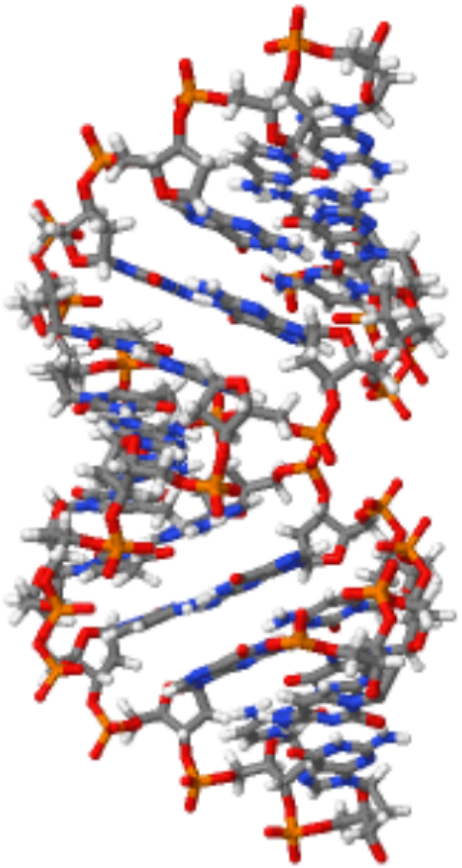
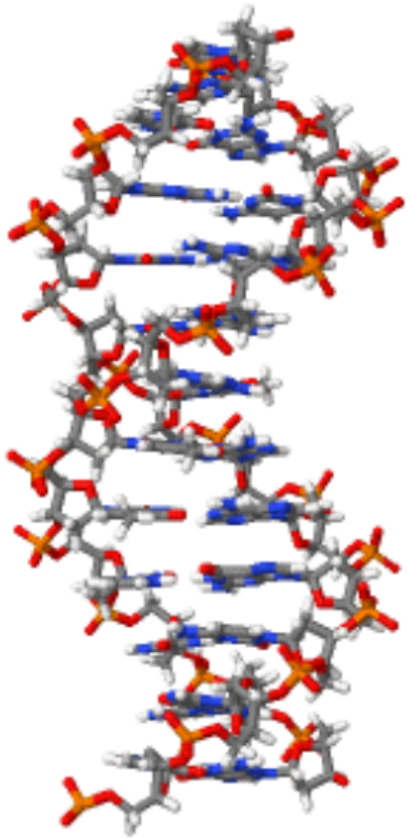
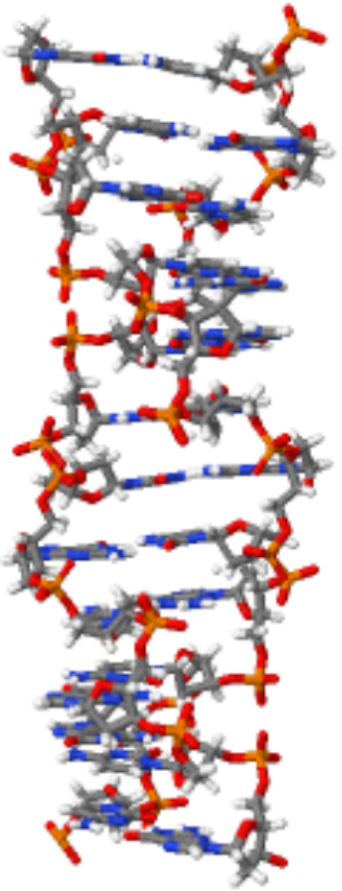
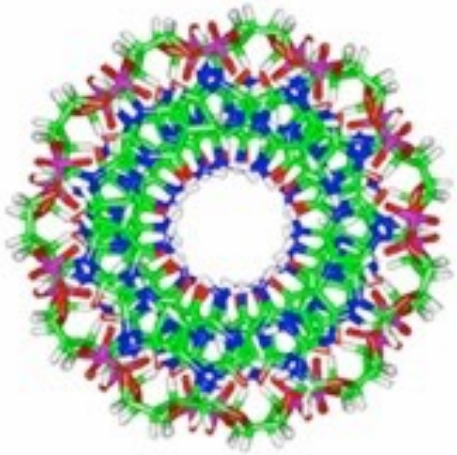
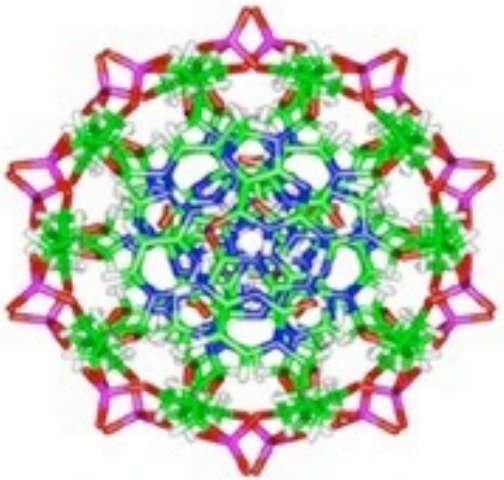
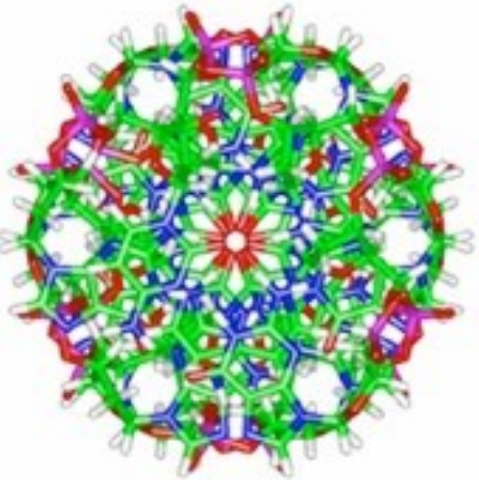
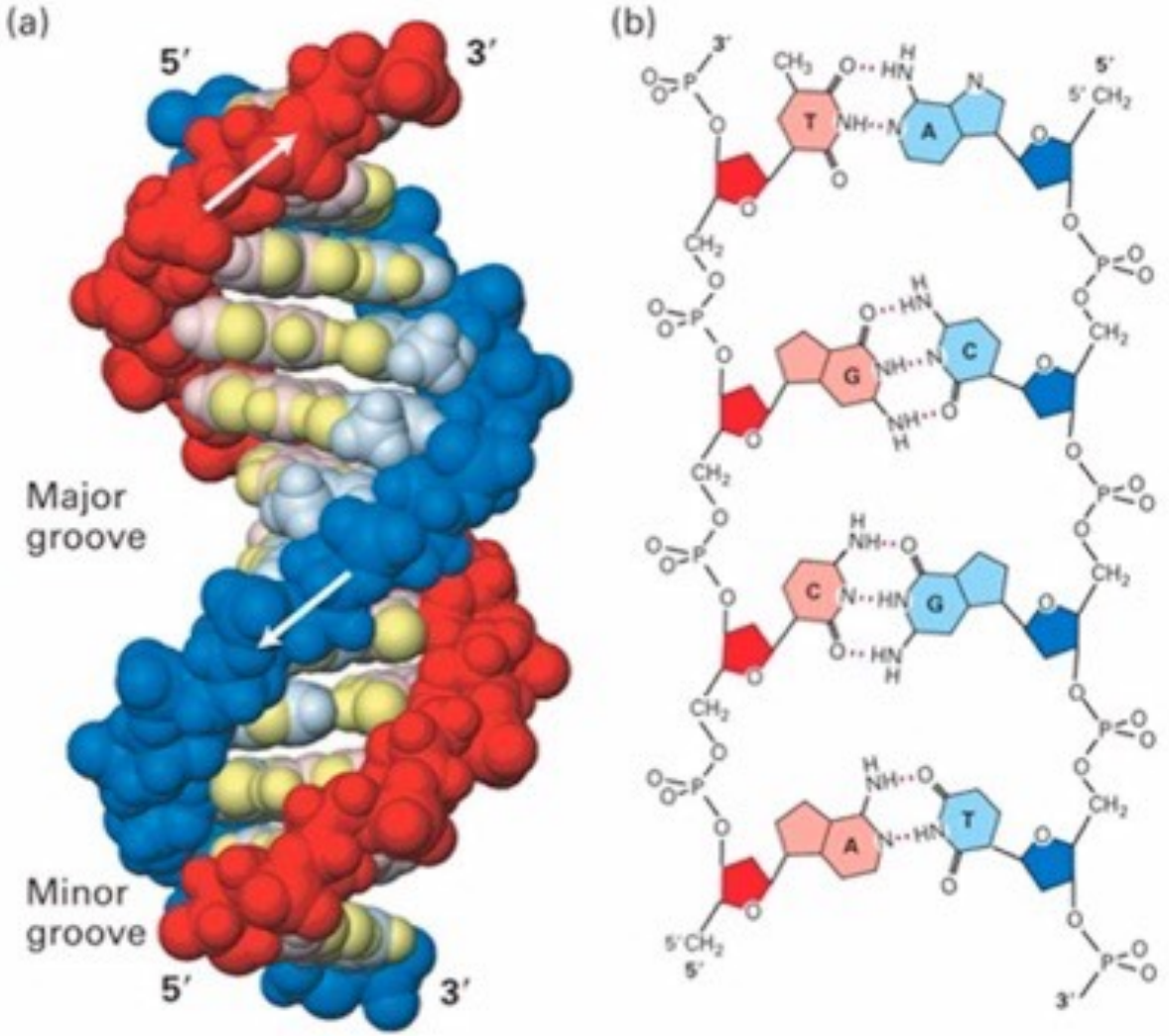
SUMMER INSTITUTE - ONLINE MODALITY CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
June 26	27	28	29	30	31	July 02
	9:00-10:00am Virtual Program Orientation for Summer Institute Online Modality					
July 03	04	05	06	07	08	09
	Holiday (Independence Day)	8:30-10:00am -Welcome Reception and Buddy Meet & Greet Event	Free Day	Classes begin! 8:30-11am: BIOL4905 INTRODUCTION 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 DNA PREPARATION 8-10:20pm: Afternoon course	
10	11	12	13	14	15	16
	8:30-11am:BIOL4905 PROTEOMICS I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS III 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 RNA PREPARATION 8-10:20pm: Afternoon course	Virtual Independence Day Activity	
17	18	19	20	21	22	23
	8:30-11am:BIOL4905 qPCR / ROBOTS 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 DNA Sequence Analysis 8-10:20pm: Afternoon course	Midterm Break	8:30-11am:BIOL4905 Next Gen. Sequencing 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Automated Microscopy /AFM	
24	25	26	27	28	29	30
	8:30-11am:BIOL4905 Microarray I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Microarray II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Nanostring 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Flow Cytometry 8-10:20pm: Afternoon course	FINALS	
31	August 01	02	03			
	9:00-10:00am: Closing Reception		Grades available in PAWS			

Legend:
Orange: Courses Blue: Activities

Capillary DNA Sequencers
(ABI/Life Technologies) Model 3500xl

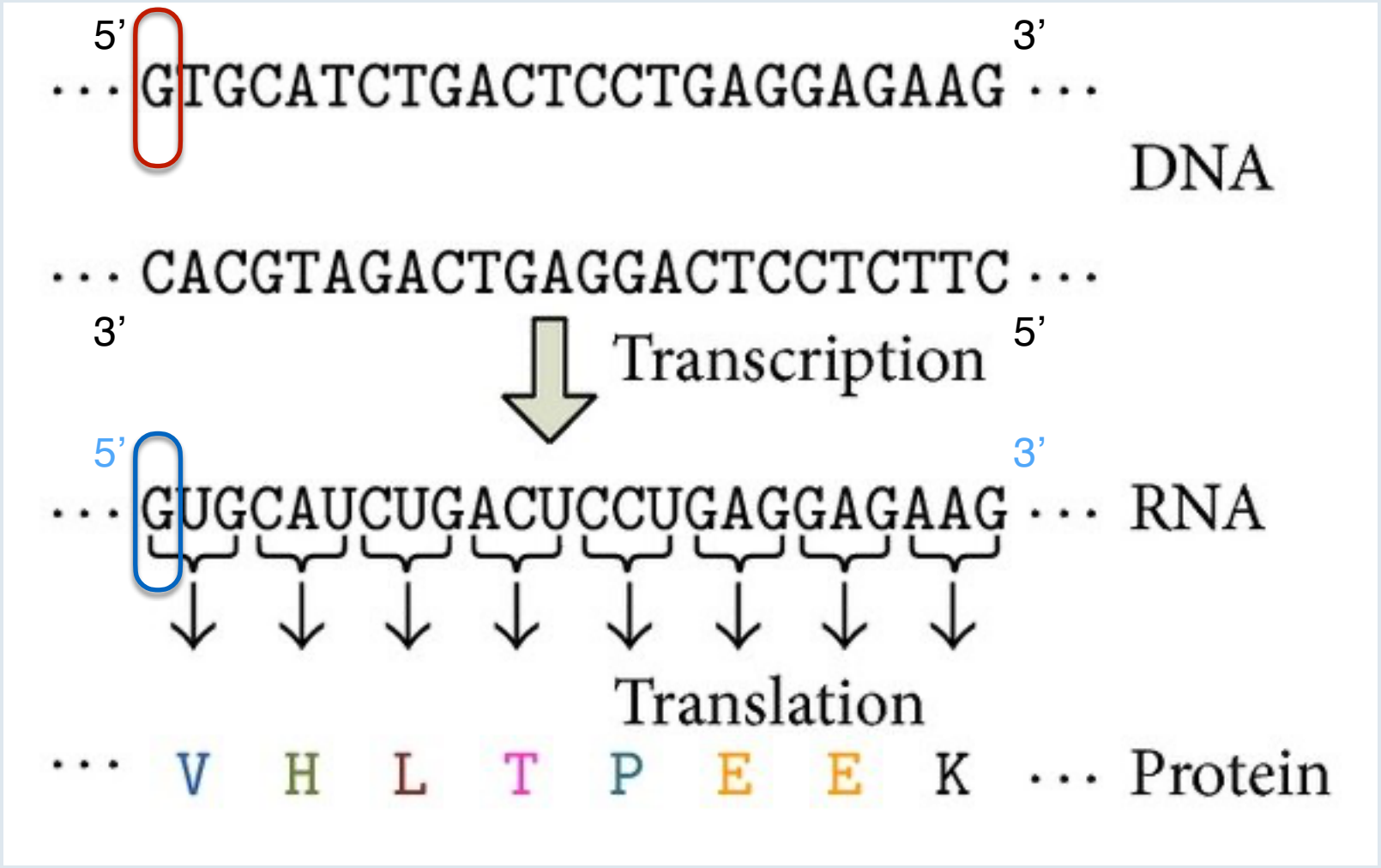


B form DNA

2.0 nM dia (20 Á)
0.36 nM (3.6 Á)
between bases
~10 bases per turn
antiparallel strands
bases perpendicular
to axis

FedEx Express

FedEx


5’
3’
···GTGCATCTGACTCCTGAGGAGAAG···
DNA
···CACGTAGACTGAGGACTCCTCTTC···
3’
5’
Transcription
5’
3’
···GUGCAUCUGACUCCUGAGGAGAAG··· RNA
Translation
··· V H L T P E E K ··· Protein

GSU Biology Core Facility

Supporting Life Sciences at GSU

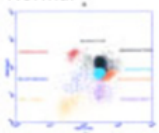
http://biotech.gsu.edu/core_facility/index.html

Georgia State University

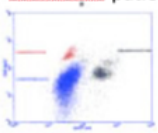
Flow Cytometry

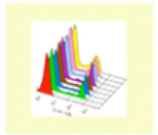
Profiling Cells

Normal



Leukaemia patient

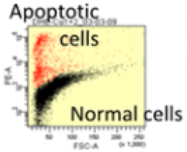




See effects of different drugs on Cell cycle

Apoptotic cells

Normal cells

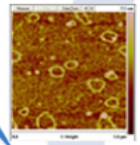


Apoptosis -programmed cell death

Atomic Force Microscopy

Imaging at the Ångström level

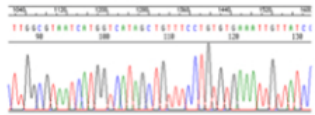
Protein structure analysis



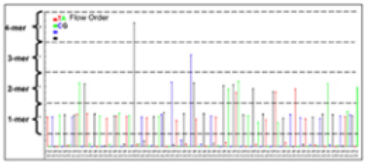
DNA Sequence Analysis:

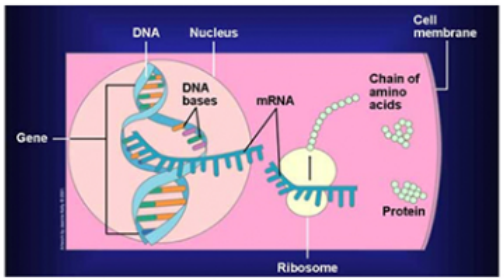
Profiling DNA

Sanger Sequencing – >800 base pairs/run



High Throughput Genomic Sequencing – 100,000,000,000 base pairs/run





DNA Replication

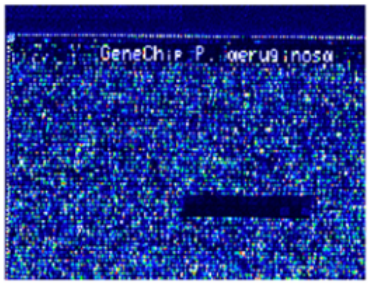
RNA Expression

Protein Expression

Cellular Functions

Microarray:Analysis

Profiling mRNA

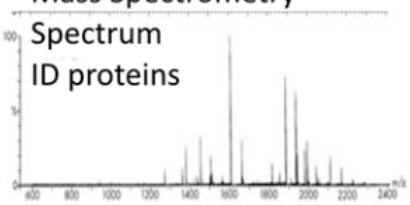


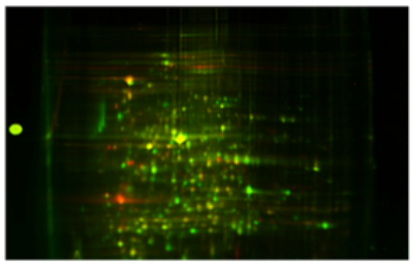
Colour of pin-point dots demonstrates the presence / absence of gene sequences

Mass Spectrometry

Spectrum

ID proteins





Proteomics

Profiling Proteins

2D Protein gel

Protein separation using

Electric charge and molecular weight

GSU Biology Core Facility

Supporting Life Sciences at GSU

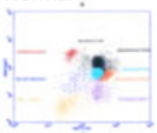
http://biotech.gsu.edu/core_facility/index.html

Georgia State University

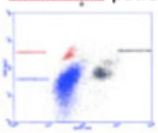
Flow Cytometry

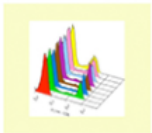
Profiling Cells

Normal



Leukaemia patient

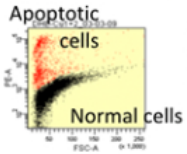




See effects of different drugs on Cell cycle

Apoptotic cells

Normal cells

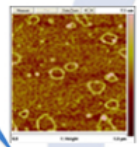


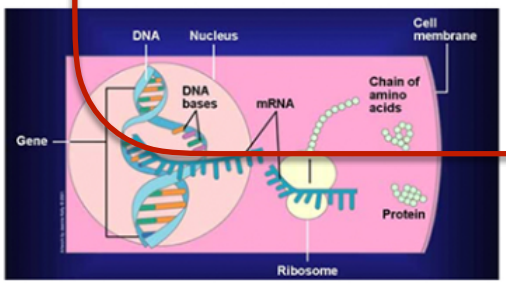
Apoptosis -programmed cell death

Atomic Force Microscopy

Imaging at the Ångström level

Protein structure analysis





DNA

Replication

RNA

Expression

Protein

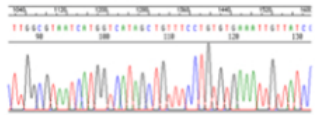
Expression

Cellular Functions

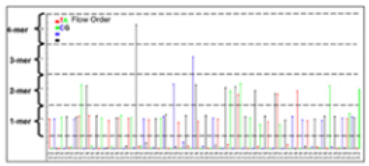
DNA Sequence Analysis:

Profiling DNA

Sanger Sequencing – >800 base pairs/run

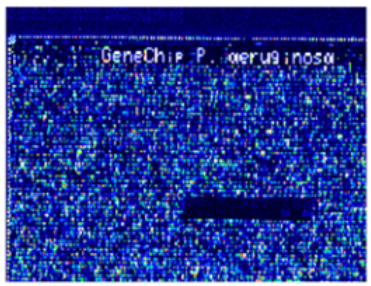


High Throughput Genomic Sequencing – 100,000,000,000 base pairs/run



Microarray:Analysis

Profiling mRNA

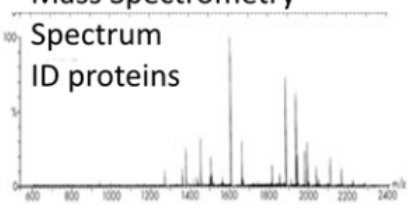


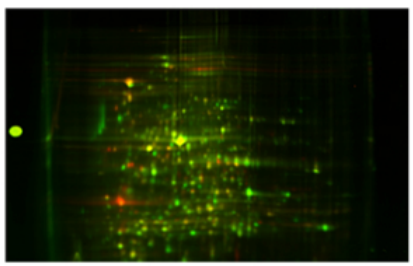
Colour of pin-point dots demonstrates the presence / absence of gene sequences

Mass Spectrometry

Spectrum

ID proteins





Proteomics

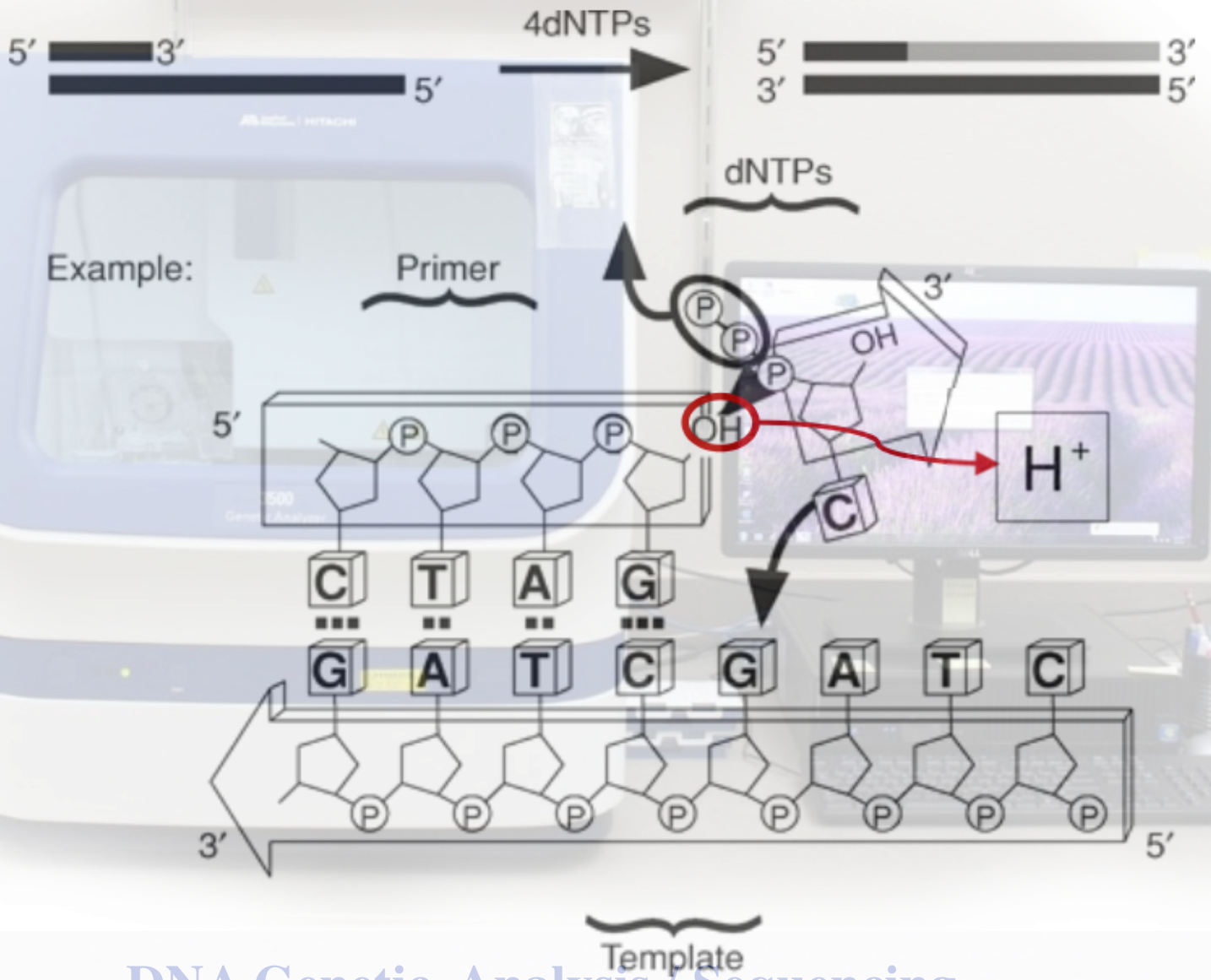
Profiling Proteins

2D Protein gel

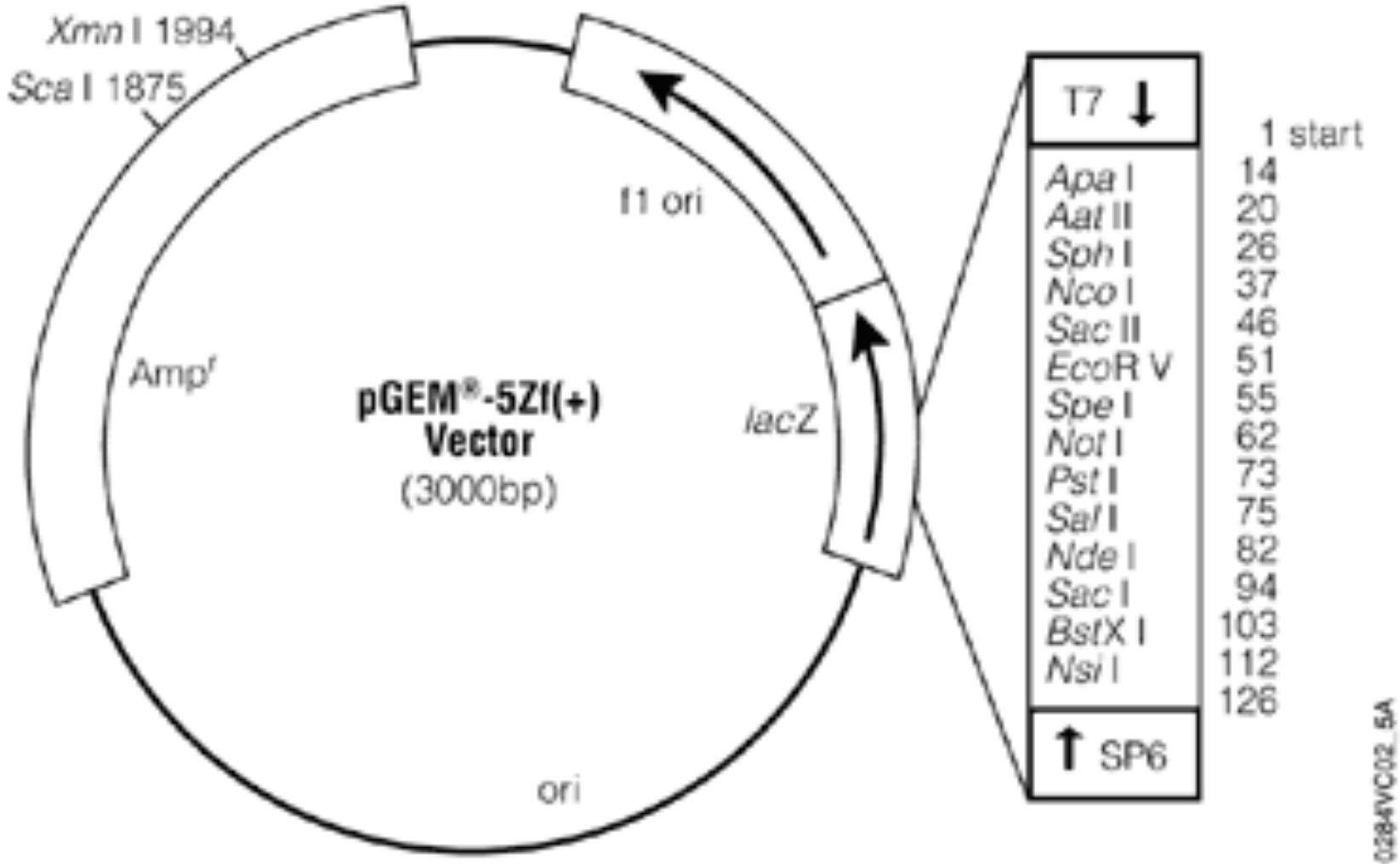
Protein separation using

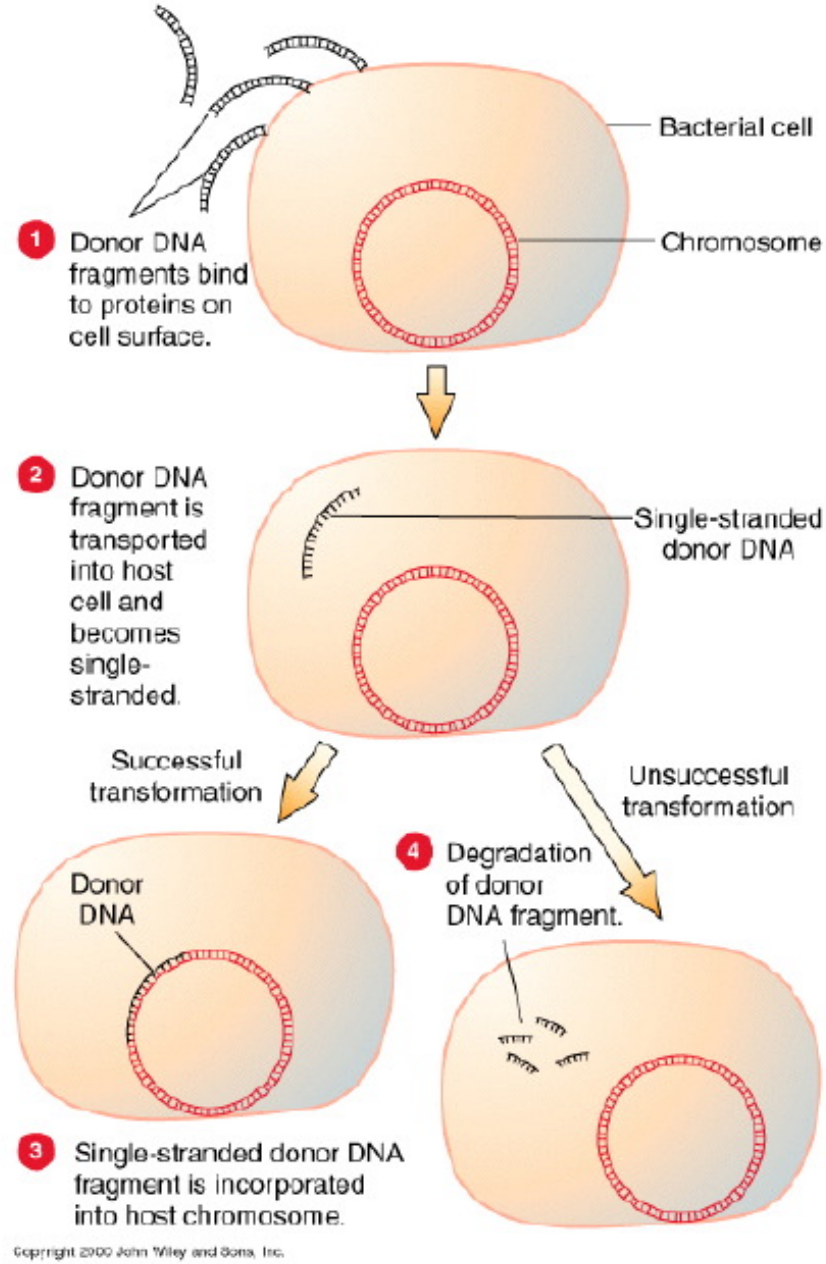
Electric charge and molecular weight

Simple, Natural Chemistry



DNA Genetic Analysis / Sequencing
(ABI/ *Life technologies*) Model 3500xl


Xmn I 1994
Sca I 1875
f1 ori
Amp^r
pGEM®-5Zf(+)
Vector
(3000bp)
lacZ
ori
T7
Apa I
Aat II
Sph I
Nco I
Sac II
EcoR V
Spe I
Not I
Pst I
Sal I
Nde I
Sac I
BstX I
Nsi I
SP6
1 start
14
20
26
37
46
51
55
62
73
75
82
94
103
112
126
0284VC02_5A

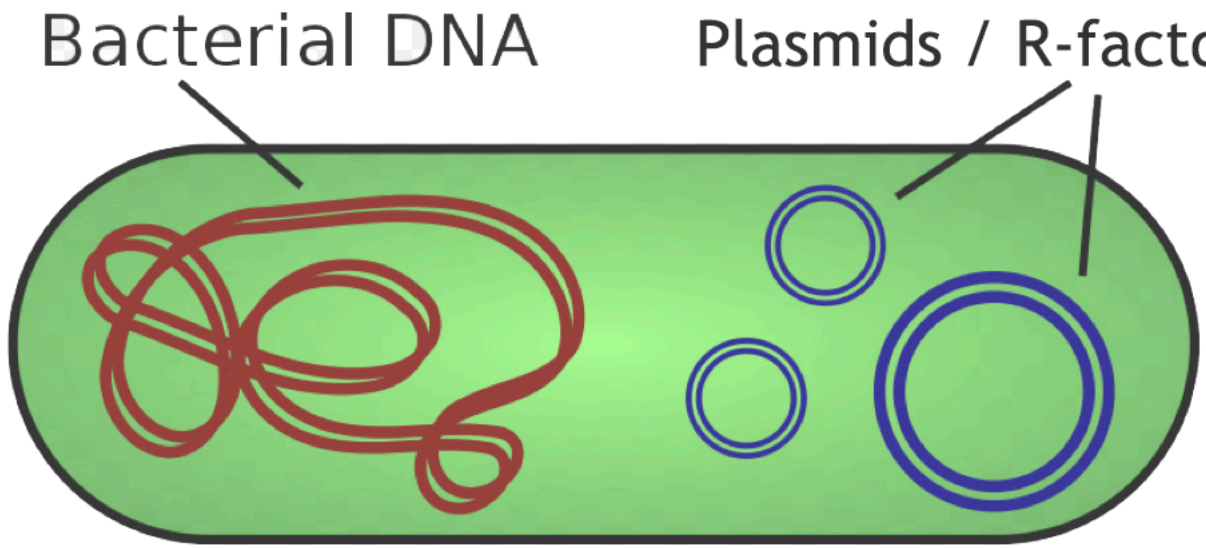


There are three basic fates of DNA as it enters the bacterial cell.

1) It is degraded rapidly due to host defense mechanisms.

2) It is integrated into the host chromosome by recombination (homologous or non-homologous).

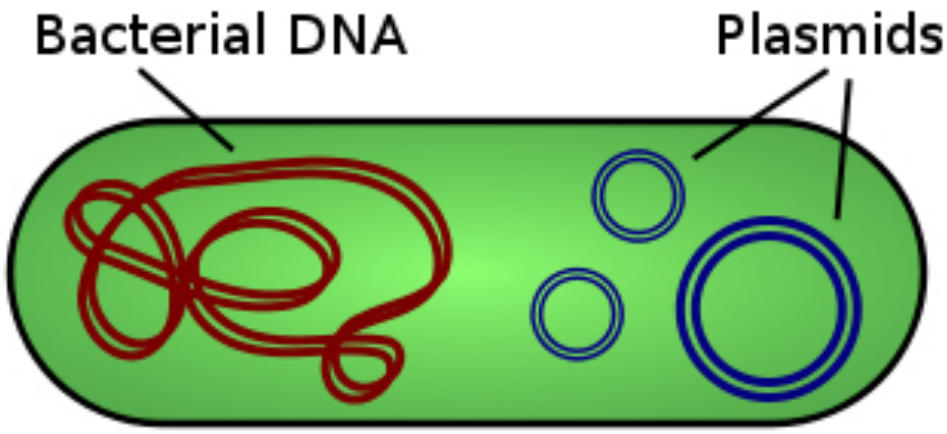
3) The DNA is able to circularize and replicate **independently** (autonomously) from the host chromosome (i.e. it contains an origin of replication that is recognized by host replicating enzymes).

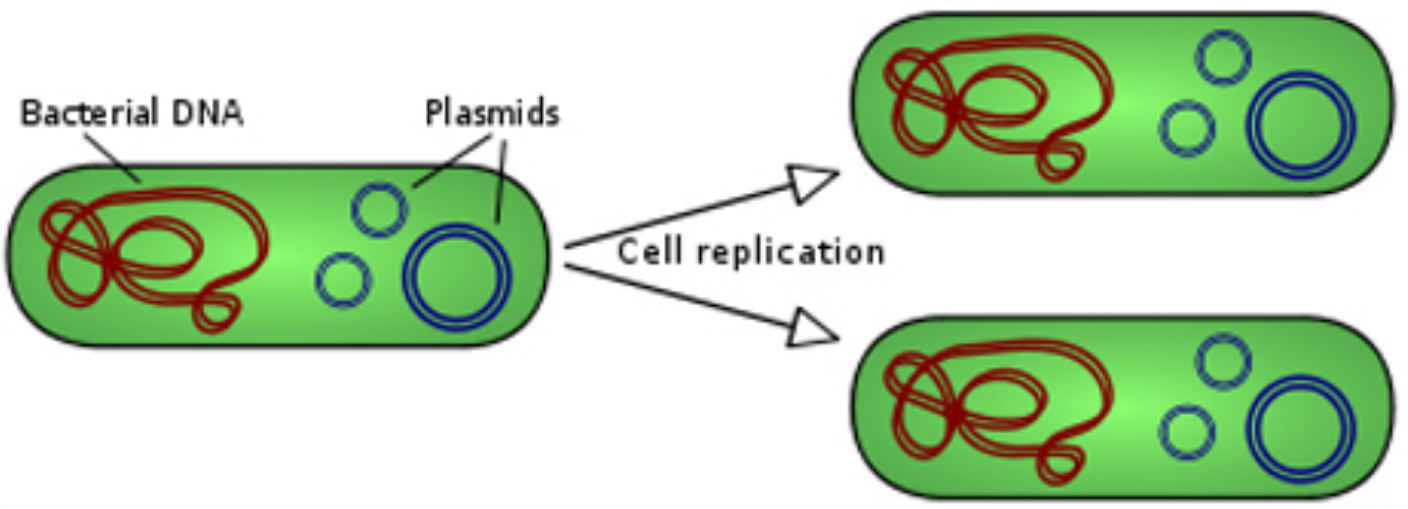


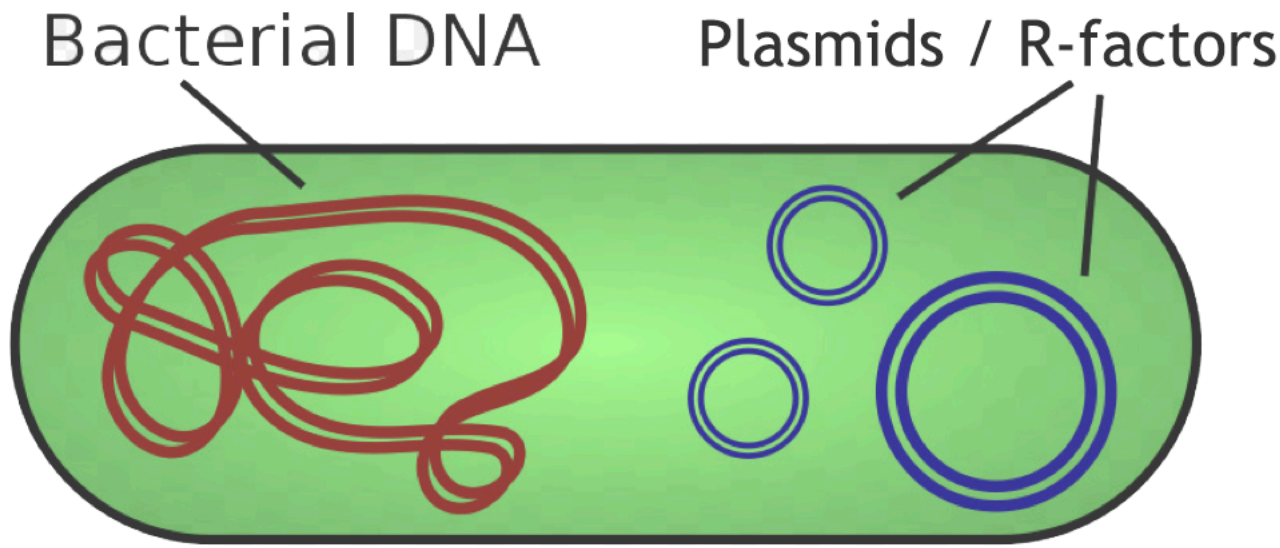
Plasmids / R-factors: Extrachromosomal, self replicating or autogenous replicating, covalently closed, circular pieces of dsDNA. They can, sometimes be integrated into the host chromosome, and if so they are often called and **episome**.

Plasmids of 3,000 - 5,000 **bp**, often have a high copy number (15 - 100 copies per cell).
Plasmids of 4,000 - 300,000 bp (300 **kbp**), are as common in nature, but less highly copied per cell(one or two per cell) and (due to these factors) are less easily manipulatable.

Conjugative plasmids invariably contain *tra* and *mob* genes, which are necessary to promote cell to cell interaction and and also to promote movement of the DNA through the "**conjugative bridge**".


Bacterial DNA
Plasmids

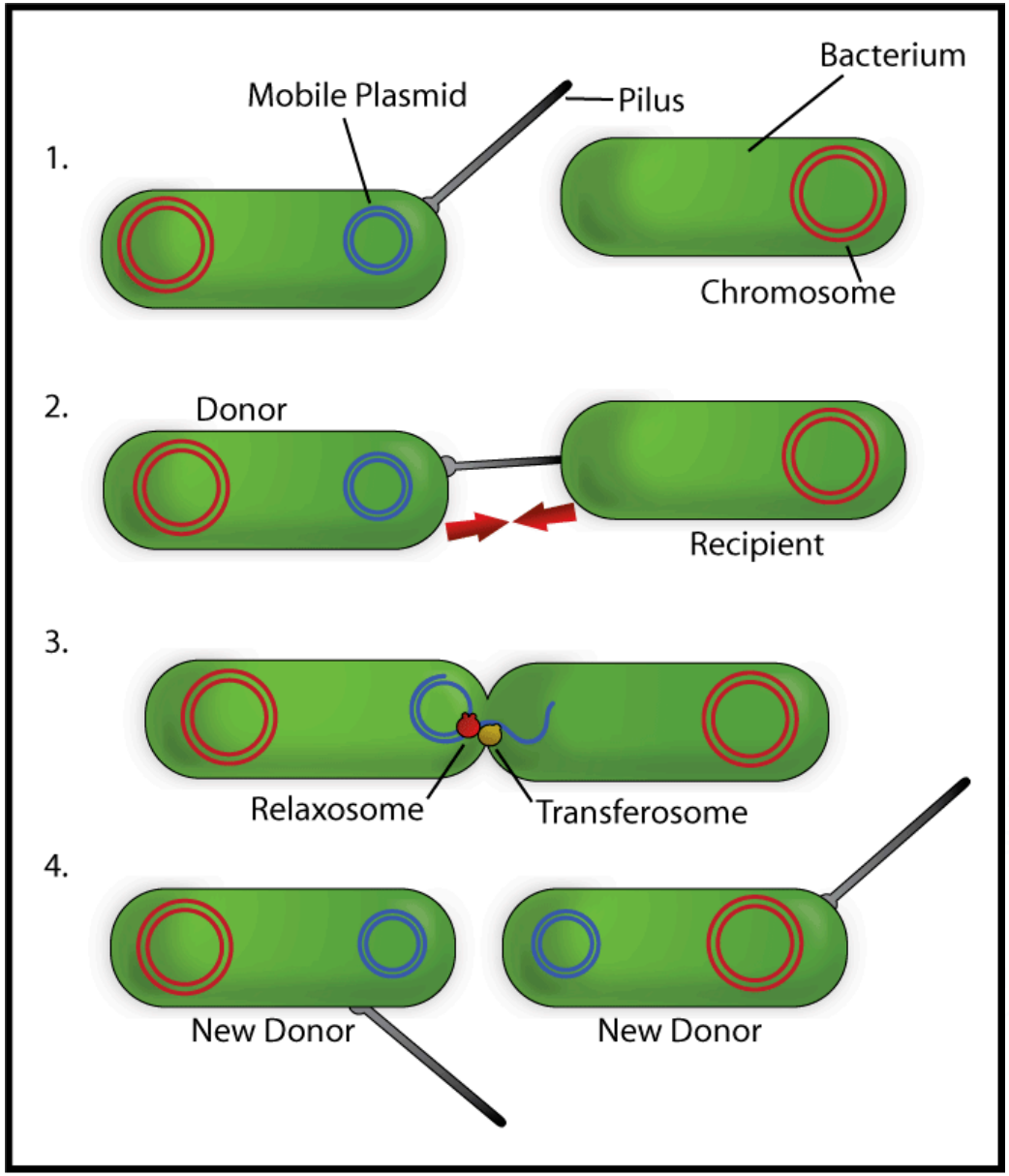

Bacterial DNA
Plasmids
Cell replication

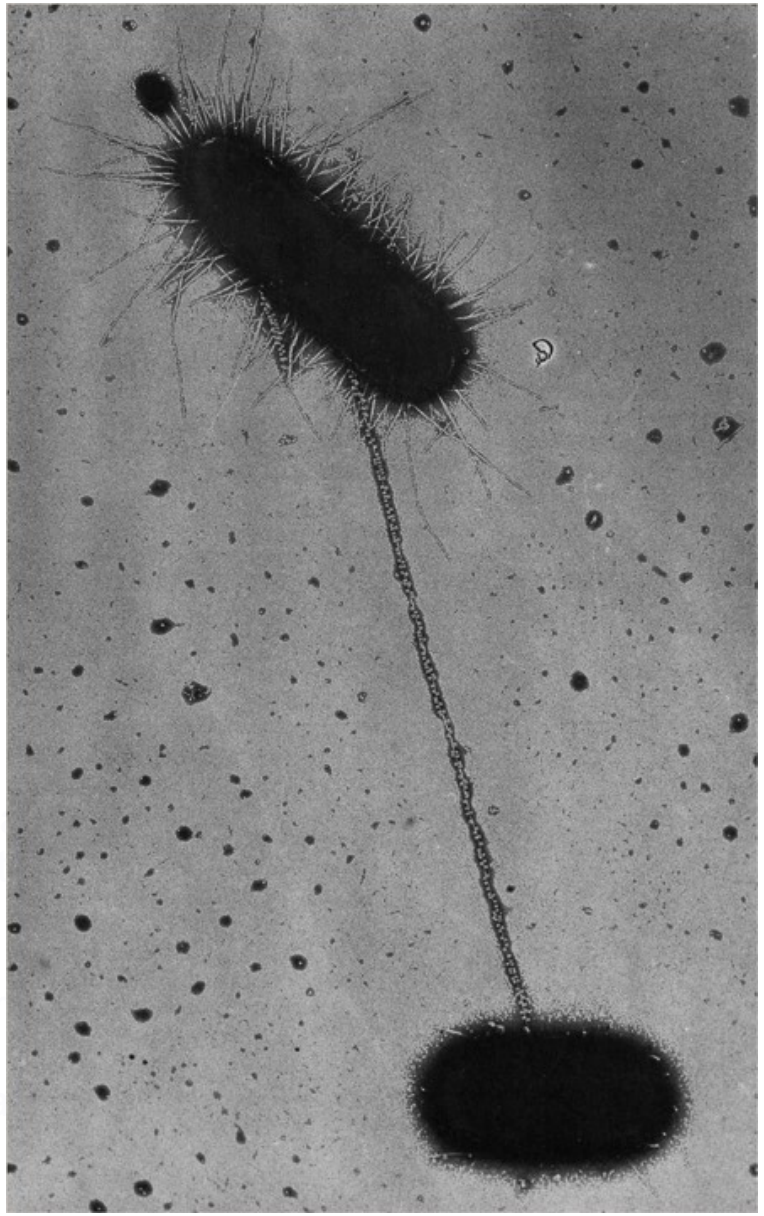


Plasmids / R-factors: Extrachromosomal, self replicating or autogenous replicating, covalently closed, circular pieces of dsDNA. They can, sometimes be integrated into the host chromosome, and if so they are often called and **episome**.

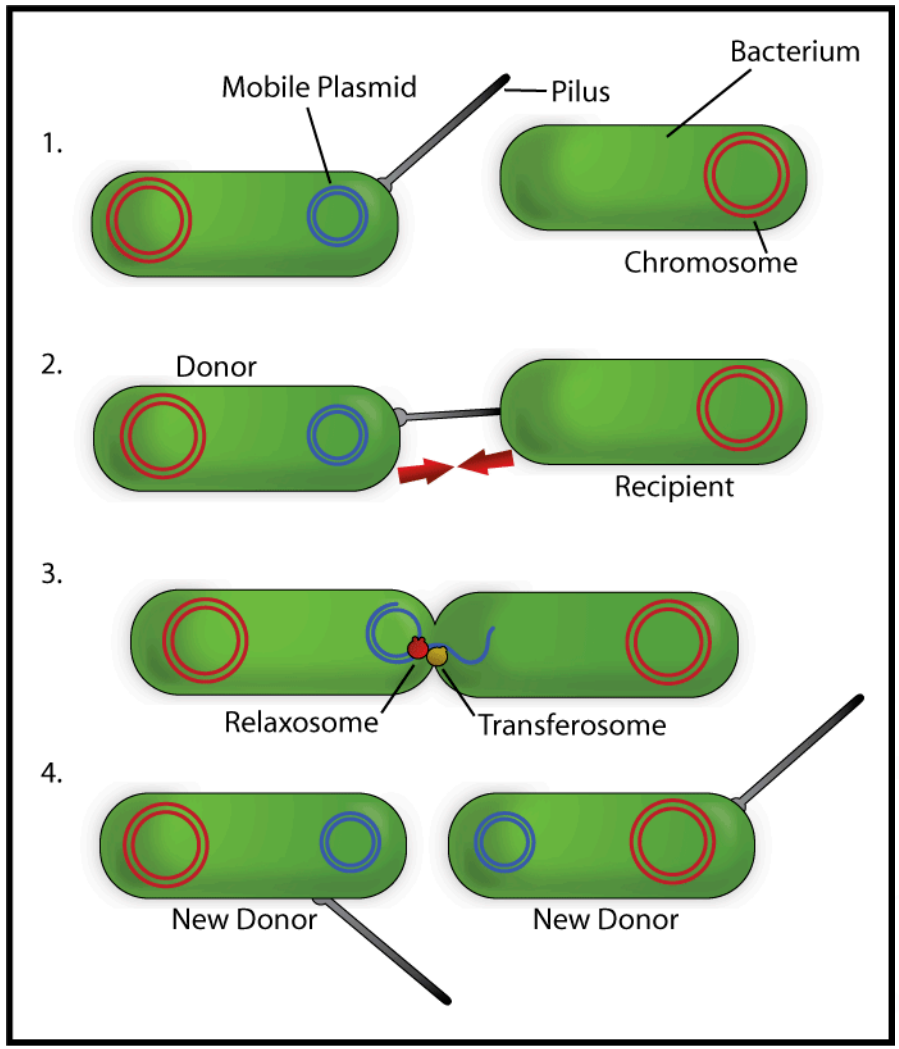
Plasmids of 3,000 - 5,000 **bp**, often have a high copy number (15 - 100 copies per cell).
Plasmids of 4,000 - 300,000 bp (300 **kbp**), are as common in nature, but less highly copied per cell(one or two per cell) and (due to these factors) are less easily manipulatable.

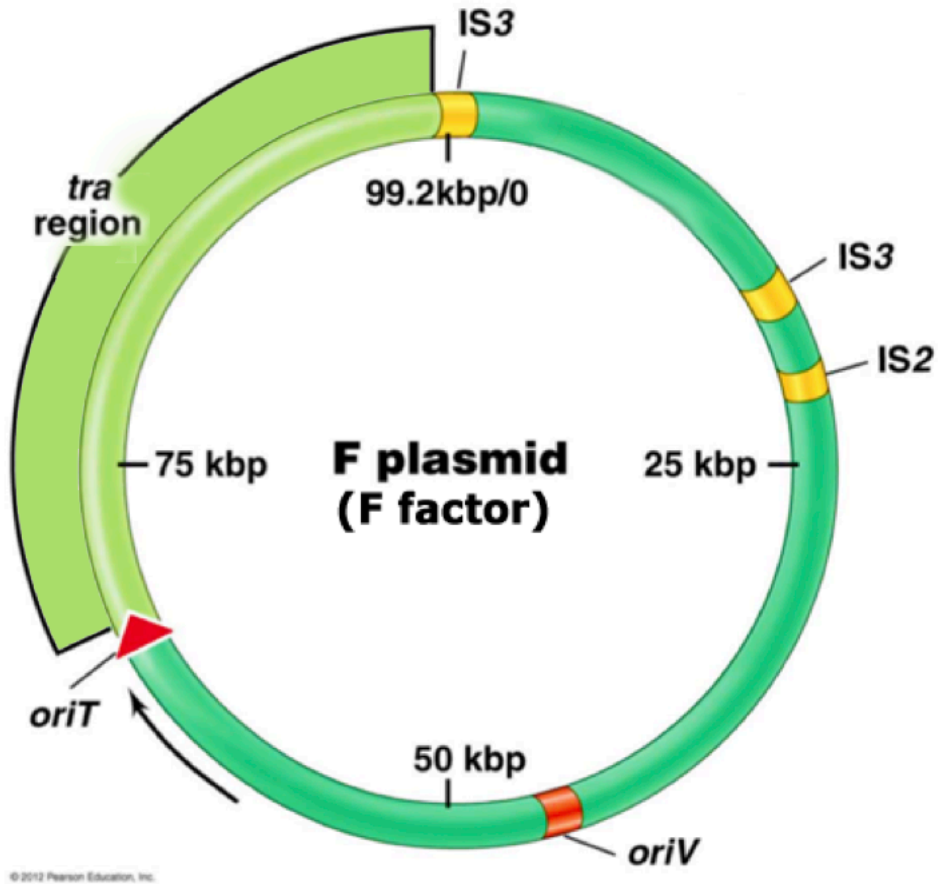
Conjugative plasmids invariably contain *tra* and *mob* genes, which are necessary to promote cell to cell interaction and and also to promote movement of the DNA through the "**conjugative bridge**".





Conjugative plasmids invariably contain *tra* and *mob* genes, which are necessary to promote cell to cell interaction and and also to promote movement of the DNA through the "**conjugative bridge**".

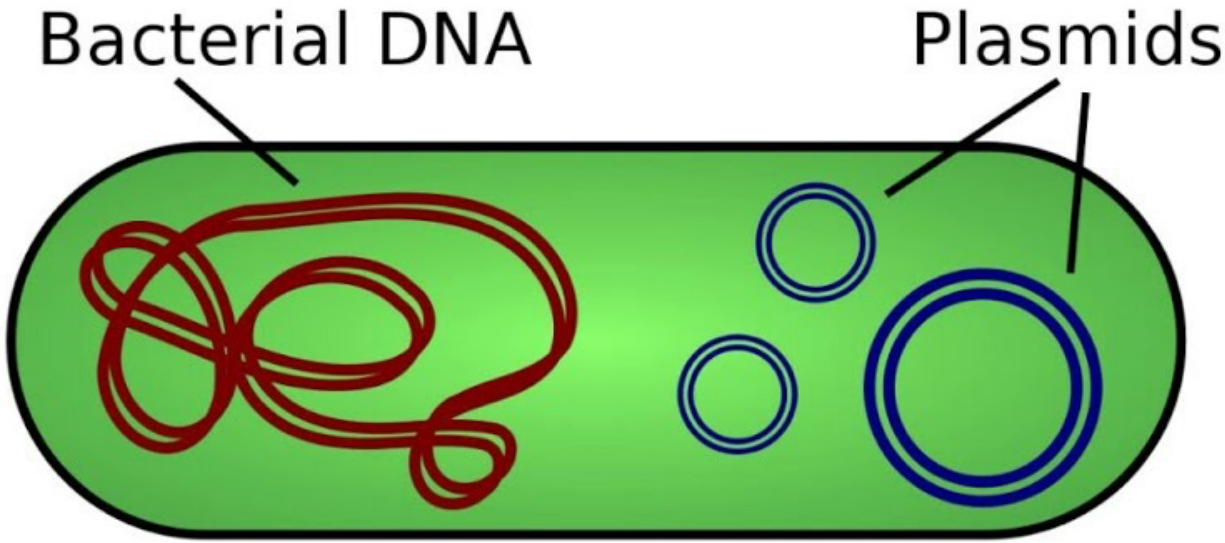




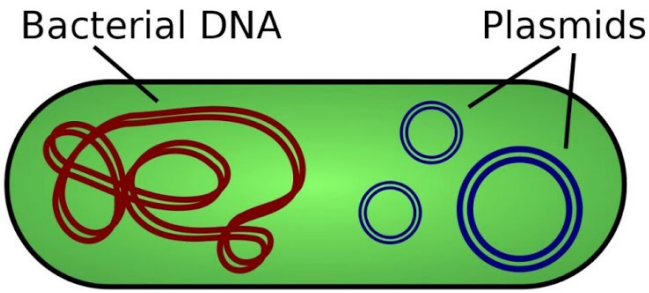
Conjugative plasmids invariably contain *tra* and *mob* genes, which are necessary to promote cell to cell interaction and and also to promote movement of the DNA through the "**conjugative bridge**".

Plasmids are <u>not</u> named and grouped by size, however, or even by DNA homology, but by.....their **“ incompatibility ” or INABILITY TO CO-EXIST**

eg. **IncP** plasmids have a broad-host range and include the **IncQ** or **IncP4** group of plasmids.

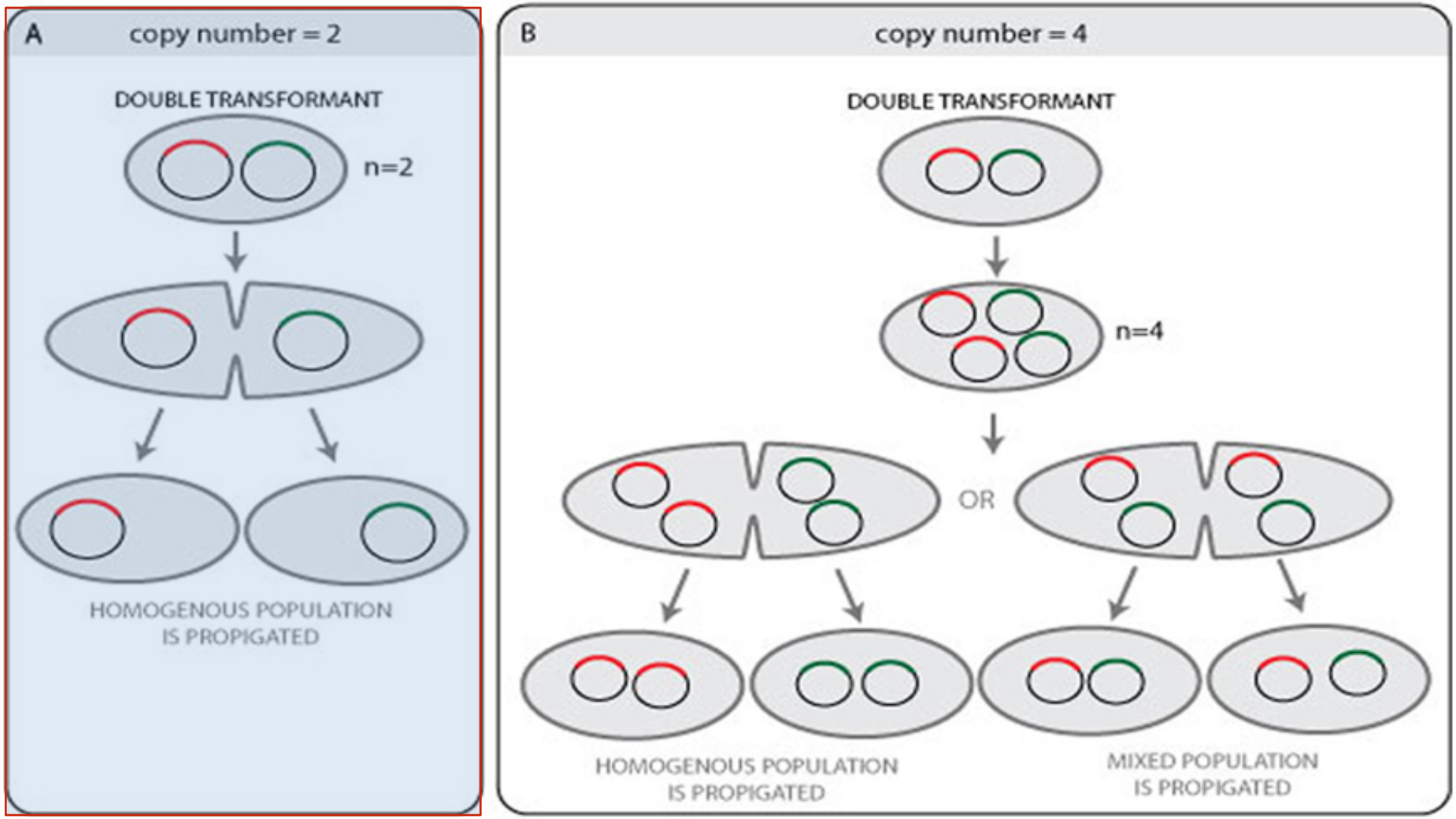


Incompatibility



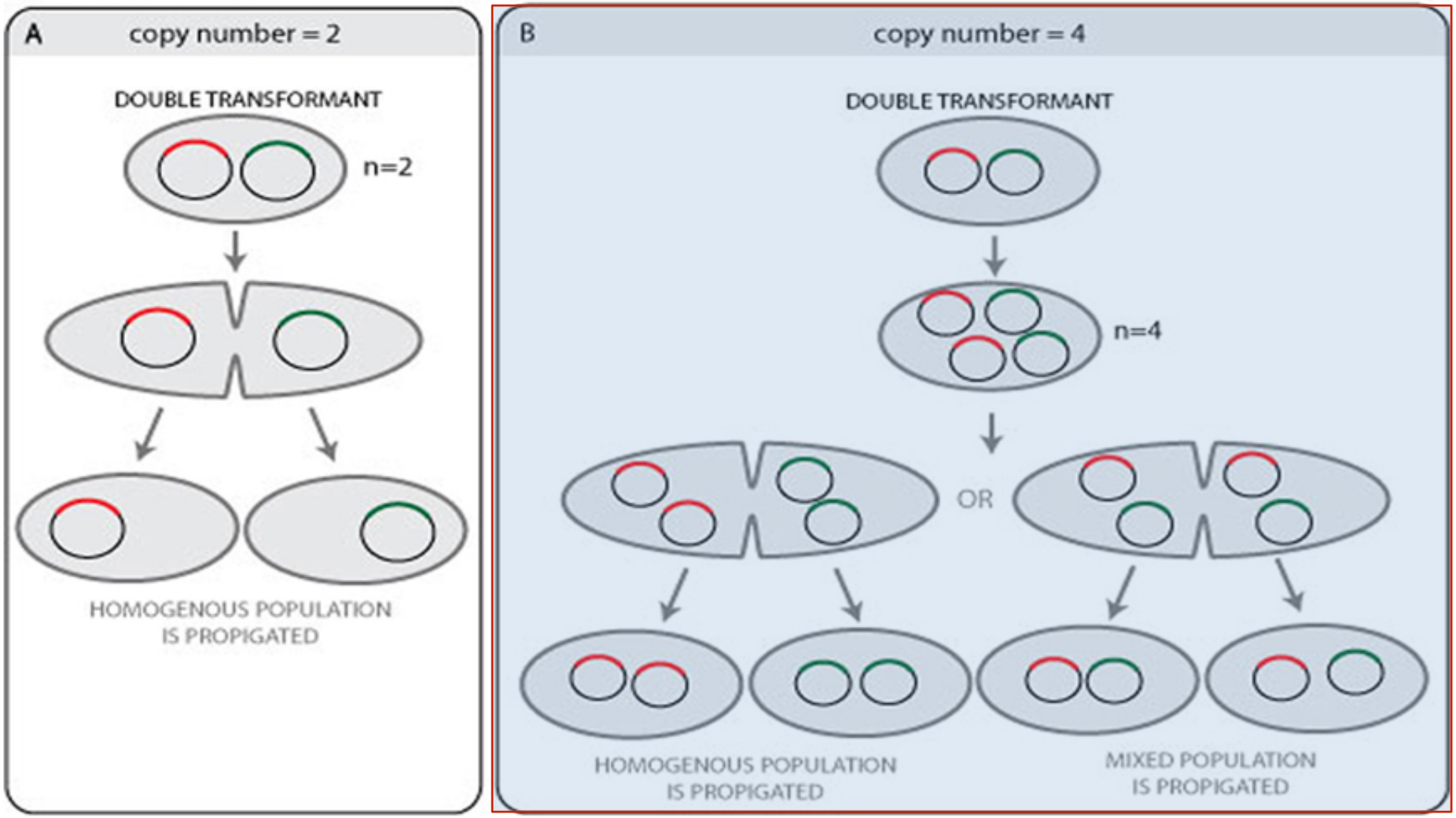
Incompatibility occurs in any number of ways, but normally affects either the initiation of replication or the control of the attachment of plasmids to the bacterial membrane (which, for some, or the transfer and/or mobilization of plasmids through the pilus during congugation. In effect, potentially any shared characteristic that is required for efficient segregation of low copy number plasmids into the two daughter cells. Thus, at its core, incompatibility can be anything that provides an element of “competition” -gives rise to selection of one “incompatible” plasmid over another

Figure 1



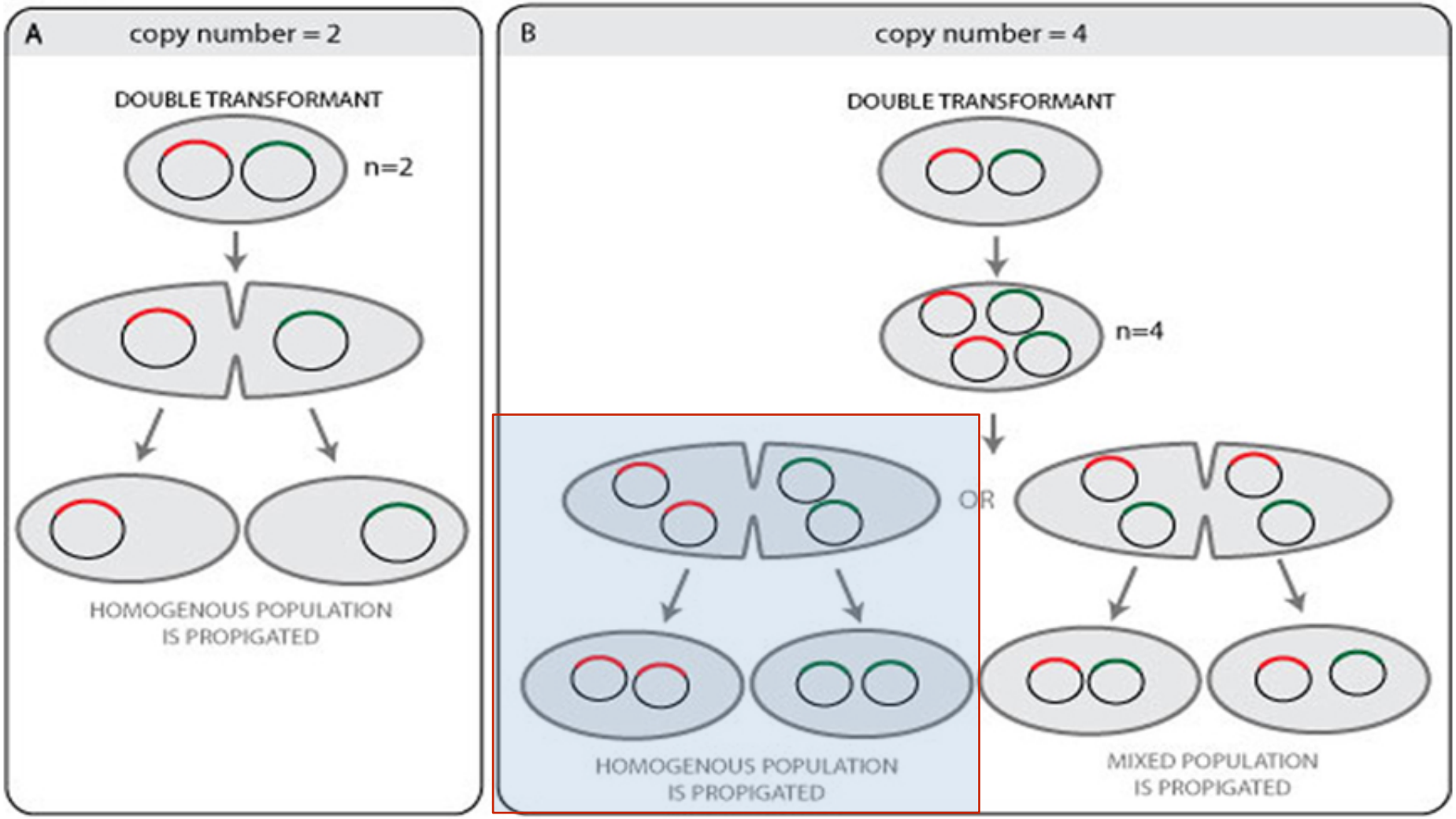
Two incompatible plasmid clones will have small differences that cause one to have a faster replication rate, or increased toxicity, over the other.
This is said to cause the plasmids to be replicated assymetrically, contributing to the eventual loss of one of the plasmids.

Figure 1

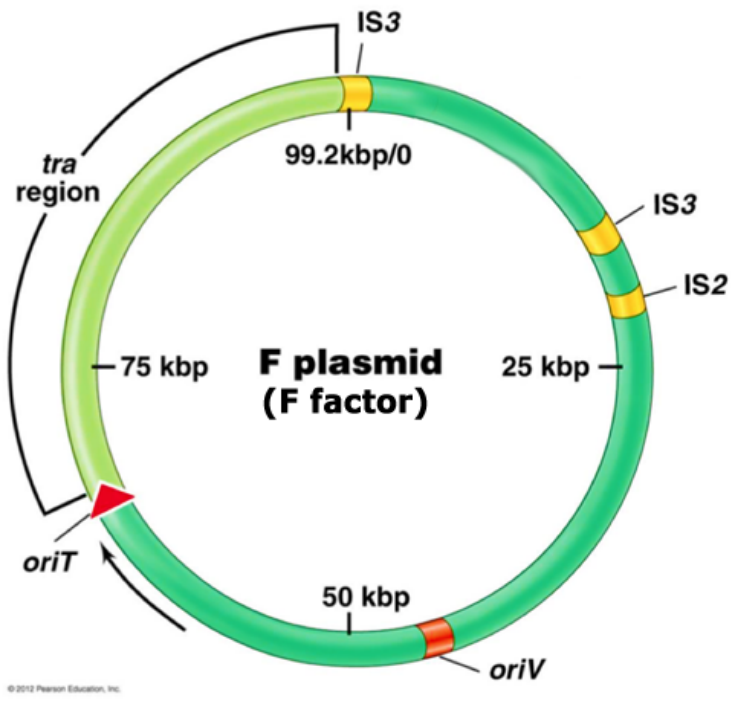


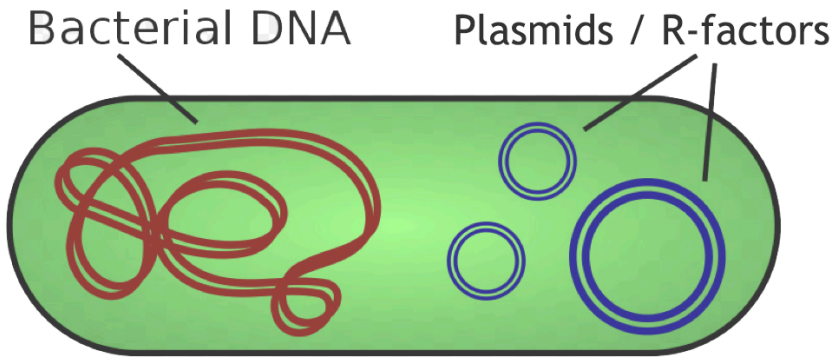
Two incompatible plasmid clones will have small differences that cause one to have a faster replication rate, or increased toxicity, over the other.
This is said to cause the plasmids to be replicated assymetrically, contributing to the eventual loss of one of the plasmids.

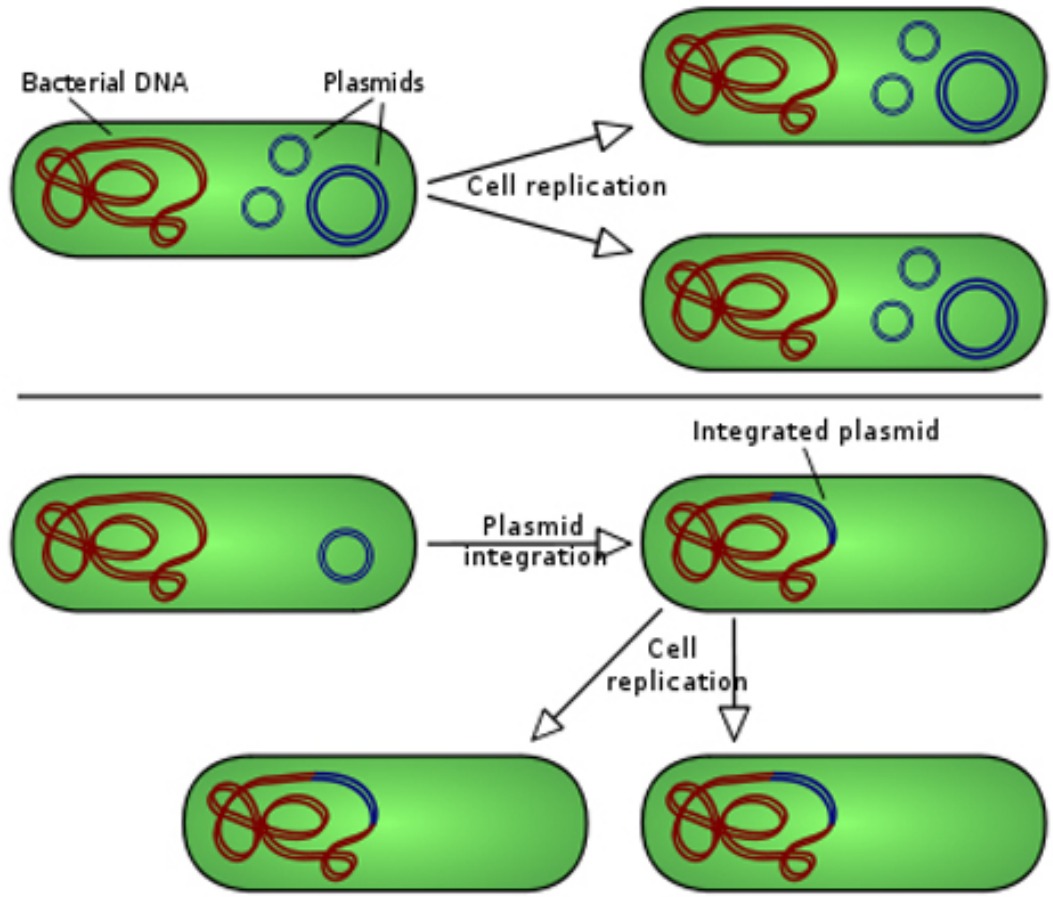
Figure 1



Two incompatible plasmid clones will have small differences that cause one to have a faster replication rate, or increased toxicity, over the other.
This is said to cause the plasmids to be replicated assymetrically, contributing to the eventual loss of one of the plasmids.

Incompatibility Group	Plasmids
FI	F, R386
	R1
	Col B-K99, Col B-K166
	R124
	R62, R64, R483 (at least 5 subgroups)
	R391
	R46
	R724
	RP4, RK2
	RSF1010
	R401
	R388, S-a


Bacterial DNA
Plasmids / R-factors


Bacterial DNA
Plasmids
Cell replication
Integrated plasmid
Plasmid integration
Cell replication

Ann. Rev. Microbiol. 1987. 41:77–101

INCOMPATIBILITY GROUP P PLASMIDS: Genetics, Evolution, and Use in Genetic Manipulation

Christopher M. Thomas and Christopher A. Smith

Department of Genetics, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, England

---

CONTENTS

Plasmid	Size (kb)	IncP-1 subgroup	Resistances	Resistance genes	Detected transposons	Reference/accession No.
pAKD1	58,246	IncP-1β	Sp, Sm, Su, Hg	*aadA, sul1, merE*	Tn*21*-like transposon	Sen et al. (2011)/JN106164.1
pB2/pB3[#]	60,732/56,167	IncP-1β	Aminoglycosides, β-lactam, Cm, Su, Tc, quaternary ammonium compounds	*aadA2, bla*$_{NPS\_2}$*, cmlA1, sul1, tetA(C), tetR(C), qacEΔ1*	Tn-*tet*, Tn*402*	Heuer et al. (2004)/NC_006388.1
pB4	79,370	IncP-1β	β-lactam, tripartite multi-drug resistance (MDR) efflux system, Sm, Em, Chr	*bla*$_{NPS\_1}$*, strAB, mexCD-oprJ, chr*	Tn*5393c*, Tn*5719*	Dröge et al. (2000), Tauch et al. (2003) /NC_003430.1
pB5	64,696	IncP-1α	Sm, Tc, Km, Gm, Su, quaternary ammonium compounds	*aacA4, aacC1, tetA, tetR, aphA, sul1, qacEΔ1*	nd	Dröge et al. (2000); Szczepanowski et al. (2011)/NC_019020.1
pB6	58	IncP-1β	Tc, Sm, Sp, Cm, Su	nd	nd	Dröge et al. (2000)
pB8	57,198	IncP-1β	Sm, Sp, β-lactam, Su, quaternary ammonium compounds	*aadA4, oxa2, sul1, qacEΔ1, qacF*	Tn*5501*, "cryptic" Tn, Tn*402*/ Tn*5090*, Tn*QAC*/(Tn*3* family), Tn*501*/Tn*21*	Schlüter et al. (2005)/NC_007502.1
pB10	64,508	IncP-1β	β-lactam, Su, Sm, Tc, quaternary ammonium compounds, Hg	*oxa2, sul1, strAB, tetA, qacEΔ1, mer*	Tn*5393c*, Tn*1721*, Tn*501*	Schlüter et al. (2003)/NC_004840.1
pB11	66,911	IncP-1α	Tc, Ap, Km, Hg	*tetA, tetR, aphA, merE*	Tn*501*, Tn*5053*	Dröge et al. (2000), Szczepanowski et al. (2011)/CP002152.1
pB12	64,393	IncP-1β	Tc, Sm, Sp, Em, β-lactam / Su, quaternary ammonium compounds	*tetA, aacA4, oxa2, sul1, qacEΔ1*	Tn*21*, Tn*402*	Dröge et al. (2000), Sen et al. (2012) /JX469826.1
pTB11	68,869	IncP-1α	Aminoglycosides, β-lactam, Tc	*aphA, aadA1, aacA4, oxa2, tetA, tetR*	Tn*402*/ (Tn*5090*), Tn*1721*,	Tennstedt et al. (2005)/NC_006352.1
pMCBF1	62,689	IncP-1ζ	Multi-drug efflux (MDE) outer membrane prot. NodT family, Hg	*oprN, merE*	Tn*5053*	Norberg et al. (2011)/AY950444.1
RP4/RK2	60,099	IncP-1α	Tc, Km, Ap	*tetA, aph*	Tn*4371*, Tn*1*	Pansegrau et al. (1994)/L27758.1
pTH10	70	IncP-1α	Tc, Km, Ap	nd	nd	Harayama et al. (1980)
R751	53,423	IncP-1β	Tp	*dhfrIIc*	Tn*402*/ Tn*5090*, Tn*501*	Thorsted et al. (1998)
pKJK5	54,383	IncP-1ε	Tc, Tp, aminoglycosides, Su, Sp, quaternary ammonium compounds	*tetA, tetR, dfrA1, aadA11b, sul1, qacEΔ1*	Tn*402*	Bahl et al. (2007)/NC_008272.1
pG527	80,762	IncP-1α	Aminoglycosides, Km, Sm, β-lactam, Tc	*aadA1, aphA, sph,* bla$_{TEM-67}$*, tetA, tetR*	Tn*3*, Tn*7*, Tn*1721*	Sen et al. (2012)/JX469830.1
pSP21	72,683	IncP-1α	Tc, Km, aminoglycosides, β-lactam	*tetA, tetR, aph, aadA1, aacA4, oxa2*	Tn*402*	Pansegrau et al. (1994), Szczepanowski et al. (2011)/NC_019021.1
pBS228	89,147	IncP-1α	Aminoglycosides, Sp, Tp, Tc, β-lactam, Hg	*aadA, dhfr, tetA,* bla$_{TEM-67}$*, aph, merE*	Tn*1013*, Tn*5718*, Tn*1*, Tn*7*	Haines et al. (2007)/NC_008357.1
BRA100	56,265	IncP-1β	Aminoglycosides, Su, quaternary ammonium compounds, Hg	*aacA4, strAB, sul1, qacEΔ1, merE*	Tn*6305*/(Tn*3* family)	Unpublished/CP003505
pWEC911	74,056	IncP-1	Tc, β-lactam, Km, Hg	*tetA,* bla$_{TEM-67}$*, aphA, merE*	nd	Sen et al. (2012)/JX469833.1
pKSP212	54,342	IncP-1	Aminoglycosides, Su, *Hg*	*aac(6)-Ib, sul1, merE*	Tn*3*	Sen et al. (2012)/JX469831.1
pBRSB222	36,880	IncP-1	Aminoglycosides, β-lactam, Su, quaternary ammonium compounds	*aadA5, oxa2, sul1, qacEΔ1*	nd	Sen et al. (2012)JX469825.1
pKS208	50,604	IncP-1	Aminoglycosides, Km, β-lactam	*aac(6)-Ib, aphA1, pEC-IMPQ_139*	Tn*1525*, Tn*5053*/Tn*402*	Sen et al. (2012)/JQ432564.1

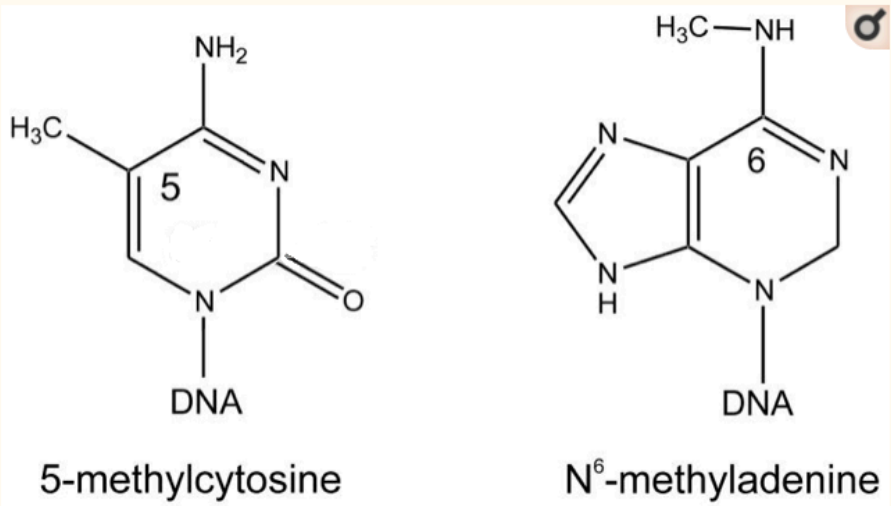
Ap, ampicillin; Cb, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Nx, nalidixic acid; Sm, streptomycin; Sp, spectinomycin; Su, sulfanilamide; Tc, tetracycline; Tp, trimethoprim; Tm, tobramycin; Hg, inorganic mercury; nd, not determined.

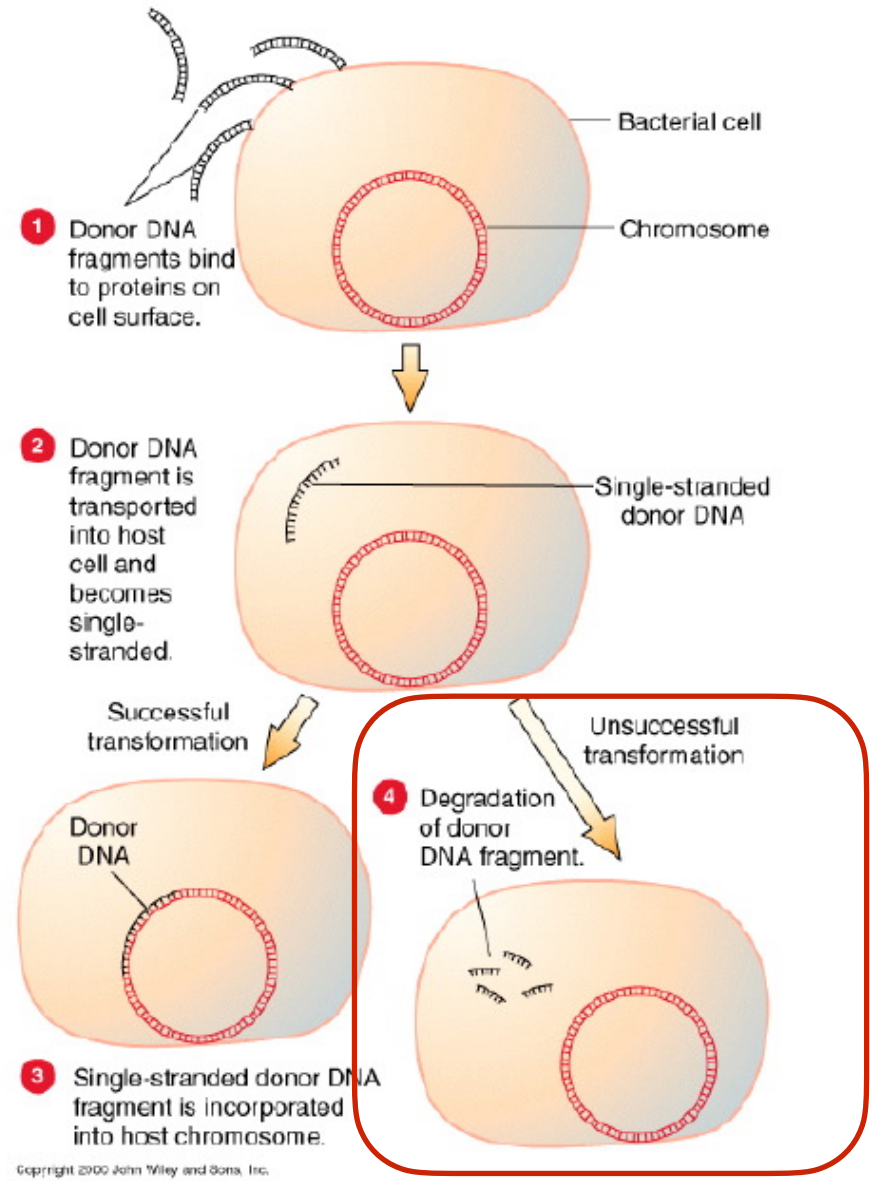
[#] Plasmids pB2 and pB3 differ only by a duplication of a tetA(C)-tetR-tnpAIS26 fragment in pB2.

Plasmid	Size (kb)	IncP-1 subgroup	Resistances	Resistance genes
pAKD1	58,246	IncP-1β	Sp, Sm, Su, Hg	*aadA, sul1, merE*
pB2/pB3[#]	60,732/56,167	IncP-1β	Aminoglycosides, β-lactam, Cm, Su, Tc, quaternary ammonium compounds	*aadA2, bla$_{NPS_2}$, cmlA1, sul1, tetA(C), tetR(C), qacEΔ1*
pB4	79,370	IncP-1β	β-lactam, tripartite multi-drug resistance (MDR) efflux system, Sm, Em, Chr	*bla$_{NPS_1}$, strAB, mexCD-oprJ, chr*
pB5	64,696	IncP-1α	Sm, Tc, Km, Gm, Su, quaternary ammonium compounds	*aacA4, aacC1, tetA, tetR, aphA, sul1, qacEΔ1*
pB6	58	IncP-1β	Tc, Sm, Sp, Cm, Su	nd
pB8	57,198	IncP-1β	Sm, Sp, β-lactam, Su, quaternary ammonium compounds	*aadA4, oxa2, sul1, qacEΔ1, qacF*
pB10	64,508	IncP-1β	β-lactam, Su, Sm, Tc, quaternary ammonium compounds, Hg	*oxa2, sul1, strAB, tetA, qacEΔ1, mer*
pB11	66,911	IncP-1α	Tc, Ap, Km, Hg	*tetA, tetR, aphA, merE*
pB12	64,393	IncP-1β	Tc, Sm, Sp, Em, β-lactam / Su, quaternary ammonium compounds	*tetA, aacA4, oxa2, sul1, qacEΔ1*

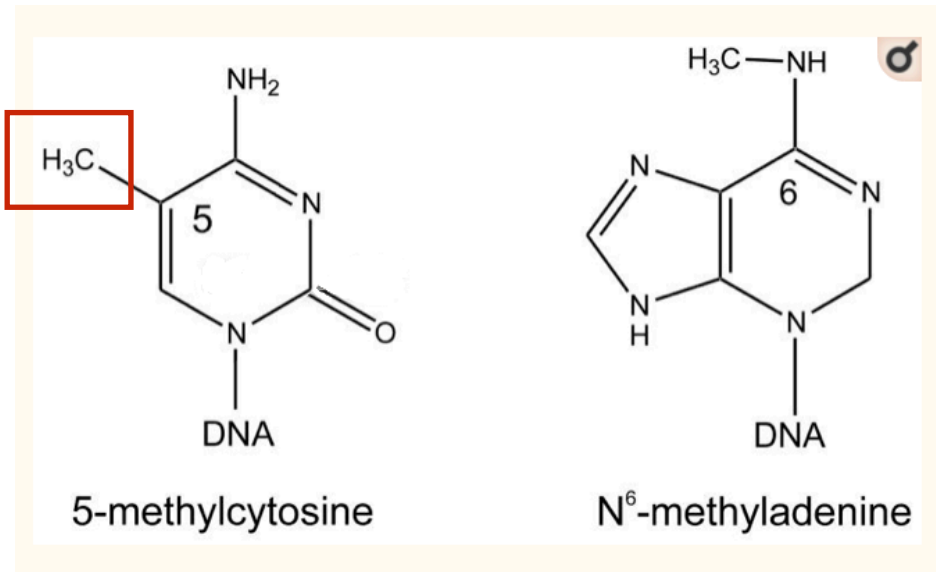
Plasmid	Size (kb)	IncP-1 subgroup	Resistances	Resistance genes
pAKD1	58,246	IncP-1β	Sp, Sm, Su, Hg	*aadA, sul1, merE*
pB2/pB3[#]	60,732/56,167	IncP-1β	Aminoglycosides, β-lactam, Cm, Su, Tc, quaternary ammonium compounds	*aadA2, bla$_{NPS_2}$, cmlA1, sul1, tetA(C), tetR(C), qacEΔ1*
pB4	79,370	IncP-1β	β-lactam, tripartite multi-drug resistance (MDR) efflux system, Sm, Em, Chr	*bla$_{NPS_1}$, strAB, mexCD-oprJ, chr*
pB5	64,696	IncP-1α	Sm, Tc, Km, Gm, Su, quaternary ammonium compounds	*aacA4, aacC1, tetA, tetR, aphA, sul1, qacEΔ1*
pB6	58	IncP-1β	Tc, Sm, Sp, Cm, Su	nd
pB8	57,198	IncP-1β	Sm, Sp, β-lactam, Su, quaternary ammonium compounds	*aadA4, oxa2, sul1, qacEΔ1, qacF*
pB10	64,508	IncP-1β	β-lactam, Su, Sm, Tc, quaternary ammonium compounds, Hg	*oxa2, sul1, strAB, tetA, qacEΔ1, mer*
pB11	66,911	IncP-1α	Tc, Ap, Km, Hg	*tetA, tetR, aphA, merE*
pB12	64,393	IncP-1β	Tc, Sm, Sp, Em, β-lactam / Su, quaternary ammonium compounds	*tetA, aacA4, oxa2, sul1, qacEΔ1*

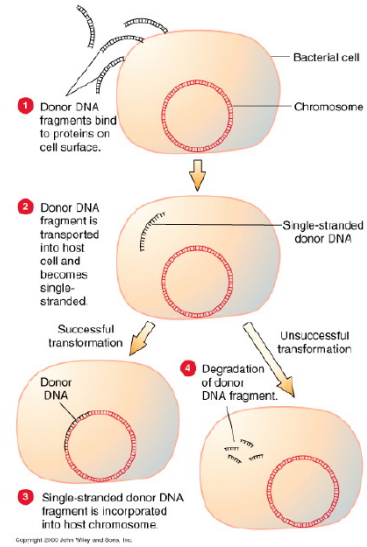
Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.





Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.

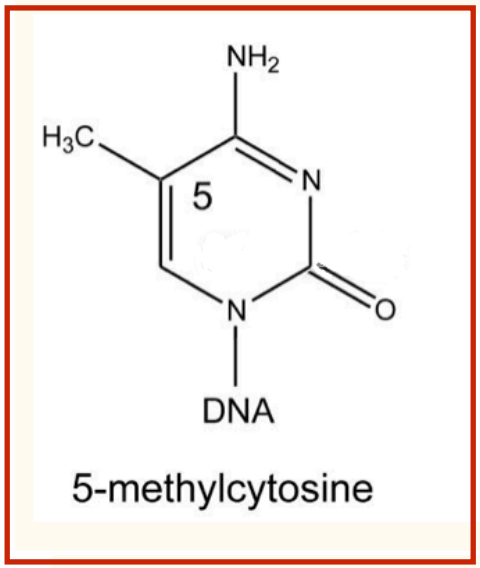


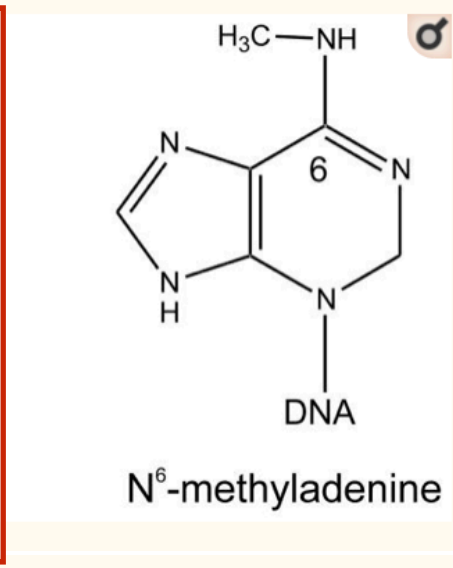


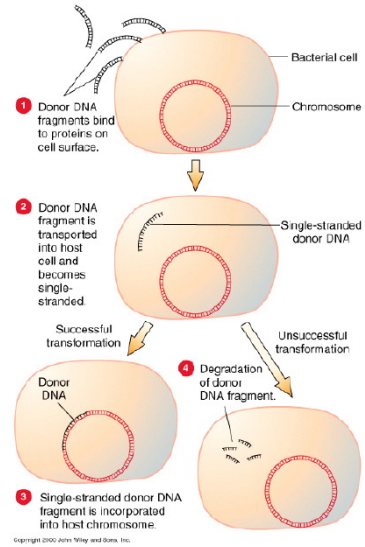
DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.



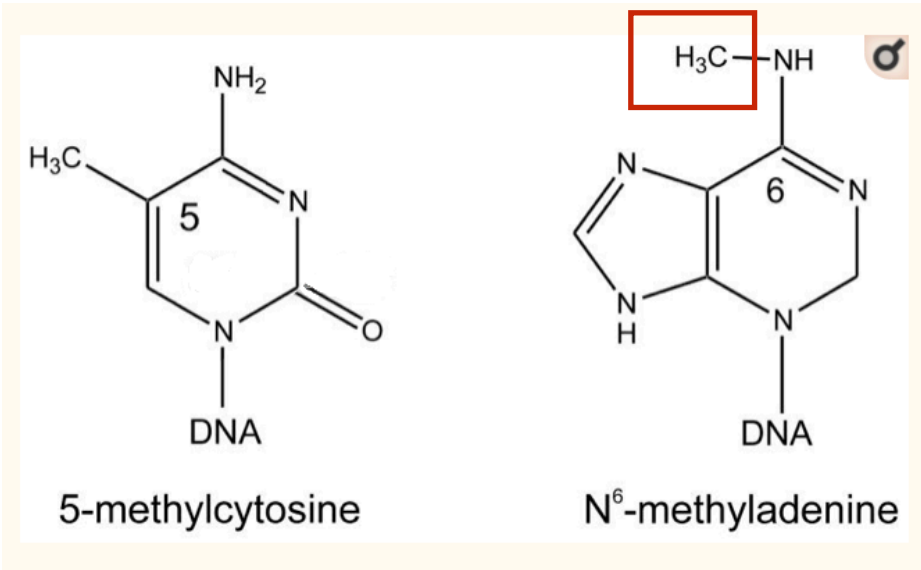


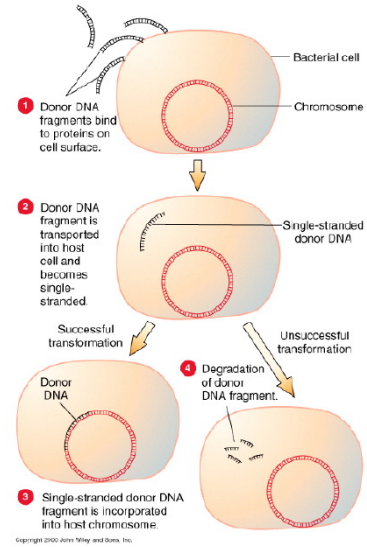


DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.

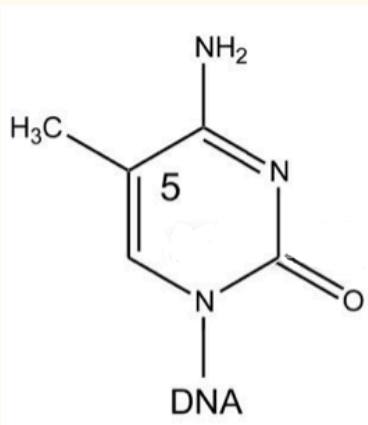




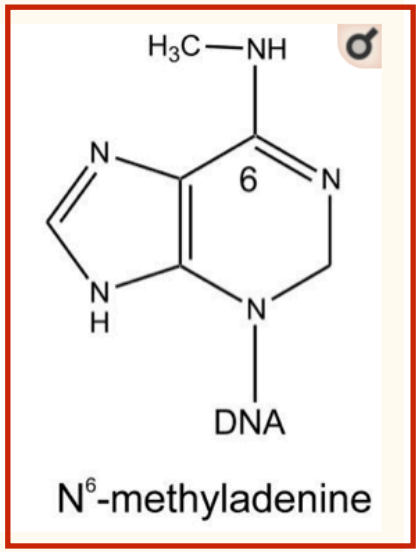
DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

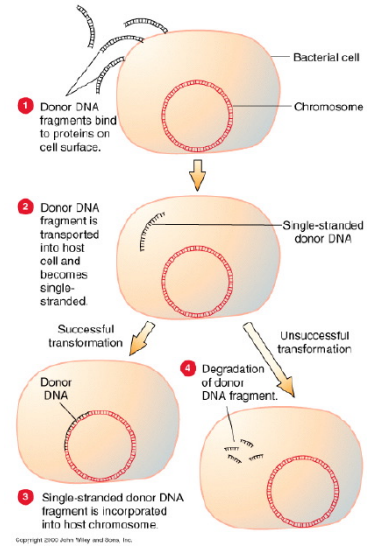
Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.



5-methylcytosine



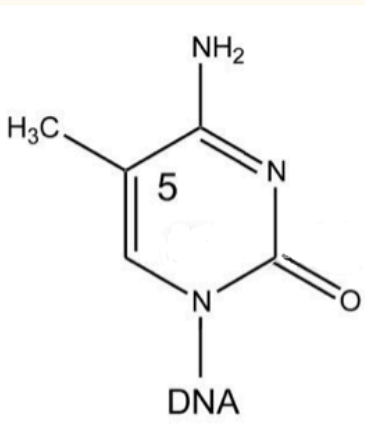
N^6-methyladenine



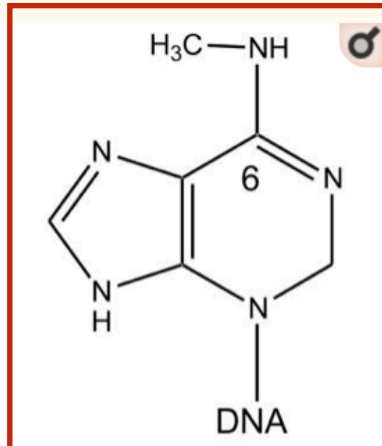
DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

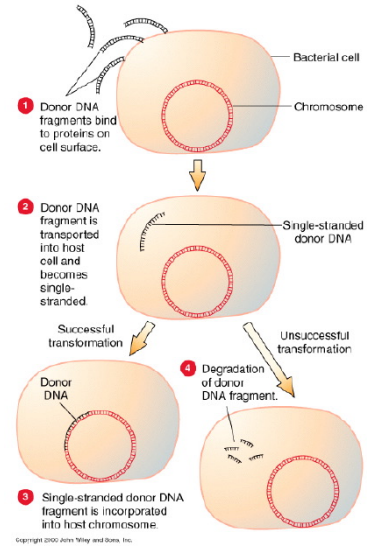
Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.



5-methylcytosine



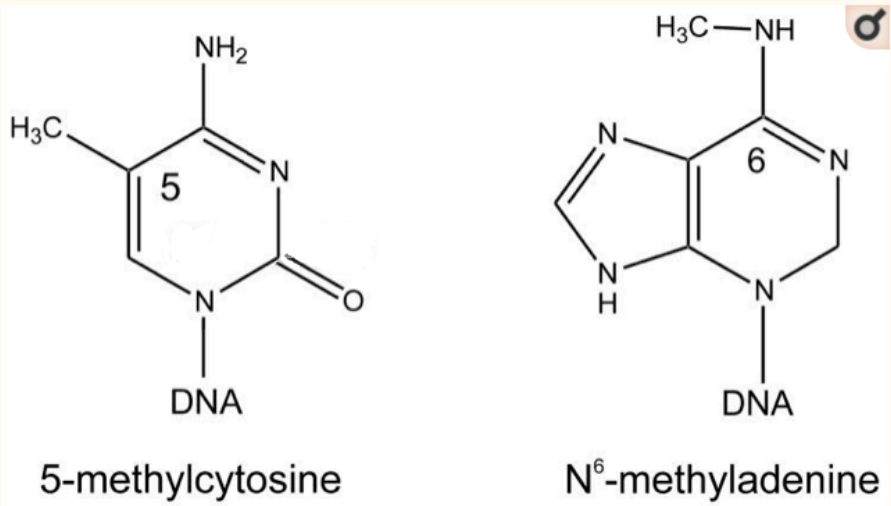
N^6-methyladenine

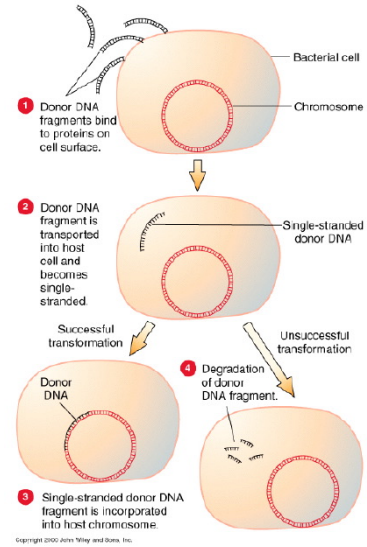


DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.

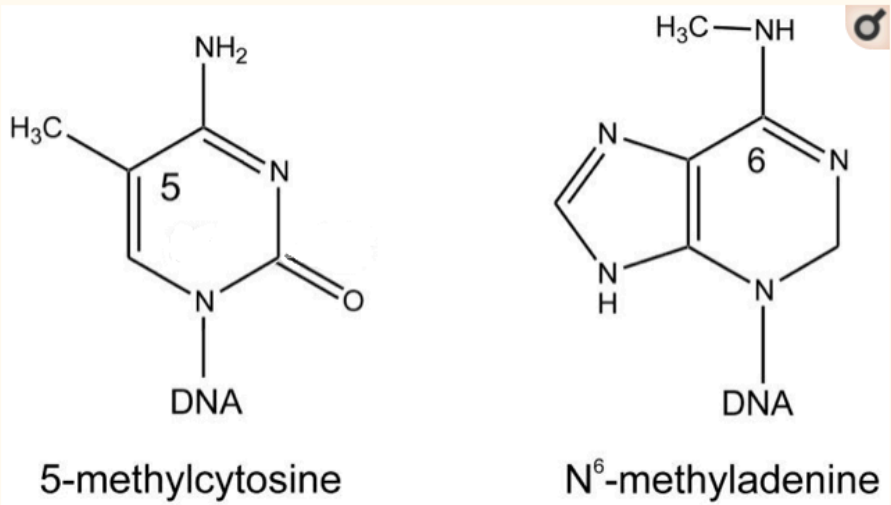


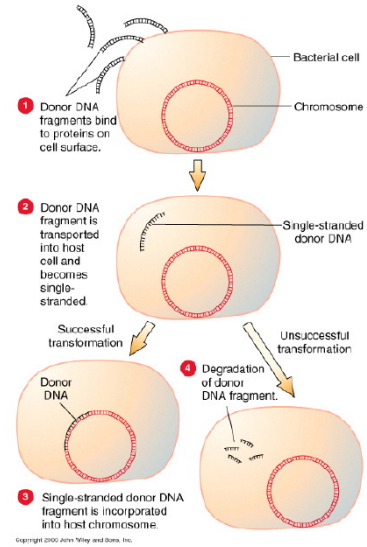


DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.

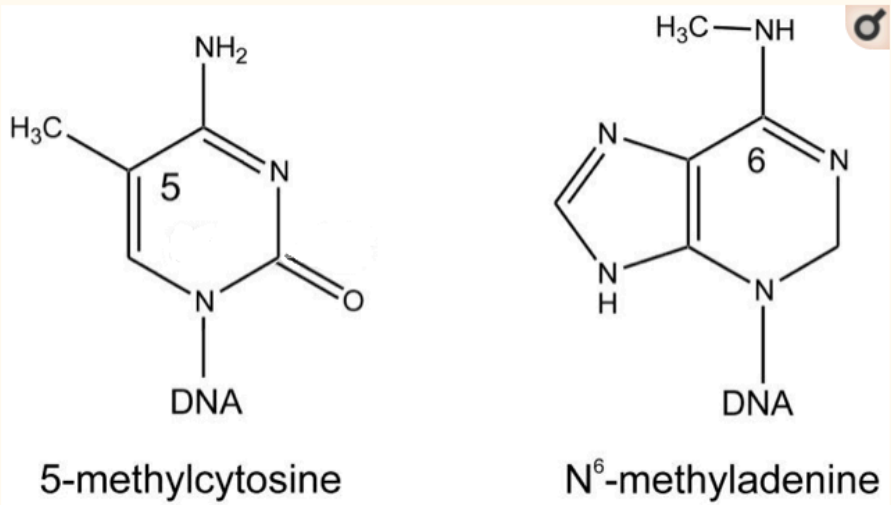


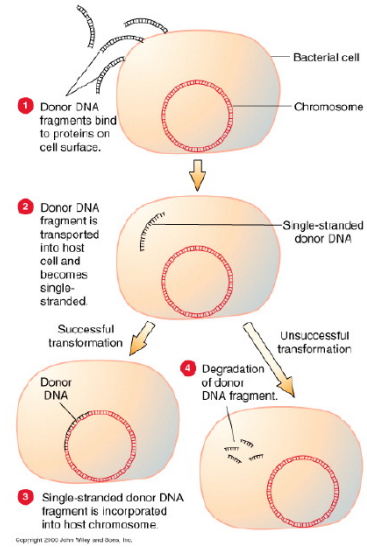


DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.





DNA methyltransferases in *E. coli* K-12

Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of "self" for the prokaryotic cell.

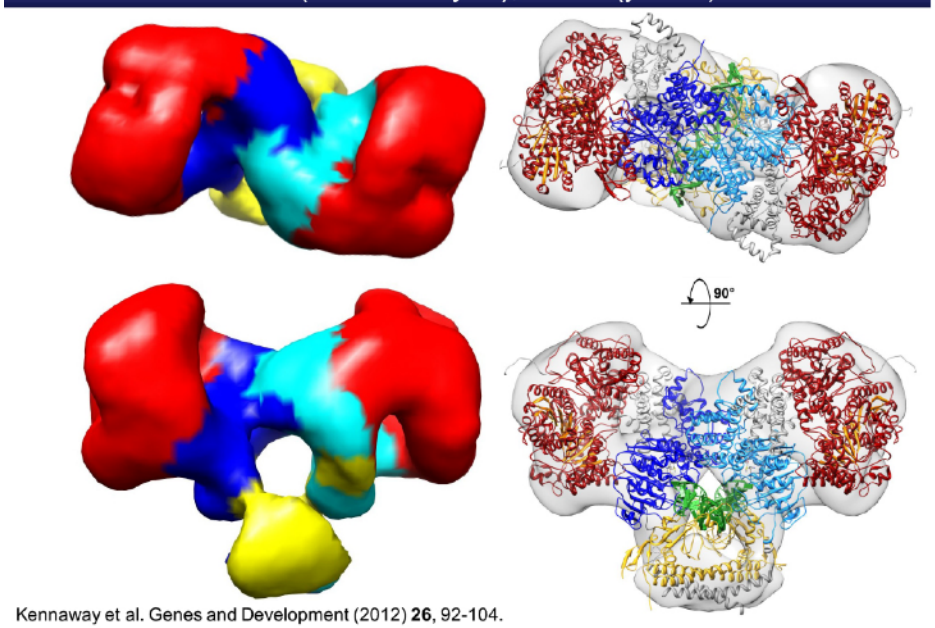
Type I restriction modification enzymes (first identified by Werner Arber and Dussoix in the1960's using lamda phage infection of *E. coli*) initially defined two different strains of *E. coli -E. coli*B and *E. coli* K12 (two *E. coli* strains that encode for slight, but specific variants of their **HSD system (Host Specificity Determinant)** -encoded by the ***hsdR***, ***hsdM***and ***hsdS*** genes).

These enymes are expressed together and generally require interactions with cofactors, such as S-Adenosyl methionine (AdoMet), hydrolyzed adenosine triphosphate (ATP), and magnesium (Mg^{2+}) ions.

eg. ***Eco*B** recognizes **TGA (N_8) TGCT**, ***Eco*K12** recognizes **AAC(N6)GTGC**

5' TG**A**NNNNNNNNTGCT 3'
3' ACTNNNNNNNN**A**CGA 5'

EM model for a Type I RM enzyme with DNA bound. HsdR (red), HsdM (blue and cyan), HsdS (yellow).



Kennaway et al. Genes and Development (2012) **26**, 92-104.

TYPE I

Restriction Endonucleases: Restriction endonuclease provide an additional tool to facilitate the creation of physical maps of DNA

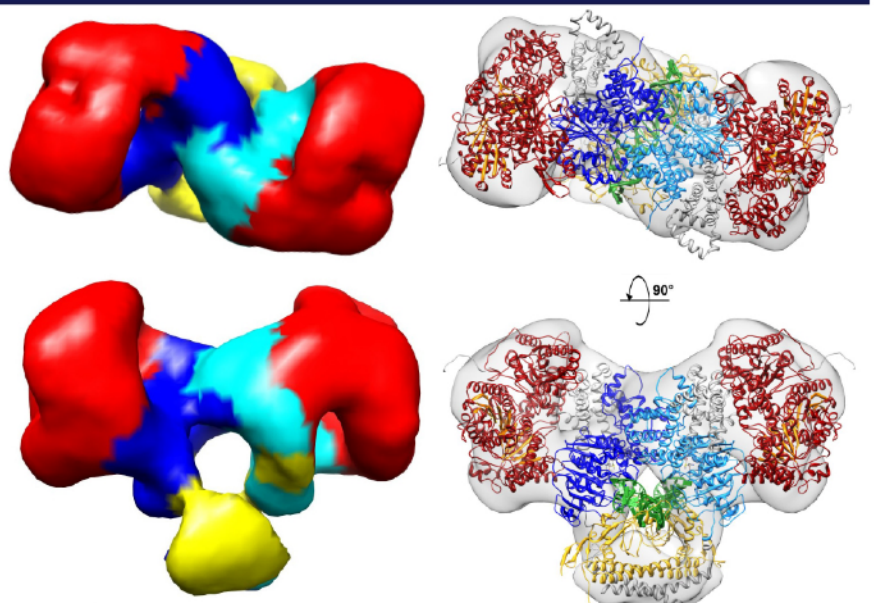
Type I restriction modification enzymes (first identified by Werner Arber and Dussoix in the1960's using lamda phage infection of *E. coli*) initially defined two different strains of *E. coli -E. coli*B and *E. coli* K12 (two *E. coli* strains that encode for slight, but specific variants of their **HSD system (Host Specificity Determinant)** -encoded by the ***hsdR***, ***hsdM***and ***hsdS*** genes).

These enymes are expressed together and generally require interactions with cofactors, such as S-Adenosyl methionine (AdoMet), hydrolyzed adenosine triphosphate (ATP), and magnesium (Mg^{2+}) ions.

eg. ***Eco*B** recognizes **TGA (N_8) TGCT**, ***Eco*K12** recognizes **AAC(N6)GTGC**

5' TG**A**NNNNNNNNTGCT 3'
3' ACTNNNNNNNN**A**CGA 5'

EM model for a Type I RM enzyme with DNA bound. HsdR (red), HsdM (blue and cyan), HsdS (yellow).



Kennaway et al. Genes and Development (2012) **26**, 92-104.

DNA methyltransferases in *E. coli* K-12

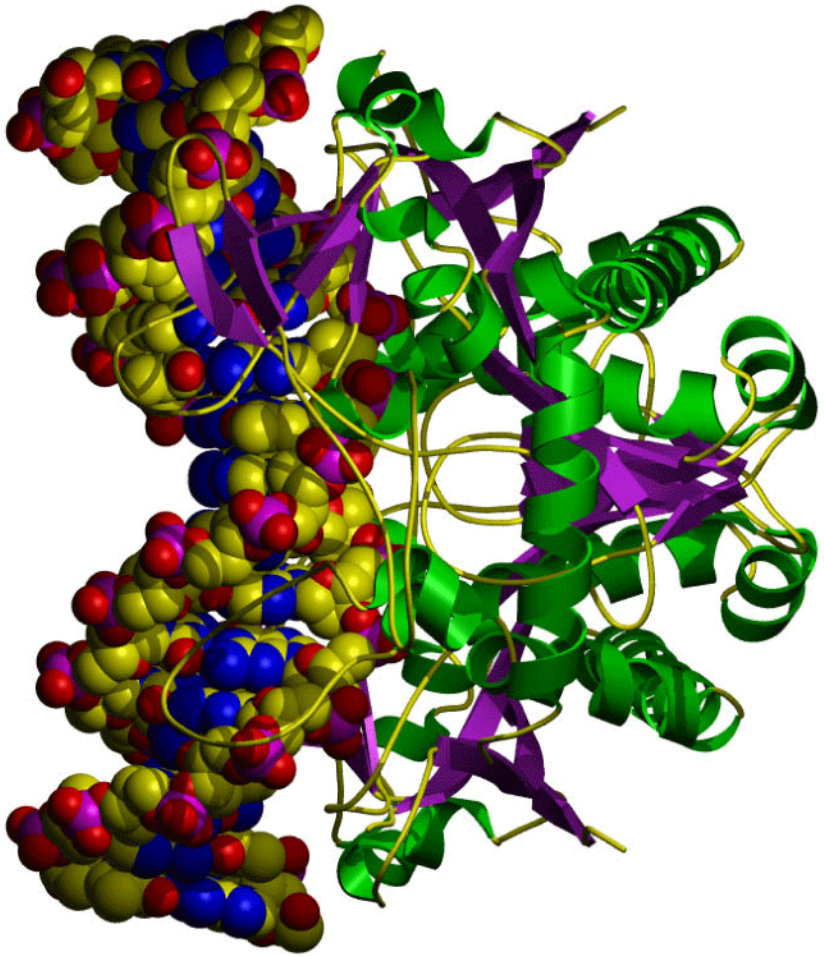
Gene(s)	Modification methyltransferase	Recognition sequence[a]	Number in genome[b]	Restriction endonucleases[c]
hsdSM	M.EcoK	-AAC(N^6)GTCG-	595	EcoKI
dam	Dam	-GATC-	19,120	DpnI, DpnII, Sau3A
dcm	Dcm	-CCWGG-	12,045	EcoRII, BstNI
yhdJ	YhdJ	-ATGCAT-	839	NsiI

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of “self” for the prokaryotic cell. In addition they provide an additional tool to facilitate the creation of physical maps of DNA

Type II restriction enzymes (most commonly used in Biotechnology) are only able to restrict DNA any methylase activity (if any) is present on a separate protein.

Type II enzymes are usually dimeric proteins, and have a variety of digest patterns.

Restriction characteristics. Blunt- , 5' and 3' "sticky- ends". *Dpn*I (meth) or *Dpn*II



*Eco*RI recognizes

“GAATTC” palindrome

TYPE II

Restriction Endonucleases: Restriction endonuclease provide an additional tool to facilitate the creation of physical maps of DNA

Type III restriction enzymes are similar to **Type I** enzymes, they also have an ATPase requirement and differ mainly in that their **M** and **S** subunits are combined into one ~75kDa subunit, with the additional R subunit being ~108kDa. Again these enzymes are BI-functional enzymes, normally as **heterodimers,** which can methylate and/or restrict **simultaneously**, although the methylase subunits can often work on its own. Methylation only occurs on one strand.

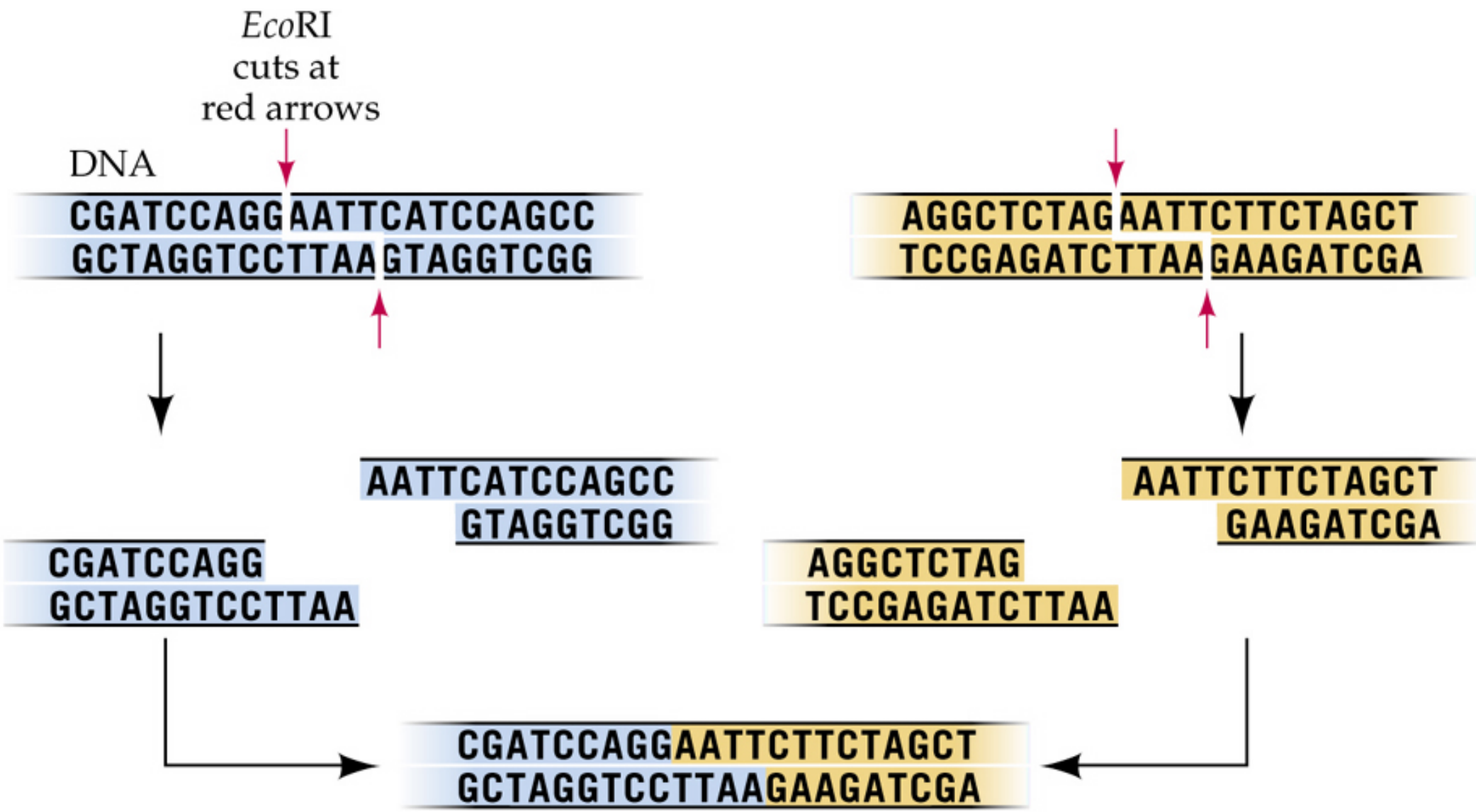
Usually the site of restriction is removed from the recognition site. with the enzyme cutting often cuttingh some 24-28 bases down from recognition site, eg. *Eco*P1 and *Eco*P15, and *Hinf* in

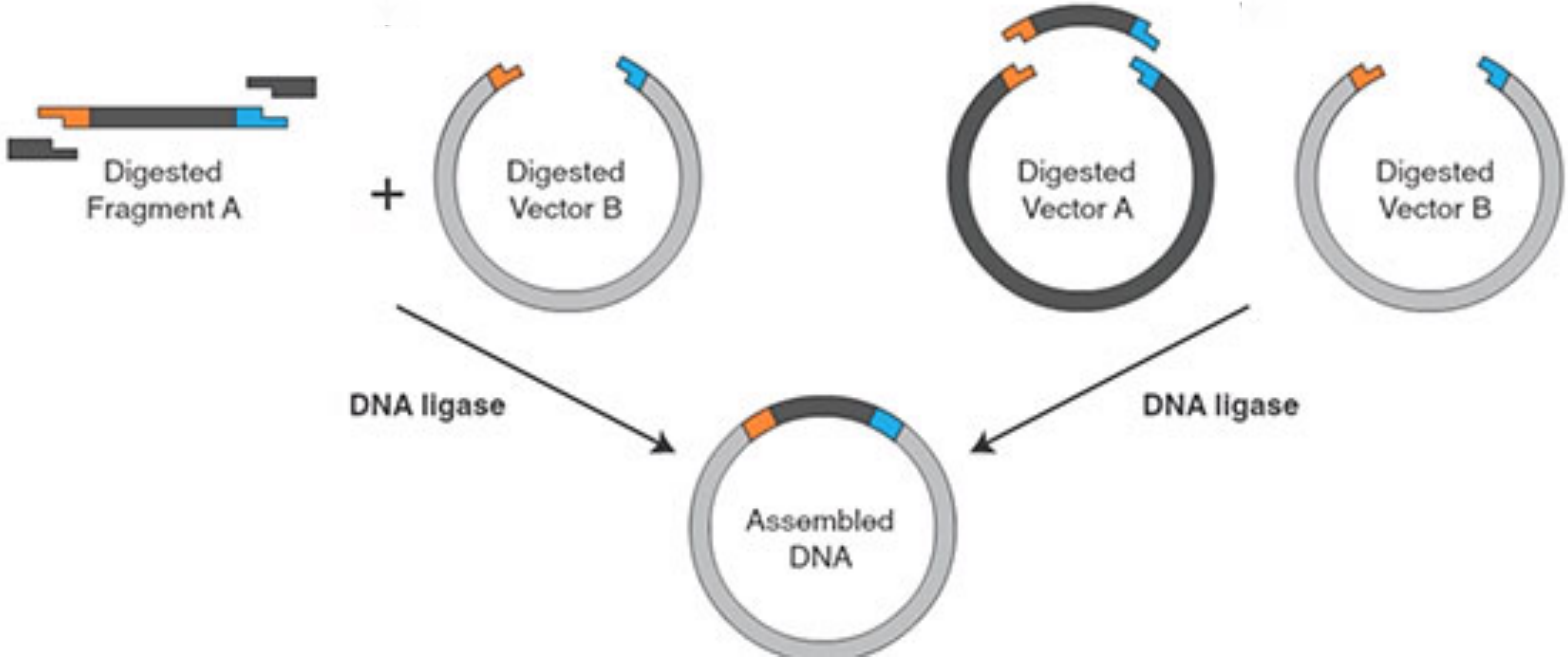
Haemophilus influenzae.

5' **AGACC - 2**3-NNN-|- N 3'
3' **TGTGG - 2**3-|-NNNNN 5'

TYPE III

Restriction Endonucleases: Restriction endonuclease provide -in part- a determination of “self” for the prokaryotic cell.





In using these Restriction enzymes to clone fragments of DNA into **cloning vectors** there are number of variables that need to be considered.

Size of restriction recognition site -will affect frequency of site within any given DNA sequence.

G/C content of restriction site vs. G/C content of DNA to be restricted.

Time

Compatability of ends

Ability to KNOW that you have stably cloned a fragment of DNA into a plasmid and that it is maintained within a cell.

42

Desirable attributes of "ideal" cloning vectors:

Use of *E.coli* as the preferred host for genetic manipulations has definitely biased the choice of vectors and choice of gene transfer.

Ideal cloning vectors do not exist in nature and, while most of the ones used are derived from bacteria in the wild, they have themselves been genetically engineered to accommodate man's purpose.

Replicates autonomously in bacterial host of choice, usually *E. coli,* and is not too large

Encodes for multiple drug resistances.

Encodes for various and numerous "single" restriction sites

Has a relatively high copy number.

pBR322 used to foot the bill.
Maintained at ~40-50 copies/cell
Enodes for **2 distinct** drug resistances
Has a number of single sites.

By convention *EcoR* I site defines "0"

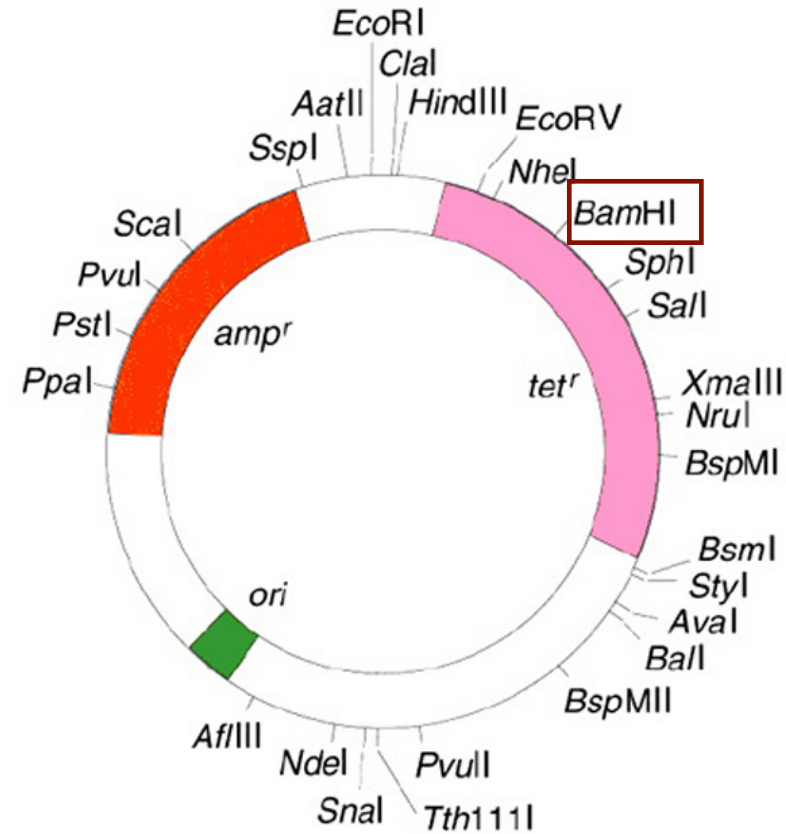
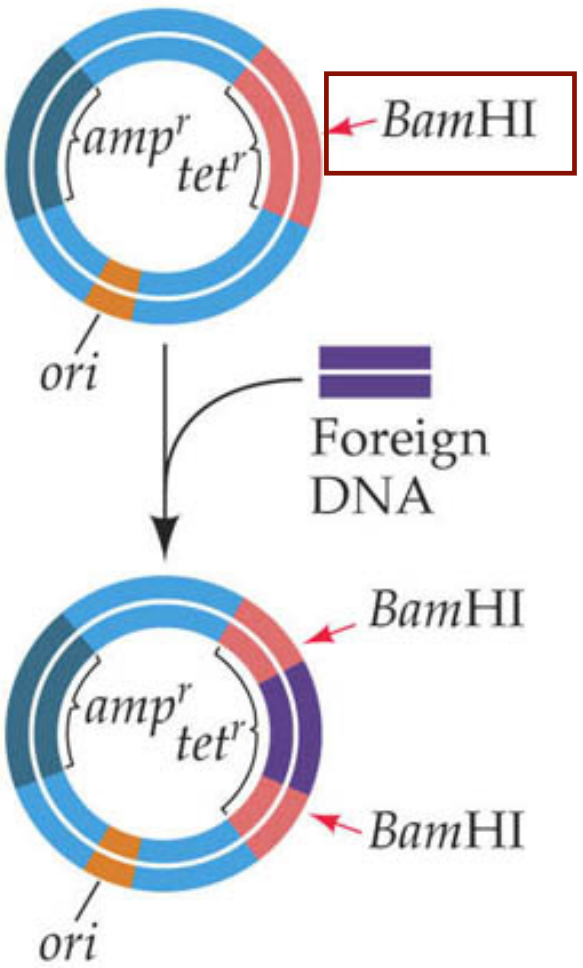


Figure 20.4 Structure of *E. coli* plasmid cloning vector pBR322, a circular DNA molecule 4.36 kb in size. The locations of unique restriction enzyme cleavage sites, the origin (*ori*) of replication, and the genes that confer resistance to the antibiotics ampicillin (amp^r) and tetracycline (tet^r) are shown on the map of the plasmid DNA molecule.

RESEARCH METHOD



DNA taken up by *amp*^{s} and *tet*^{s} *E. coli*	Phenotype for ampicillin	Phenotype for tetracycline
None	Sensitive	Sensitive
Foreign DNA only	Sensitive	Sensitive
pBR322 plasmid	Resistant	Resistant
pBR322 recombinant plasmid	Resistant	Sensitive

LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 16.6 Marking Recombinant DNA by Inactivating a Gene

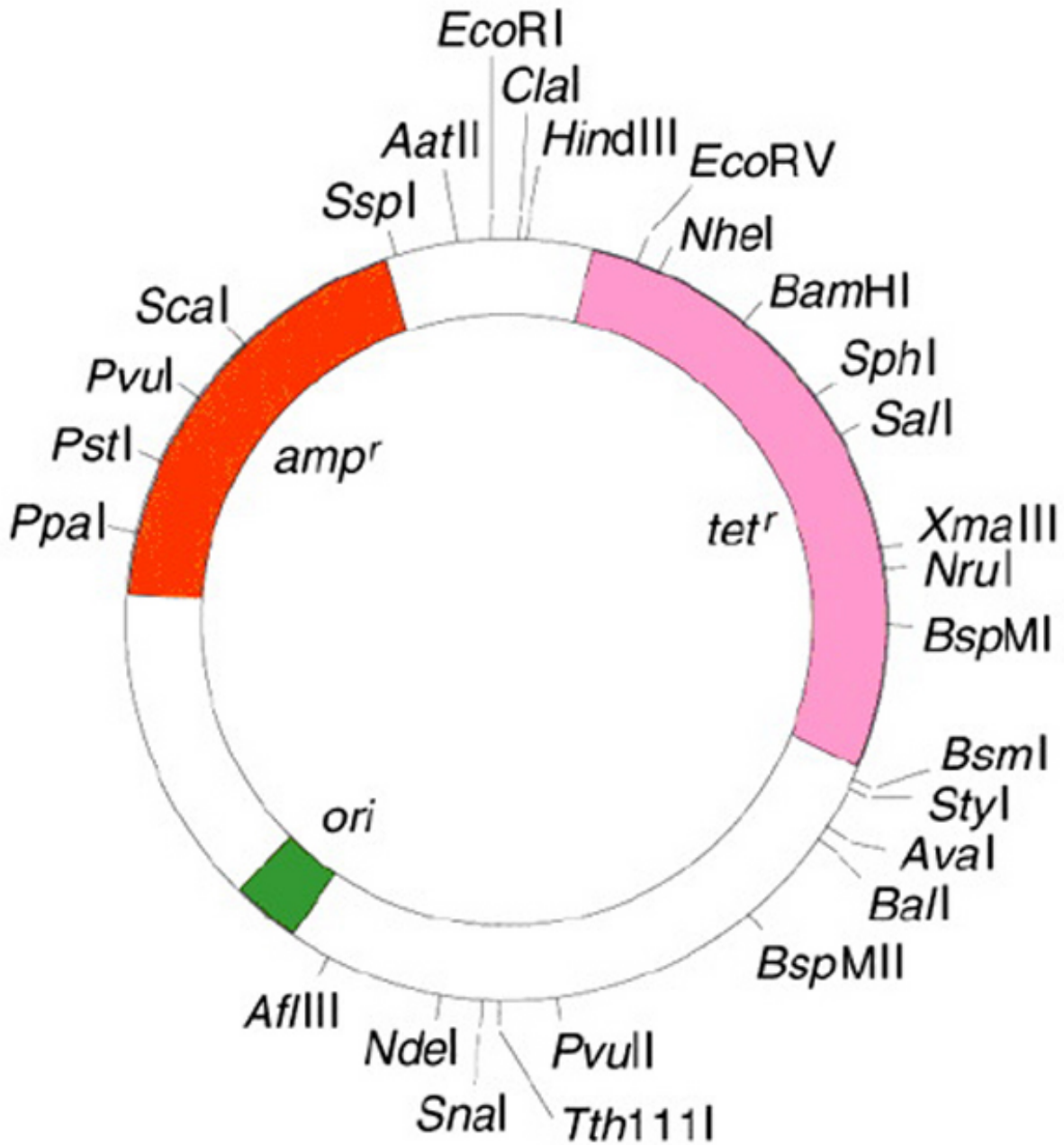
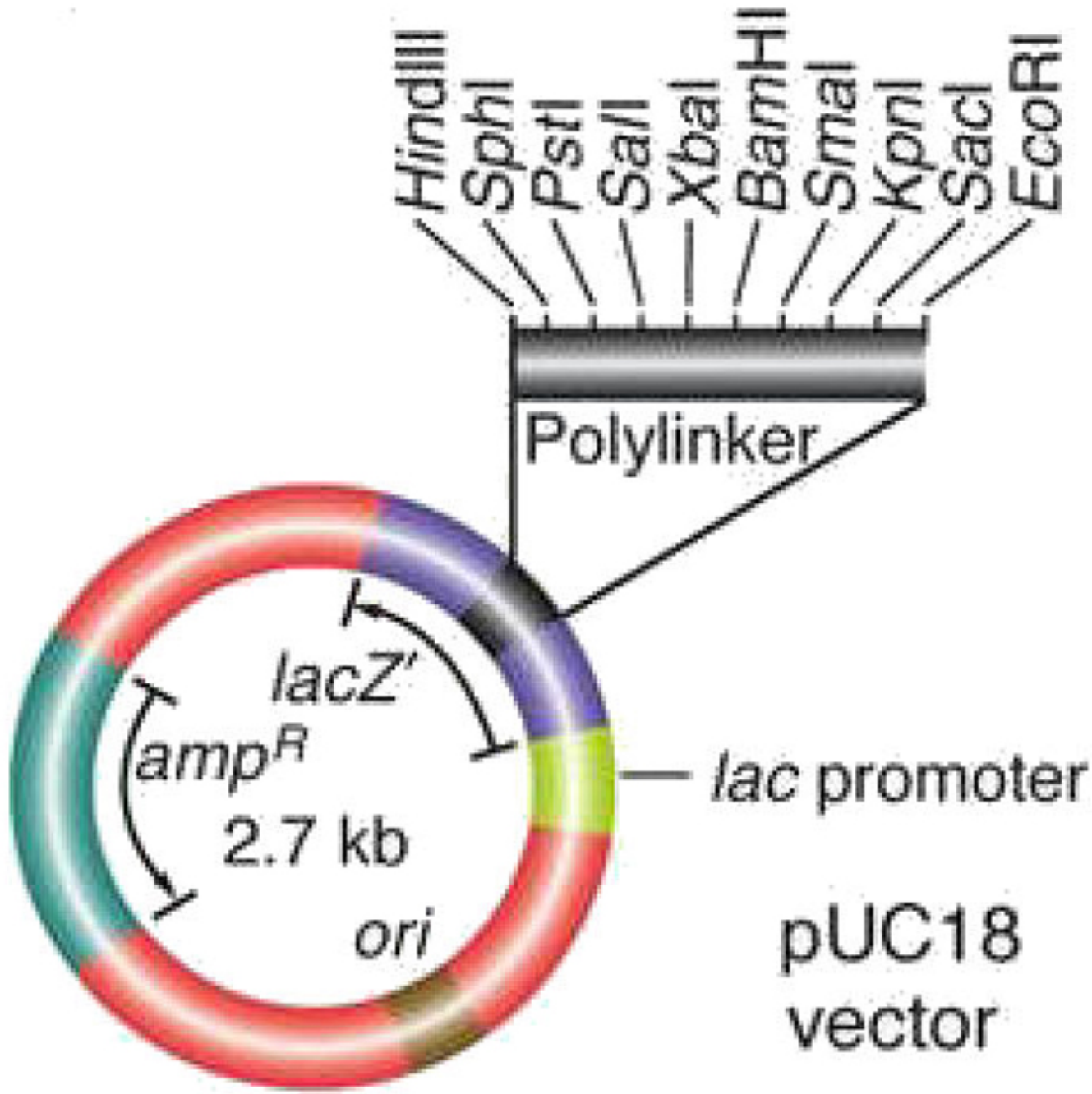
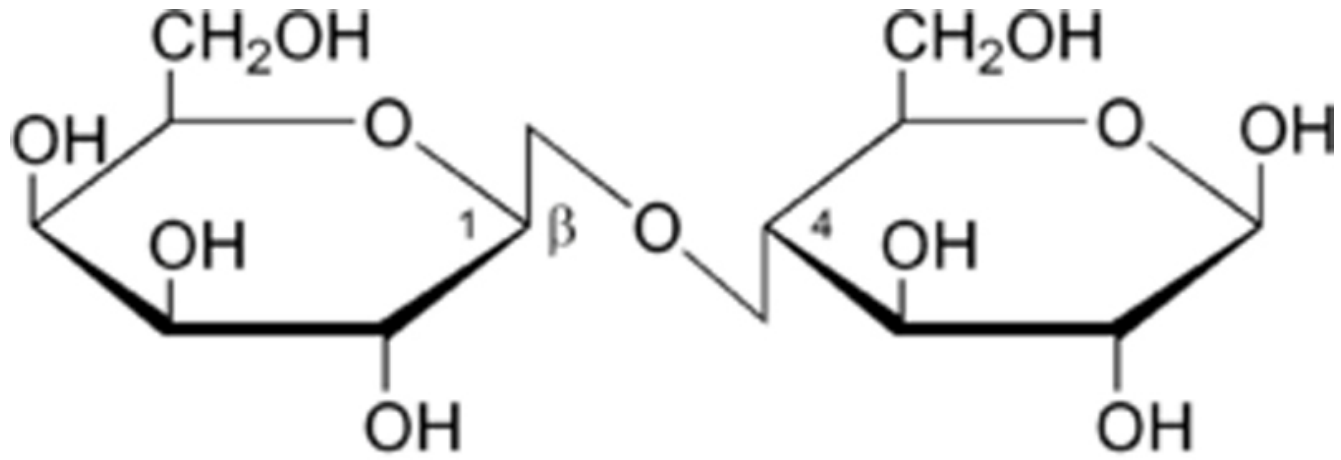
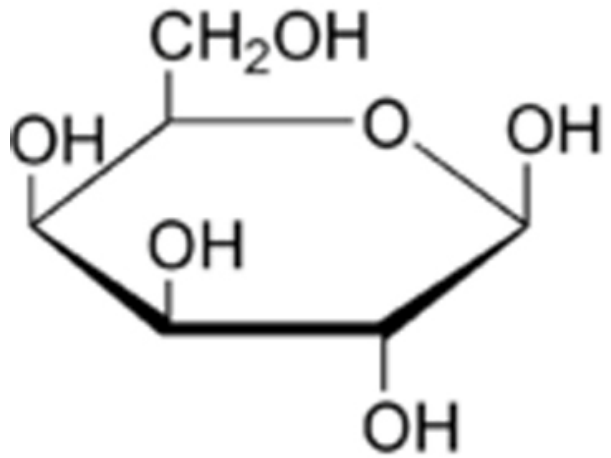


Figure 20.4 Structure of *E. coli* plasmid cloning vector pBR322, a circular DNA molecule 4.36 kb in size. The locations of unique restriction enzyme cleavage sites, the origin (*ori*) of replication, and the genes that confer resistance to the antibiotics ampicillin (amp^r) and tetracycline (tet^r) are shown on the map of the plasmid DNA molecule.

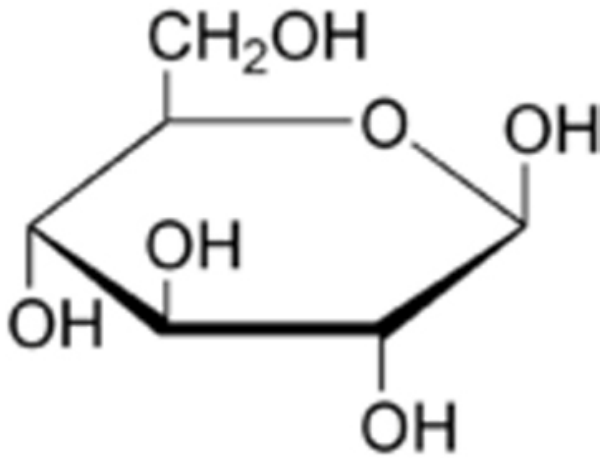

HindIII
SphI
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
Polylinker
lacZ'
ampR
2.7 kb
ori
lac promoter
pUC18
vector



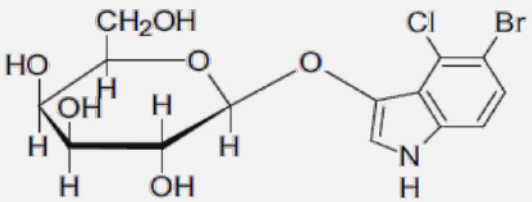
Lactose



D-galactose



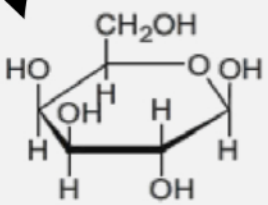
D-glucose



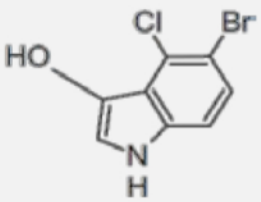
5-bromo-4-chloro-3-indolyl-
β-D-galactopyranoside
(X-gal)

β–D-galactosidase

H_2O



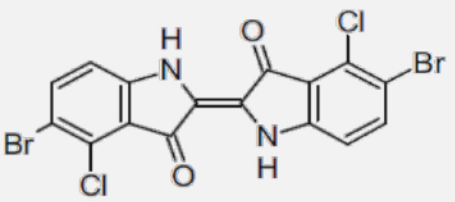
β-D-galactose



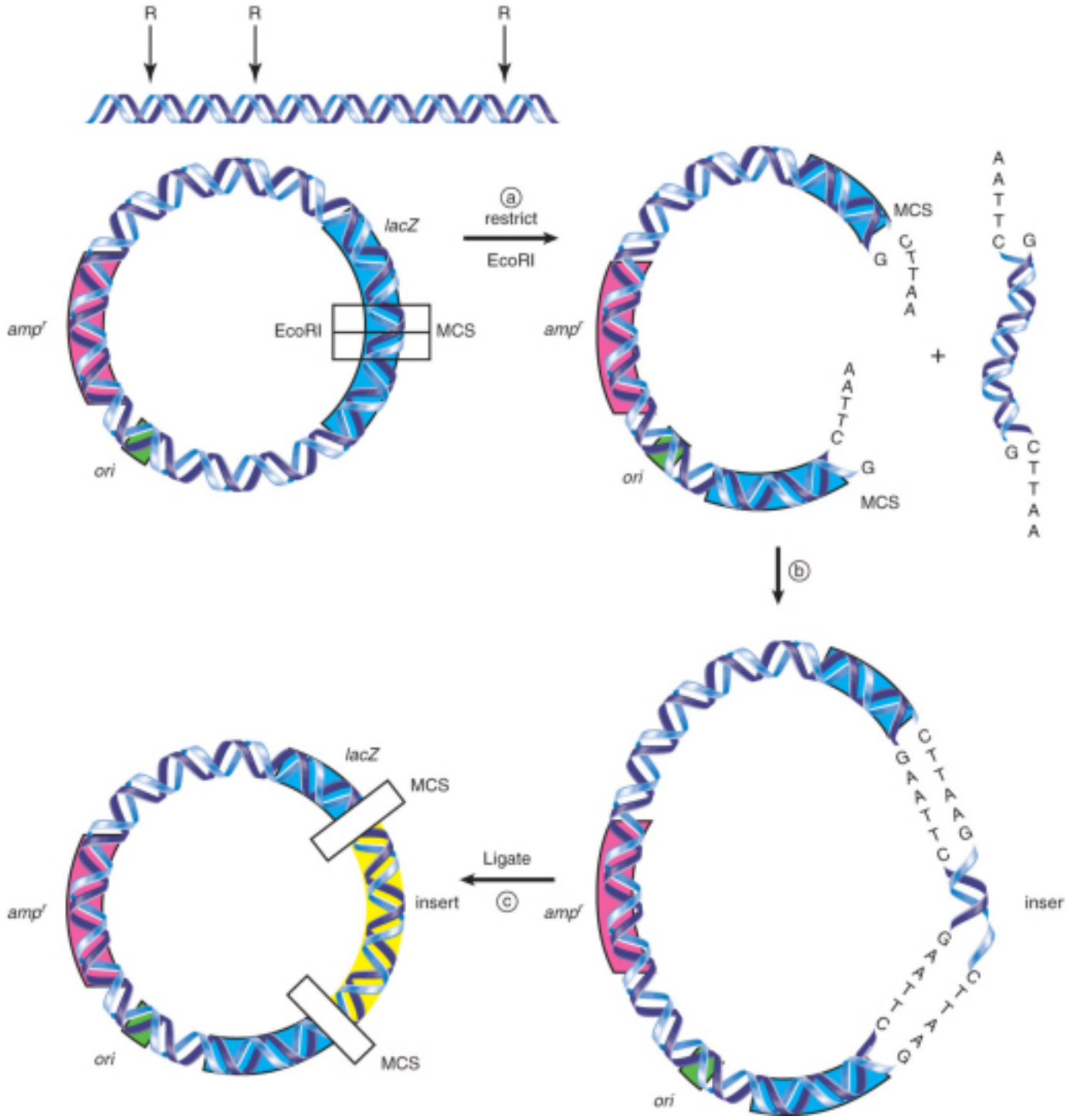
5-bromo-4-chloro-3-hydroxyindol

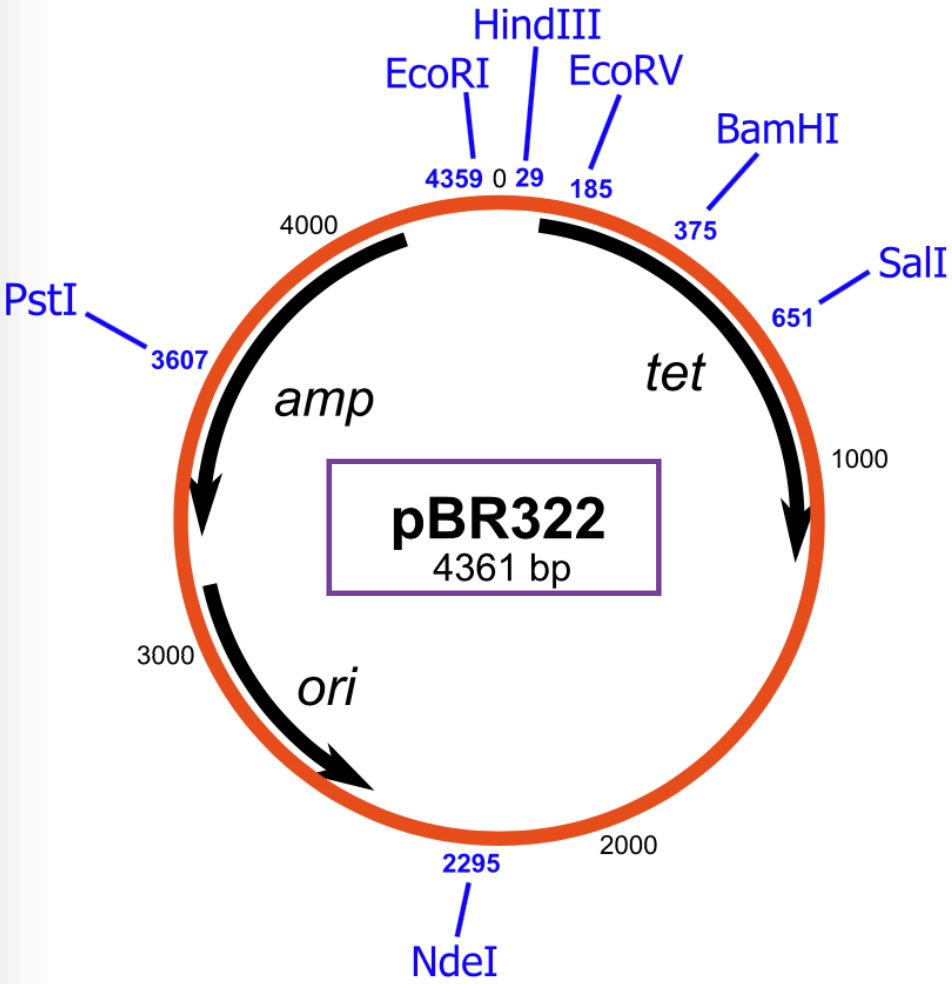
O_2

dimerization

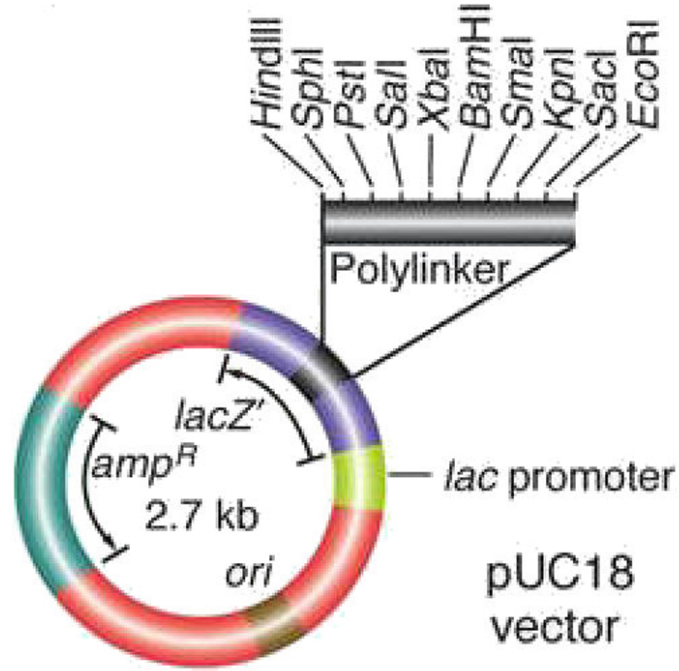


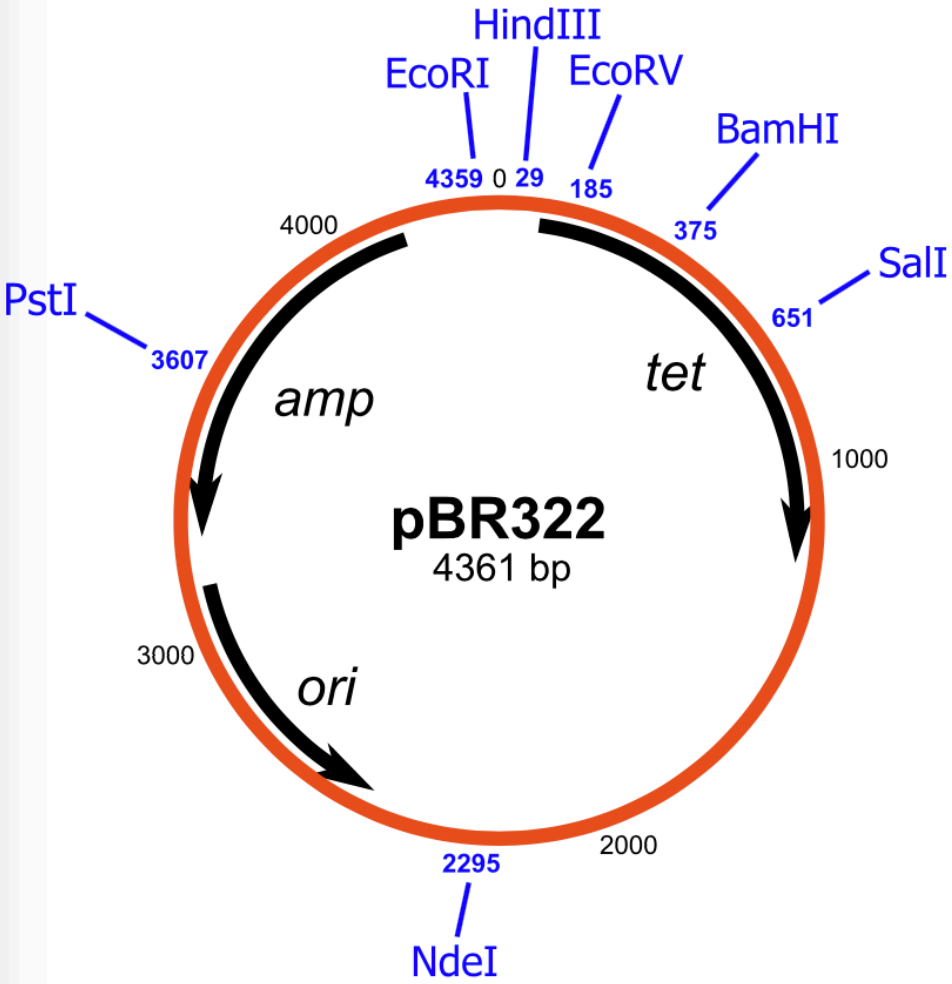
5,5'-dibromo-4,4'-dichloro-indigo
(Blue precipitate)


R
R
R
lacZ
ampr
EcoRI
MCS
ori
a
restrict
EcoRI
MCS
G
CTTAA
AATTC
G
ampr
ori
AATTC
G
MCS
+
G
CTTAA
b
lacZ
MCS
ampr
insert
ori
MCS
Ligate
c
ampr
ori
inser



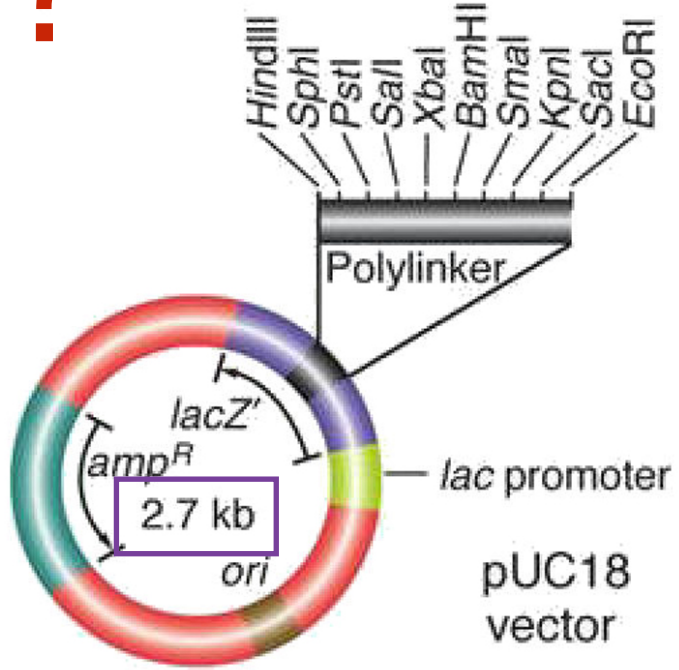
lacZ is 3,075 bp

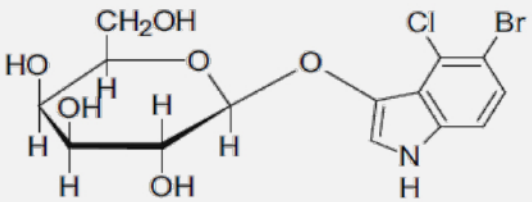




lacZ is 3,075 bp

???

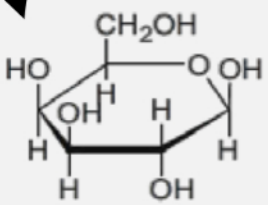




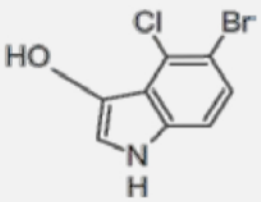
5-bromo-4-chloro-3-indolyl-
β-D-galactopyranoside
(X-gal)

β–D-galactosidase

H_2O



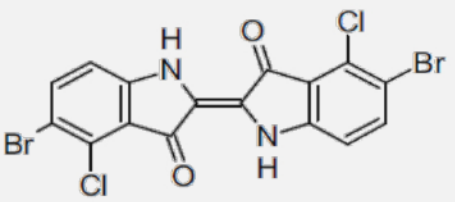
β-D-galactose



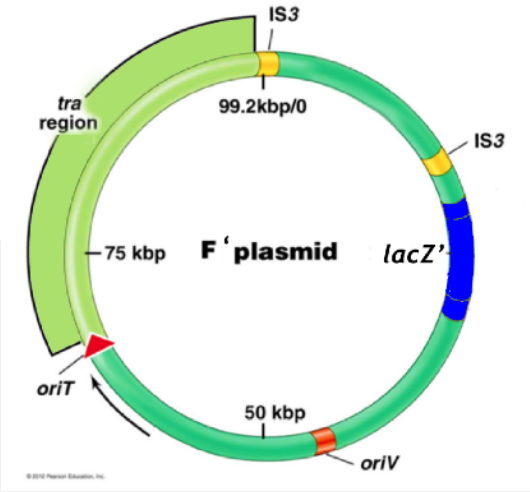
5-bromo-4-chloro-3-hydroxyindol

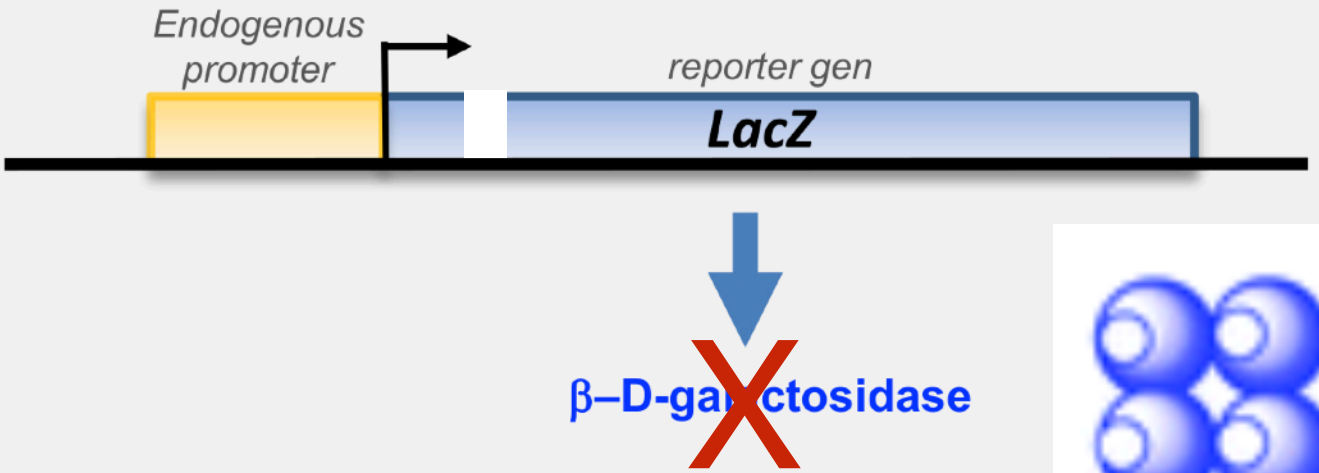
O_2

dimerization



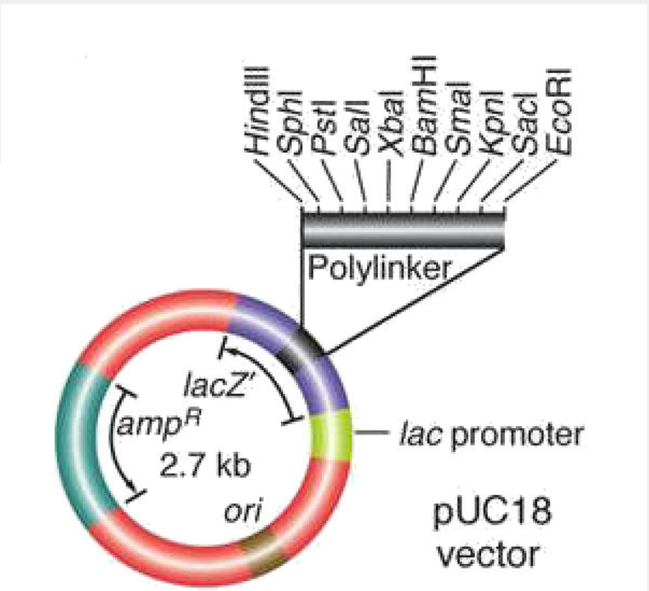
5,5'-dibromo-4,4'-dichloro-indigo
(Blue precipitate)



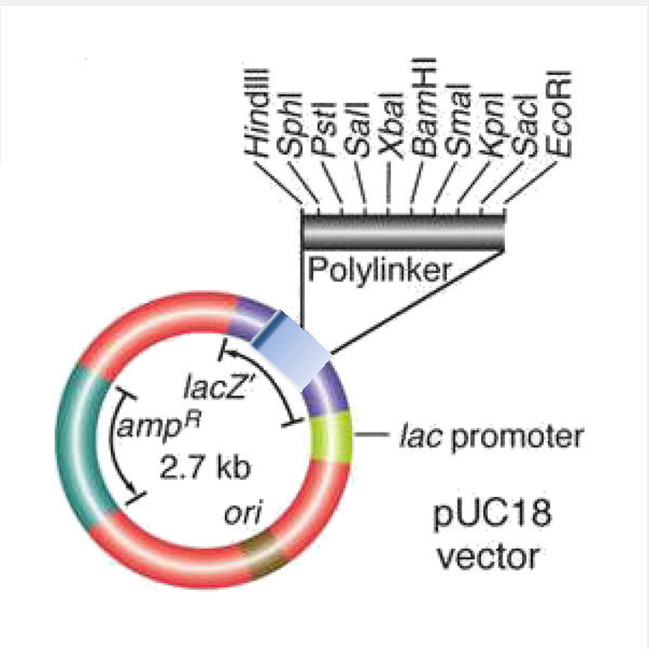


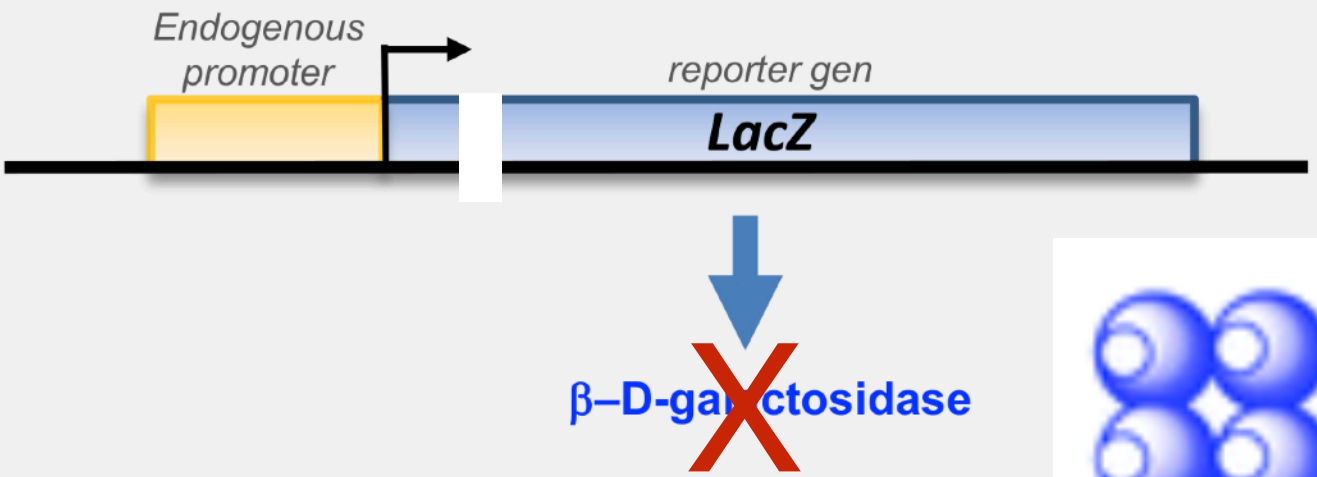
Inactive
ß-Galactosidase

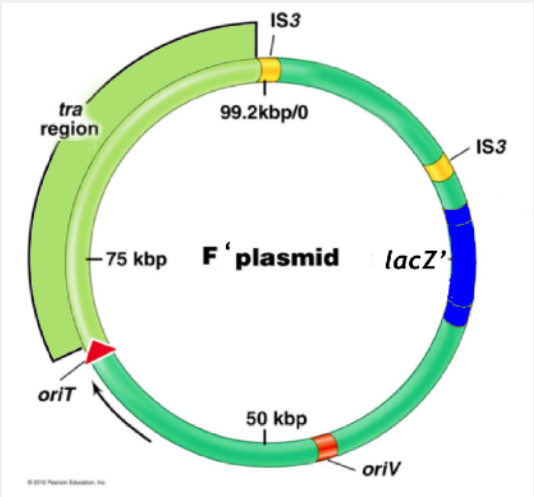

Endogenous
promoter
lacZ'


HindIII
SphI
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
Polylinker
lacZ'
ampR
2.7 kb
ori
lac promoter
pUC18
vector

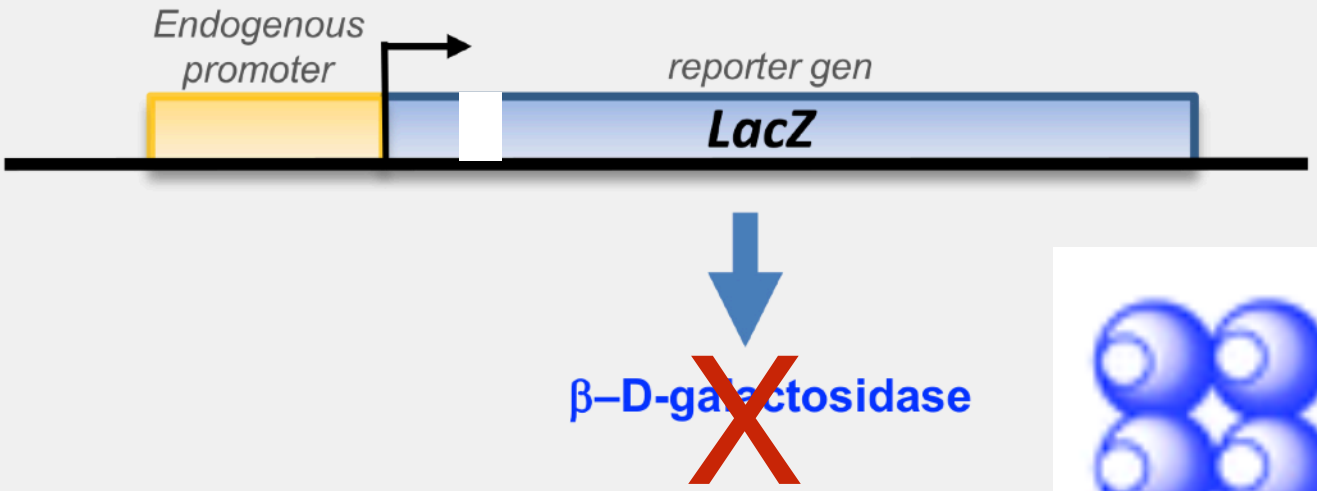

Endogenous
promoter
lacZ'

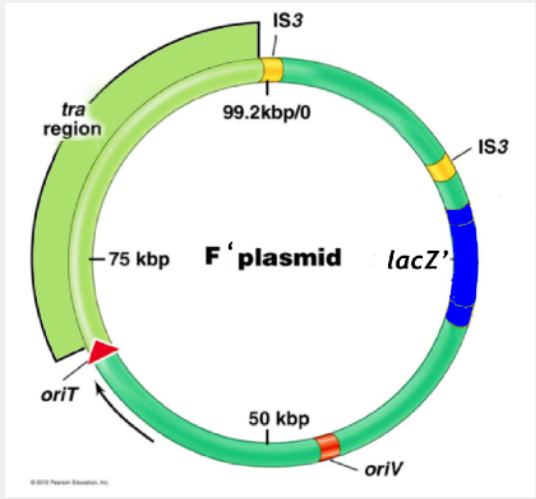

HindIII
SphI
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
Polylinker
lacZ'
ampR
2.7 kb
ori
lac promoter
pUC18
vector



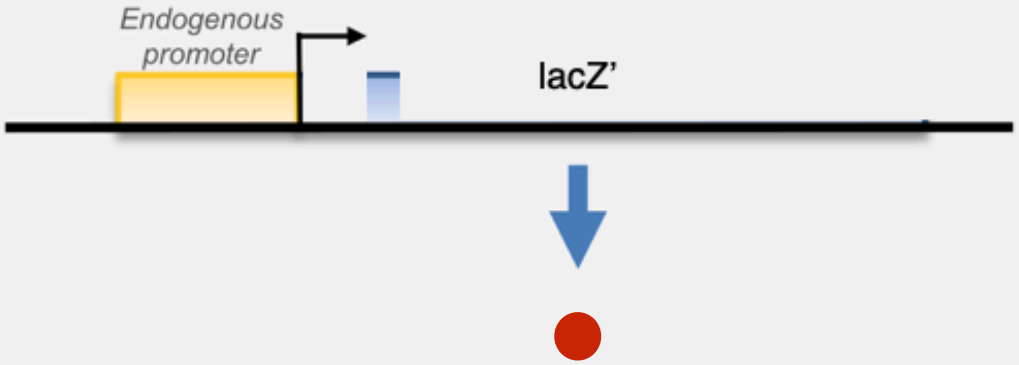


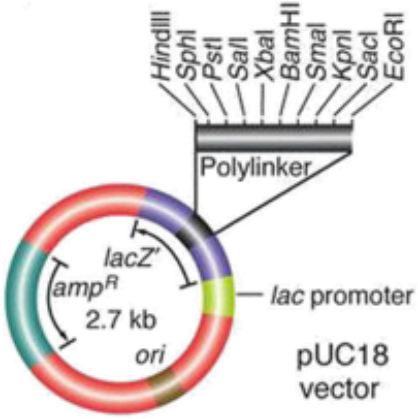
Inactive
ß-Galactosidase

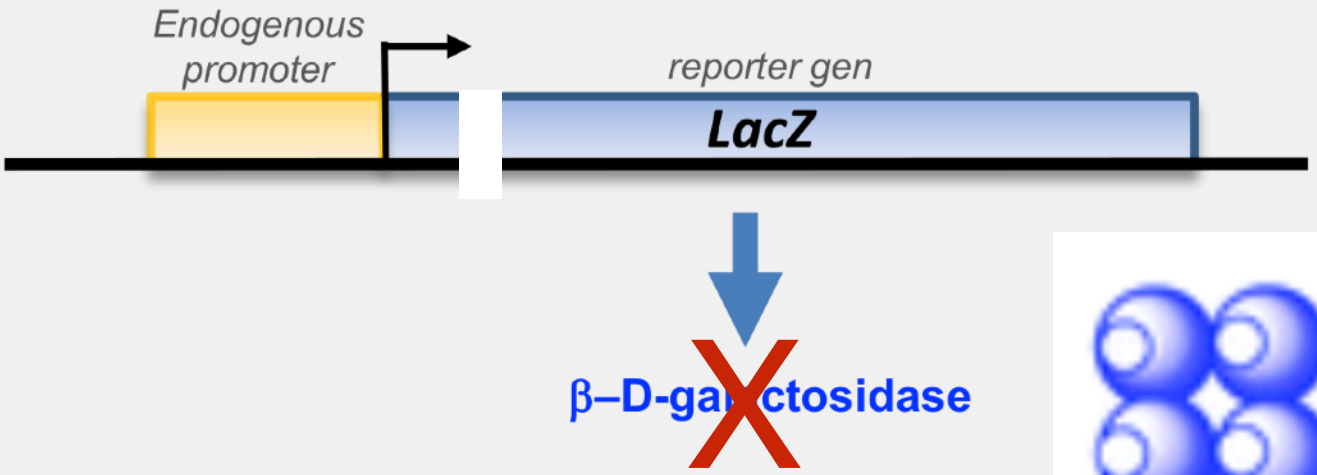


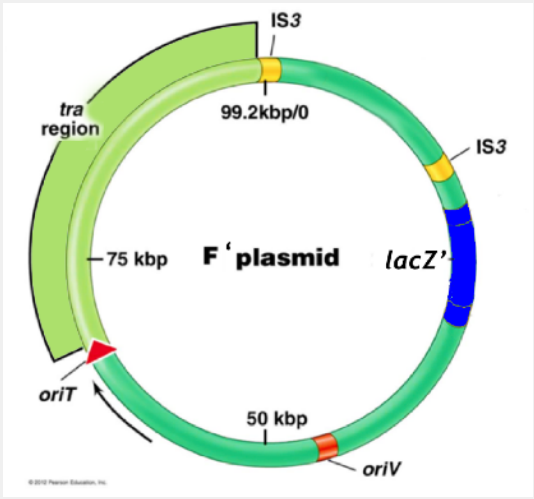


Inactive
ß-Galactosidase

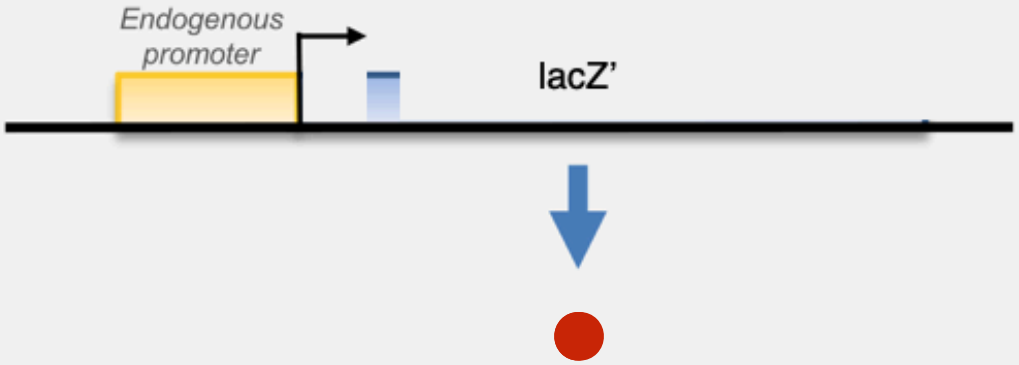


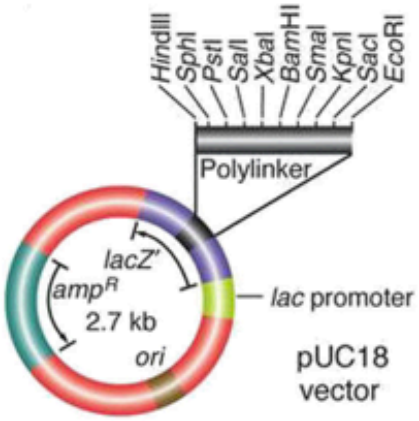


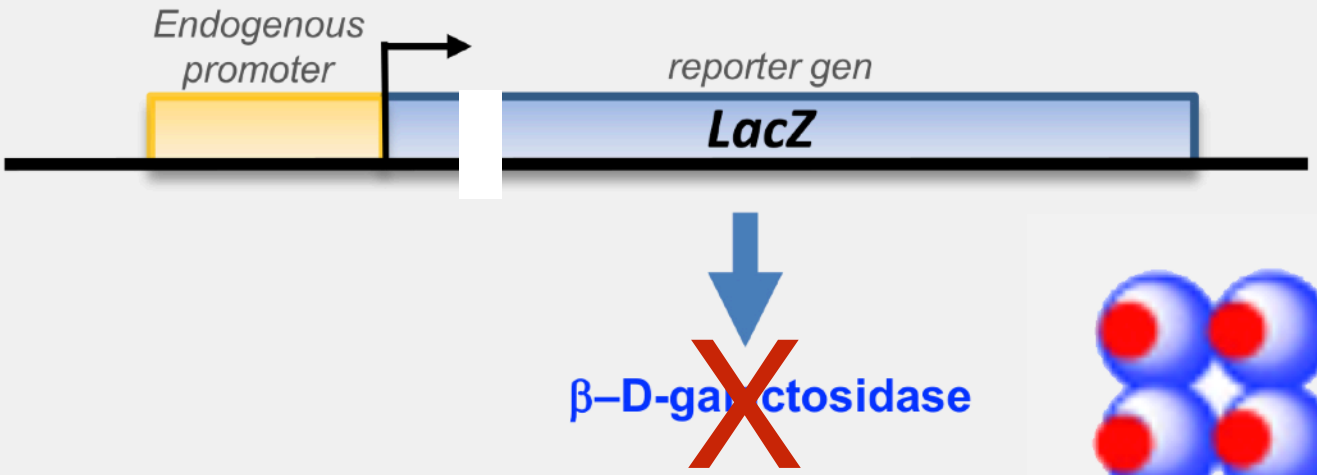


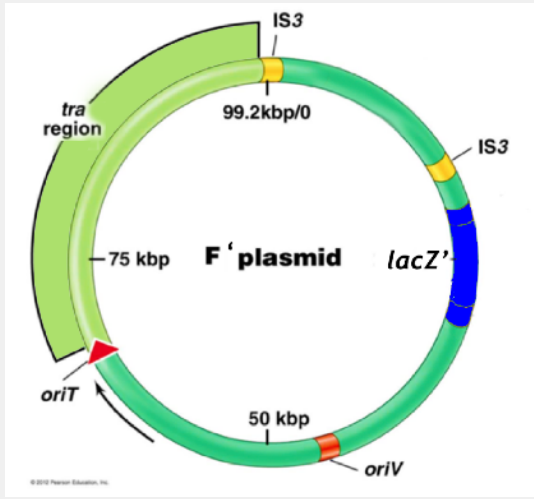


Inactive
ß-Galactosidase



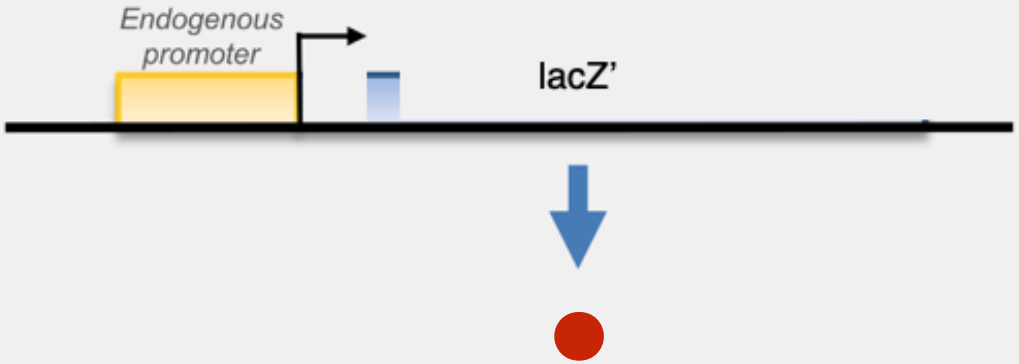


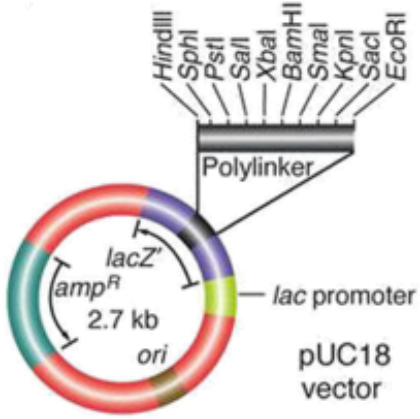


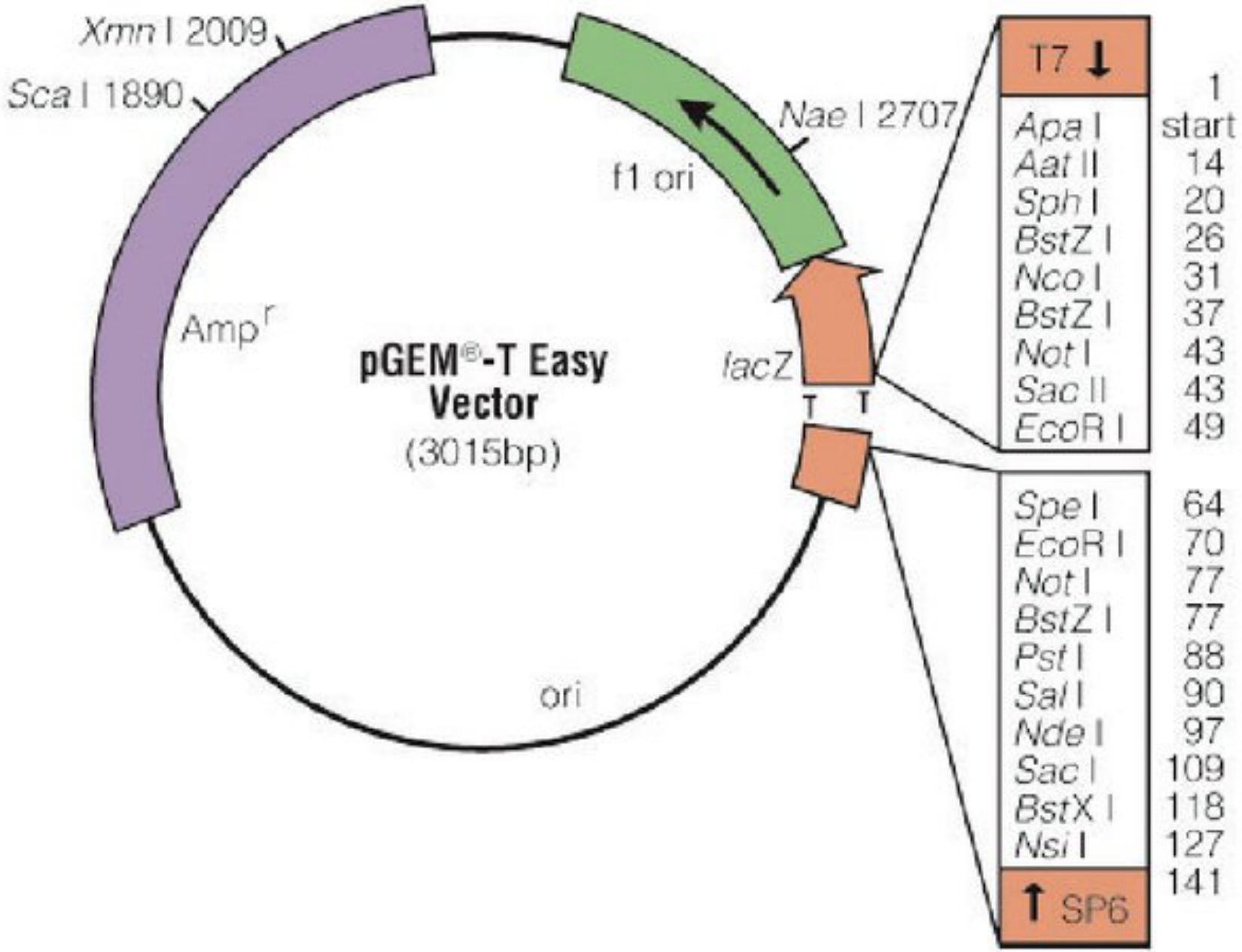


β–D-galactosidase

through alpha - complementation






Xmn I 2009
Sca I 1890
Nae I 2707
f1 ori
Amp^r
pGEM®-T Easy
Vector
(3015bp)
lacZ
T
T
ori
T7
Apa I 1
Aat II 14
Sph I 20
BstZ I 26
Nco I 31
BstZ I 37
Not I 43
Sac II 43
EcoR I 49
Spe I 64
EcoR I 70
Not I 77
BstZ I 77
Pst I 88
Sal I 90
Nde I 97
Sac I 109
BstX I 118
Nsi I 127
SP6 141
start
1473VA05_6B

DNA Sequencing Core Facility

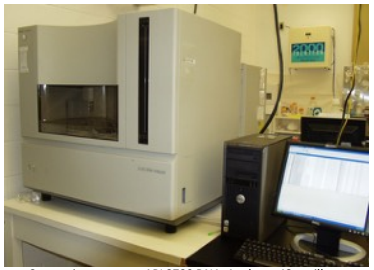
Ping L Jiang (pjiang@gsu.edu) /4044135370

Georgia State University

Principle of DNA sequencing

- Sanger sequencing (dideoxy/enzymatic method)
- Cyclic sequencing, PCR steps, one primer
- Dideoxynucleotides stop the new strand (terminator)
- Fluorescent labeling or **ddNTPs**
- Fragments separated by gel or capillary
- Electrophoresis

Instruments & applications



Current instrument—ABI 3730 DNA, Analyzer, 48 capillary array.
reading *length—750 bp.*

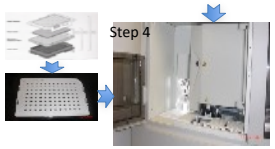
ABI 3100 DNA, Analyzer, 16 capillary array.

Biomek NX
dye and salt removal

Applications
- Plasmid DNA
- PCR amplicons
- BAC/PAC/Cosmid DNA
- Gene walking

What happens to Your DNA

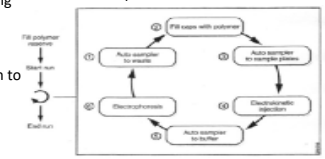
Step1. Sequencing reaction:
– DNA template + BigDye v3.1 (dNTPs, labeled ddNTPs, DNA polymerase) + Sequencing primer (+ DMSO for high GC and repeats) + Sequencing buffer and DD Water



Step 2. Cyclic sequencing
– PCR cycles
– Primer Tm optimization annealing 60ºC for BigDye v3.1.

Step 3. Purification of the reaction to get rid of extra dye and salts.
– Biomek NX CleanSEQ magnetic bead.
– Ethanol Precipitation

Step 4. Sequencing run
– Capillary electrophoresis
– 96 samples in one run



Step 5. After run
– Checking the files with sequencing analysis program.
– Sending the results.

How to read your sequence

Data sent either as a .Seq file (chromatogram) or a .txt file (Text File)

- Chromatogram = trace file
  -when doing sequencing assembly, use trace files!
  -when doing mutation studies, use trace files!
- Text file has everything including the "bad resolution" area
  -usually reliable sequence is 600-750 nucleotides, sometimes more.
  -there might be mistakes in text file, especially places of heterozygotes

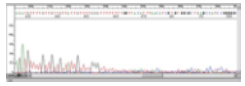
Software to view chromatogram:
Chromas (http://www.technelysium.com.au/chromas.html)
BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html)

Ideal Results

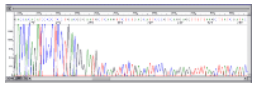
Chrmatogram

Text file

Results that are not so great

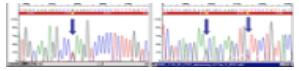


High GT



Possible secondary structure

Insertion, deletion or multiple colonies

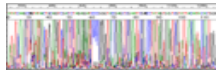


Heterozygotes / multiple colonies

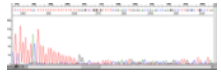
Troubleshooting 1: Template problems

No template

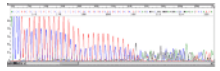
Too little template



Too much template



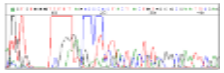
Poly-T



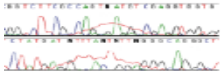
Too many repeats

- Contamination problems
- salt, detergent, ethanol, phenol, chloroform, PCR primer Proteins

Troubleshooting 3: Cleaning problems



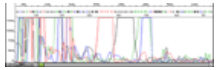
residual ddNTPs after sequencing reaction cleanup



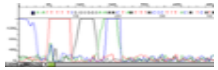
T-blob

G-blob

Troubleshooting 2: Primer problems

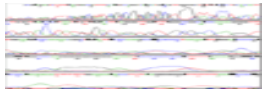


Primer dimer



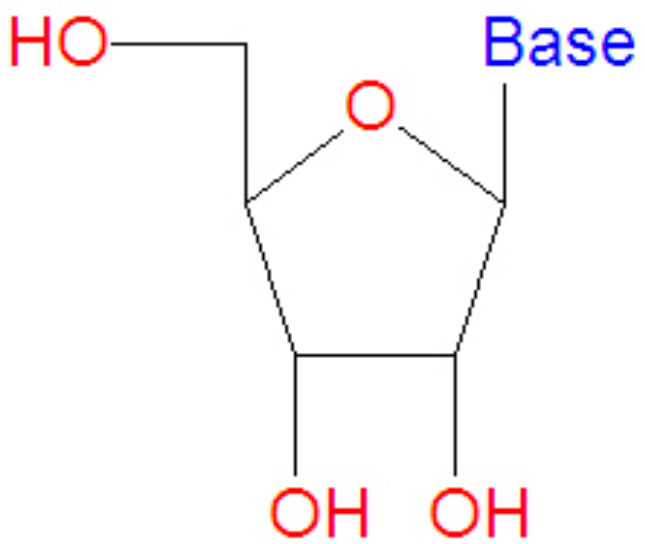
No binding site

Troubleshooting 4: Sequencer problems

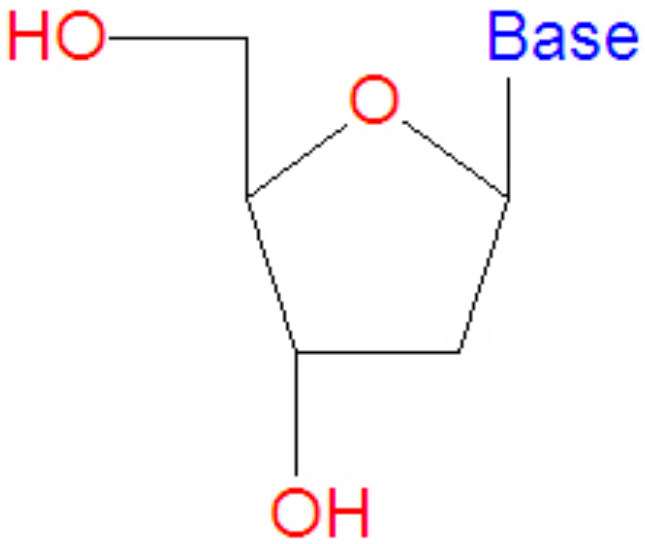


Bad resolution in the capillary

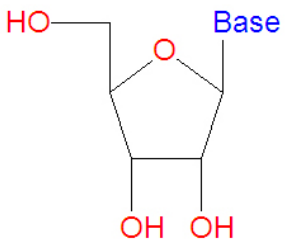
When you identify problems in your results, please discuss possible solutions with us.



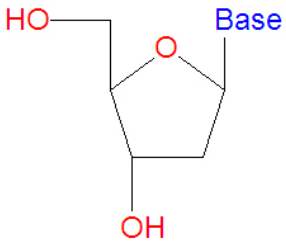
Ribonucleic acid (RNA)
Base= A, C, G or T



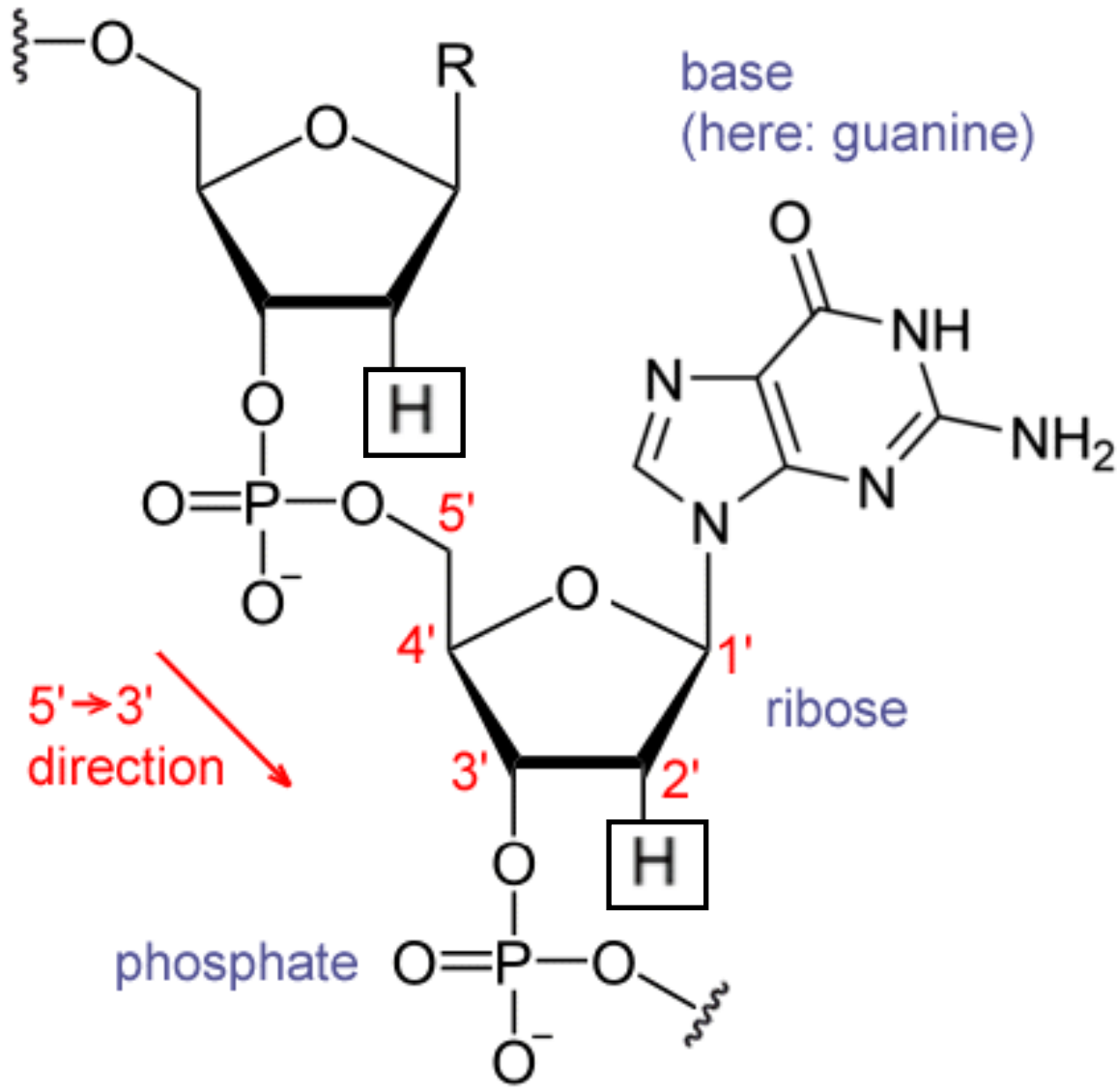
Deoxyribonucleic acid (DNA)
Base = A, C, G or U



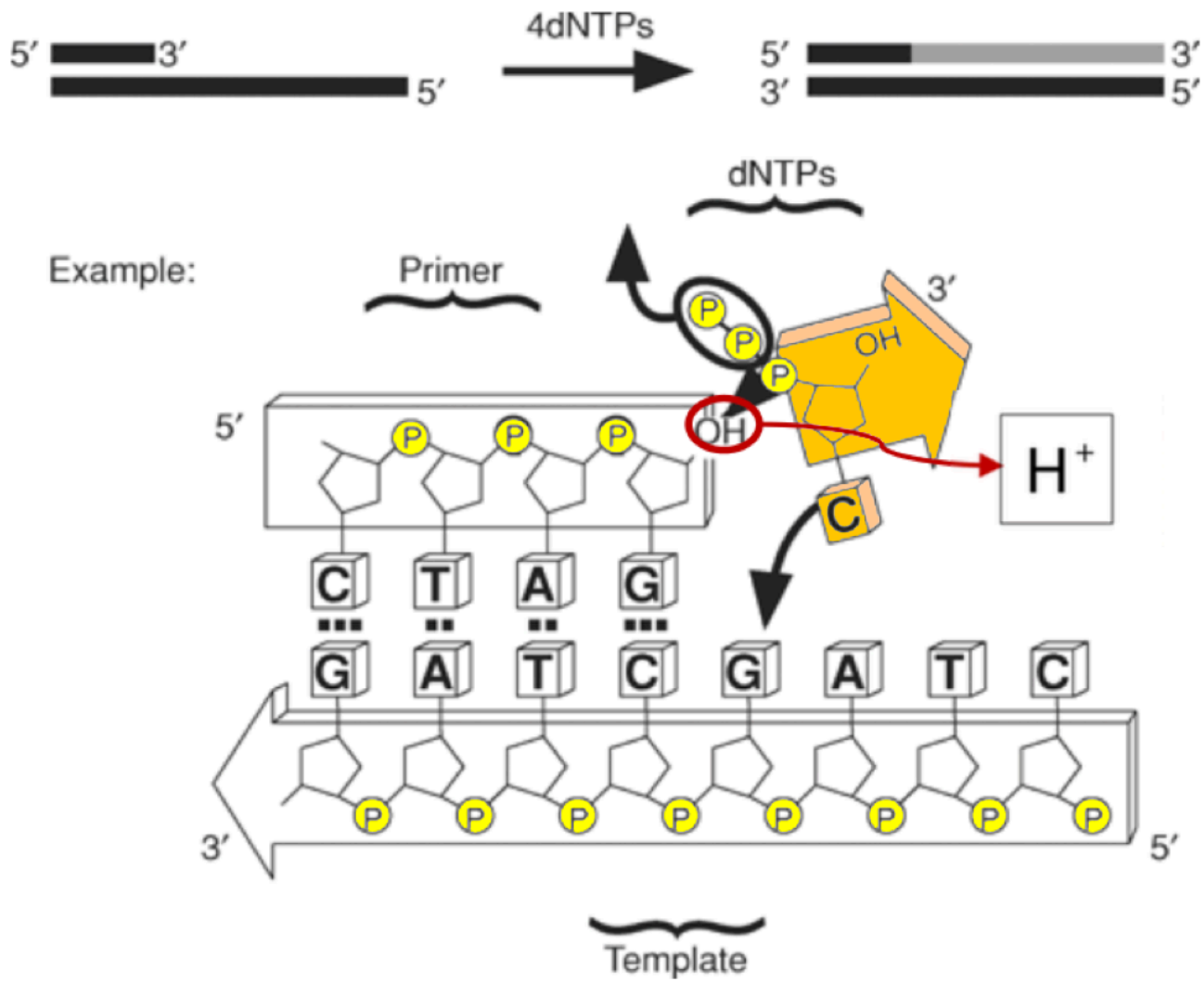
Ribonucleic acid (RNA)
Base= A, C, G or T

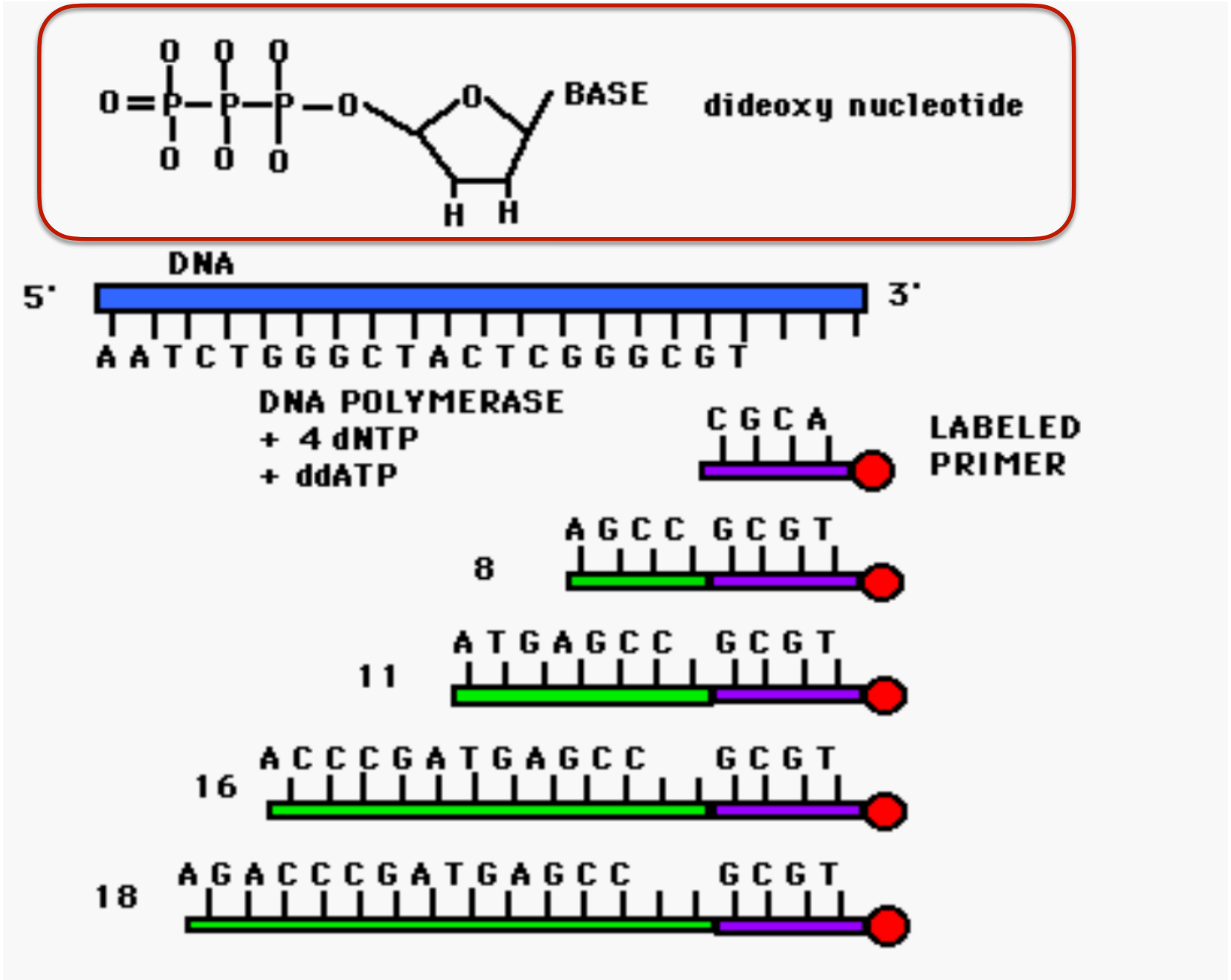


Deoxyribonucleic acid (DNA)
Base = A, C, G or U



Simple, Natural Chemistry

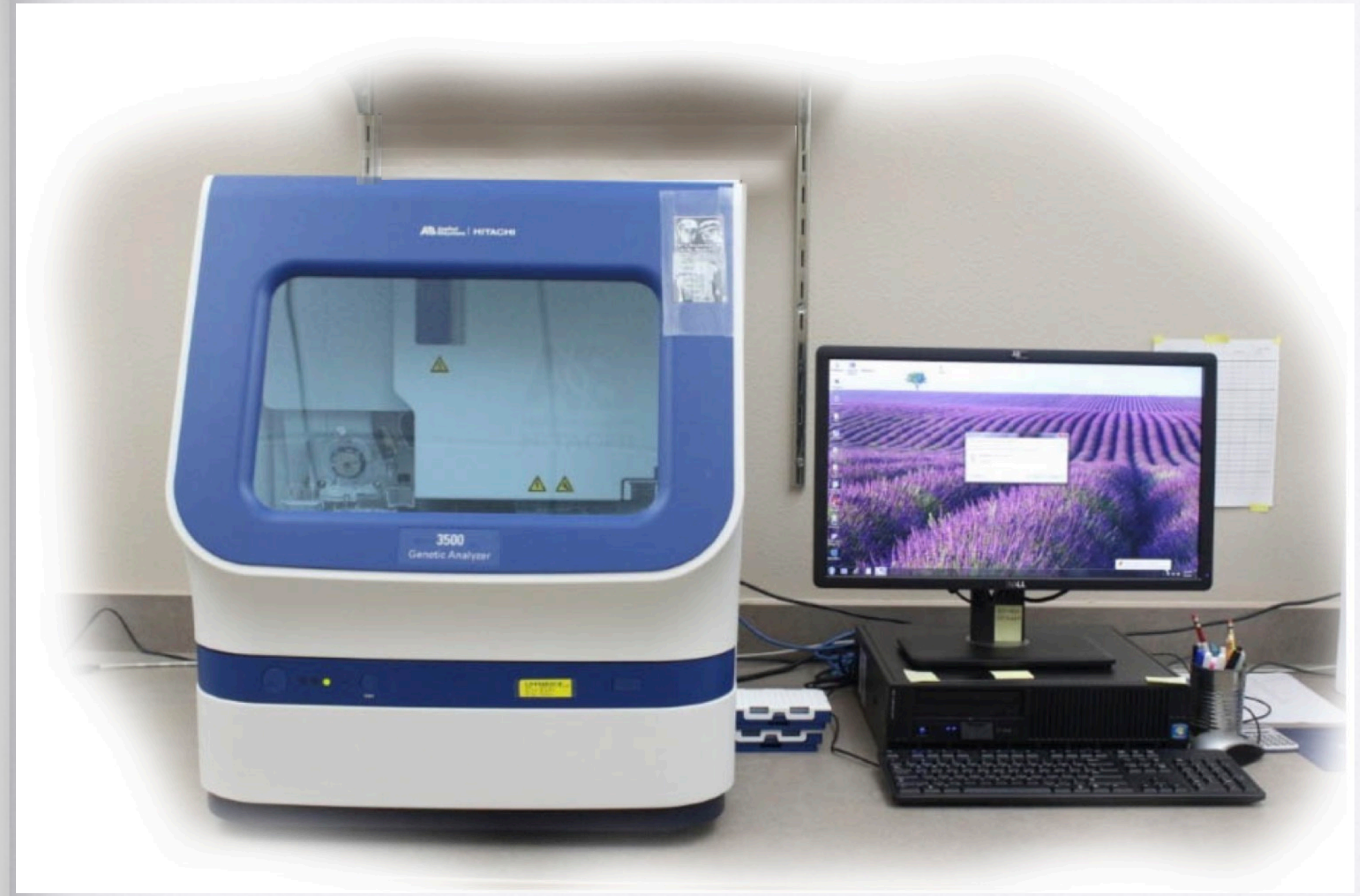



BASE
dideoxy nucleotide
DNA
5'
3'
A A T C T G G G C T A C T C G G G C G T
DNA POLYMERASE
+ 4 dNTP
+ ddATP
C G C A
LABELED
PRIMER
8
A G C C G C G T
11
A T G A G C C G C G T
16
A C C C G A T G A G C C G C G T
18
A G A C C C G A T G A G C C G C G T

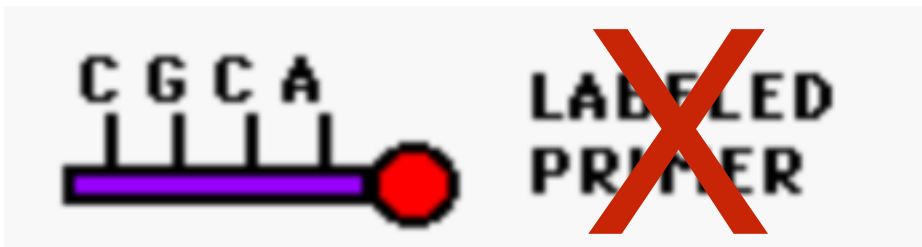
DNA sequencing

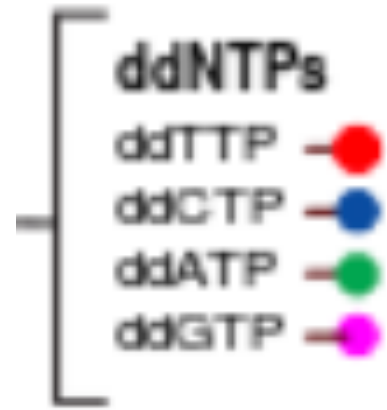
The dideoxy approach

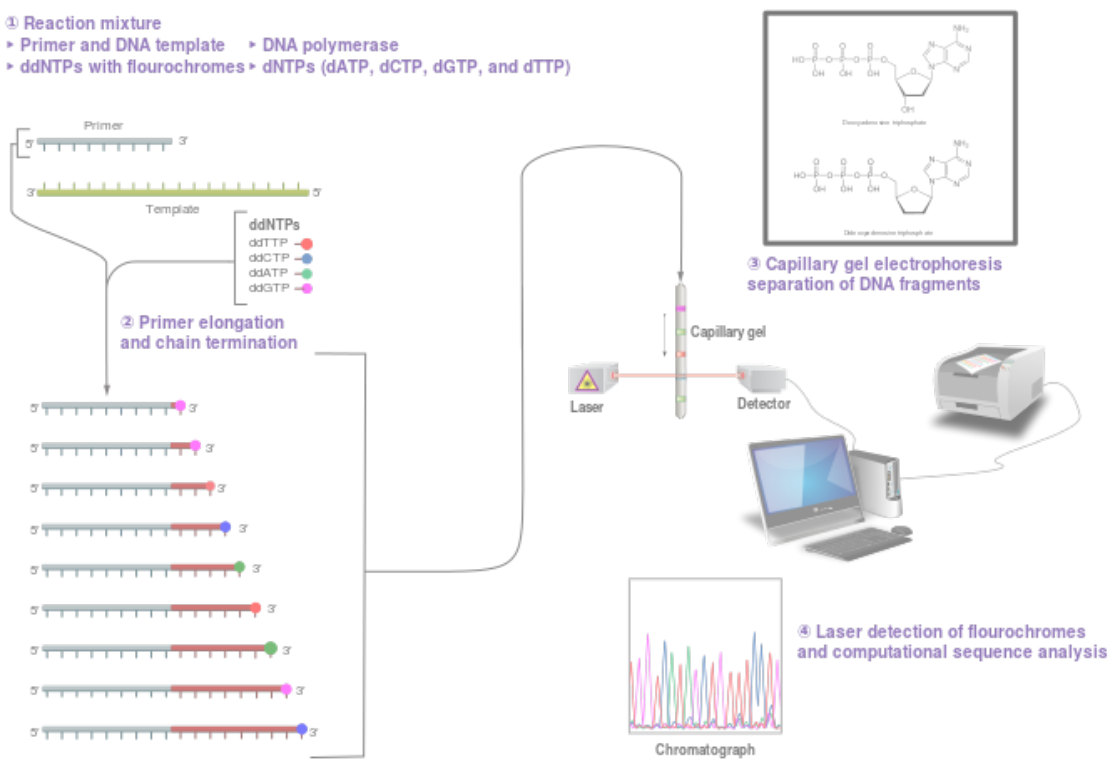
https://www.youtube.com/watch?v=bEFLBf5WEtc



DNA Genetic Analysis / Sequencing
(ABI/ *Life technologies*) Model 3500xl

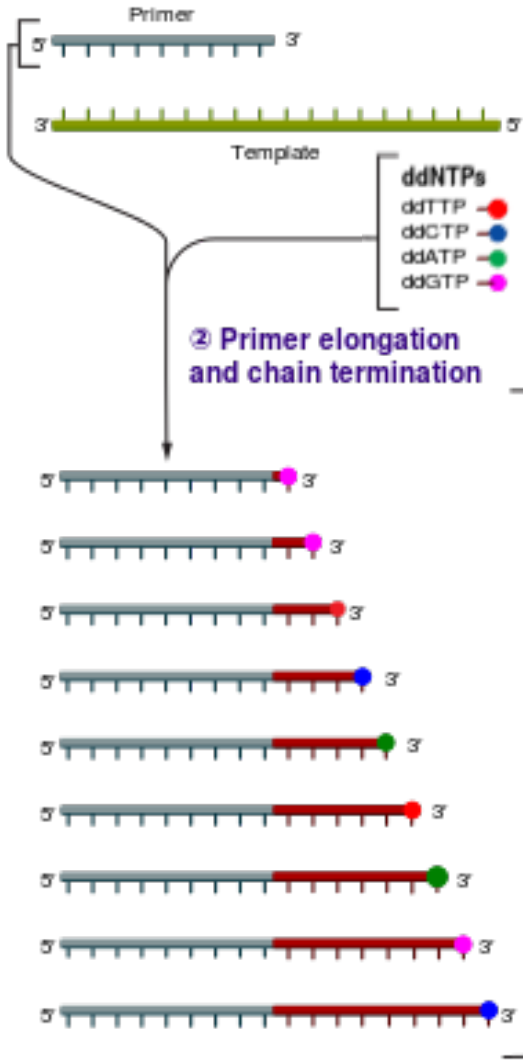

C G C A
LABELED
PRIMER


ddNTPs
ddTTP
ddCTP
ddATP
ddGTP

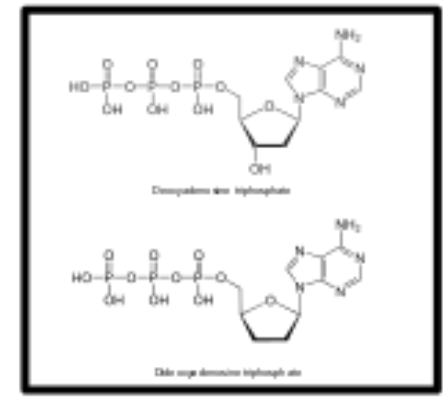

① Reaction mixture
‣ Primer and DNA template
‣ DNA polymerase
‣ ddNTPs with flourochromes
‣ dNTPs (dATP, dCTP, dGTP, and dTTP)
Primer
Template
ddNTPs
ddTTP
ddCTP
ddATP
ddGTP
② Primer elongation and chain termination
③ Capillary gel electrophoresis separation of DNA fragments
Capillary gel
Laser
Detector
④ Laser detection of flourochromes and computational sequence analysis
Chromatograph

① Reaction mixture

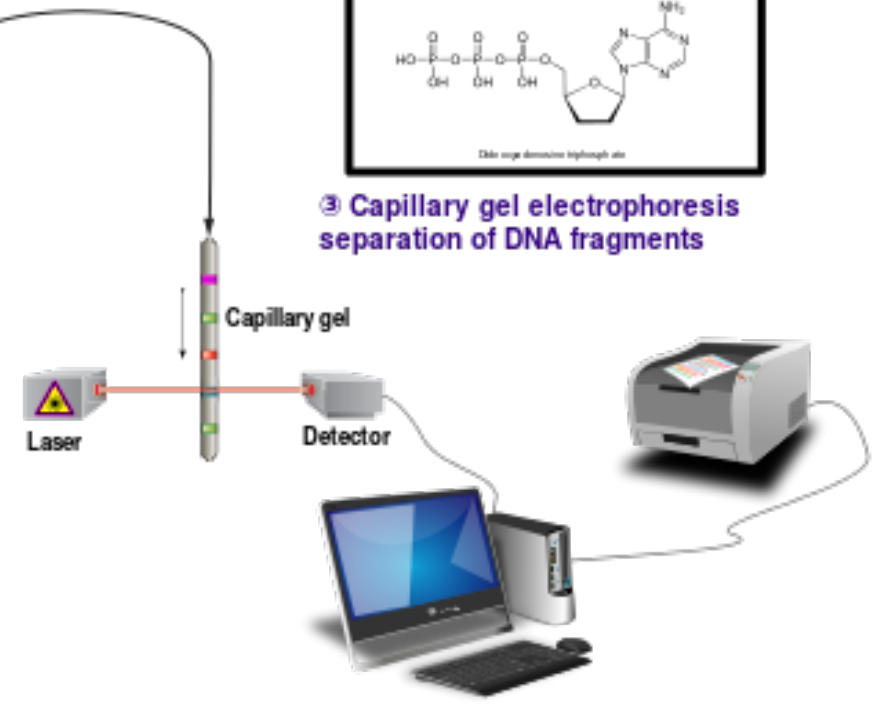
- Primer and DNA template
- DNA polymerase
- ddNTPs with flourochromes
- dNTPs (dATP, dCTP, dGTP, and dTTP)

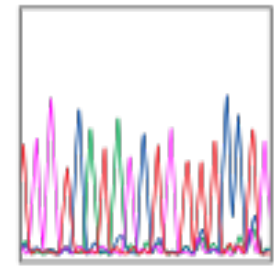


② Primer elongation and chain termination

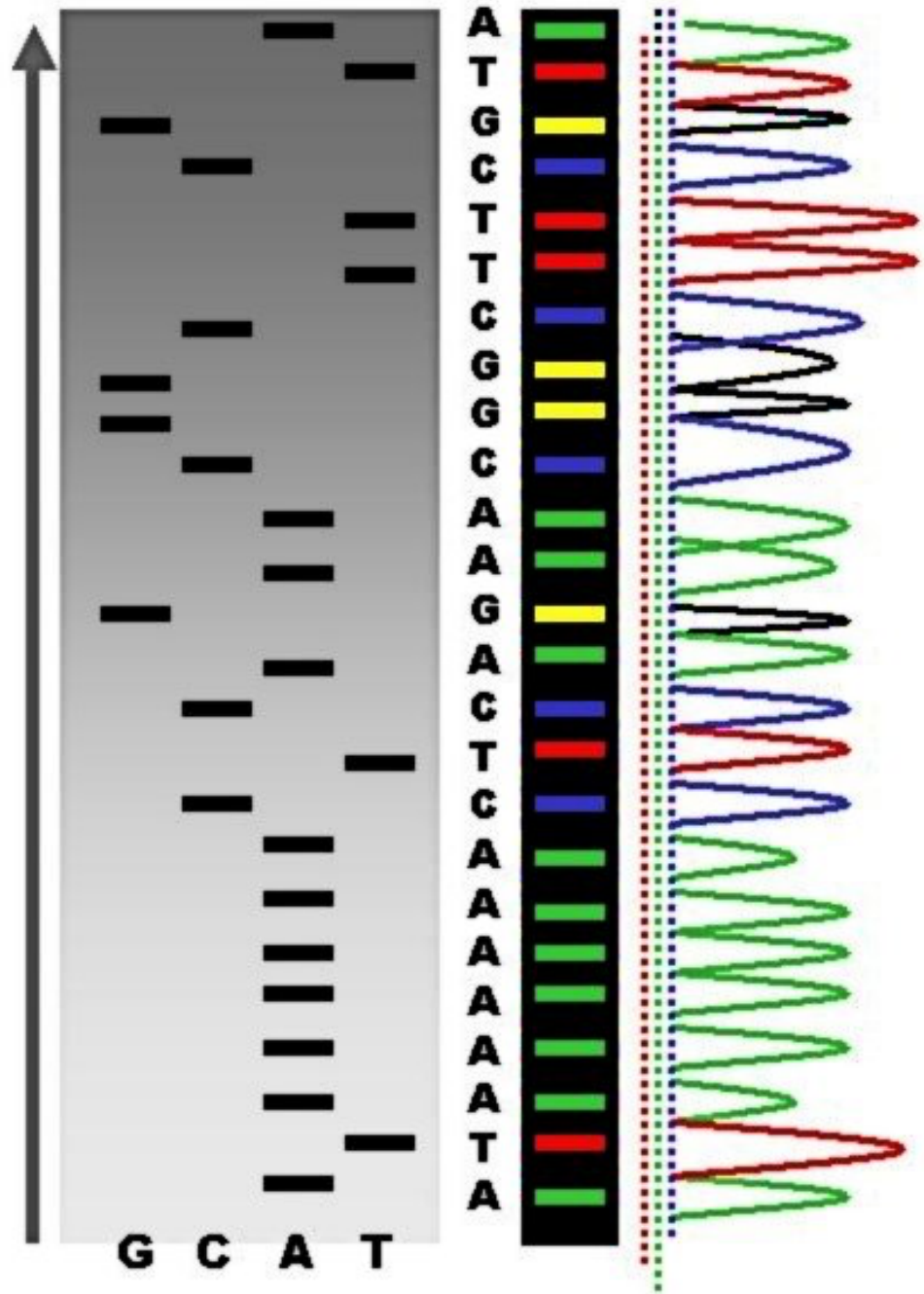


③ Capillary gel electrophoresis separation of DNA fragments





④ Laser detection of flourochromes and computational sequence analysis


G C A T
A T G C T T C G G C A A G A C T C A A A A A A A T A

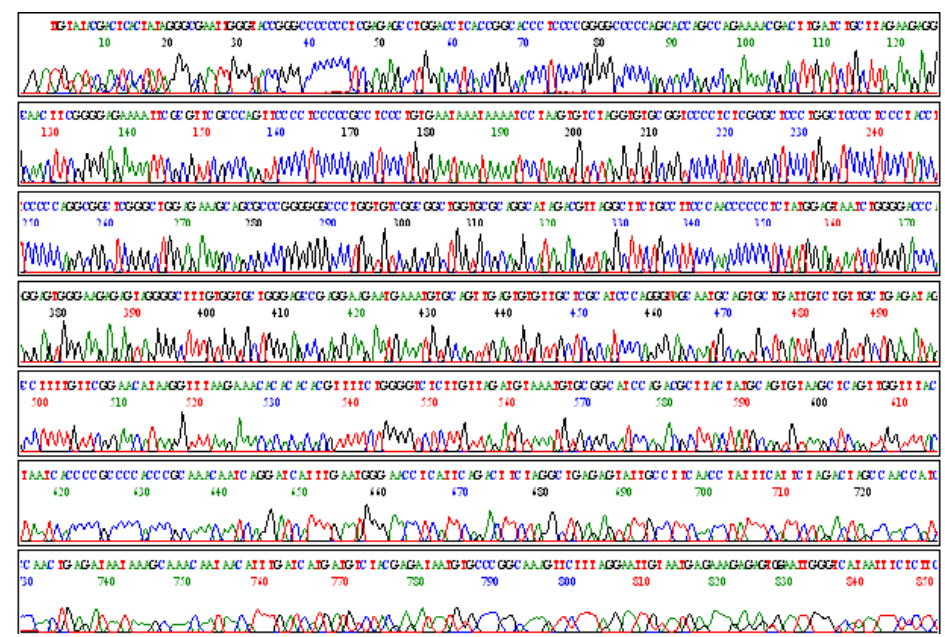
Sanger Sequencing

- Ideal for single gene assays
- Target gene candidates

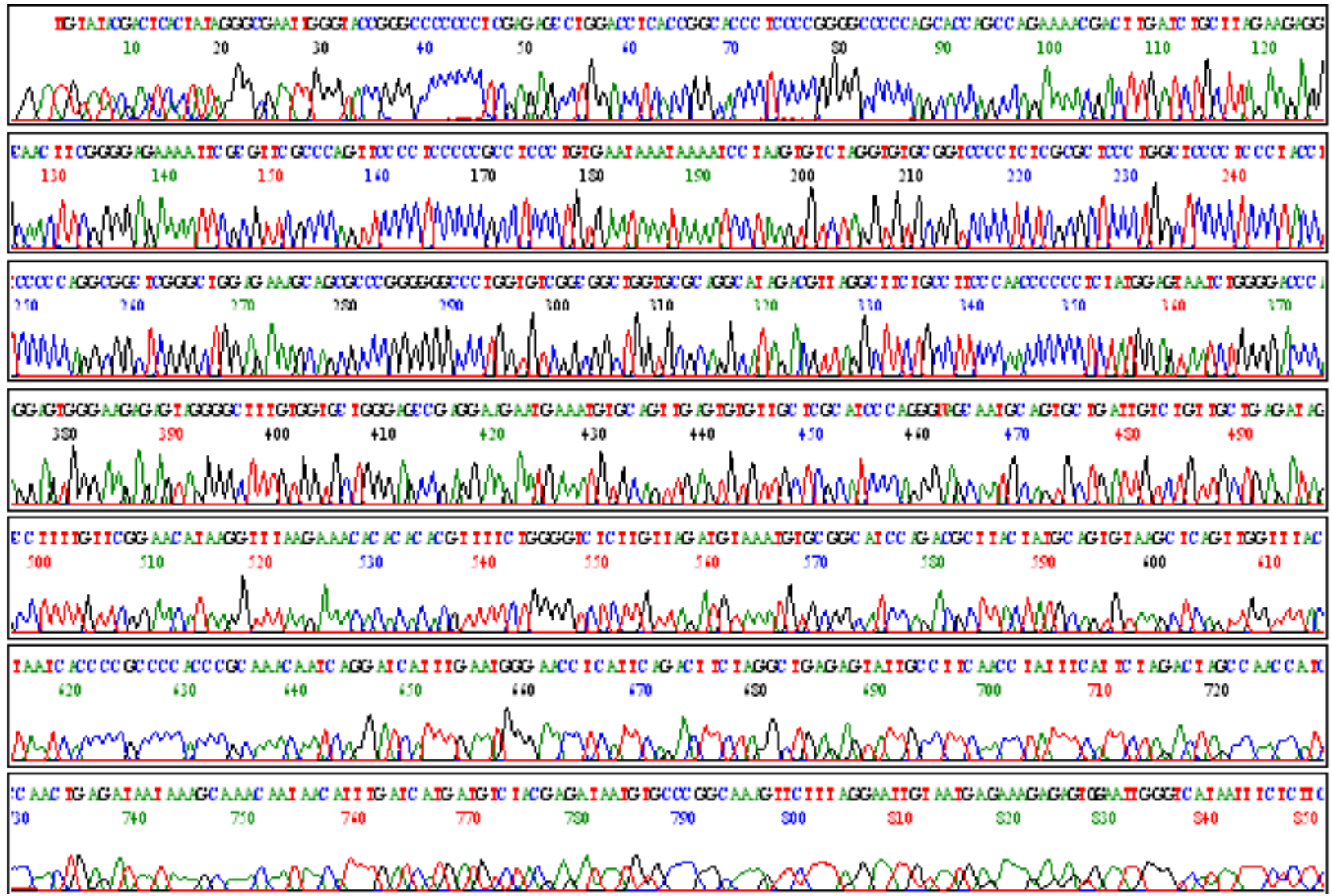
Single-gene (few amplicons)

p53

- Few amplicons, few samples
- Bidirectional sequencing
- Can be used to confirm variants from PGM



life technologies



Sanger Sequencing

- Ideal for single gene assays
- Target gene candidates

Single-gene (few amplicons)

p53

- Few amplicons, few samples
- Bidirectional sequencing
- Can be used to confirm variants from PGM

life technologies

Automated Sanger Sequencing using the 3500xl Genetic Analyzer

Last Updated: Monday, 14 April 2003, 16:01 GMT 17:01 UK

E-mail this to a friend Printable version

Human genome finally complete

By Ivan Noble
BBC News Online science staff

The biological code crackers sequencing the human genome have said they have finished the job - two years ahead of schedule.

Their announcement came less than three years after a "rough draft" was published to worldwide acclaim.

Decoding using the power of robotics and computers (*Image by The Wellcome Trust Sanger Institute*)

When UK Prime Minister Tony Blair and then US President Bill Clinton hailed the publication of the draft in June 2000, 97% of the "book of life" had been read.

The decoding is now close to 100% complete. The remaining tiny gaps are considered too costly to fill and those in charge of turning genomic data into medical and scientific progress have plenty to be getting on with.

The Wellcome Trust Sanger Institute, the only British institution taking part in the international effort, completed almost a third of the sequence - the biggest contribution by a single institution.

VIDEO AND AUDIO NEWS

The BBC's Sue Nelson
"British scientists contributed almost one third of the human genome"

Watch

SEE ALSO

- 'Secret of life' discovery turns 50
  27 Feb 03 | Science/Nature
- DNA databases 'no use to terrorists'
  15 Jan 03 | Science/Nature
- Mouse clues to human genetics
  04 Dec 02 | Science/Nature
- Genome man to create new life
  21 Nov 02 | Science/Nature
- Scientists crack human code
  26 Jun 00 | Science/Nature

RELATED INTERNET LINKS

- DNA 1953-2003
- Nature: 50 years of DNA
- Wellcome Trust Sanger Institute
- NIH National Human Genome Research Institute
- Celera Genomics

The BBC is not responsible for the content of external internet sites

TOP SCIENCE & ENVIRONMENT STORIES


397-387