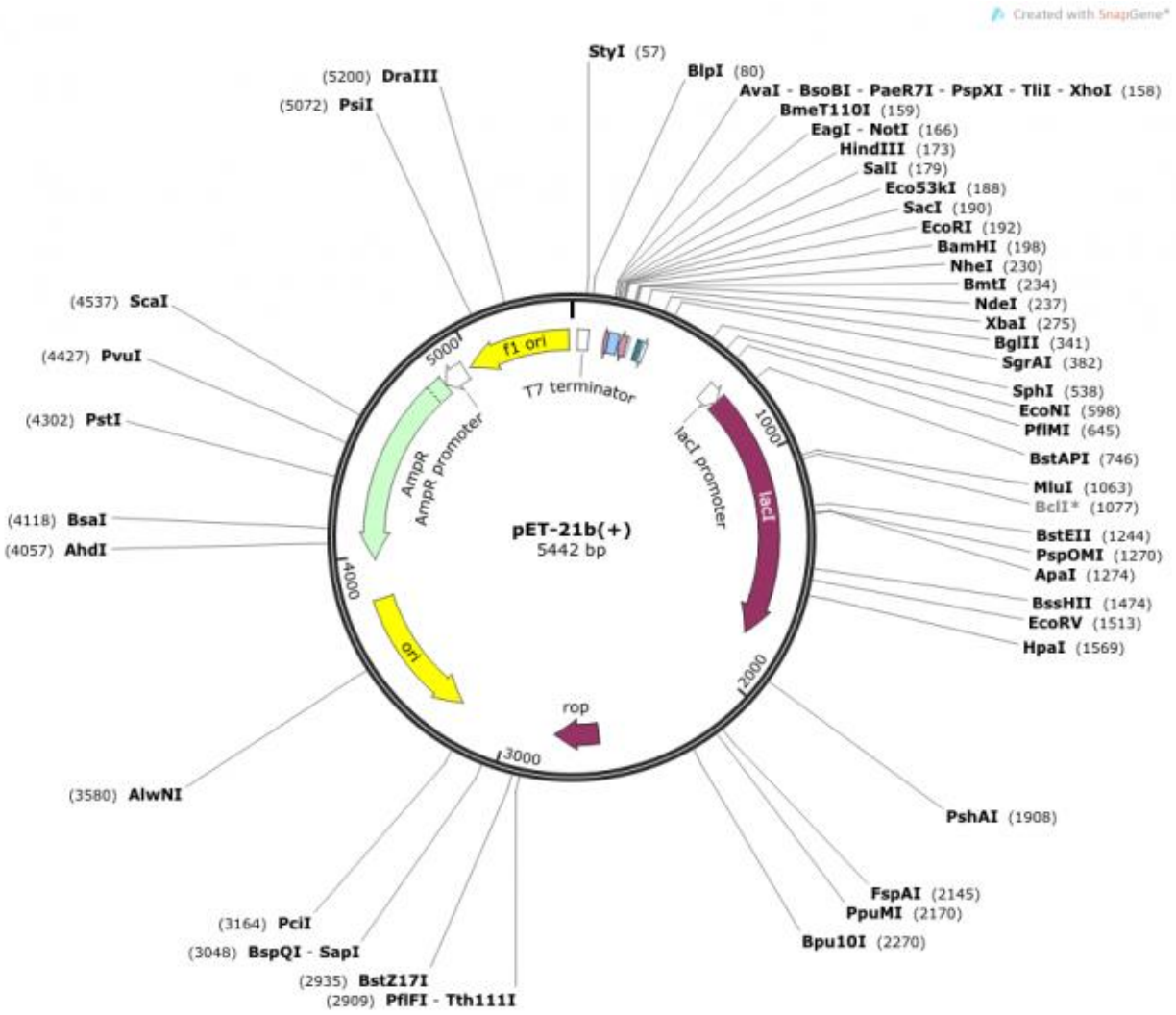
DNA synthesis & molecular cloning

Concepts
Methods
Applications



NdeI

Recognition Site:

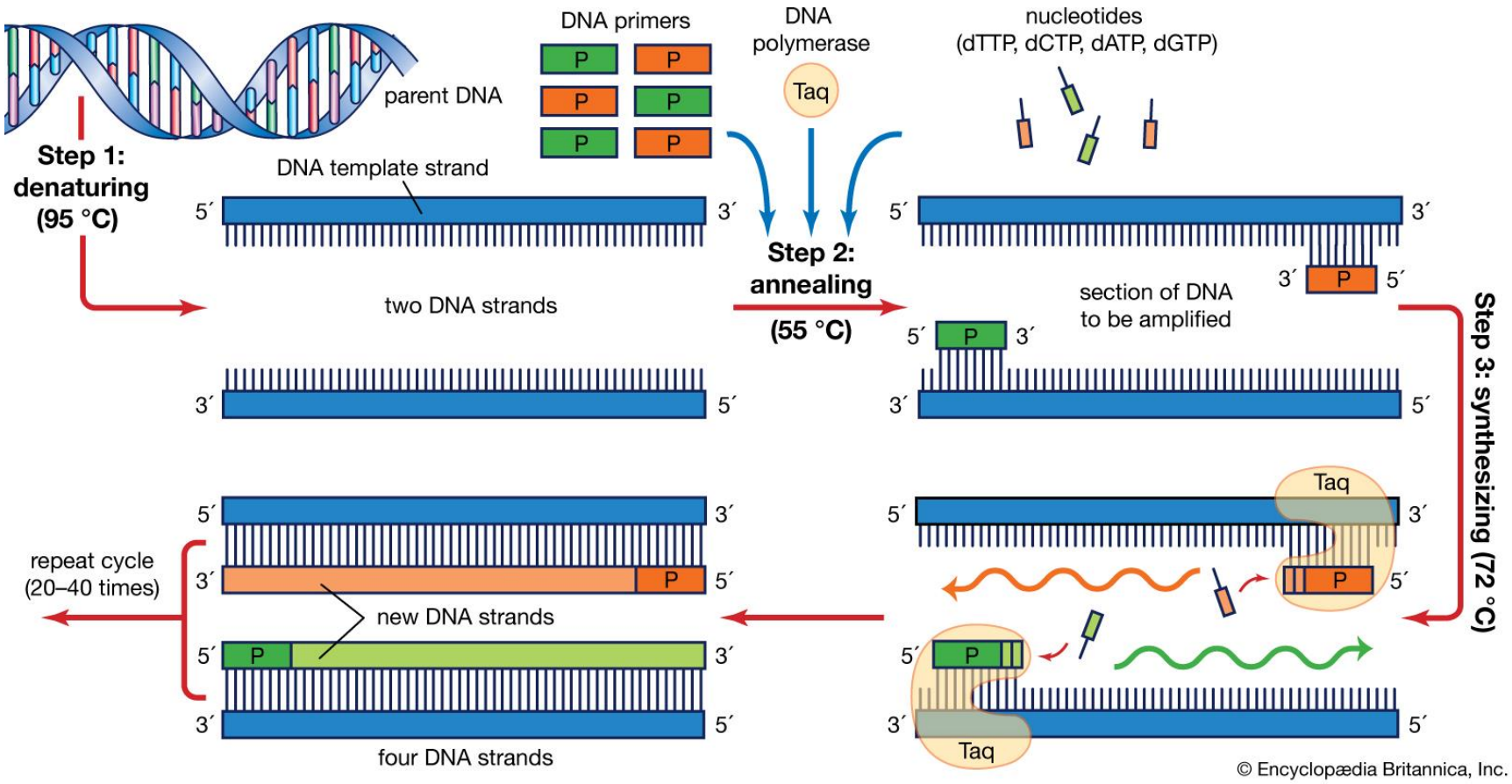
5’ . . . C A▼T A T G . . . 3’
3’ . . . G T A T▲A C. . . 5’

BamHI

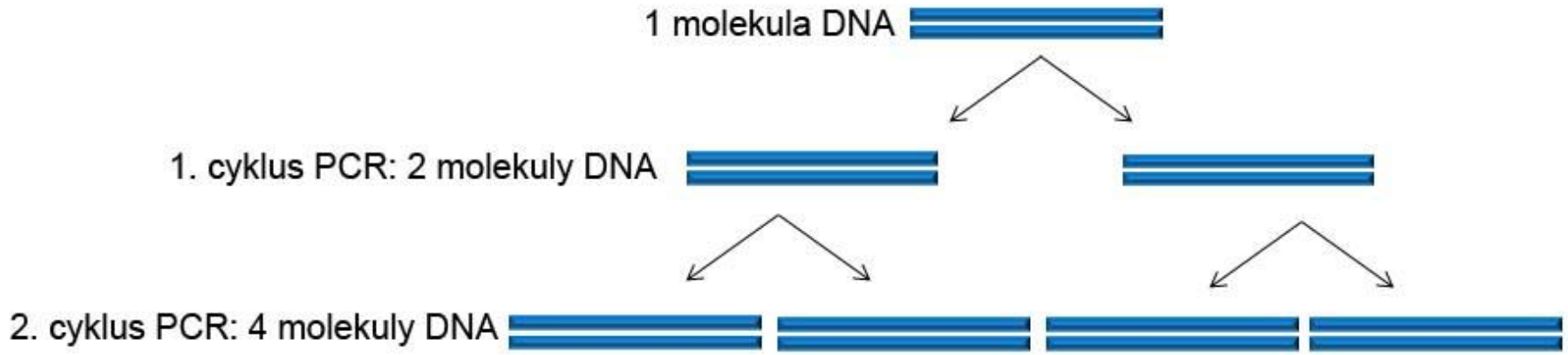
Recognition Site:

5’ . . . G▼G A T C C. . . 3’
3’ . . . C C T A G▲G . . . 5’

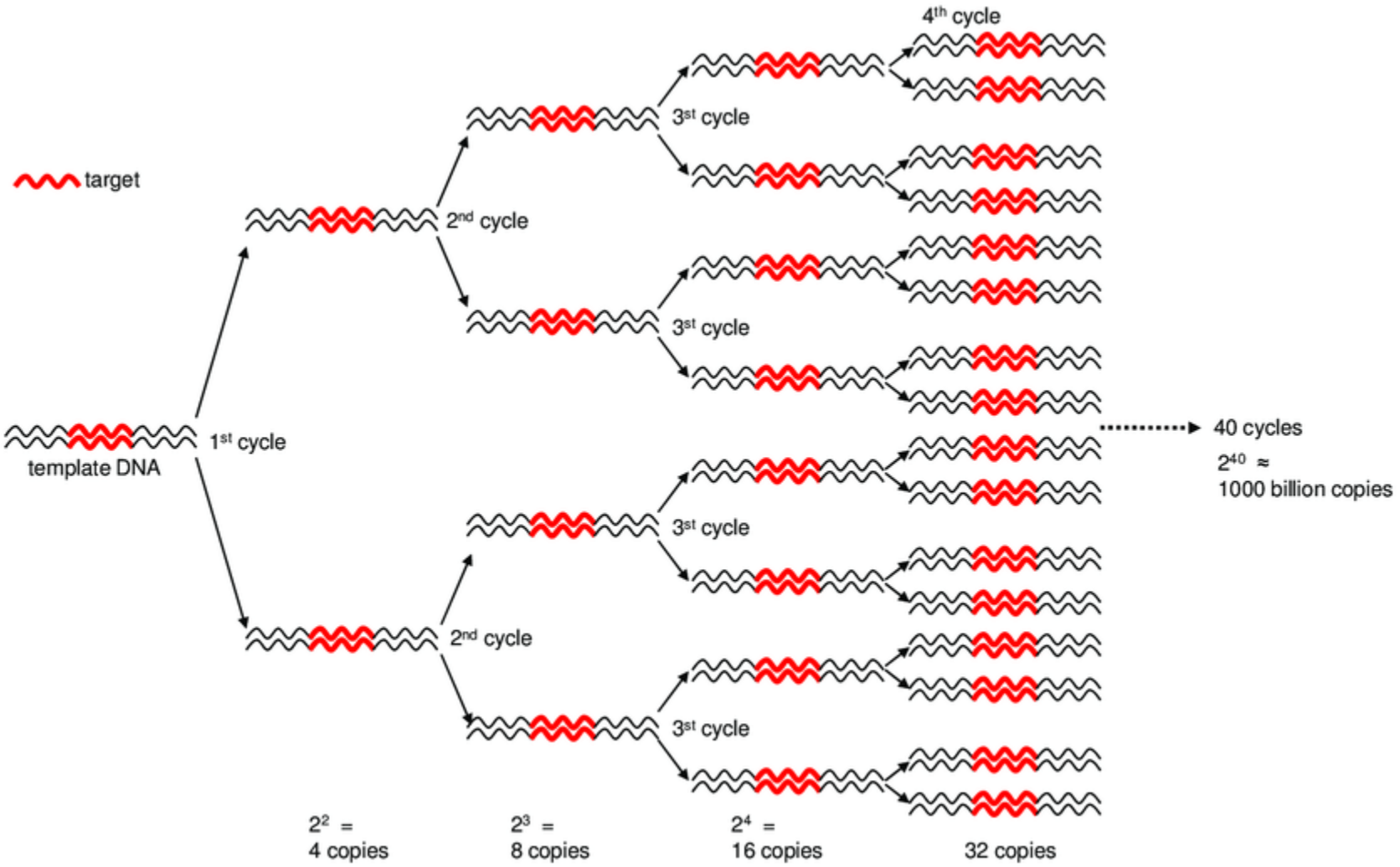
Enzymatic DNA synthesis: polymerase chain reaction (PCR)

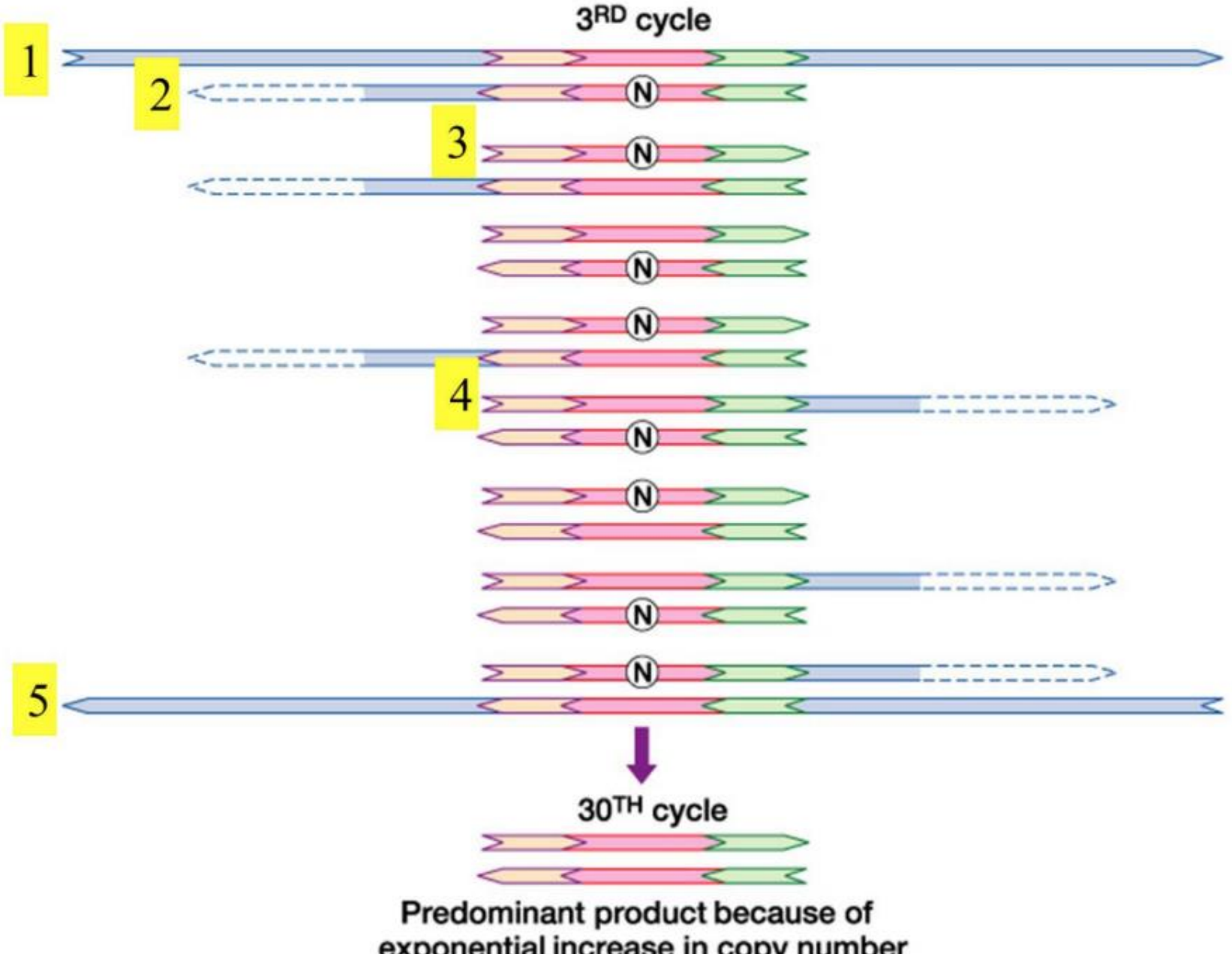


Amplification of up to 20 kbp DNA fragment from pre-existing template (genomic loci, cDNA library, cloned fragment etc.)



po *n* cyklech: 2^n molekul DNA


4th cycle
target
3st cycle
2nd cycle
3st cycle
1st cycle
template DNA
40 cycles
2^40 ≈
1000 billion copies
3st cycle
2nd cycle
3st cycle
2^2 =
4 copies
2^3 =
8 copies
2^4 =
16 copies
32 copies


3RD cycle
1
2
3
4
5
N
30TH cycle
Predominant product because of
exponential increase in copy number

Jaký je celkový počet polynukleotidových DNA řetězců po *n* cyklech?

Kolik je nově syntetizovaných polynukleotidových DNA řetězců po *n* cyklech?

Kolik je nově syntetizovaných cílových polynukleotidových DNA řetězců po *n* cyklech?

Kolik je cílových dvouvláknových DNA fragmentů po *n* cyklech?

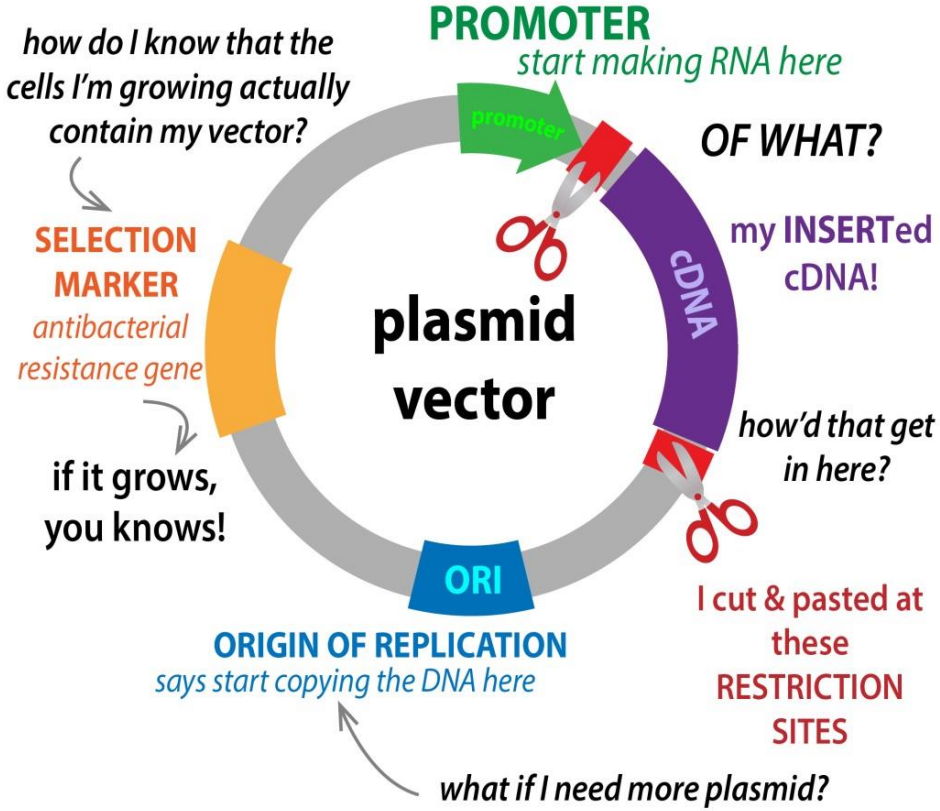
Kolik se spotřebuje primerů po *n* cyklech?

Kolik se zabuduje nukleotidů po *n* cyklech?

Kolik mikrogramů DNA se nasyntetizuje po *n* cyklech?

Questions

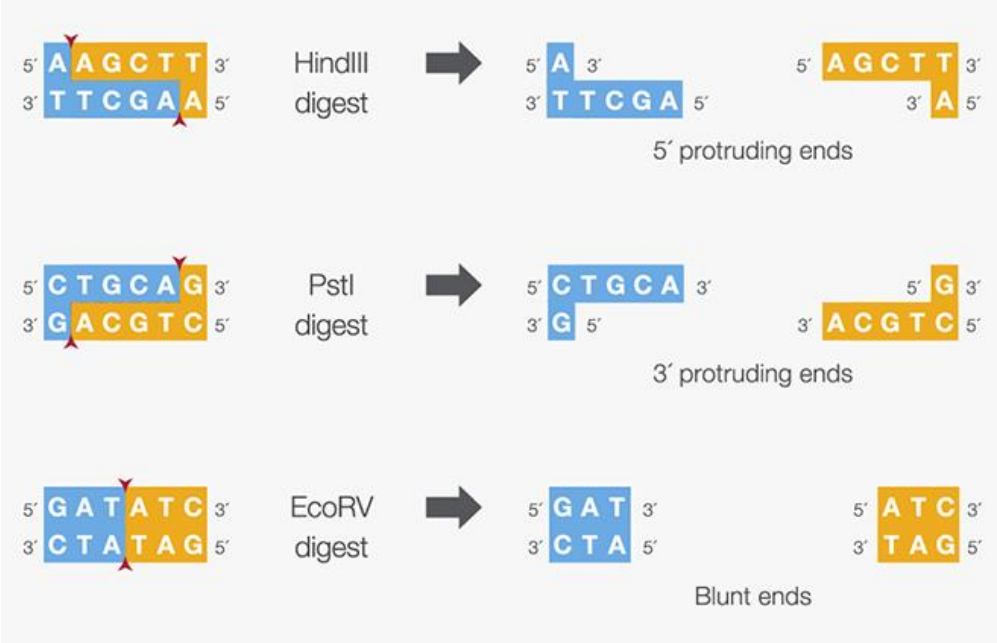
What is a plasmid?



- PLASMIDS are "extrachromosomal" (not part of the chromosomes), circular pieces of DNA.
- Similarly to chromosomes, they are double-stranded, which means they can easily be "unzipped" and copied (replicated).
- Plasmids use the host's machinery (DNA polymerase), but they don't have to wait for the host to divide to copy themselves → lots of copies of themselves.
- When the cell does divide, these copies will get split between the daughter cells, so they'll inherit the plasmid as well.
- The plasmid can act as a VECTOR – a vehicle for taking genes we want to deliver into cells.

Always sequence your plasmid to double-check that the gene is correctly inserted!

Restriction endonucleases (restriction enzymes)



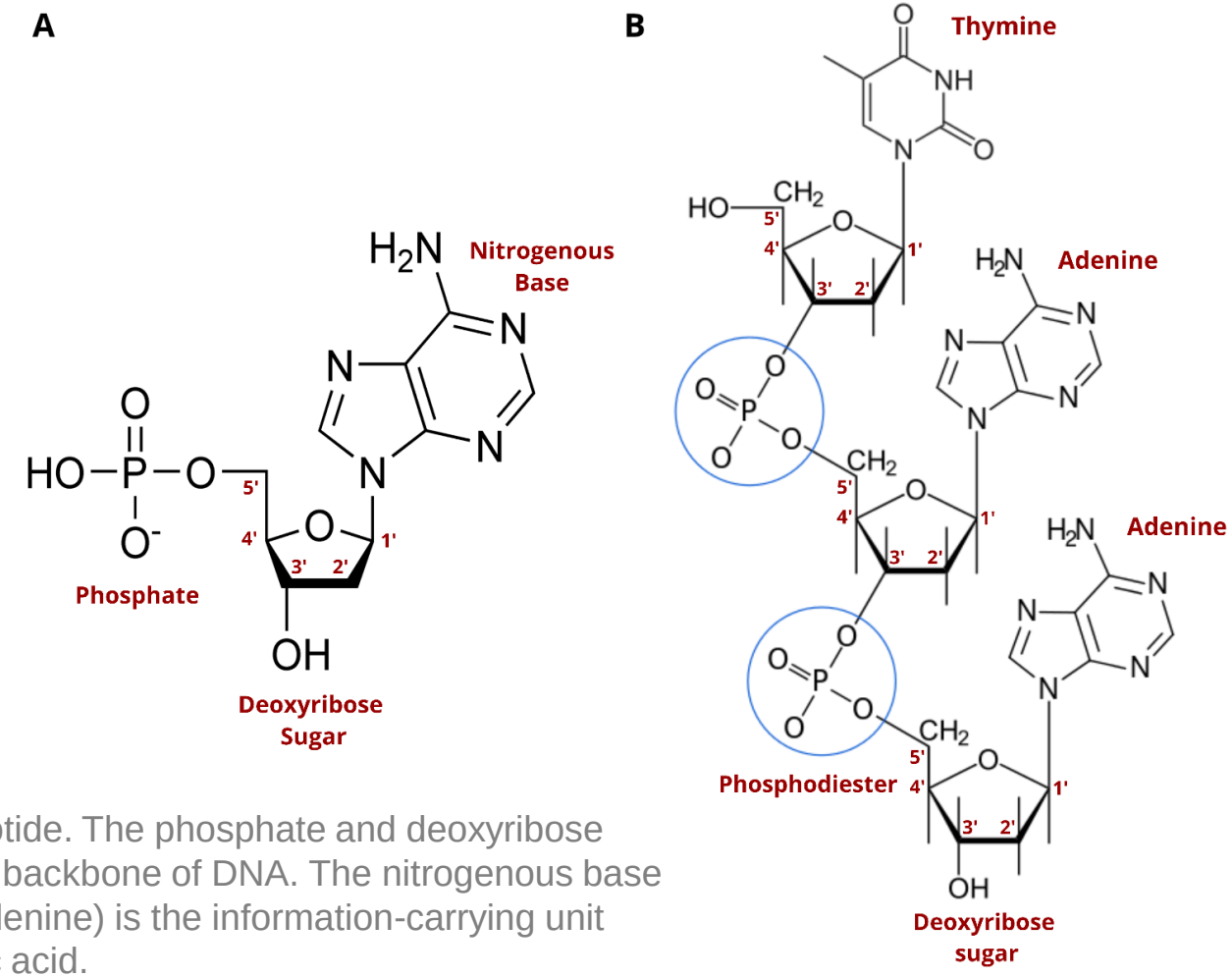
Type I enzymes cleave at sites remote from a recognition site; require both ATP and S-adenosyl-L-methionine to function; multifunctional protein with both restriction and methylase activities.

Type II enzymes cleave within or at short specific distances from a recognition site; most require magnesium; single function (restriction) enzymes independent of methylase.

Type III enzymes cleave at sites a short distance from a recognition site; require ATP (but do not hydrolyze it); S-adenosyl-L-methionine stimulates the reaction but is not required; it exists as part of a complex with a modification methylase.

Type IV enzymes target modified DNA, e.g. methylated, hydroxymethylated and glucosyl-hydroxymethylated DNA.

Structure of DNA

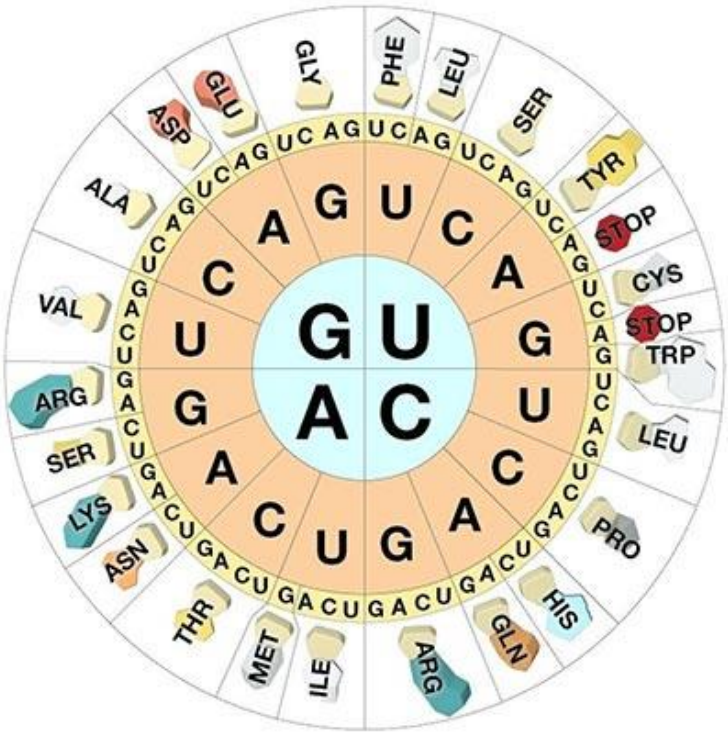


A) A single nucleotide. The phosphate and deoxyribose sugar form the backbone of DNA. The nitrogenous base (in this case adenine) is the information-carrying unit of each nucleic acid.

B) The structure of single-stranded DNA. In nature, enzymes form phosphodiester bonds (blue circles) that link the 5th position and 3rd position of adjacent deoxyribose sugars. Due to the modular nature of nucleotides, this chain can grow indefinitely.

Redundancy of the genetic code

- Degeneracy of codons is the redundancy of the genetic code, exhibited as the multiplicity of three-base pair codon combinations that specify an amino acid
- The genetic code is degenerate mainly at the third codon position
- The genetic code consists of 64 triplet codons specifying 20 canonical amino acids and 3 stop signals



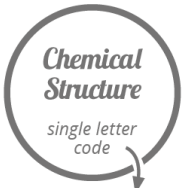
Second Letter

First Letter	T	C	A	G	Third Letter
T	TTT Phe, TTC Phe, TTA Leu, TTG Leu	TCT Ser, TCC Ser, TCA Ser, TCG Ser	TAT Tyr, TAC Tyr, TAA Stop, TAG Stop	TGT Cys, TGC Cys, TGA Stop, TGG Trp	T C A G
C	CTT Leu, CTC Leu, CTA Leu, CTG Leu	CCT Pro, CCC Pro, CCA Pro, CCG Pro	CAT His, CAC His, CAA Gln, CAG Gln	CGT Arg, CGC Arg, CGA Arg, CGG Arg	T C A G
A	ATT Ile, ATC Ile, ATA Ile, ATG Met	ACT Thr, ACC Thr, ACA Thr, ACG Thr	AAT Asn, AAC Asn, AAA Lys, AAG Lys	AGT Ser, AGC Ser, AGA Arg, AGG Arg	T C A G
G	GTT Val, GTC Val, GTA Val, GTG Val	GCT Ala, GCC Ala, GCA Ala, GCG Ala	GAT Asp, GAC Asp, GAA Glu, GAG Glu	GGT Gly, GGC Gly, GGA Gly, GGG Gly	T C A G

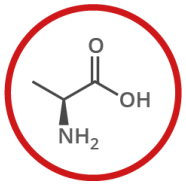
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

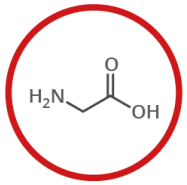
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



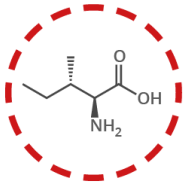
NAME (A)
three letter code
DNA codons



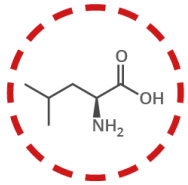
ALANINE (A)
Ala
GCT, GCC, GCA, GCG



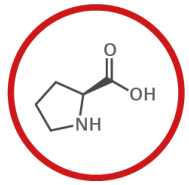
GLYCINE (G)
Gly
GGT, GGC, GGA, GGG



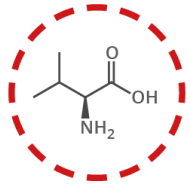
ISOLEUCINE (I)
Ile
ATT, ATC, ATA



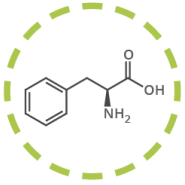
LEUCINE (L)
Leu
CTT, CTC, CTA, CTG, TTA, TTG



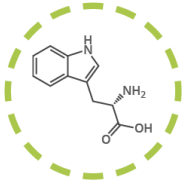
PROLINE (P)
Pro
CCT, CCC, CCA, CCG



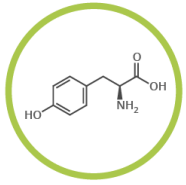
VALINE (V)
Val
GTT, GTC, GTA, GTG



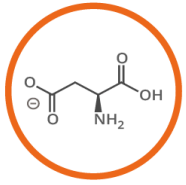
PHENYLALANINE (F)
Phe
TTT, TTC



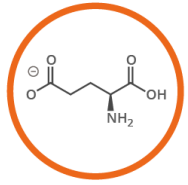
TRYPTOPHAN (W)
Trp
TGG



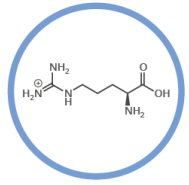
TYROSINE (Y)
Tyr
TAT, TAC



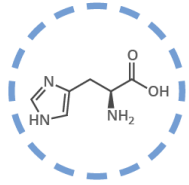
ASPARTIC ACID (D)
Asp
GAT, GAC



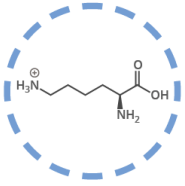
GLUTAMIC ACID (E)
Glu
GAA, GAG



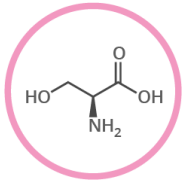
ARGININE (R)
Arg
CGT, CGC, CGA, CGG, AGA, AGG



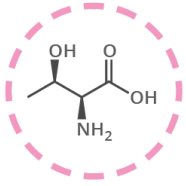
HISTIDINE (H)
His
CAT, CAC



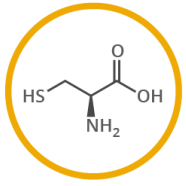
LYSINE (K)
Lys
AAA, AAG



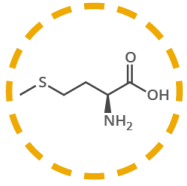
SERINE (S)
Ser
TCT, TCC, TCA, TCG, AGT, AGC



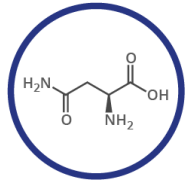
THREONINE (T)
Thr
ACT, ACC, ACA, ACG



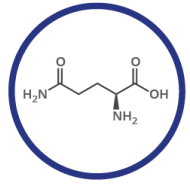
CYSTEINE (C)
Cys
TGT, TGC



METHIONINE (M)
Met
ATG



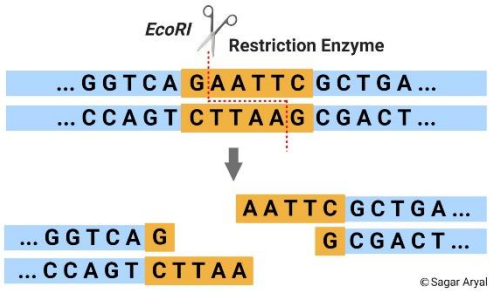
ASPARAGINE (N)
Asn
AAT, AAC

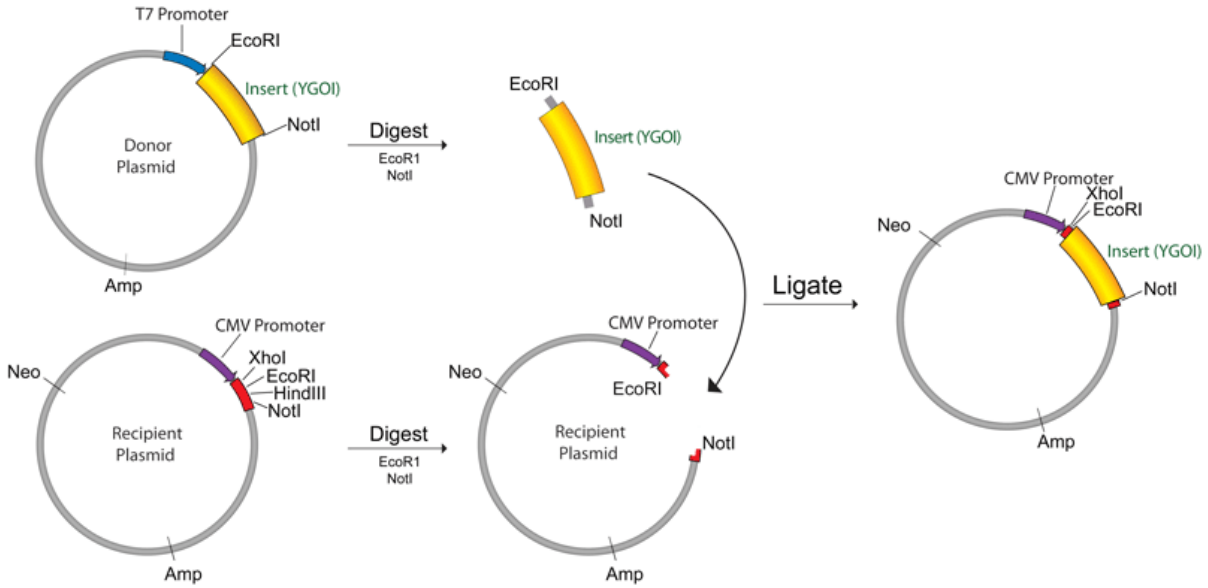


GLUTAMINE (Q)
Gln
CAA, CAG

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

Restriction cloning





In vitro

The production of exact copies of a particular gene or DNA sequence using genetic engineering techniques is called gene cloning.

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