DNA Construct for Plant Gene Recombination

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Discussion questions

1. What basic elements should be included in the design and construction of an efficient ubiquitous and constitutive plant gene expression vector?
2. Discuss the advantages and disadvantages of recombination cloning technologies versus traditional restriction digestion and ligation technology.
3. Describe a novel strategy to generate a T-DNA vector that allows the expression of several genes from a single position in the genome.
4. Discuss the advantages and disadvantages of using plastid vectors for plant transformation and gene expression.
5. Describe ways in which transgene technology could be made more acceptable to the public.

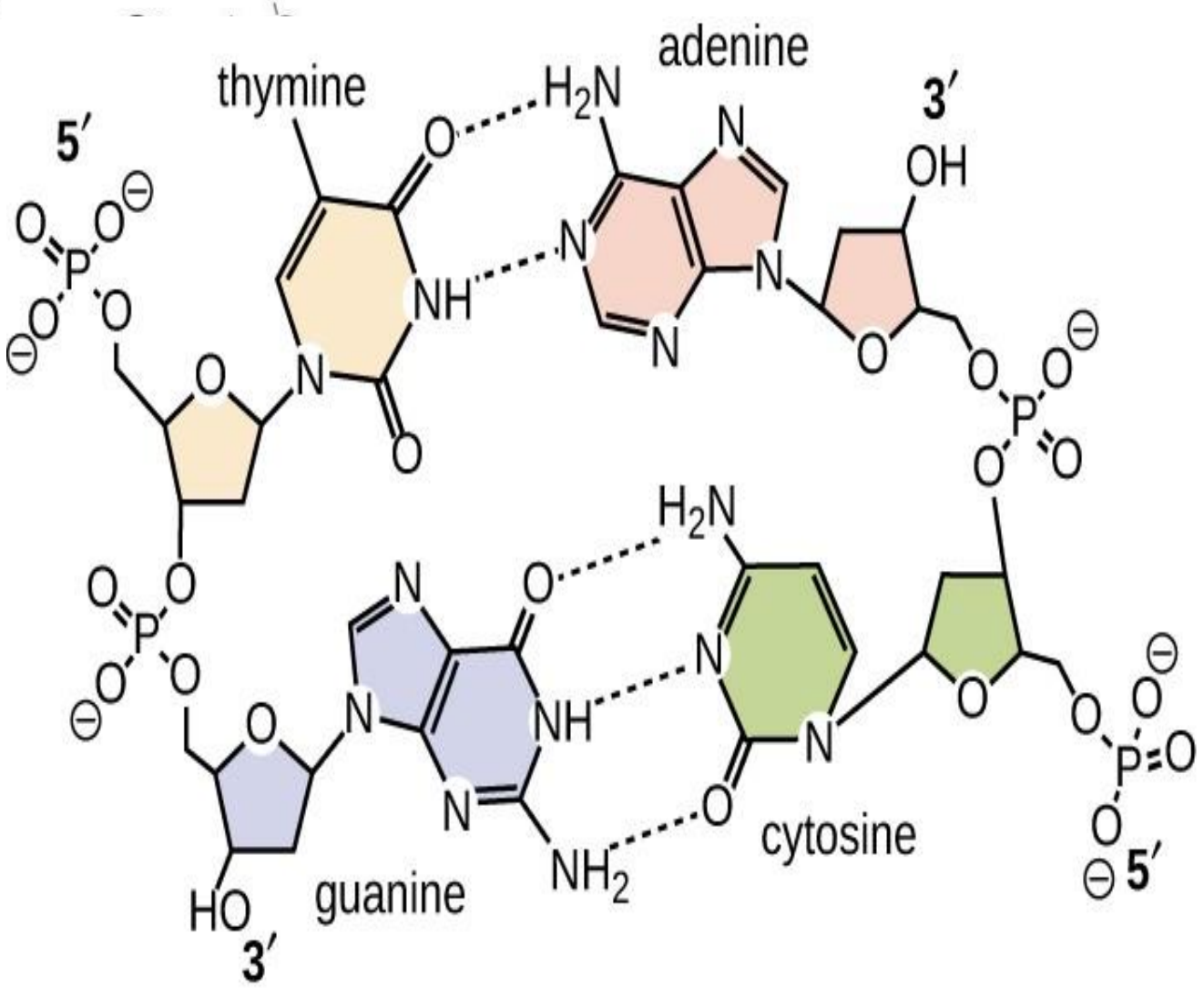
Nucleotide base pairing

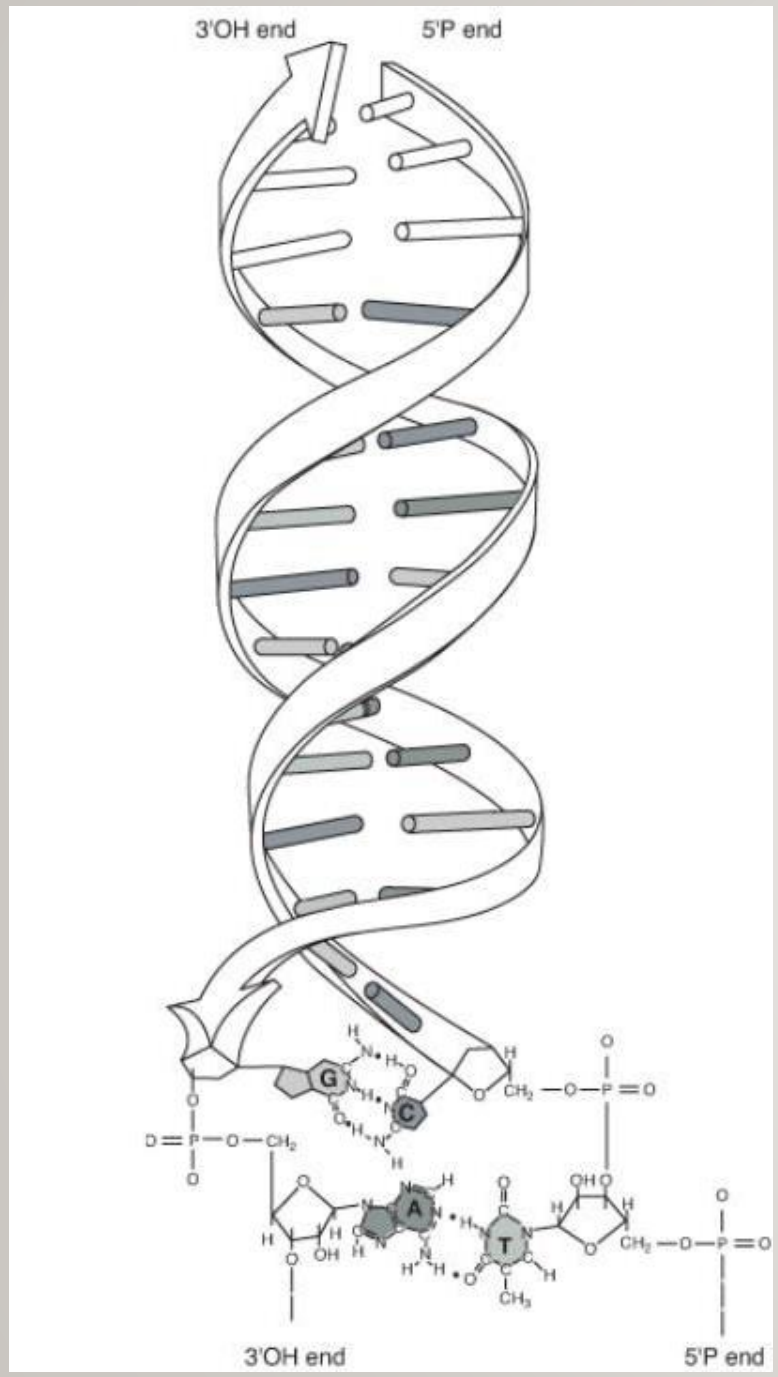
A's pair with T's

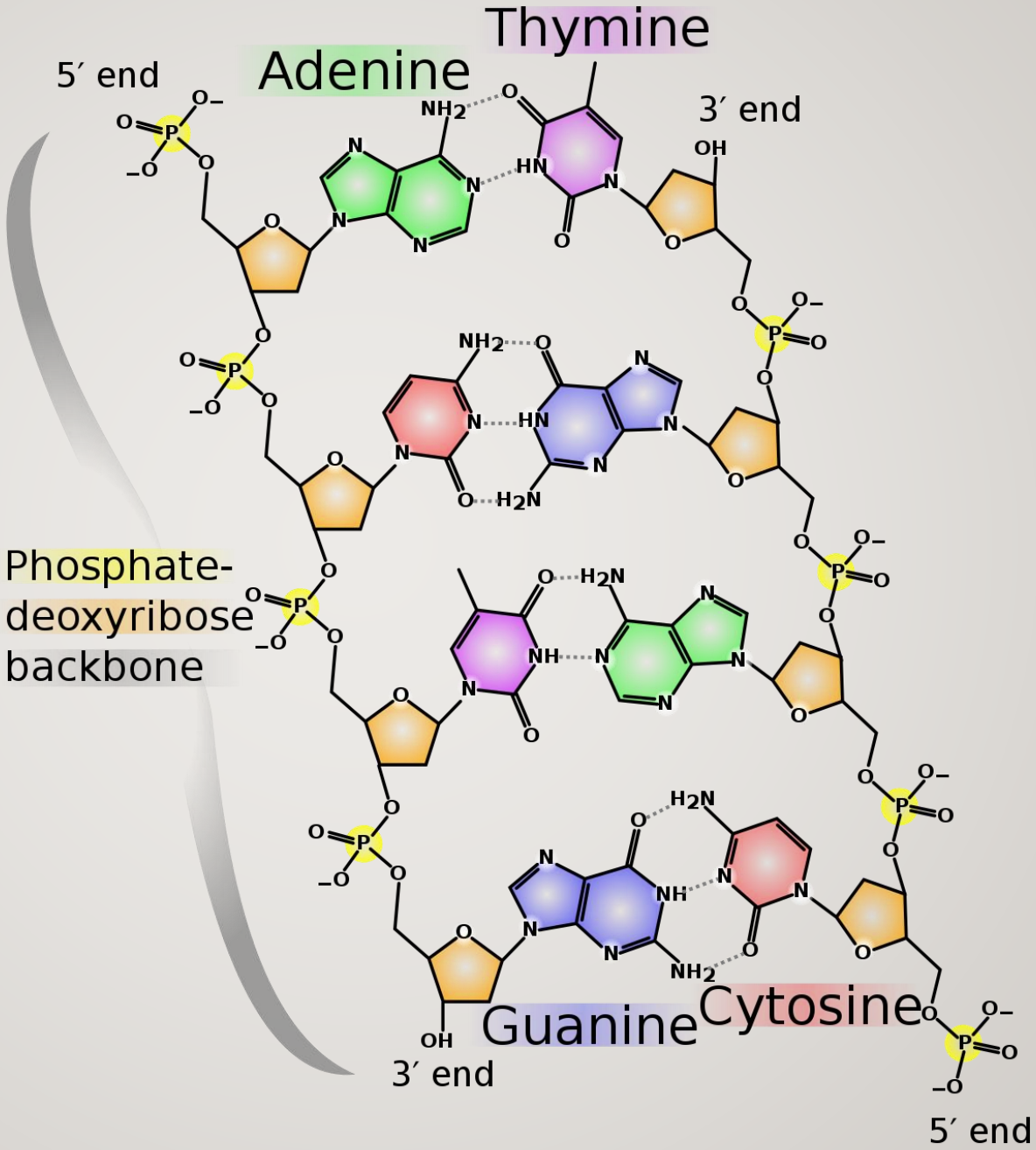
G's pair with C's

Nucleotide base pairing occurs through "hydrogen bonding"

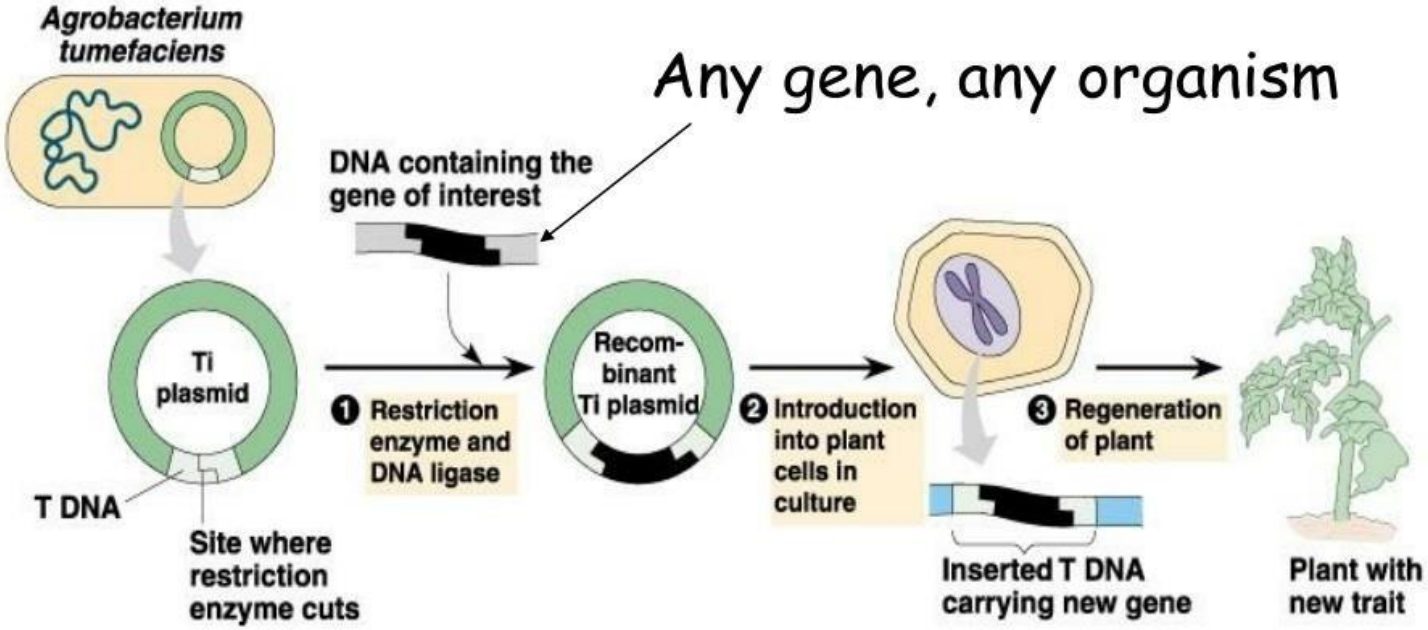
Strands have directionality from 5' to 3' and when paired strands are in "antiparallel" orientation




3'OH end
5'P end
G
C
A
T
3'OH end
5'P end

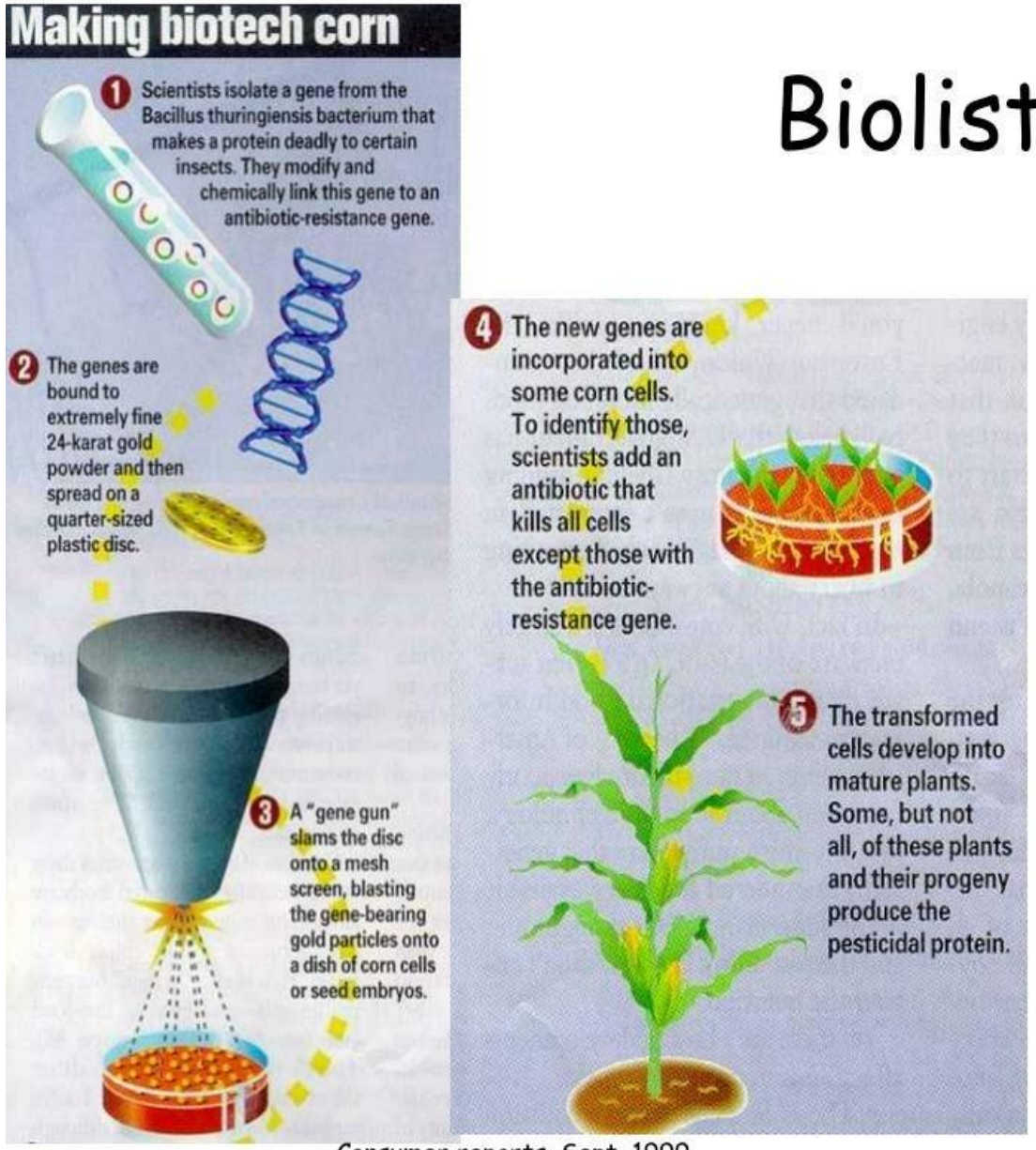

Thymine
Adenine
5′ end
3′ end
Phosphate-
deoxyribose
backbone
Guanine
Cytosine
3′ end
5′ end

Transgenic plants- *Agrobacterium*

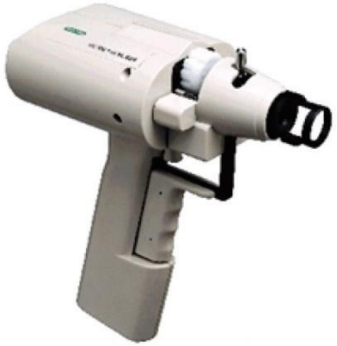


The new plant will pass the transgene to its progeny through seed.

Biolistics



Consumer reports, Sept. 1999



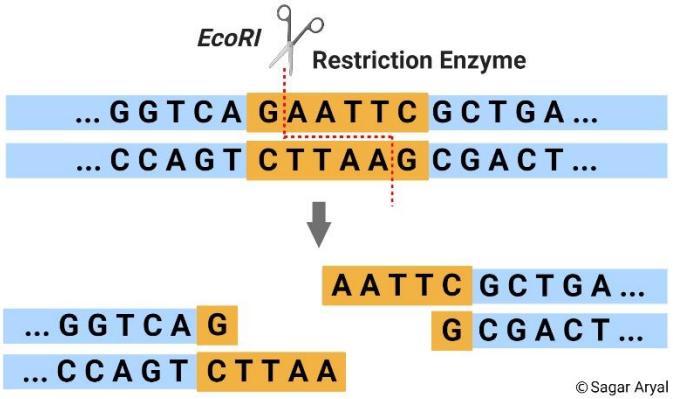
Steps to make Transgenic plants

Construct	• Construct transformation cloning plasmid vector
Transform	• Transform to bacteria (usually Escherichia coli) for maintaining clone
Characterize	• Characterize plasmid (restriction digest and sequencing)
Transform	• Transform to Agrobacterium (if using biological method) and characterize
Transform	• Transform to plant

Recombinant DNA history

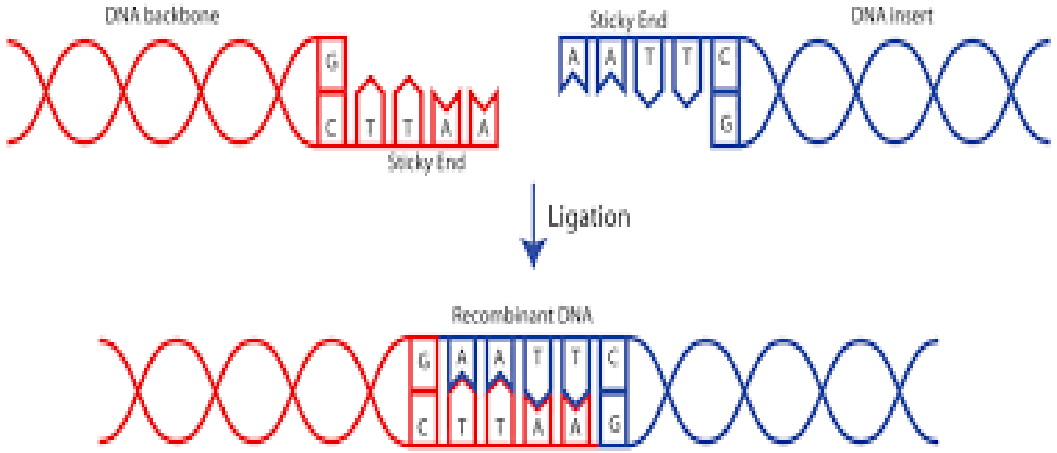
1966 The genetic code is deciphered when biochemical analysis reveals which codons determine which amino acids.
1970 Hamilton Smith, at Johns Hopkins Medical School, isolates the first restriction enzyme, an enzyme that cuts DNA at a very specific nucleotide sequence. Over the next few years, several more restriction enzymes will be isolated.
1972 Stanley Cohen and Herbert Boyer combine their efforts to create recombinant DNA. This technology will be the beginning of the biotechnology industry.
1976 Herbert Boyer cofounds Genentech, the first firm founded in the United States to apply recombinant DNA technology
1978 Somatostatin, which regulates human growth hormones, is the first human protein made using recombinant technology.
1983 Kary Mullis does PCR. 1985 Kary Mullis publishes method. Patents follow.
2000 Gateway cloning

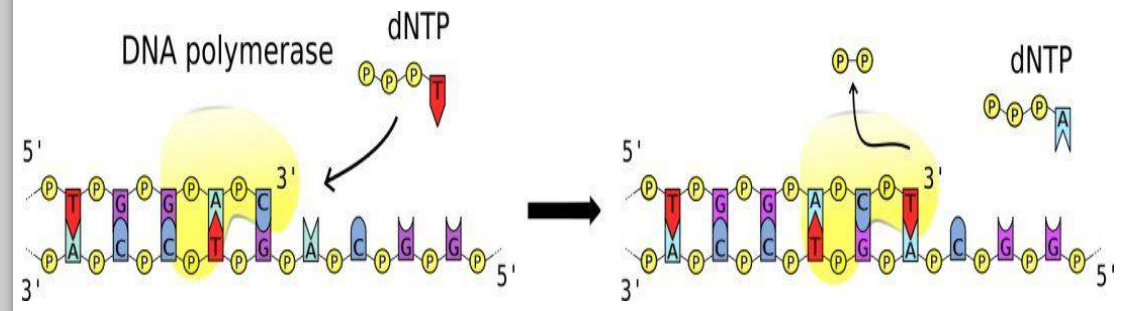
http://www.accessexcellence.org/RC/AB/WYW/wkbooks/SFTS/sidebarmilestone.html



Key enzymes

- Restriction endonuclease
- DNA ligase
- Taq DNA polymerase





Cloning Platforms

- Restriction enyzmes/ ligase
- PCR-Based methods
- Gateway and other site-specific recombination methods

Restriction Enzyme - Ligation

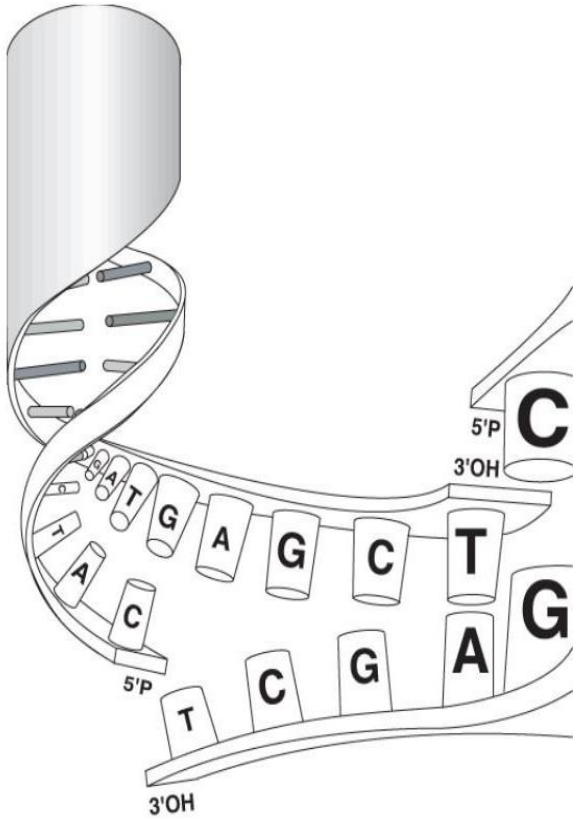
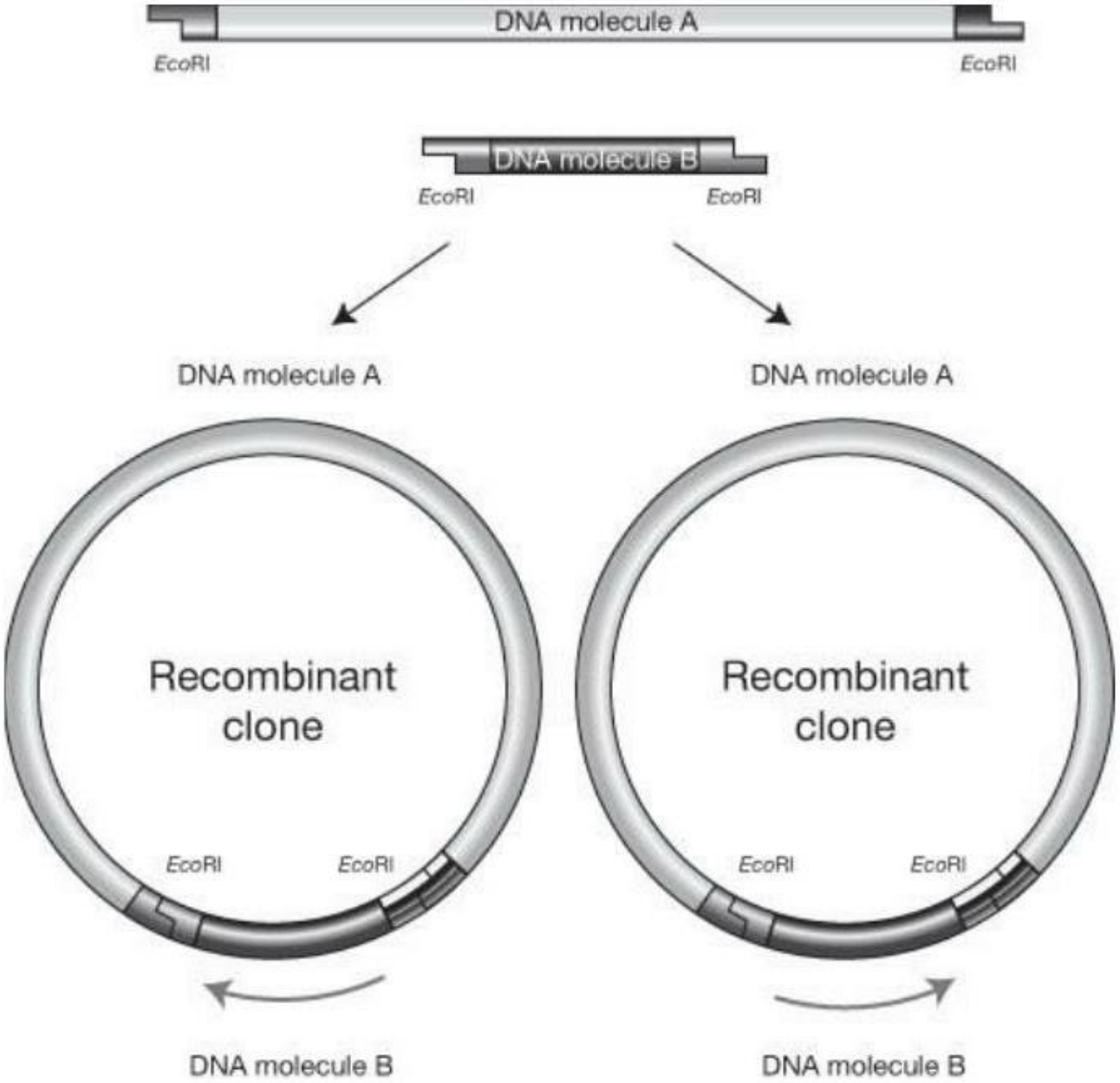
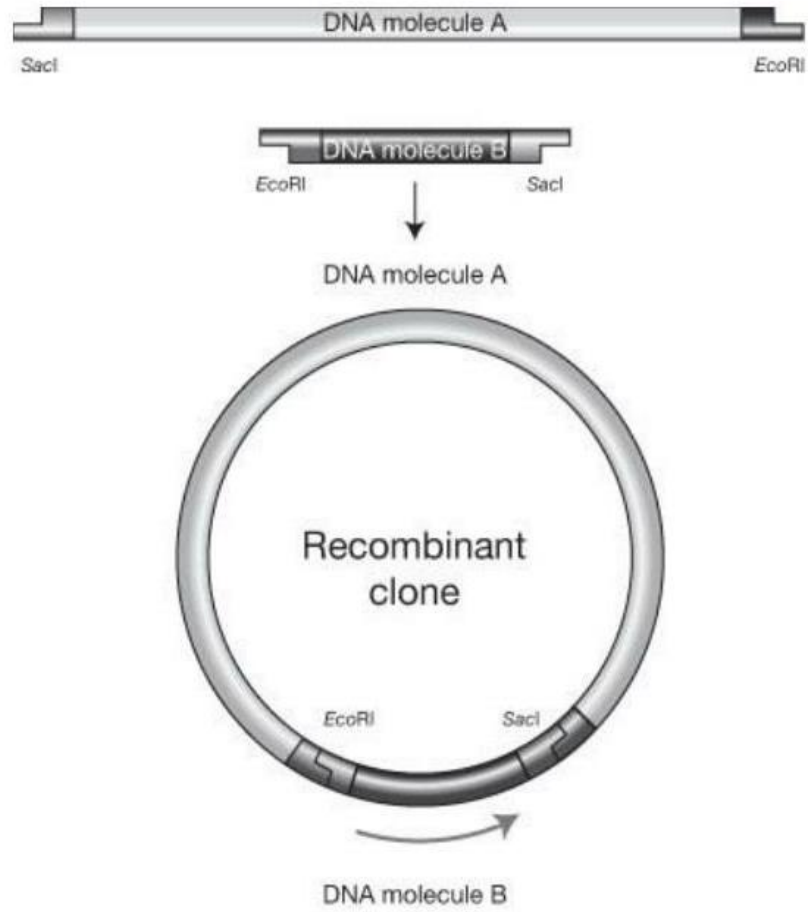


TABLE 7.1. Restriction Endonucleases

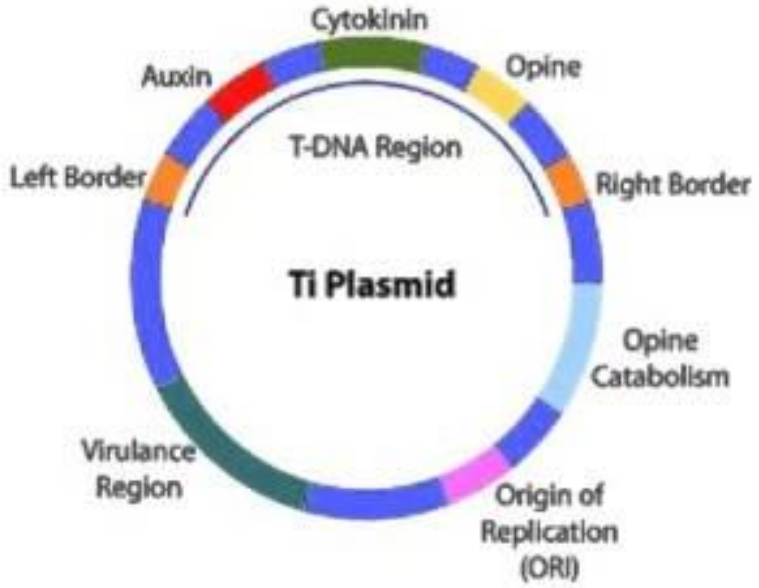
Enzyme	Source	Recognition sequence	Cut		Ends
*Eco*RI	*Escherichia coli* RY13	GAATTC	G	AATTC	5′overhangs
		CTTAAC	CTTAA	G	
*Bam*HI	*Bacillus amyloliquefaciens* H	GGATCC	G	GATCC	5′overhangs
		CCTAGG	CCTAG	G	
*Hin*dIII	*Haemophilus inflenzae* Rd	AAGCTT	A	AGCTT	5′overhangs
		TTCGAA	TTCGA	A	
*Kpn*I	*Klebsiella pneumoniae*	GGTACC	GGTAC	C	3′overhangs
		CCATGG	C	CATGG	
*Not*I	*Nocardia otitidis*	GCGGCCGC	GC	CGCCGG	5′overhangs
		CGCCGGCG	GGCCGC	CG	
*Pst*I	*Providencia stuartii*	CTGCAG	CTGCA	G	3′overhangs
		GACGTC	G	ACGTC	
*Sma*I	*Serratia marcescens*	CCCGGG	CCC	GGG	Blunt ends
		GGGCCC	GGG	CCC	
*Sac*I	*Streptomyces achromogenes*	GAGCTC	GAGCT	C	3′overhangs
		CTCGAG	C	TCGAG	
*Sst*I	*Streptomyces stanford*	GAGCTC	GAGCT	C	3′overhangs
		CTCGAG	C	TCGAG	
*Taq*I	*Thermophilus aquaticus*	TCGA	T	CGA	5′overhangs
		AGCT	AGC	T	
*Xba*I	*Xanthomonas campestris pv. badrii*	TCTAGA	T	CTAGA	5′overhangs
		AGATCT	AGATC	T	


DNA molecule A
EcoRI
EcoRI
DNA molecule B
EcoRI
EcoRI
DNA molecule A
DNA molecule A
Recombinant clone
EcoRI
EcoRI
Recombinant clone
EcoRI
EcoRI
DNA molecule B
DNA molecule B


DNA molecule A
SacI
EcoRI
DNA molecule B
EcoRI
SacI
DNA molecule A
Recombinant clone
EcoRI
SacI
DNA molecule B

Transformation vector requirements

- Origin of replication
- Bacterial selectable marker
- Gene constructs of interest
- T-DNA borders and other *Agrobacterium* genes if using *Agrobacterium*
- Compatible with helper plasmid if using *Agrobacterium*


Cytokinin
Auxin
Opine
T-DNA Region
Left Border
Right Border
Ti Plasmid
Opine Catabolism
Virulance Region
Origin of Replication (ORI)

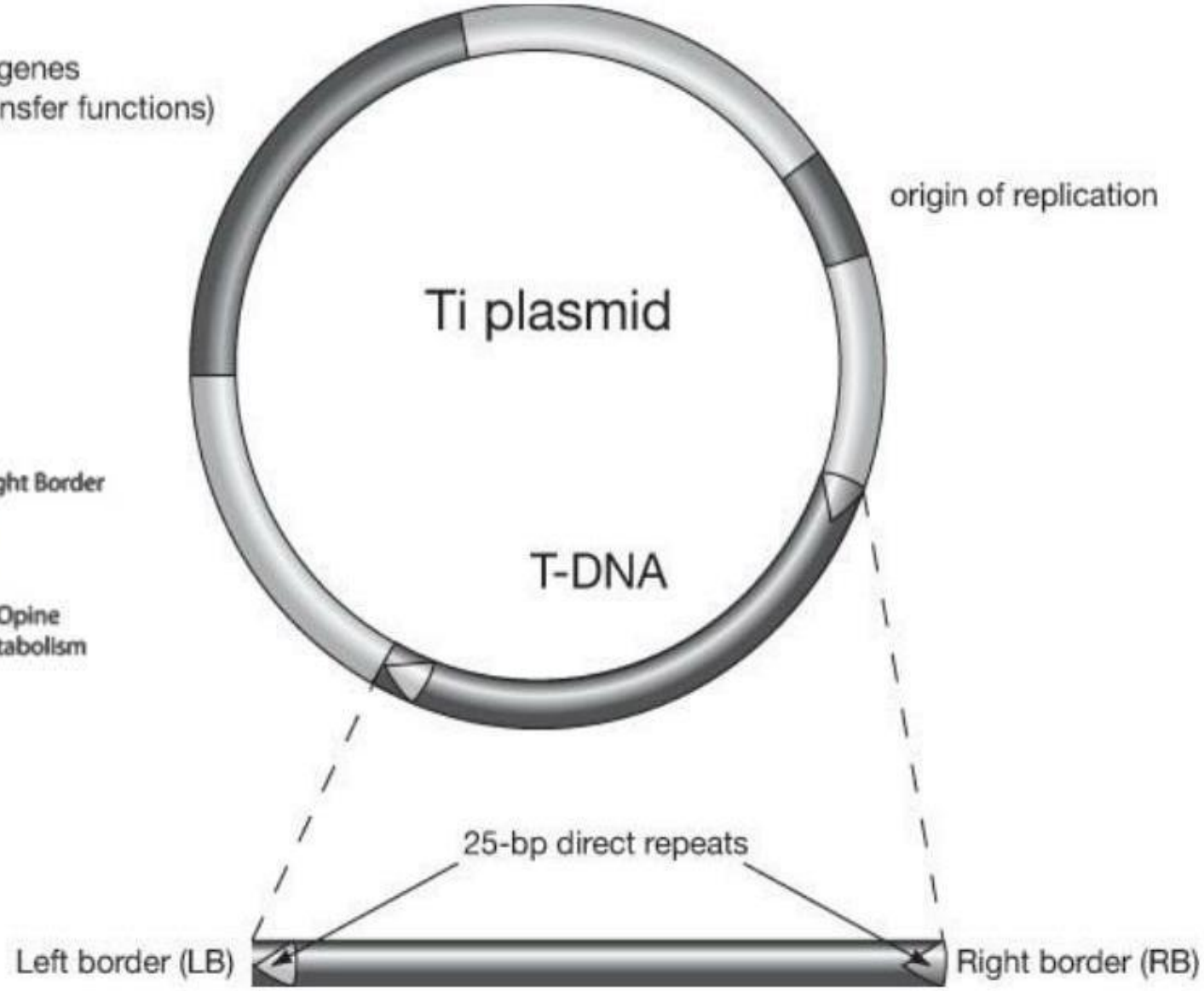
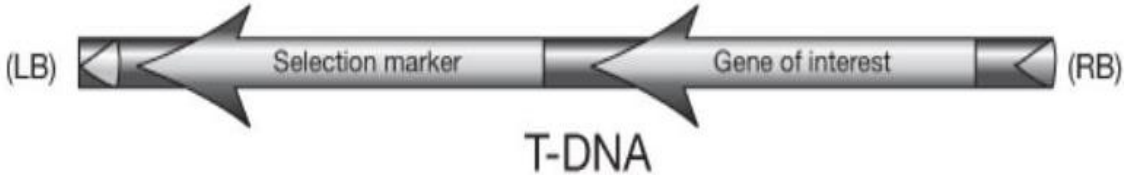

vir genes
(transfer functions)
origin of replication
Ti plasmid
T-DNA
25-bp direct repeats
Left border (LB)
Right border (RB)

TABLE 7.2. Commonly Used Bacterial Selectable Marker Genes

Antibiotic	Antibiotic Resistance Gene	Gene	Source Organism
Streptomycin/Spectinomycin	Aminoglycoside adenyl transferase gene	*aadA*	*E. coli*
kanamycin	Neomycin phospho transferase gene	*nptII* (*neo*)	*E. coli* Tn5
Chloramphenicol	Chloramphenicol acetyl transferase gene	*cat*	*E. coli* Tn5
Ampicillin	β-Lactamase	*bla*	*E. coli* Tn3
Tetracycline	Tetracycline/H^+antiporter	*tet*	*E. coli* Tn10

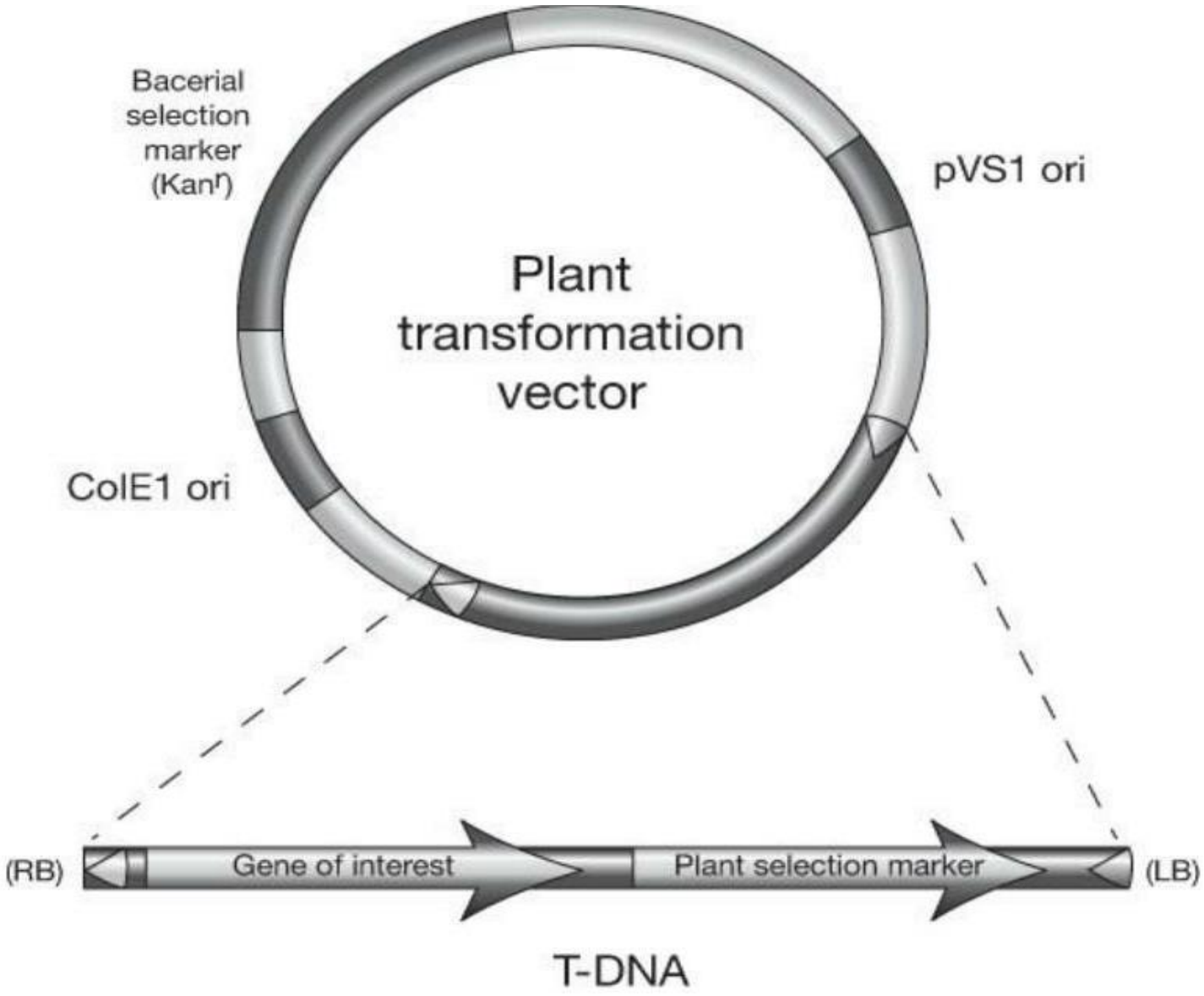
Typical component of transformation Vector

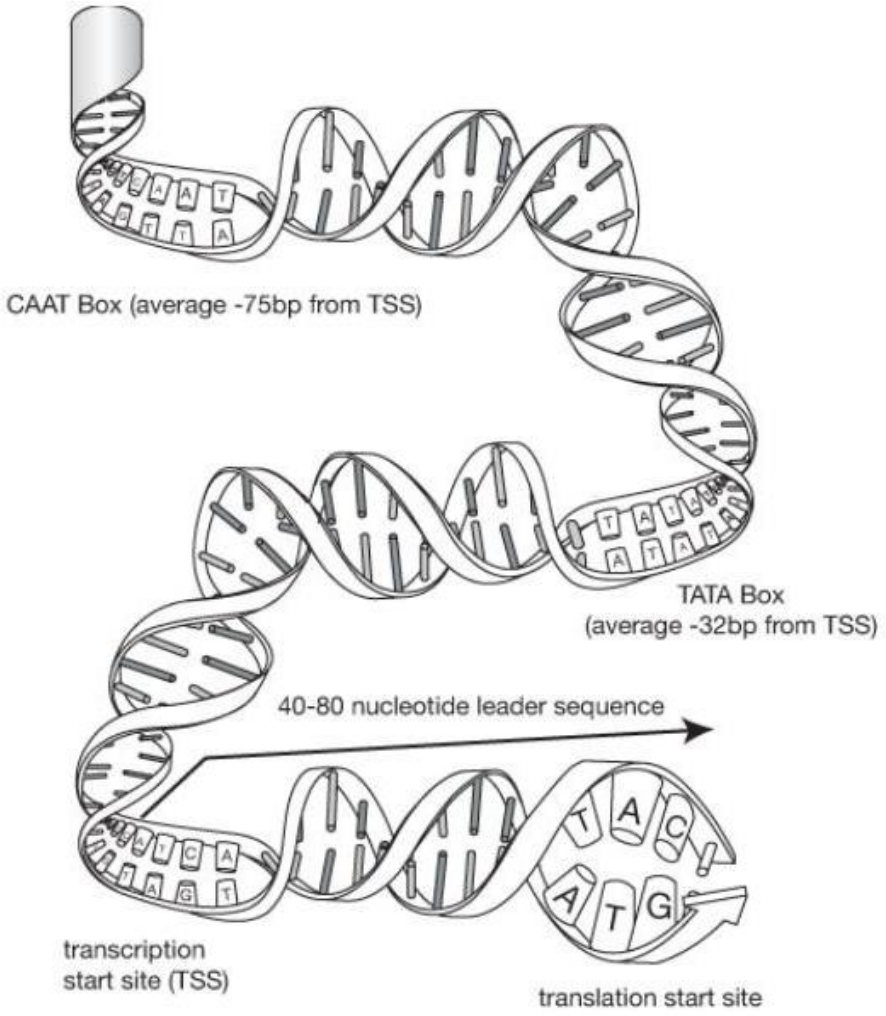


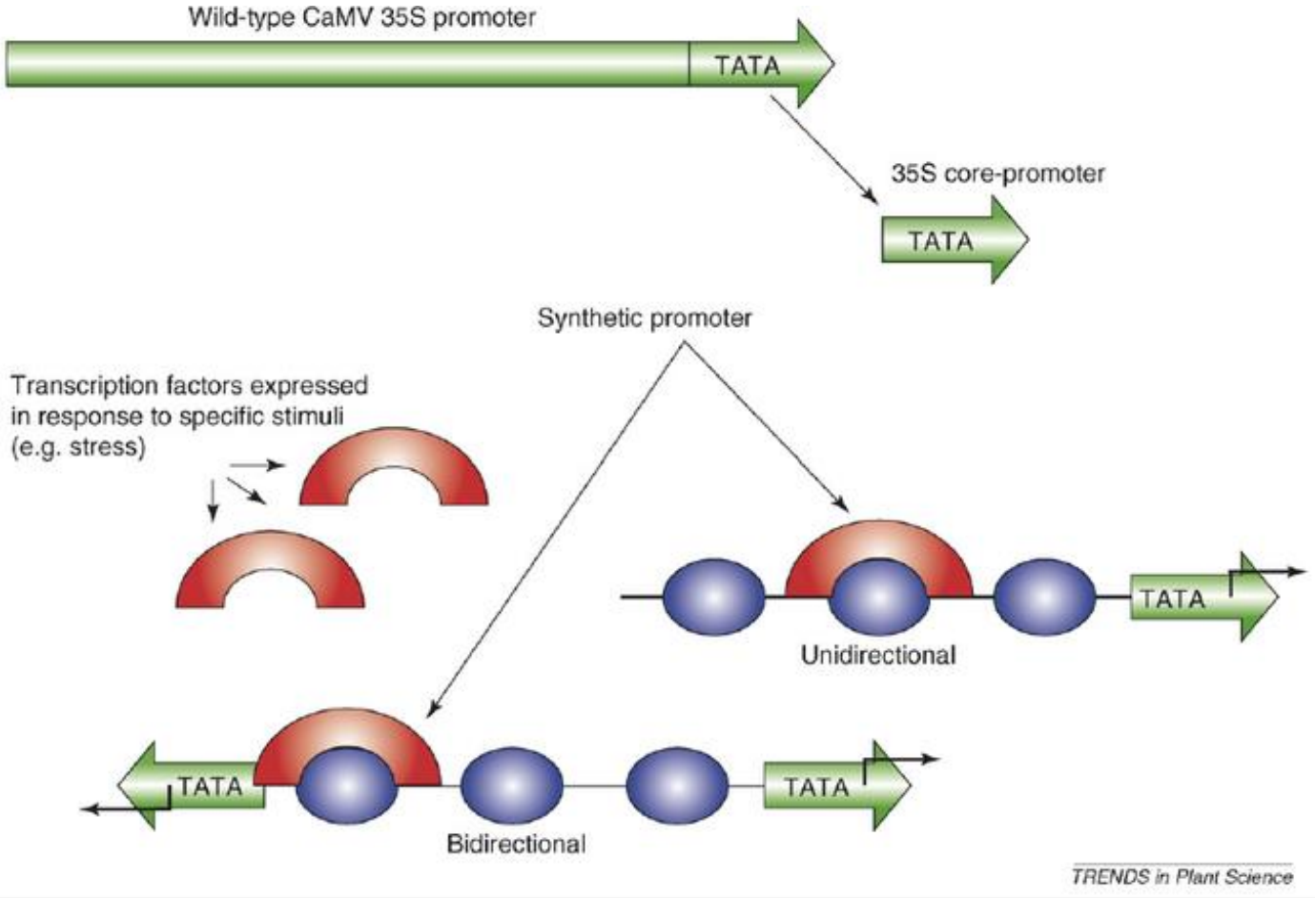
- Selectable marker cassette (with promoter and terminator)
- Gene of interest cassette (with promoter and terminator)
- Scorable marker cassette (with promoter and terminator)

What happens if the promoter is missing?

Is there ever a time when a promoterless construct is desirable?


Bacerial selection marker (Kanʳ)
pVS1 ori
Plant transformation vector
ColE1 ori
(RB)
Gene of interest
Plant selection marker
(LB)
T-DNA


CAAT Box (average -75bp from TSS)
TATA Box
(average -32bp from TSS)
40-80 nucleotide leader sequence
transcription
start site (TSS)
translation start site


Wild-type CaMV 35S promoter
TATA
35S core-promoter
TATA
Synthetic promoter
Transcription factors expressed
in response to specific stimuli
(e.g. stress)
TATA
Unidirectional
TATA
TATA
Bidirectional
TRENDS in Plant Science

Vector Preparation

- Restriction digestion
- Dephosphorylation
- Blunt-end creation (optional)
- Purification

Insert preparation

- Restriction digestion
- Blunt-end creation (optional)
- Purification

Ligation

- T4 DNA Ligase
- PEG
- Vector to insert ratio
- Reaction time and temperature
- Purification / heat-inactivation (optional)

Transformation

- Transformation efficiencies
- Competent cell choices
- Chemical and electrocompetent cells

Colony screening

- Blue / white colonies
- Positive selection
- Restriction digestion
- Colony PCR
- Sanger sequencing


Thank You