



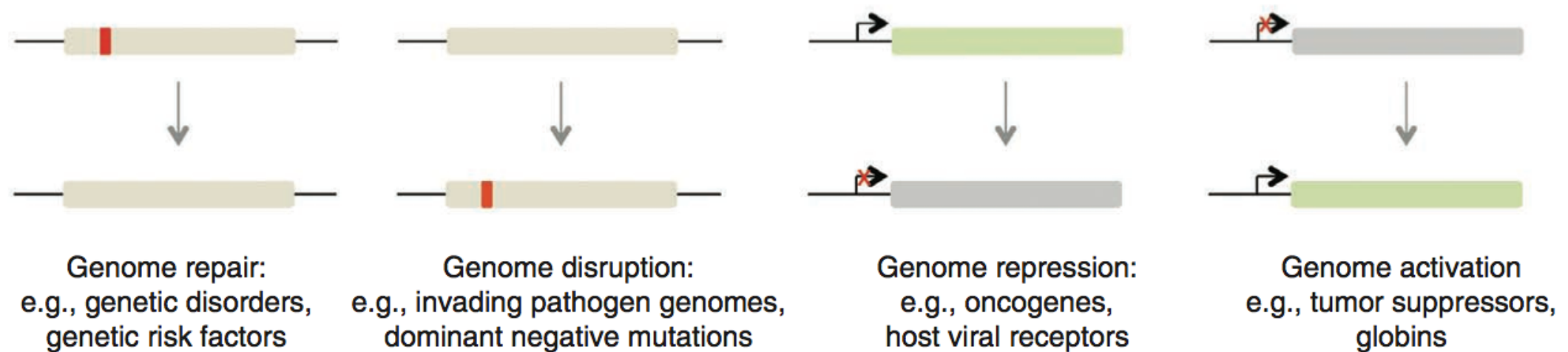
Genetic Manipulation

Samuel Wagner



Principle applications

- Cloning of genes for protein production
- Mutagenesis of organisms
 - Deletions
 - Point mutants
 - Insertions
 - Chimeras
- Reprogramming of gene expression
- Gene-therapy





Part I: Molecular basics

Avery–MacLeod–McCarty experiment

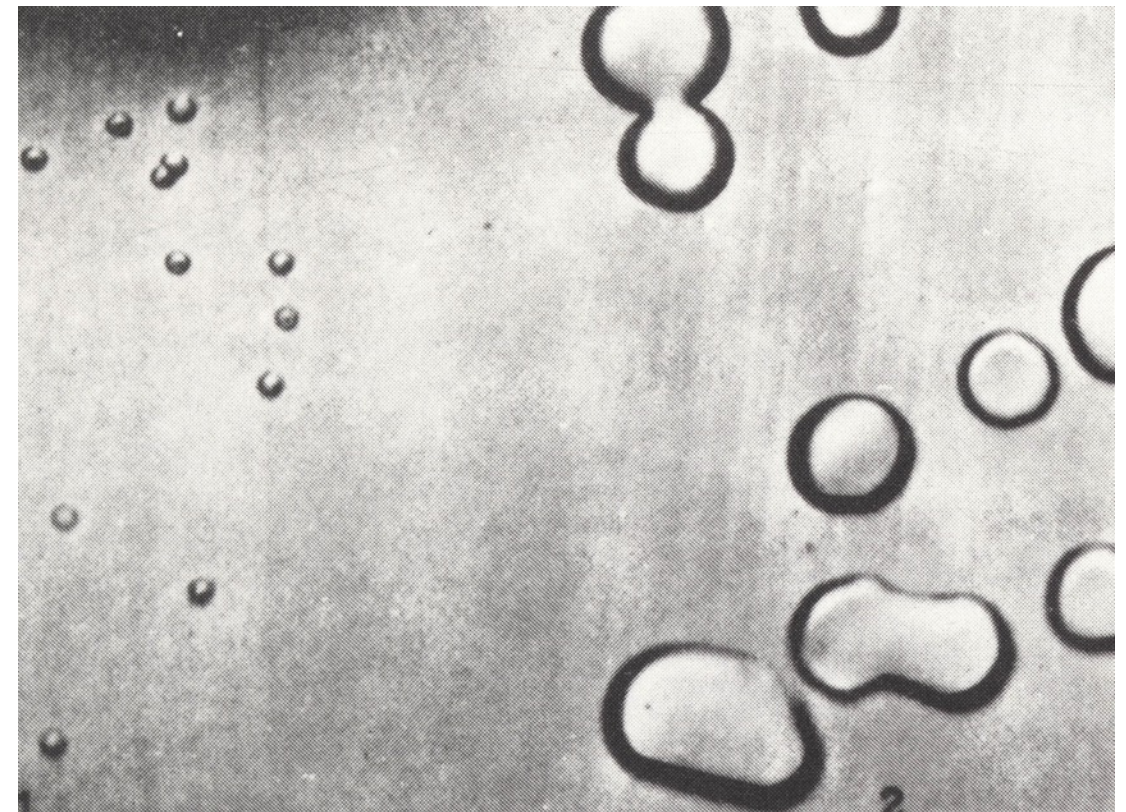
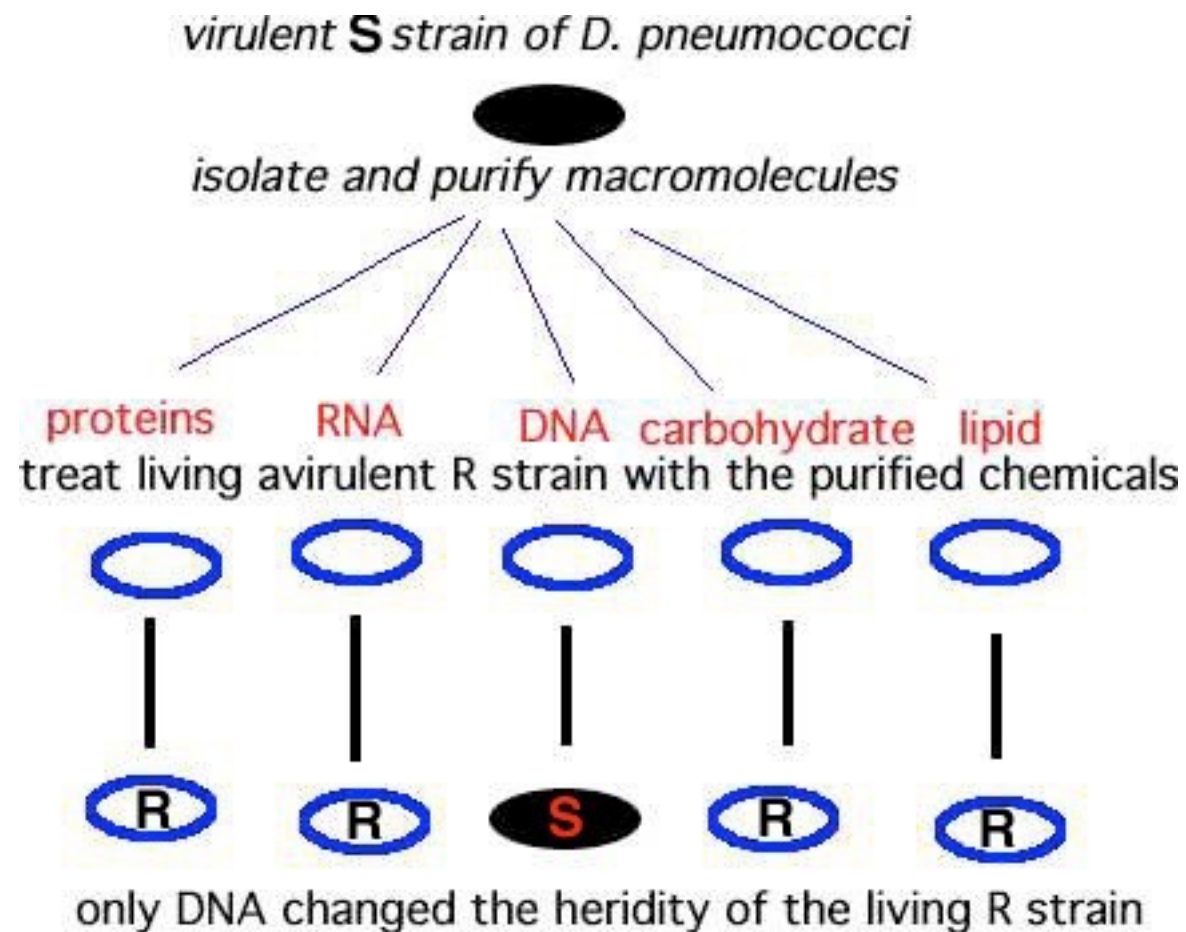


figure: J Exp Med (1944) 79:158

This is naturally occurring genetic “auto-manipulation”

Genetic elements

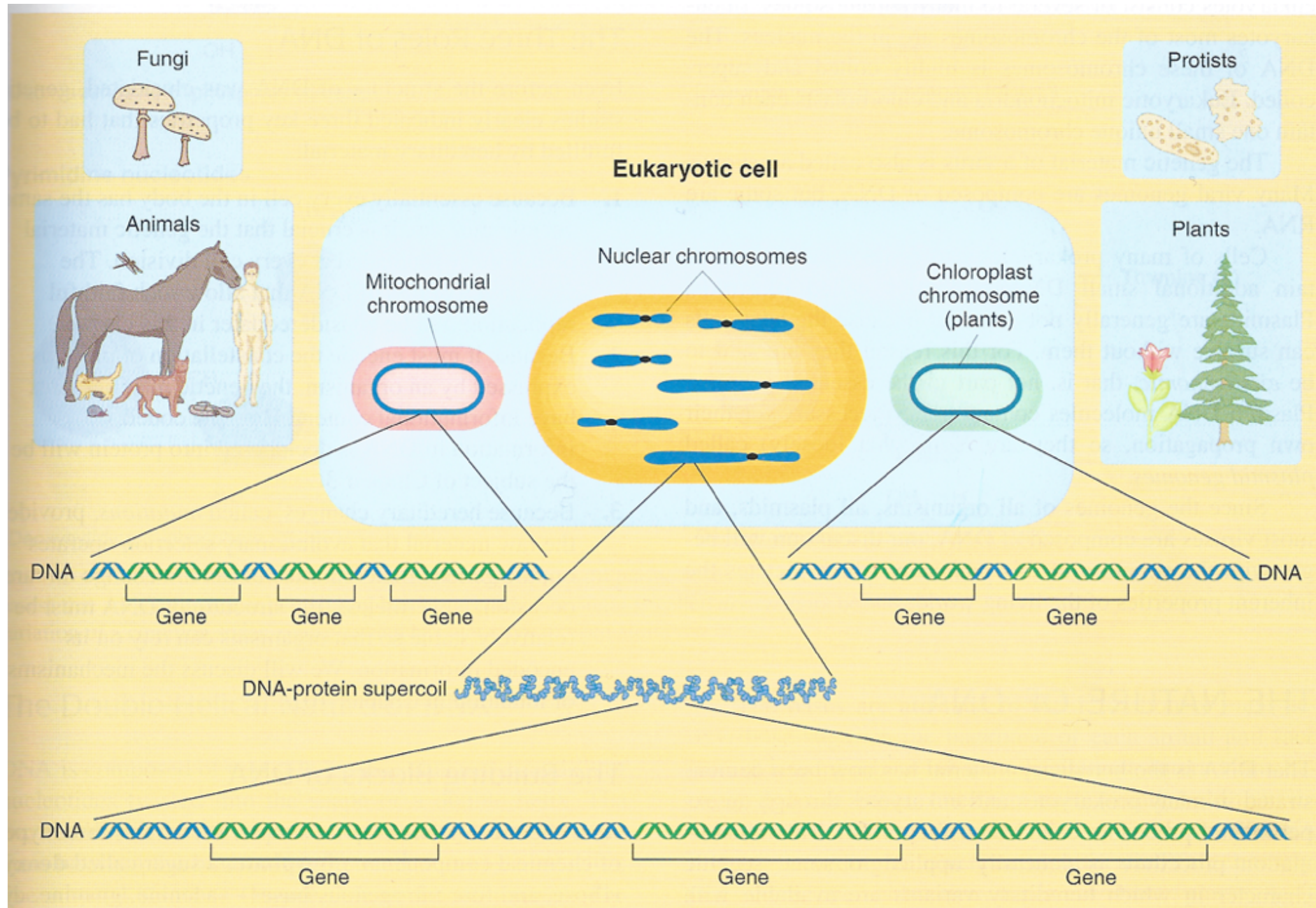
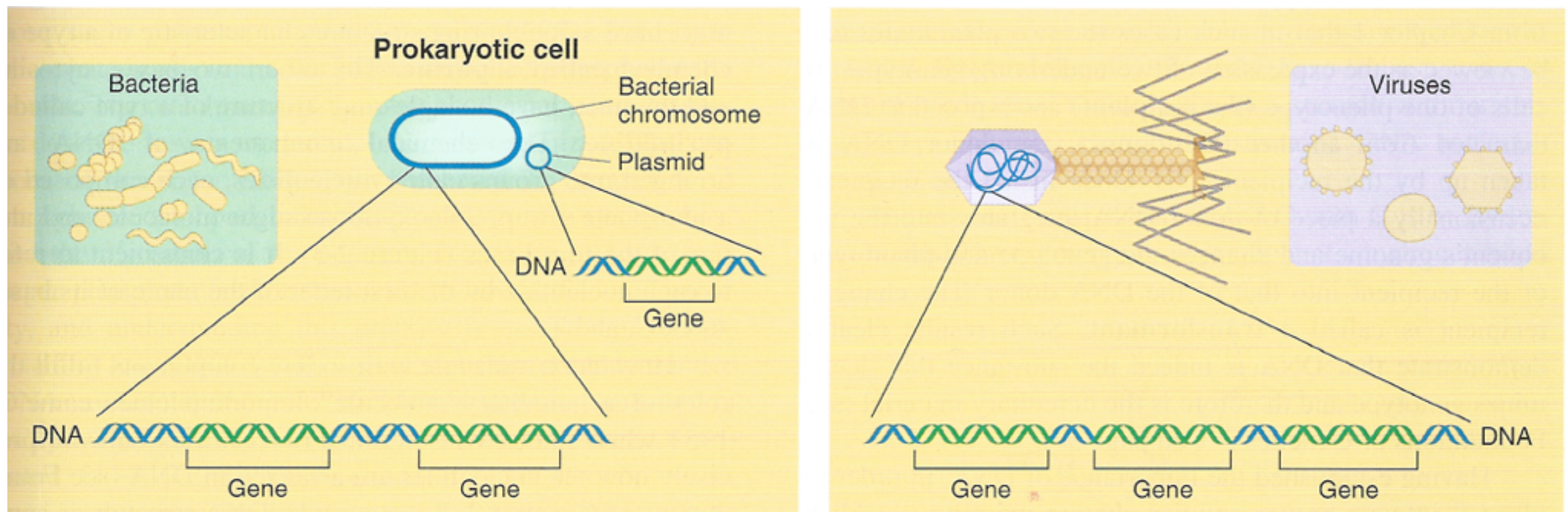


figure: Modern Genetic Analysis Fig 2-2

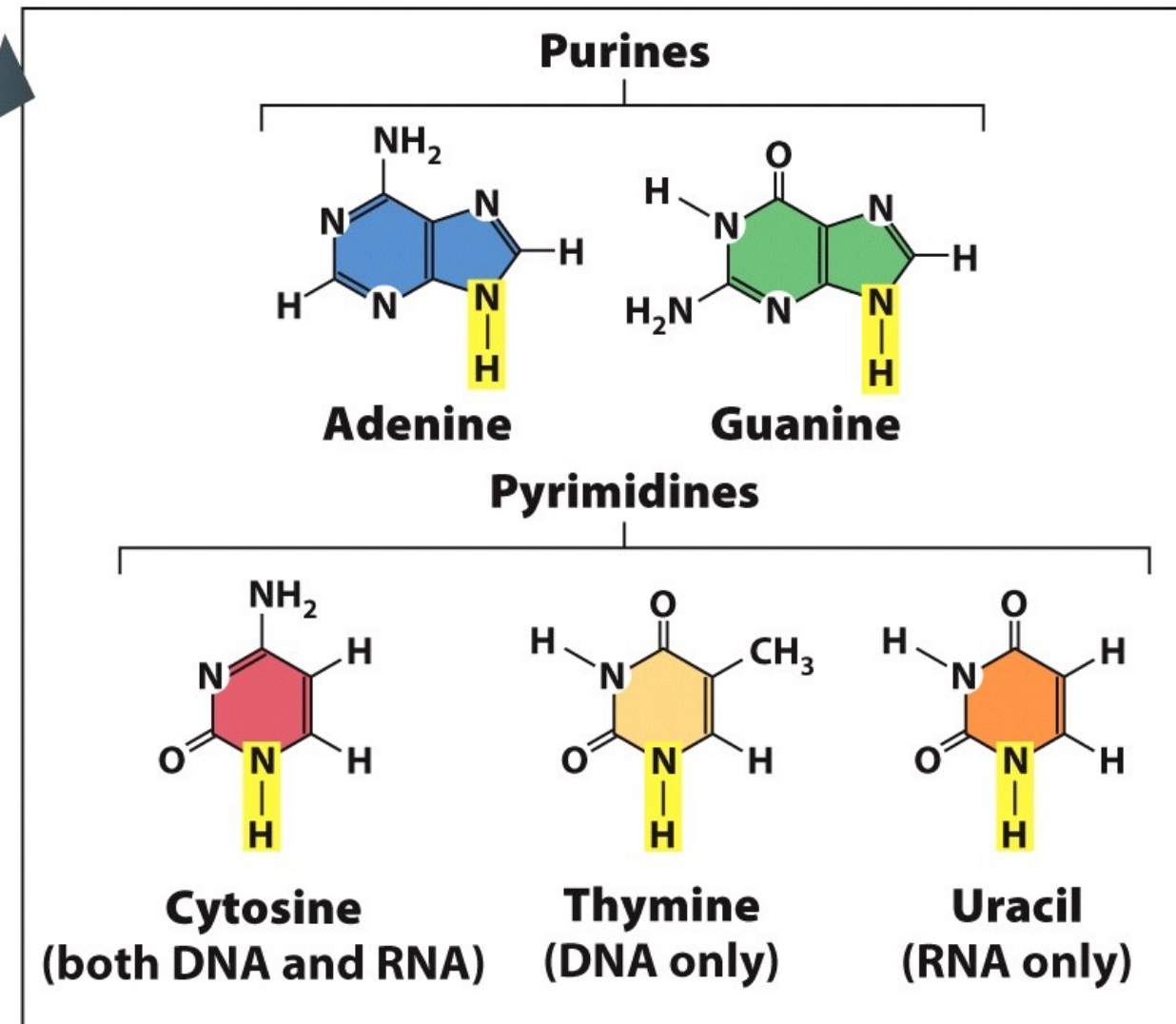
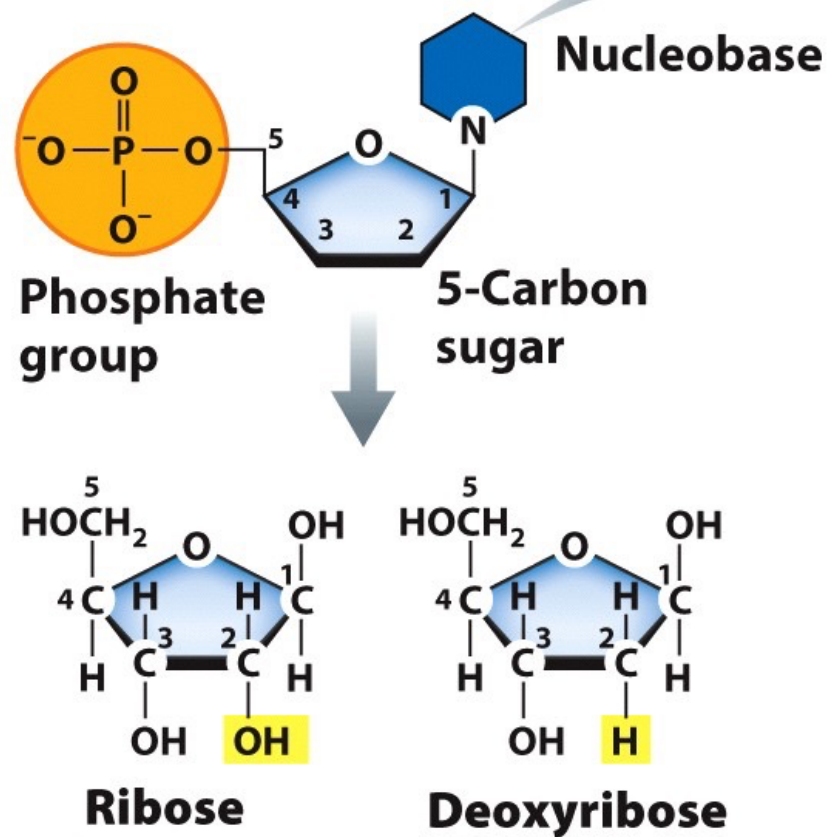
Genetic elements



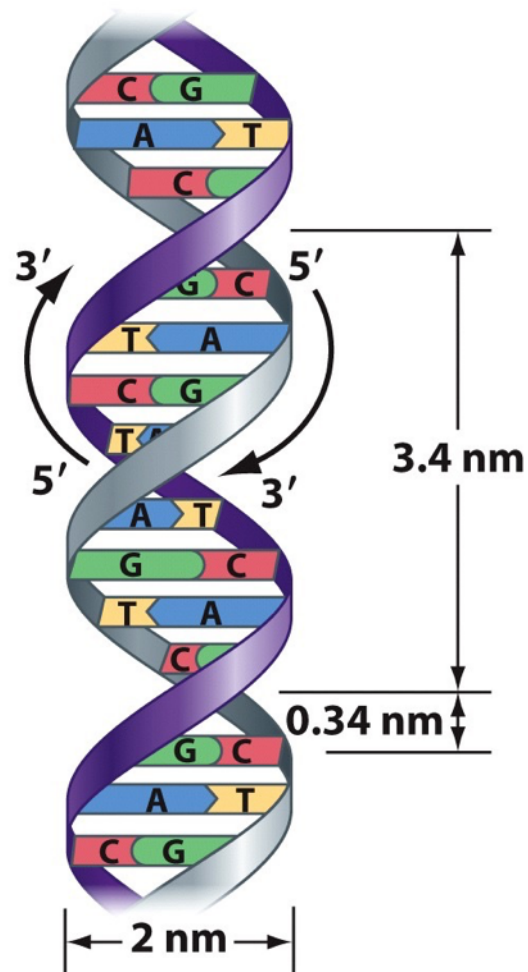
Nucleotide and DNA structure

Nucleotide and DNA structure

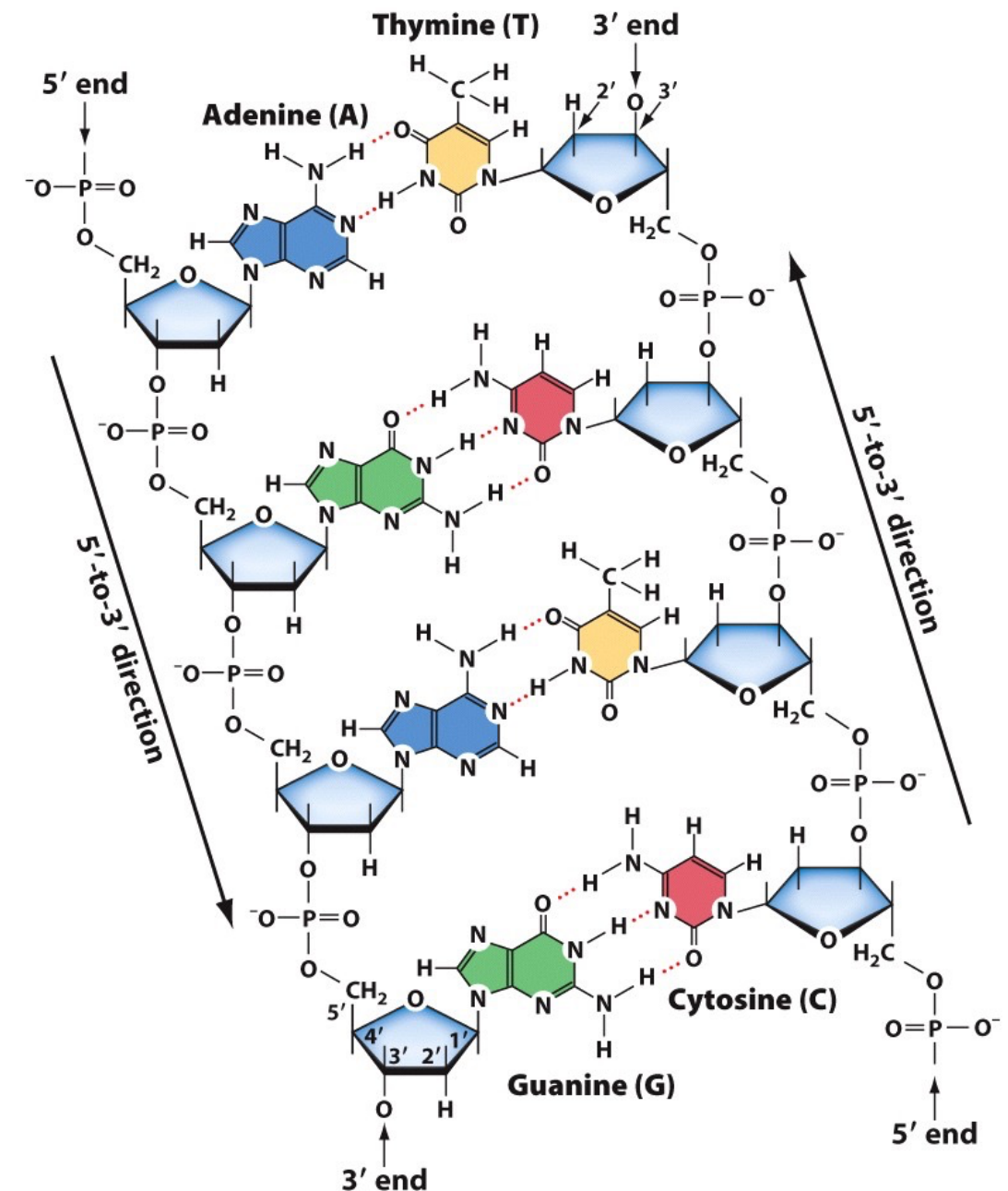
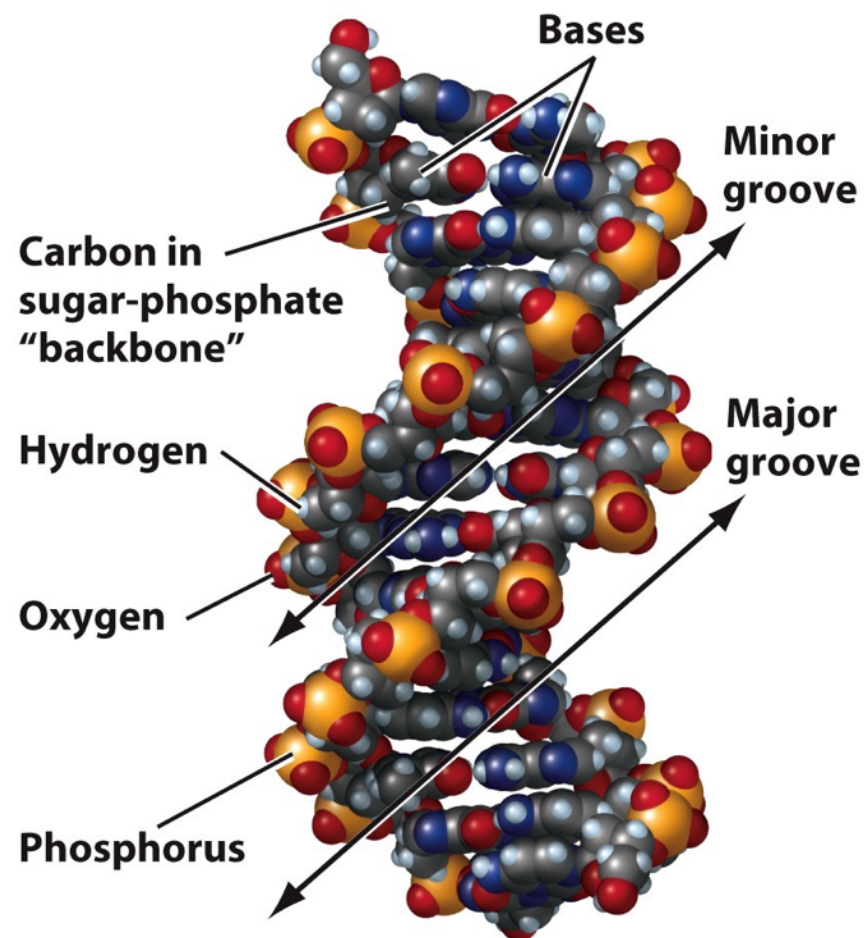
Nucleotide



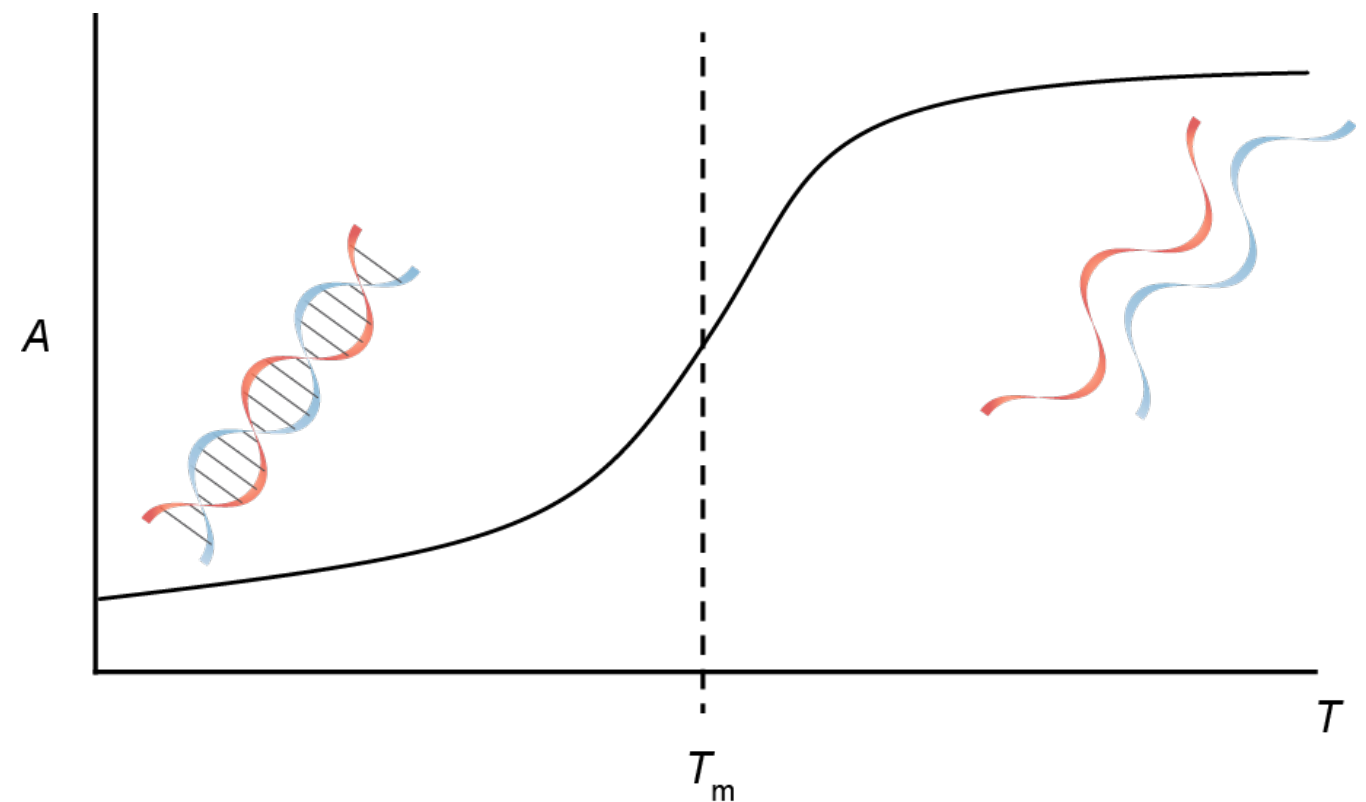
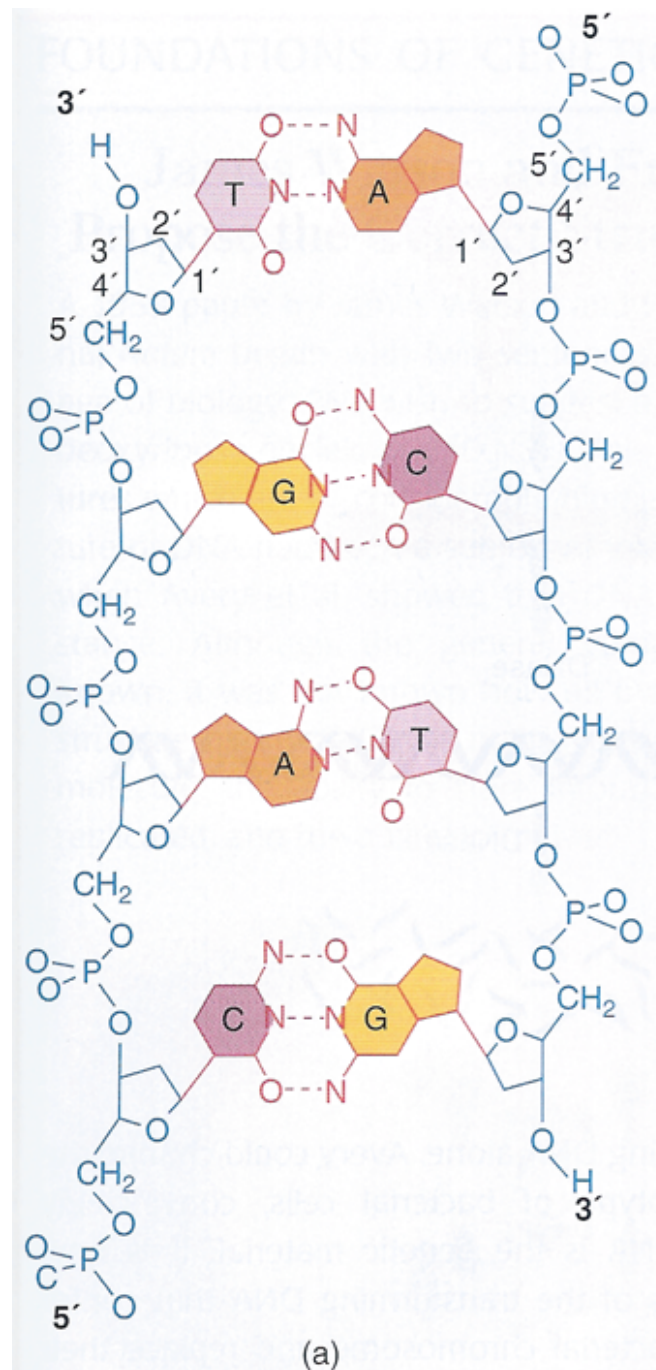
Nucleotide and DNA structure



Microbiology: An Evolving Science, Third Edition Figure A1.16
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Melting of DNA



rule-of-thumb for T_m : 4°C for G/C, 2°C for A/T

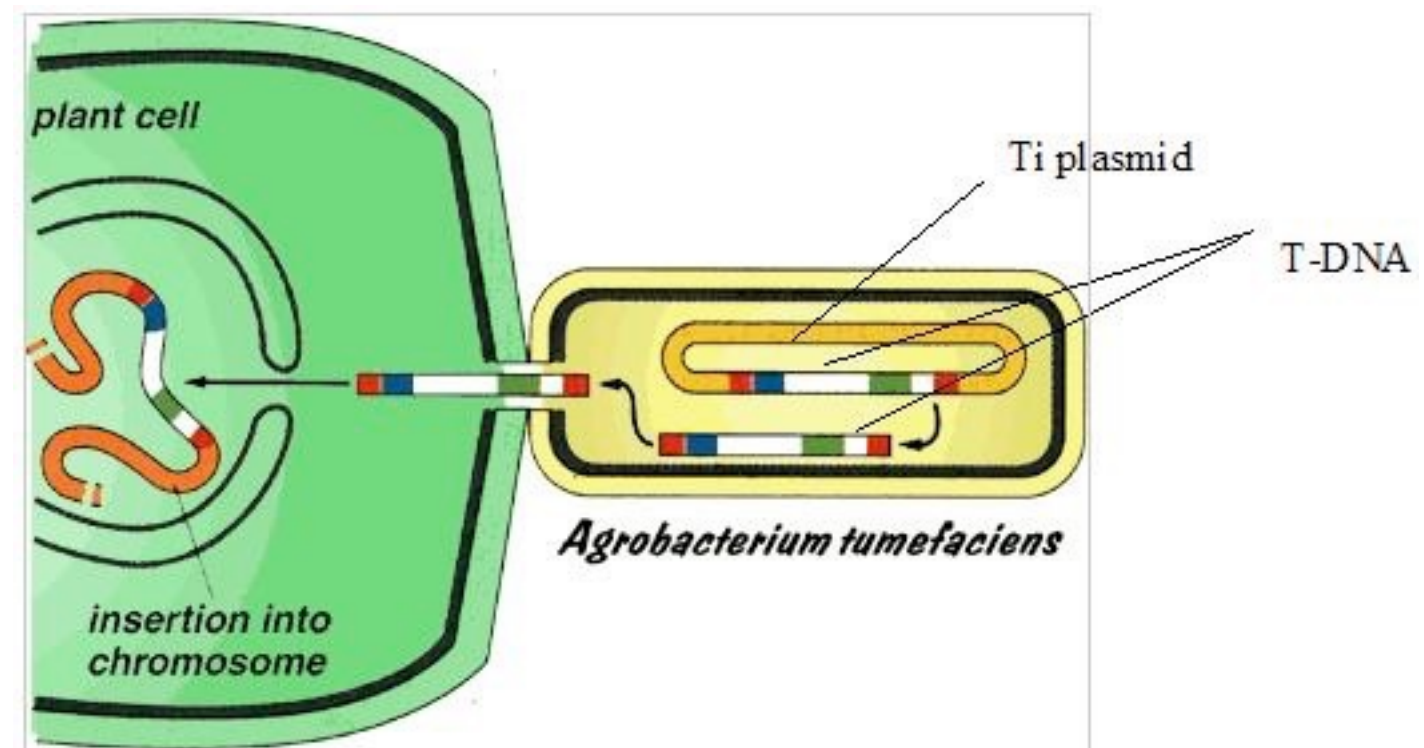


Part II: Genetic manipulation

Natural genetic manipulation



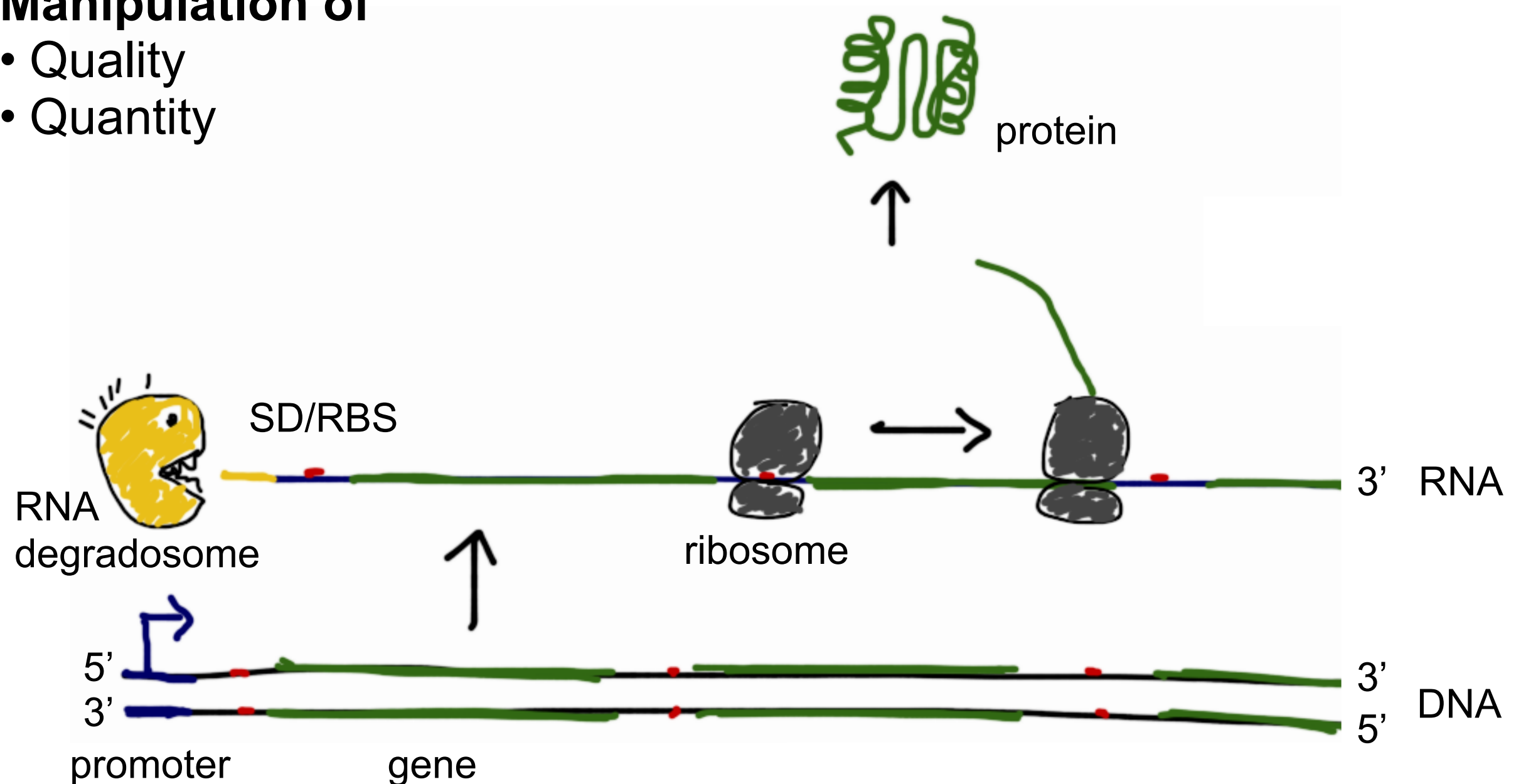
figures: lifesciencefoundation.org



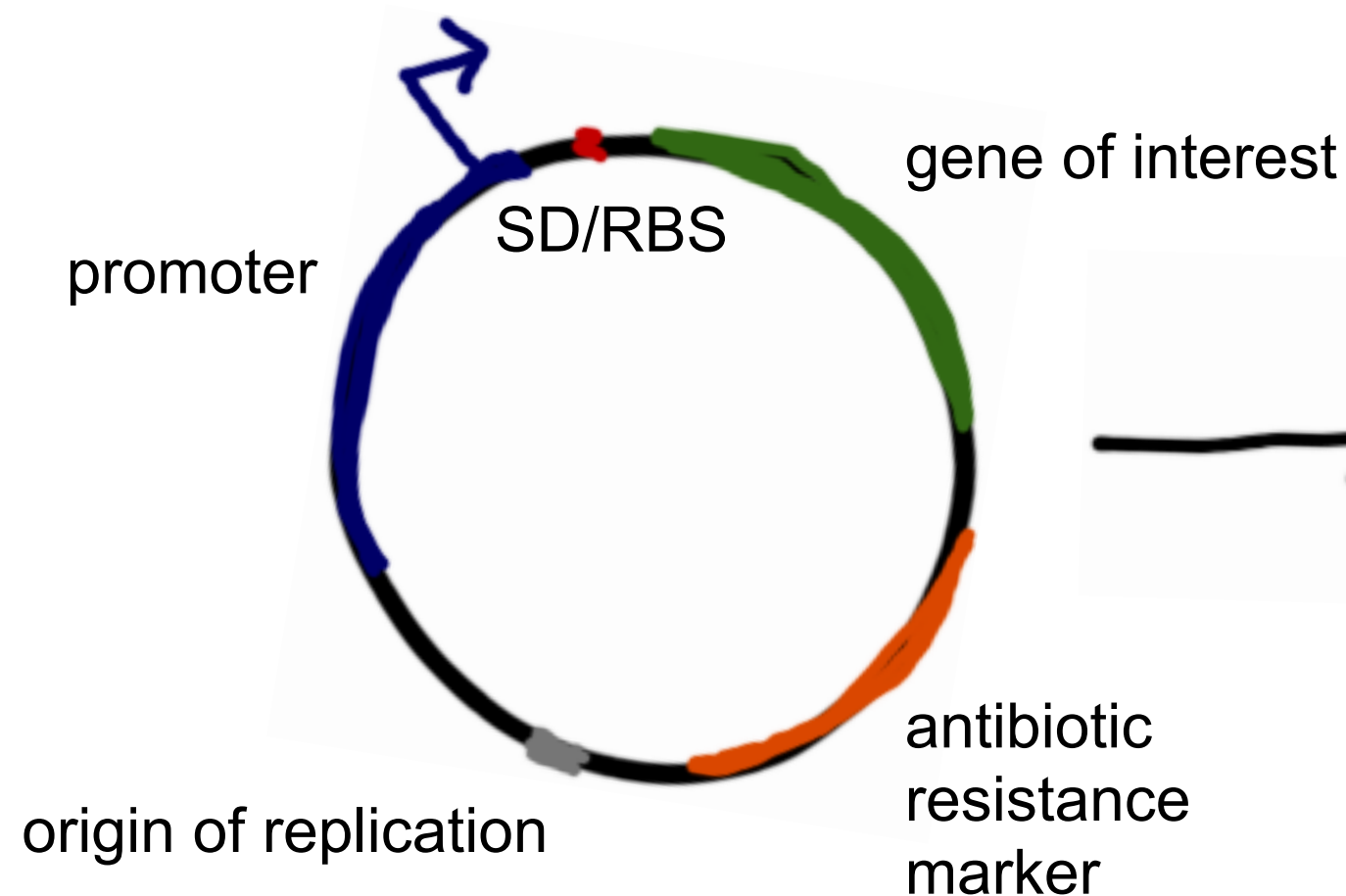
In the end it is all about making proteins...

Manipulation of

- Quality
- Quantity

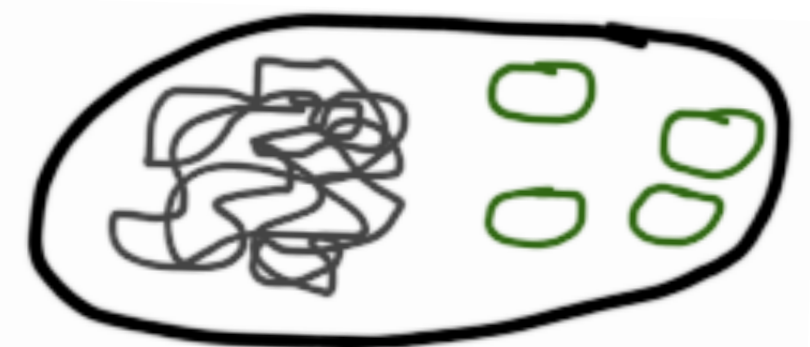


Most simple thing: cloning of gene on plasmid



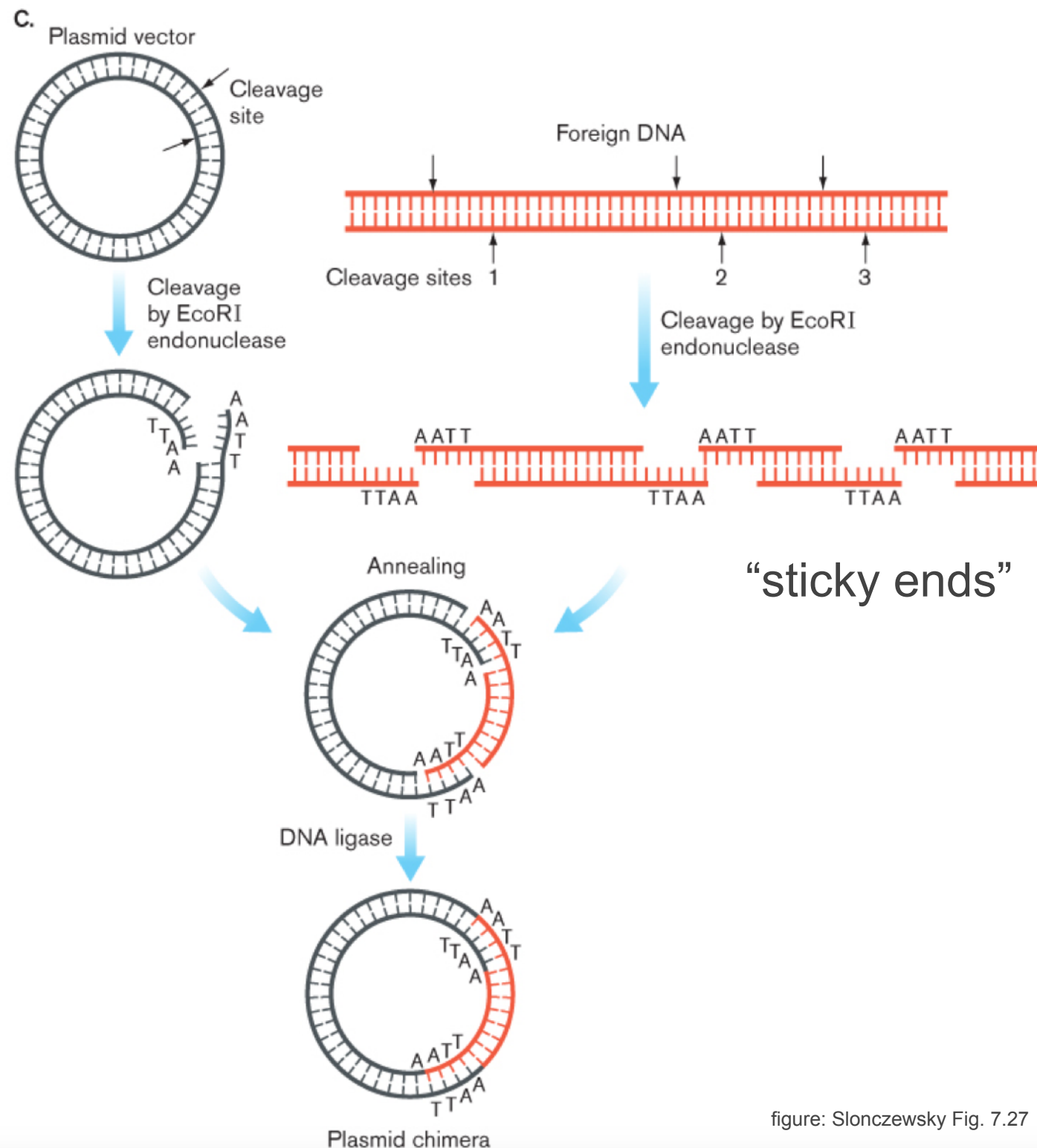
Two options:

- amplification of plasmid
- expression of gene/protein

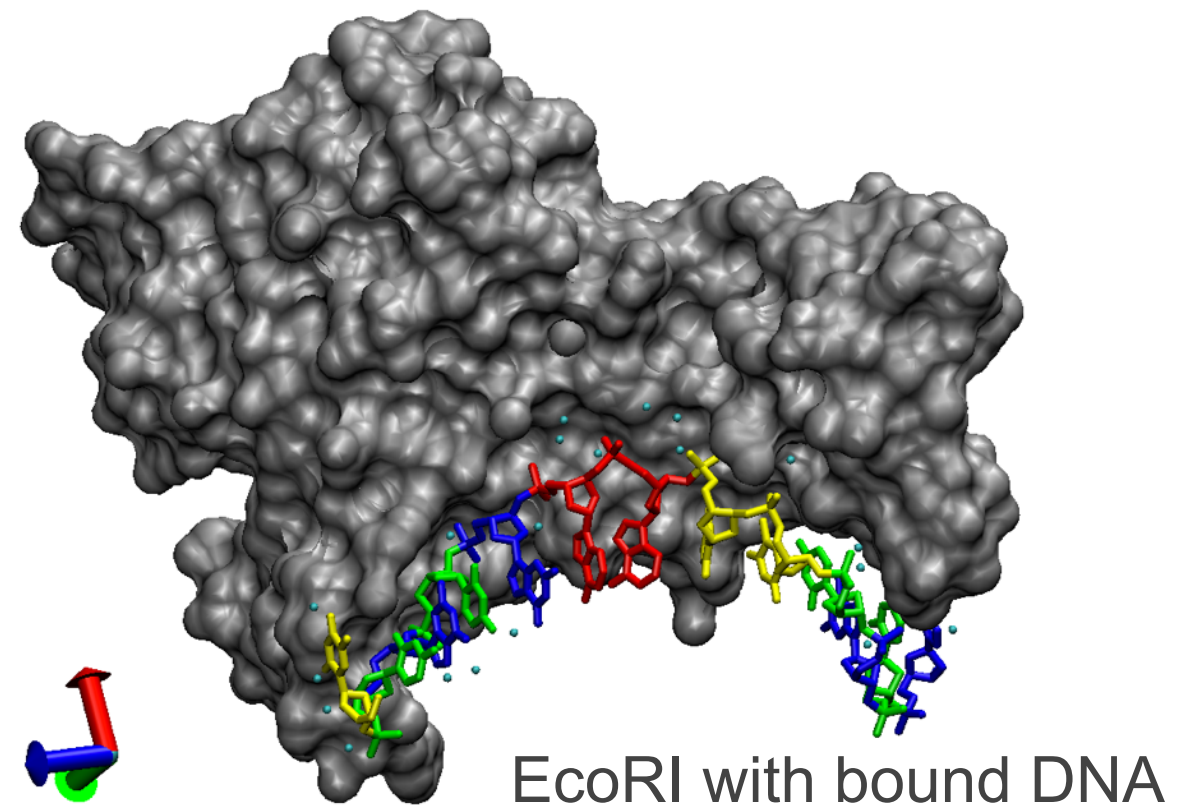


bacterium, often *Escherichia coli*

Cloning by using restriction endonuclease (old school)



cleave palindromic DNA sequence:
EcoRI: GAATTC

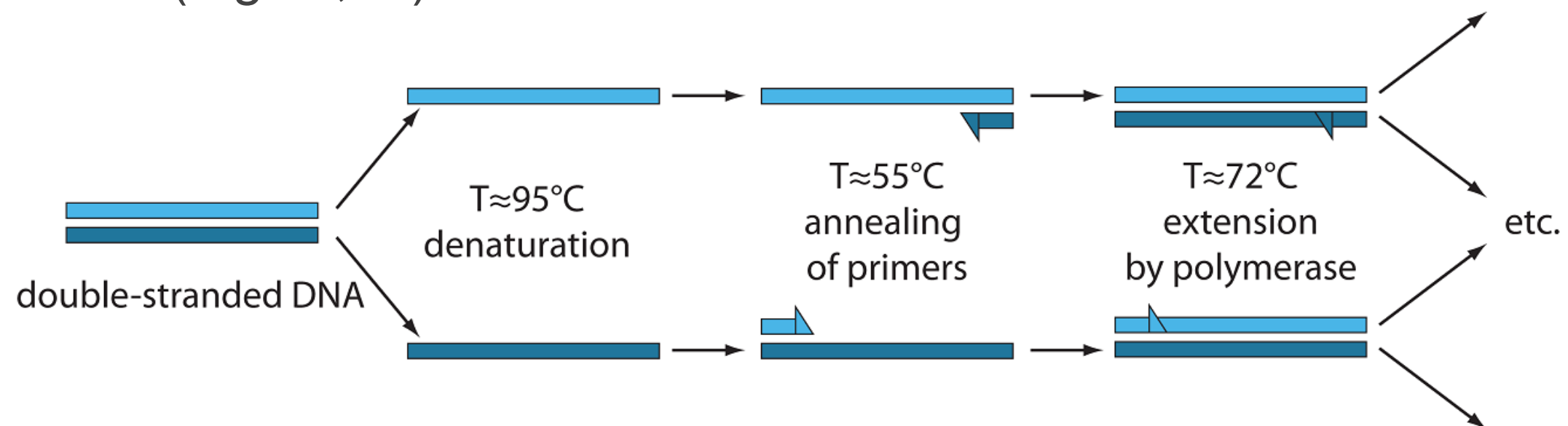


Excursus: Amplification of DNA by PCR

PCR: polymerase chain reaction

What's in the mix:

- DNA template
- DNA primer
- DNA polymerase
- dNTPs
- Buffer (MgCl₂, ...)



35 cycles: $2^{35} = 34$ billion copies



PCR – primer design

ACAGTATATG GACATTGCAA CATGACATCA TTACGAGACG GGATAGTTAA
TGTCATATAC CTCTAACGTT GTACTGTAGT AATGCTCTGC CCTATCAATT

ATGGATGATT TAGTGTTTGC AGG
ATGGATGATT TAGTGTTTGC AGGTAATAAG GCGCTCTATC TTGTTTTGAT
TACCTACTAA ATCACAAACG TCCATTATTC CGCGAGATAG AACAAAACCTA

CCTGTCAGGG TGGCCGACGA TTGTCGCAAC GATTATCGGC CTCCTGGTAG
GGACAGTCCC ACCGGCTGCT AACAGCGTTG CTAATAGCCG GAGGACCATC

GGTTATTCCA GACGGTAACG CAATTACAGG AACAGACGCT GCCTTTTGGC
CCAATAAGGT CTGCCATTGC GTTAATGTCC TTGTCTGCGA CGGAAAACCG

ATTAAATTAC TTGGCGTGTG TTTATGCTTG TTTTACTGT CTGGCTGGTA
TAATTTAATG AACCGCACAC AAATACGAAC AAAAATGACA GACCGACCAT

TGGCGAAGTT TTA CTCTCTT ACGGGCGTCA GGTGATATTC CTGGCGTTGG
ACCGCTTCAA AATGAGAGAA TGCCCGCAGT CCACTATAAG GACCGCAACC
CCGCAACC

CTAAGGGGTA A
GATTCCCAT T
GATTCCCAT T

Forward: 5' – ATGGATGATTTAGTGTTTGCAGG – 3'
Reverse: 5' – TTACCCCTTAGCAGGTGAT – 3'



Cloning a PCR product

ACAGTATATG GACATTGCAA CATGACATCA TTACGAGACG GGATAGTTAA
TGTCATATAC CTCTAACGTT GTACTGTAGT AATGCTCTGC CCTATCAATT

ATGGATGATT TAGTGTTTGC AGG
ATGGATGATT TAGTGTTTGC AGGTAATAAG GCGCTCTATC TTGTTTTGAT
TACCTACTAA ATCACAAACG TCCATTATTC CGCGAGATAG AACAAAAC TA

CCTGTCAGGG TGGCCGACGA TTGTCGCAAC GATTATCGGC CTCCTGGTAG
GGACAGTCCC ACCGGCTGCT AACAGCGTTG CTAATAGCCG GAGGACCATC

GGTTATTCCA GACGGTAACG CAATTACAGG AACAGACGCT GCCTTTTGGC
CCAATAAGGT CTGCCATTGC GTTAATGTCC TTGTCTGCGA CGGAAAACCG

ATTAAATTAC TTGGCGTGTG TTTATGCTTG TTTTACTGT CTGGCTGGTA
TAATTTAATG AACCGCACAC AAATACGAAC AAAAATGACA GACCGACCAT

TGGCGAAGTT TTA CTCTCTT ACGGGCGTCA GGTGATATTC CTGGCGTTGG
ACCGCTTCAA AATGAGAGAA TGCCCGCAGT CCACTATAAG GACCGCAACC
CCGCAACC

CTAAGGGGTA A
GATTCCCAT T
GATTCCCAT T

Addition of a restriction site: EcoRI

Forward: 5' – GAATTC ATGGATGATTTAGTGTTTGCAGG – 3'
Reverse: 5' – GAATTC TTACCCCTTAGCAGGTGAT – 3'



Cloning a PCR product

PCR Product:

GAATTC ATGGATGATT TAGTGTTTGC AGGTAATAAG GCGCTCTATC ...
CTTAAG TACCTACTAA ATCACAAACG TCCATTATTC CGCGAGATAG

ACGGGCGTCA GGTGATATTC CTGGCGTTGG CTAAGGGGTA A GAATTC
TGCCCGCAGT CCACTATAAG GACCGCAACC GATTCCCAT T CTTAAG

After EcoRI digest:

AATTC ATGGATGATT TAGTGTTTGC AGGTAATAAG GCGCTCTATC ...
G TACCTACTAA ATCACAAACG TCCATTATTC CGCGAGATAG

ACGGGCGTCA GGTGATATTC CTGGCGTTGG CTAAGGGGTA A G
TGCCCGCAGT CCACTATAAG GACCGCAACC GATTCCCAT T CTTAA

=> into EcoRI-cut vector

Gibson Assembly

no restriction site but
complementary to the plasmid

T5 exonuclease

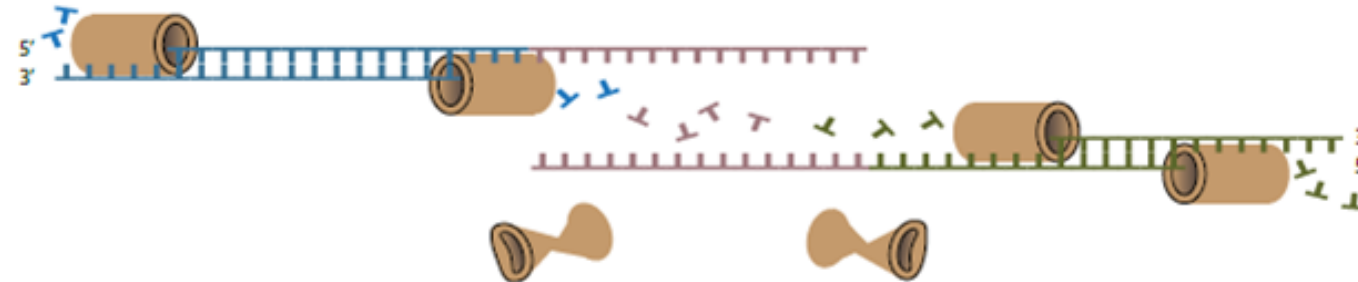
polymerase

Taq ligase

Step 1.



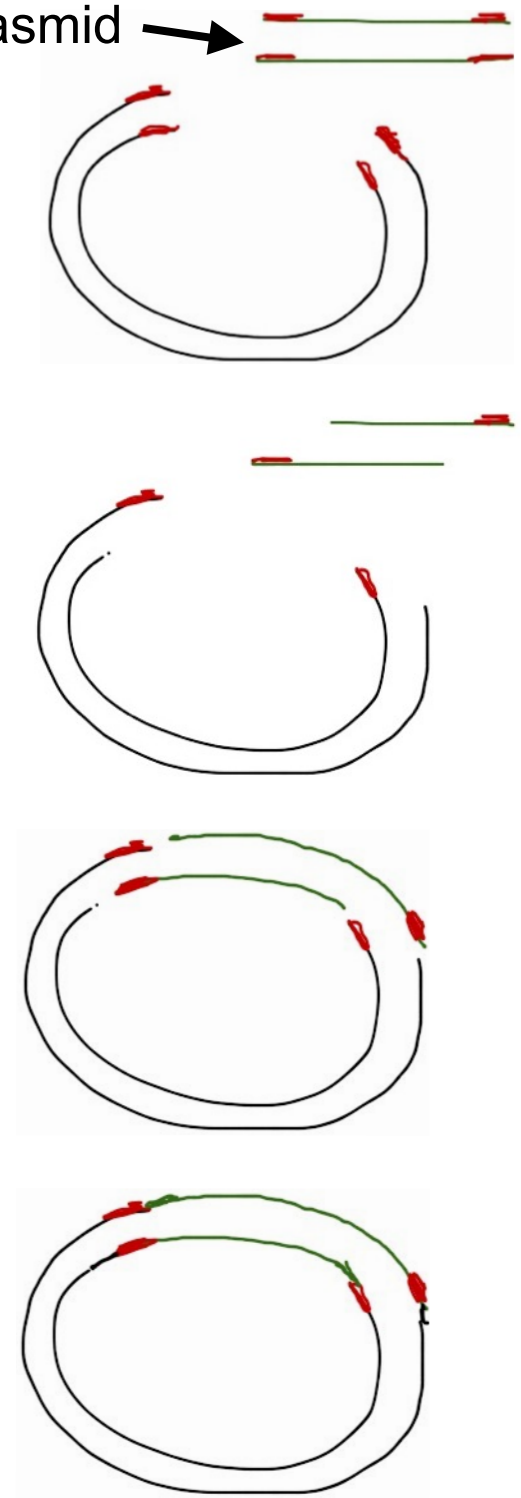
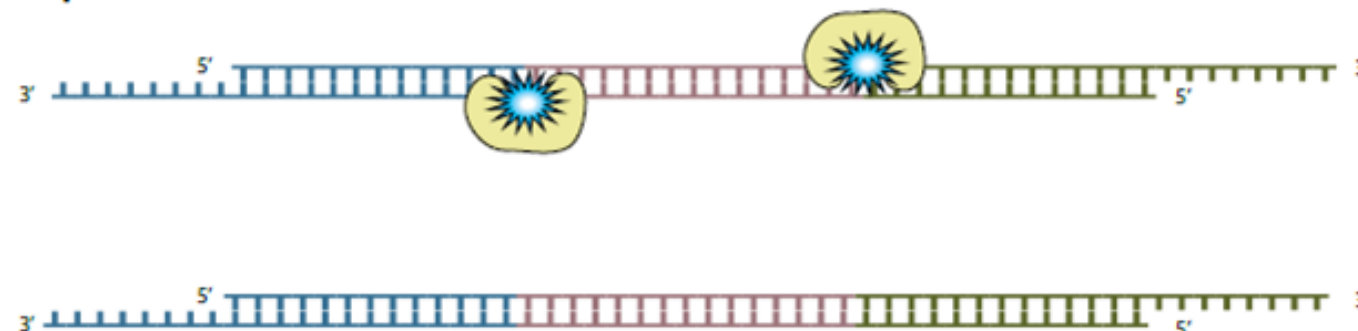
Step 2.



Step 3 and 4.

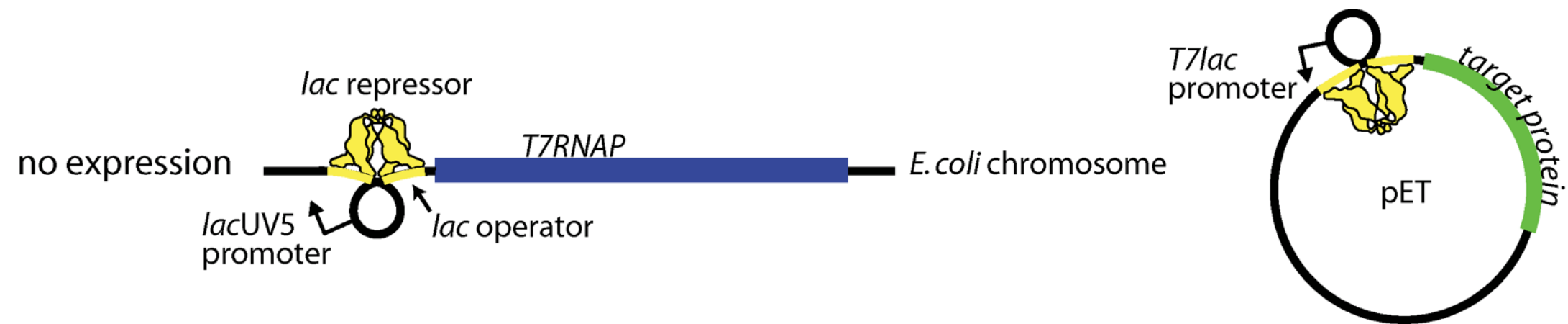


Step 5.



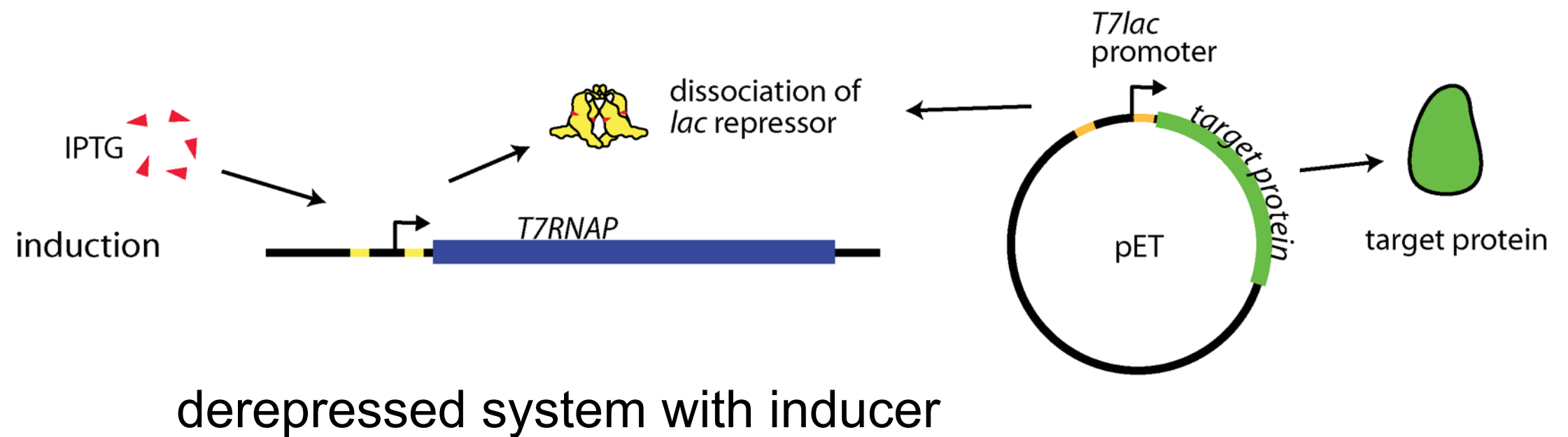
all at 50°C => isothermal assembly, just 10 - 30 min

Example: Protein expression system Lemo21

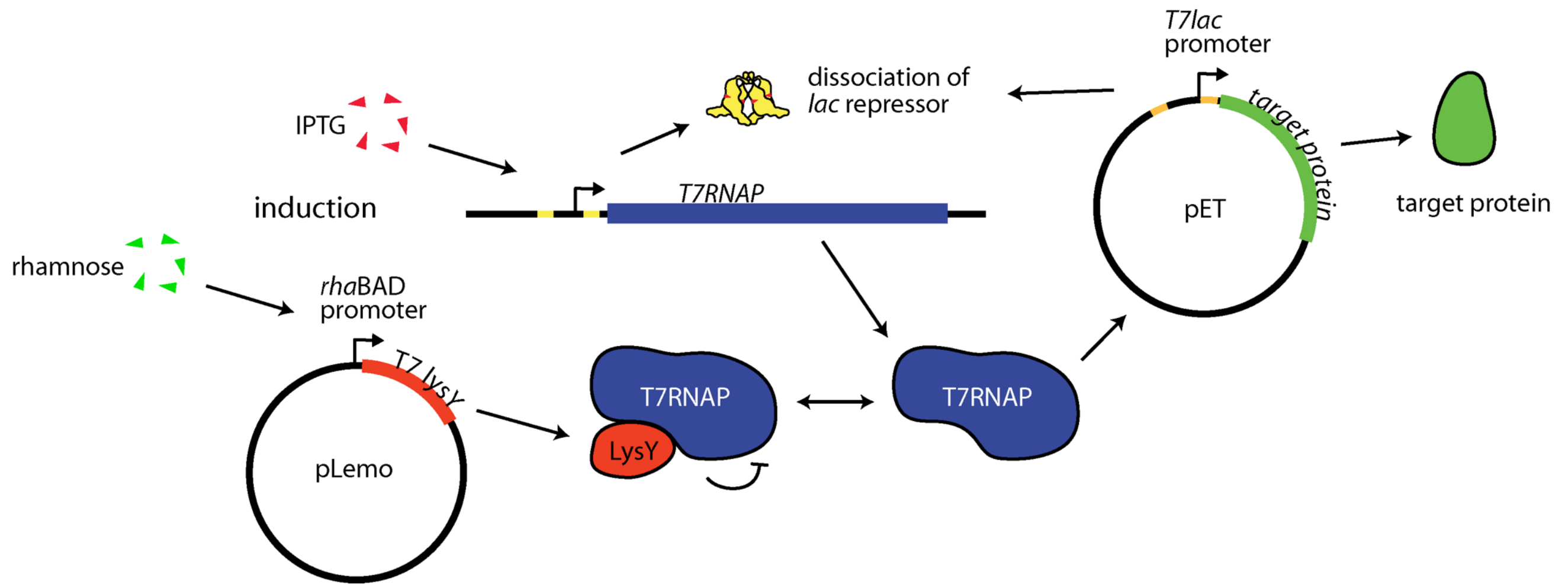


repressed system without inducer

Example: Protein expression system Lemo21



Example: Protein expression system Lemo21

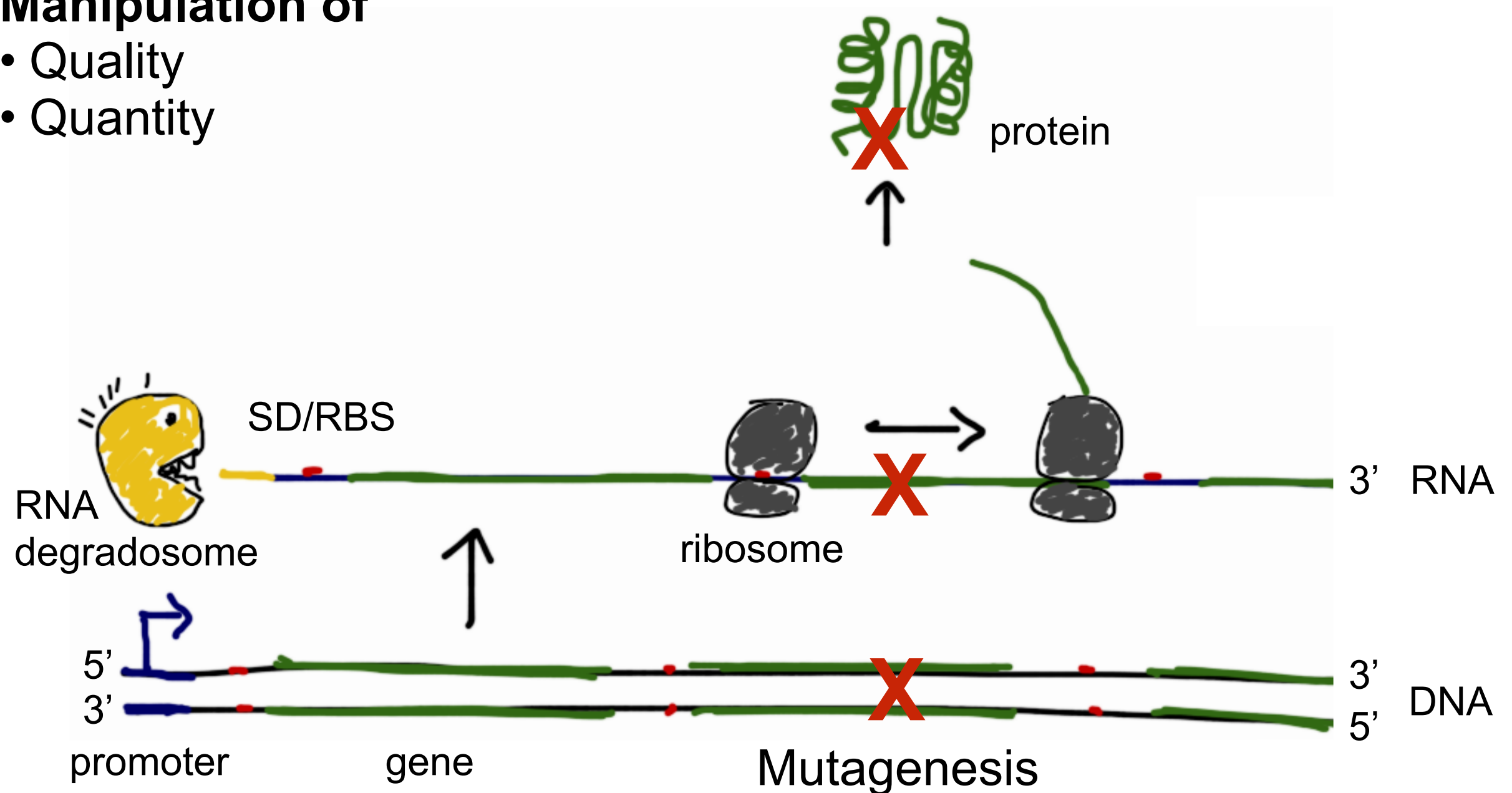


titratable system with two inducers

In the end it is all about making proteins...

Manipulation of

- Quality
- Quantity



Mutagenesis

random mutagenesis vs. **site-directed** mutagenesis

random:

- PCR products – error prone PCR, e.g. addition of Mn^{2+} , disbalance of dNTPs
- Plasmids – amplification in strains with impaired DNA repair mechanisms
- Organisms – addition of mutagens, infection with virus

site-directed:

- PCR amplification with primers containing specific mutation

ATG	GAT	GAT	TTA	GTG	TTT	GCA	GGT	AAT	AAG	GCG	CTC	TAT	CTT	GTT
TAC	CTA	CTA	AAT	CAC	AAA	CGT	CCA	TTA	TTC	CGC	GAG	ATA	GAA	CAA
M	D	D	L	V	F	A	G	N	K	A	L	Y	L	V

TTG	ATC	CTG	TCA	GGG	TGG	CCG	ACG	ATT	GTC	GCA	ACG	ATT	ATC	GGC
AAC	TAG	GAC	AGT	CCC	ACC	GGC	TGC	TAA	CAG	CGT	TGC	TAA	TAG	CCG
L	I	L	S	G	W	P	T	I	V	A	T	I	I	G

Mutation at N9

Mutagenesis

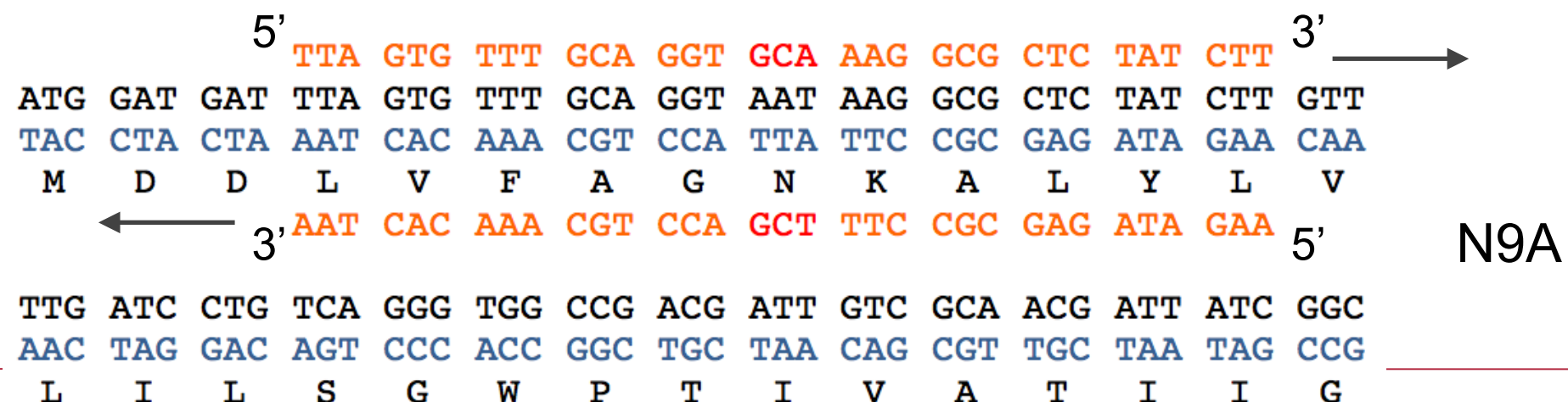
random mutagenesis vs. **site-directed** mutagenesis

random:

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site-directed:

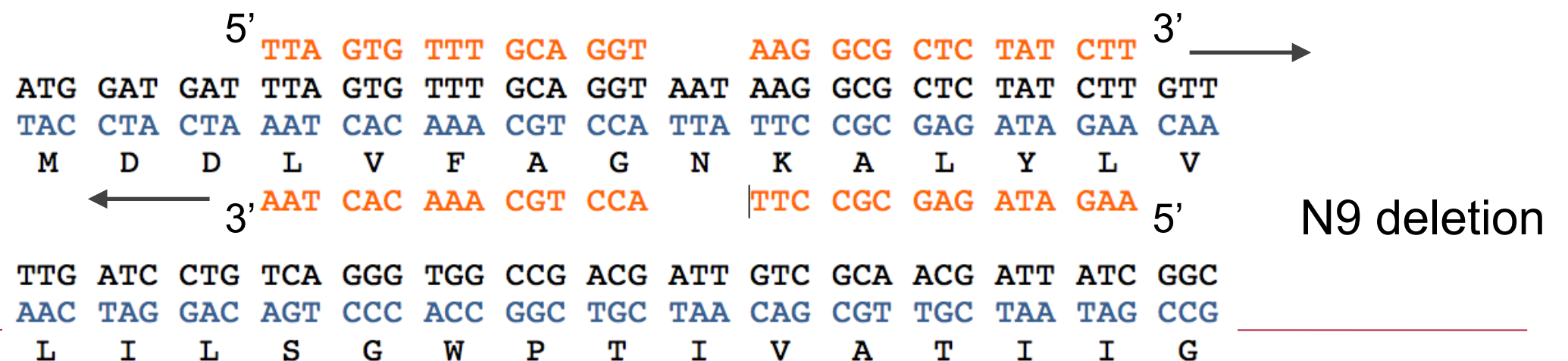
- PCR amplification with primers containing specific mutation



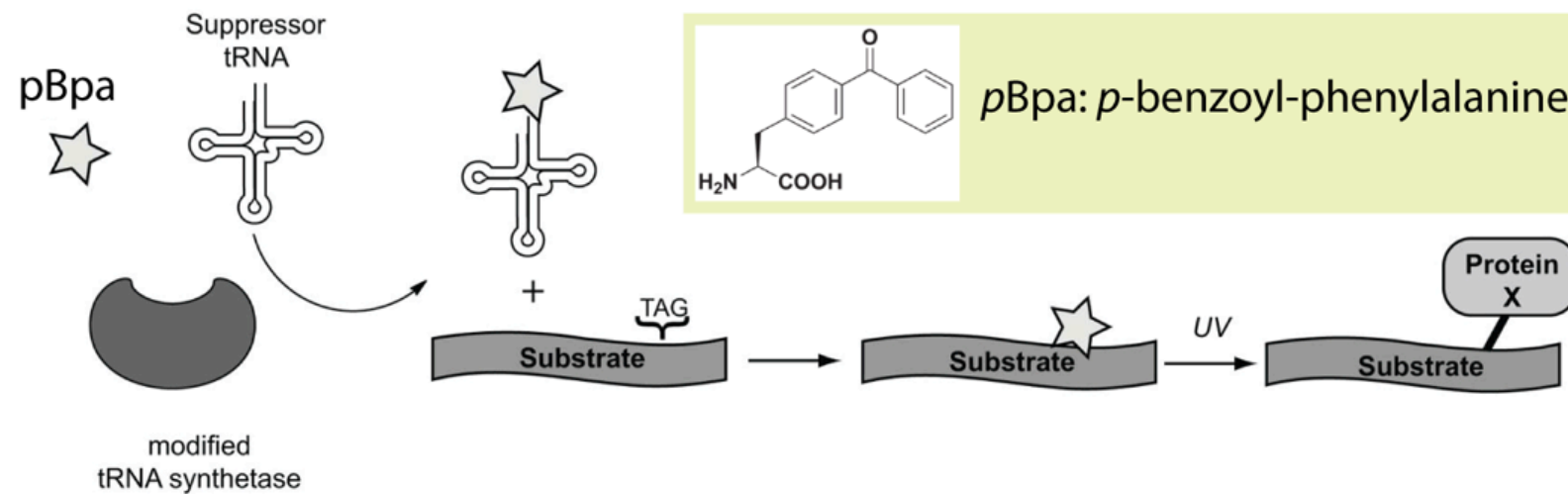
random mutagenesis vs. site-directed mutagenesis

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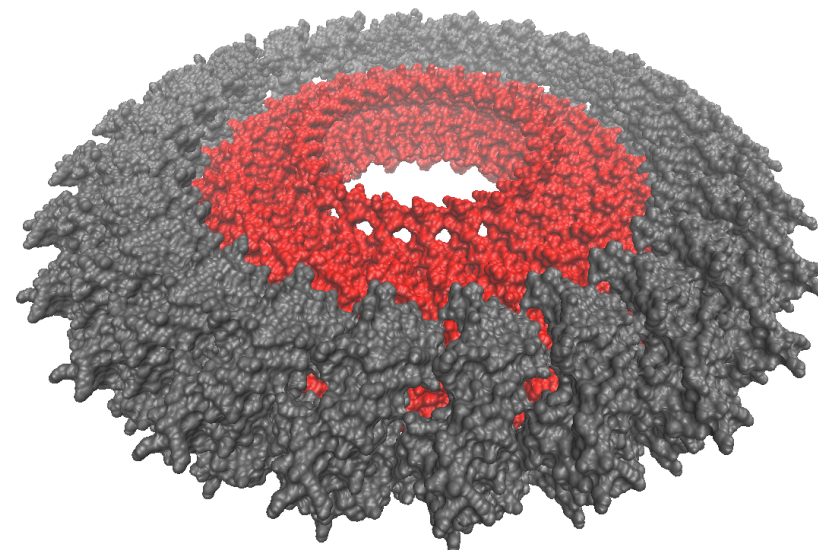
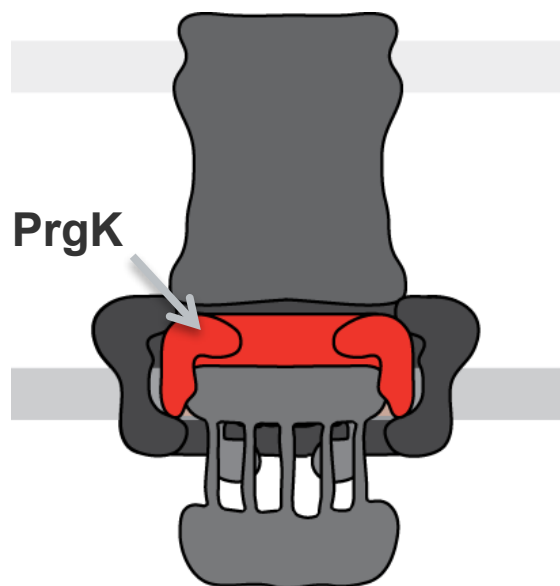
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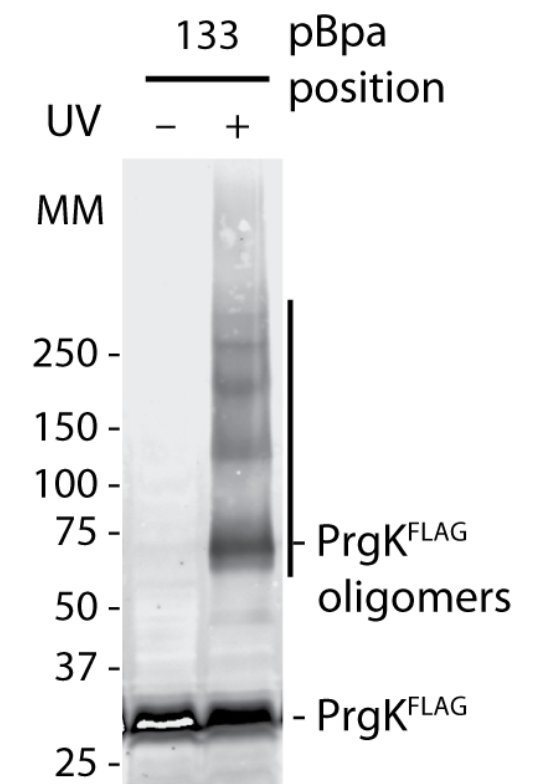
Example: Site-specific *in vivo* photocrosslinking



Adapted from Stanley et al. 2011. FEBS Lett



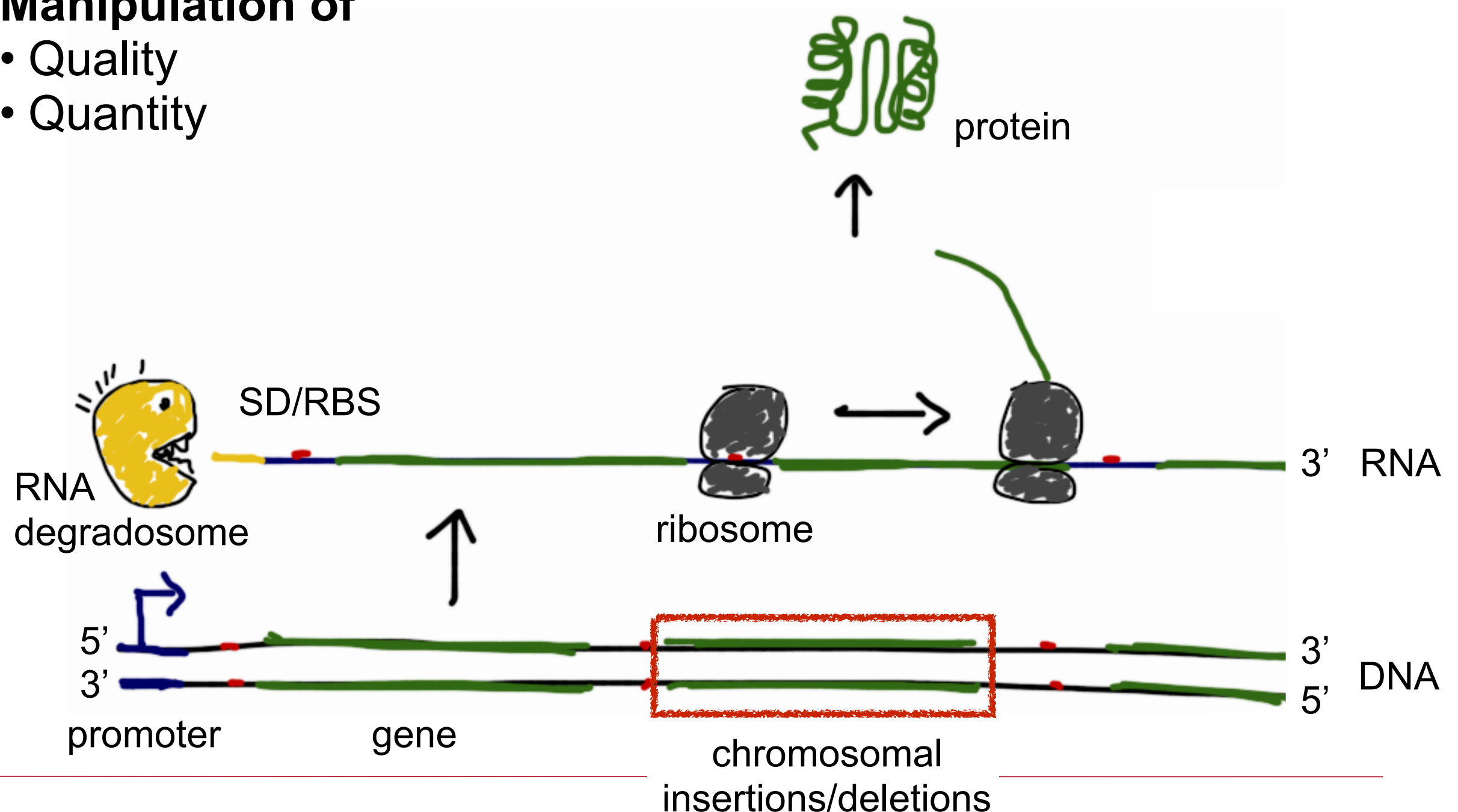
Schraidt, Marlovits. 2011. Science



In the end it is all about making proteins... or not making

Manipulation of

- Quality
- Quantity





Next week :

