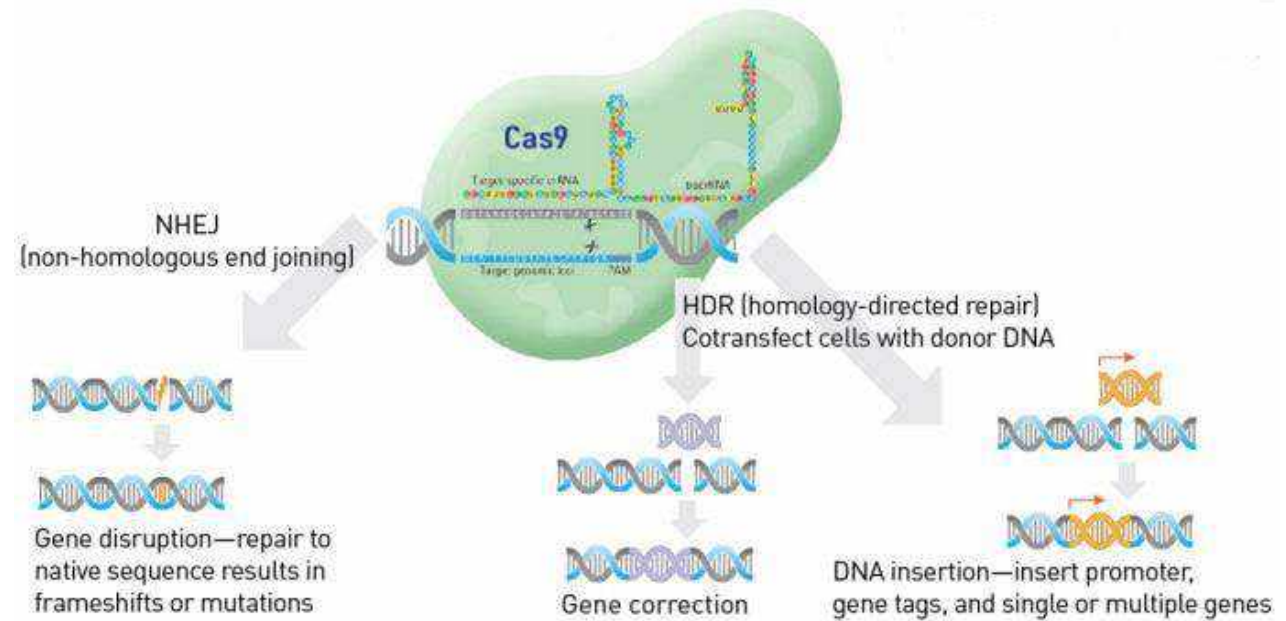
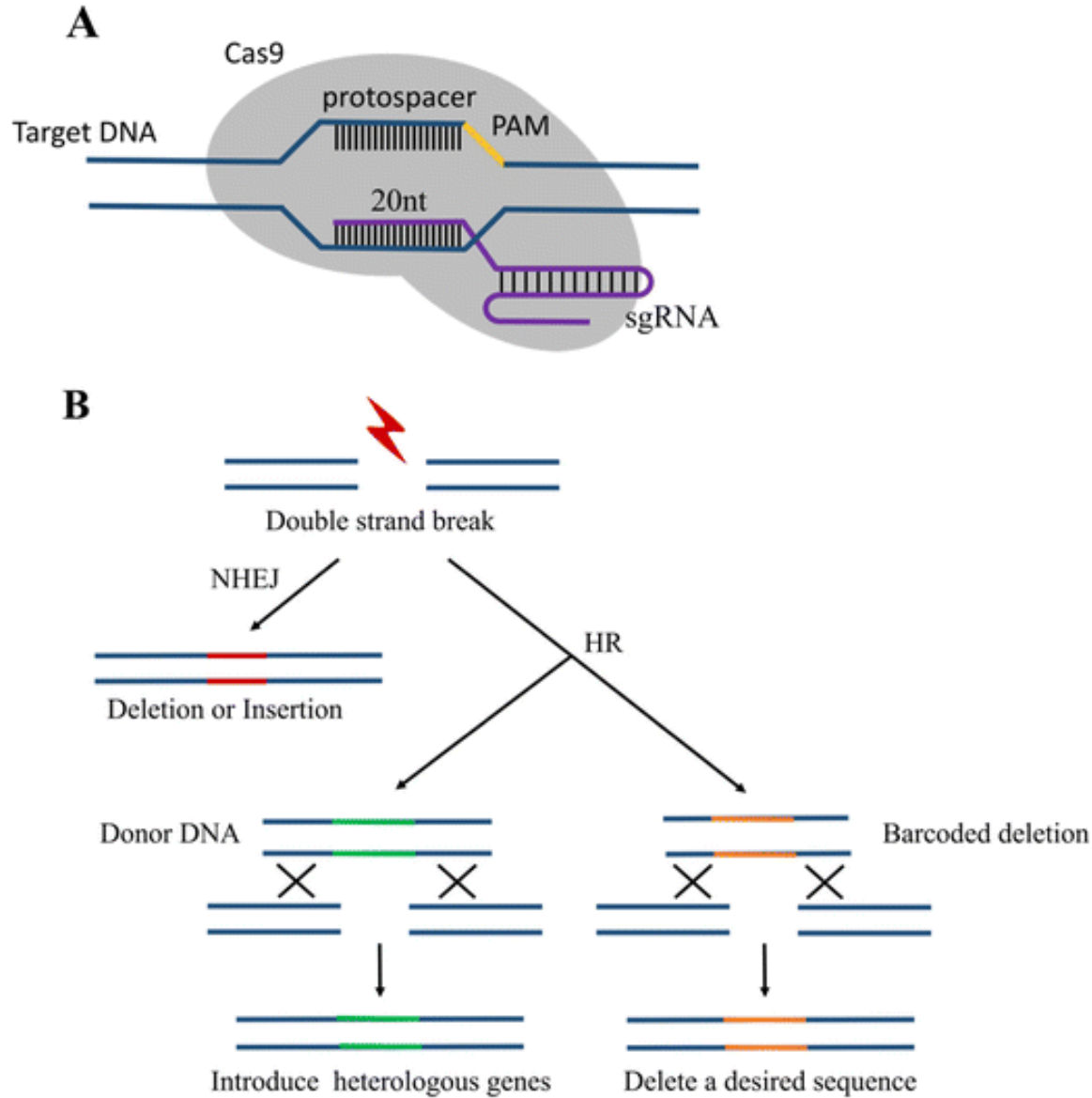
Homologous Recombination



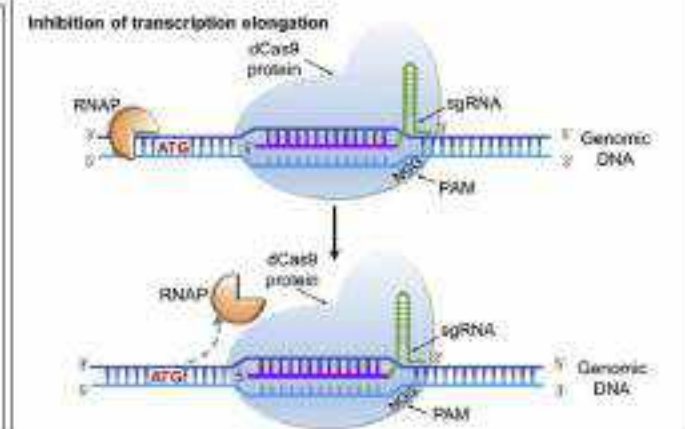
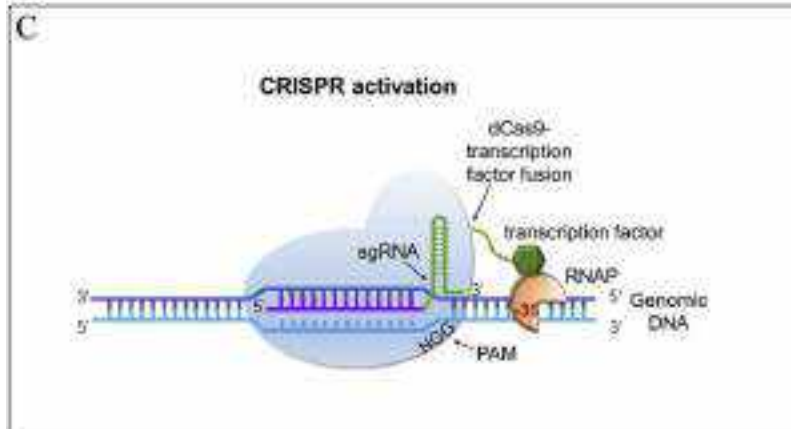
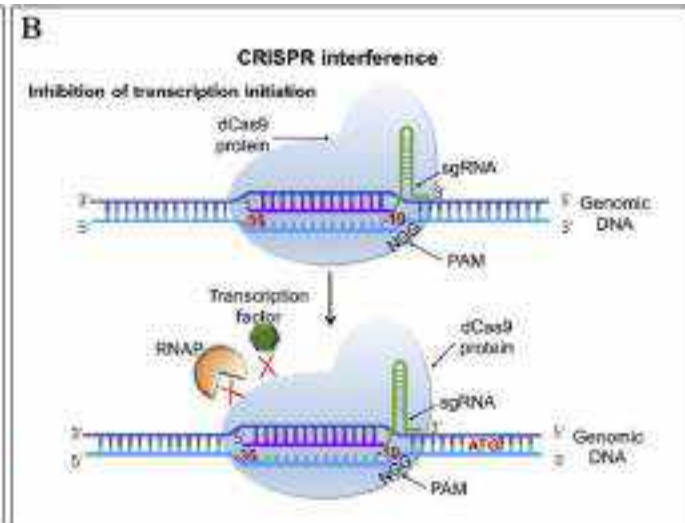
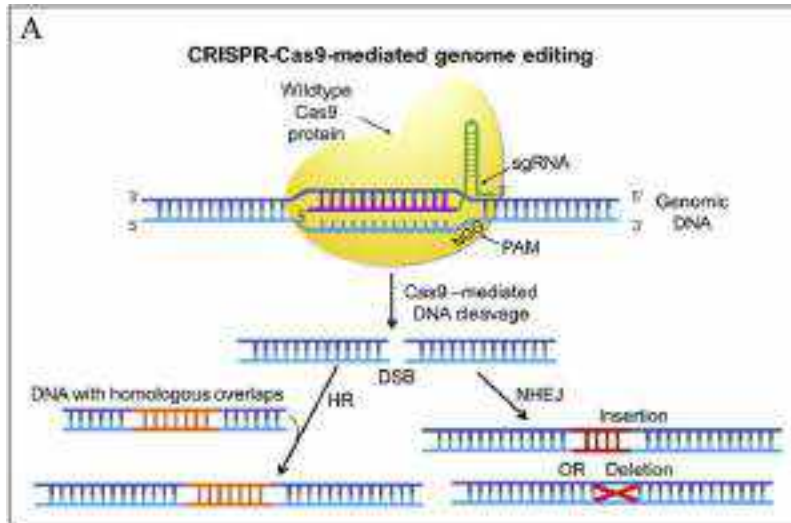


CAS9 PROTEIN DOMAIN AND COMPLEX ASSEMBLY

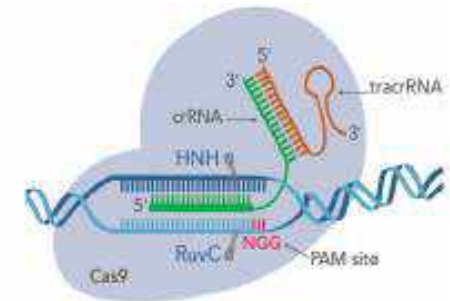
CRISPR/CAS9 APPLICATION



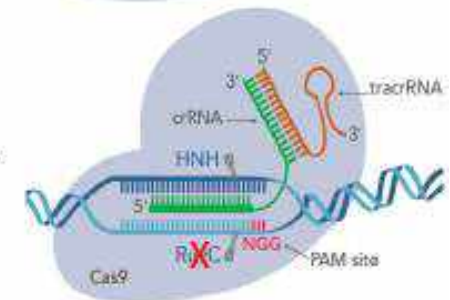
CRISPR/CAS9 APPLICATION



WT Cas9
Cleaves both strands



Cas9 D10A nickase
Inactivated RuvC domain
Cleaves target strand



Cas9 H840A nickase
Inactivated HNH domain
Cleaves non-target strand

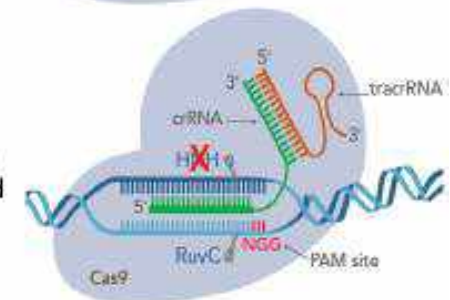
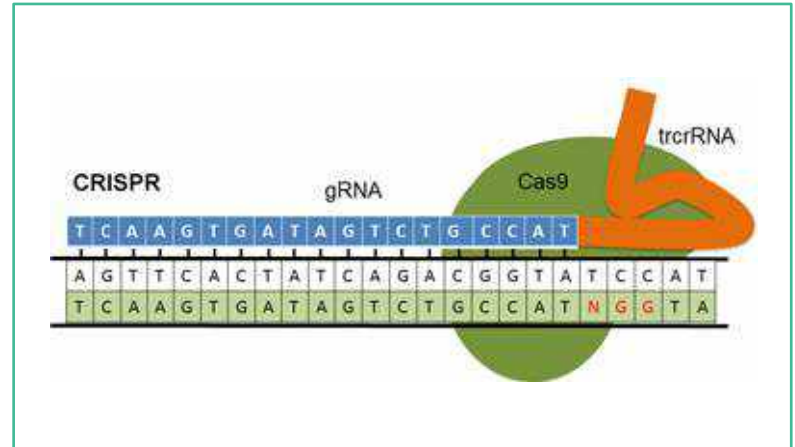
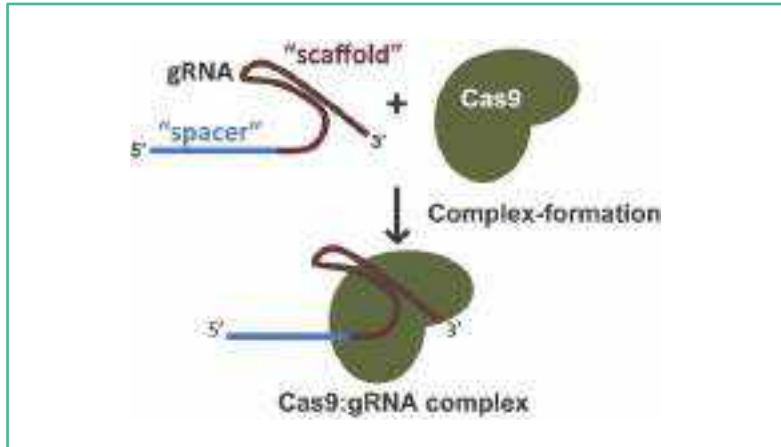
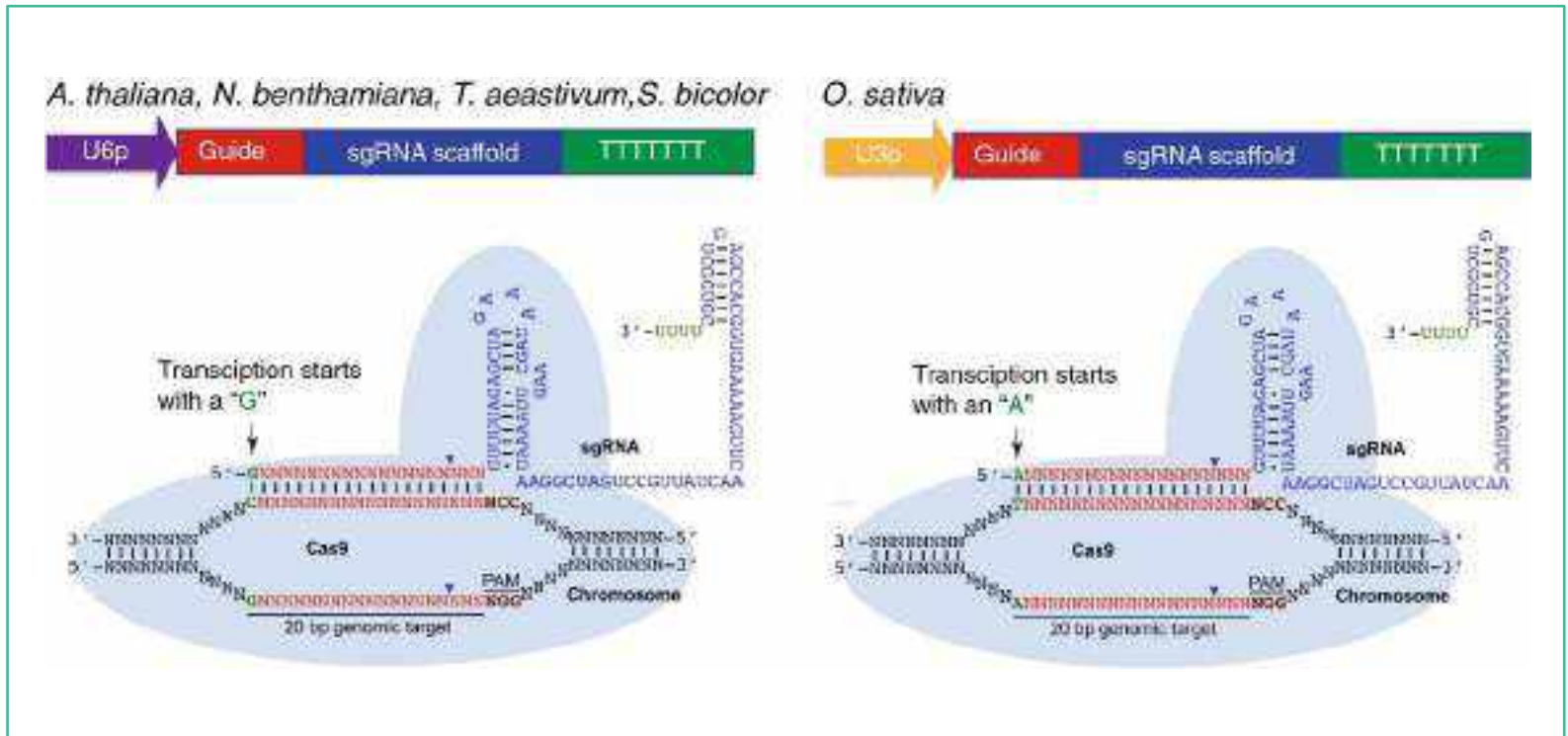


Figure 1. Cleavage capabilities of Cas9 nickases.

CRISPR/CAS9 COMPONENT FOR GENOME EDITING

Plant species	Cas9	Reference
<i>A. thaliana</i>	3x3P1CR4 (NLS (N1E) intro) plant codon optimized Cas9 (NLS)	[11]
	2x FLAG (NLS (N1E) intro) plant codon optimized Cas9 (NLS)	[11]
	2x FLAG (C. reinhardtii codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[12]
	2x355x (NLS human codon optimized Cas9 (NLS))	[13]
	2x355x (NLS (N1E) intro) plant codon optimized Cas9 (NLS)	[11]
<i>N. benthamiana</i>	3x3P1CR4 (NLS (N1E) intro) plant codon optimized Cas9 (NLS)	[11]
	2x FLAG (NLS (N1E) intro) plant codon optimized Cas9 (NLS)	[11]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
<i>T. aestivum</i>	2x355x (NLS rice codon optimized Cas9 (NLS))	[10]
	2x355x (NLS human codon optimized Cas9 (NLS))	[12]
	2x355x (NLS human codon optimized Cas9 (NLS))	[14]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
<i>O. sativa</i>	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[10]
	2x355x (NLS human codon optimized Cas9 (NLS))	[13]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
	2x355x (NLS human codon optimized Cas9 (NLS))	[16]
<i>S. bicolor</i>	2x355x (NLS human codon optimized Cas9 (NLS))	[16]





TERIMA KASIH