GENE CLONING

Part II: Manipulating the DNA

Manipulation of the DNA

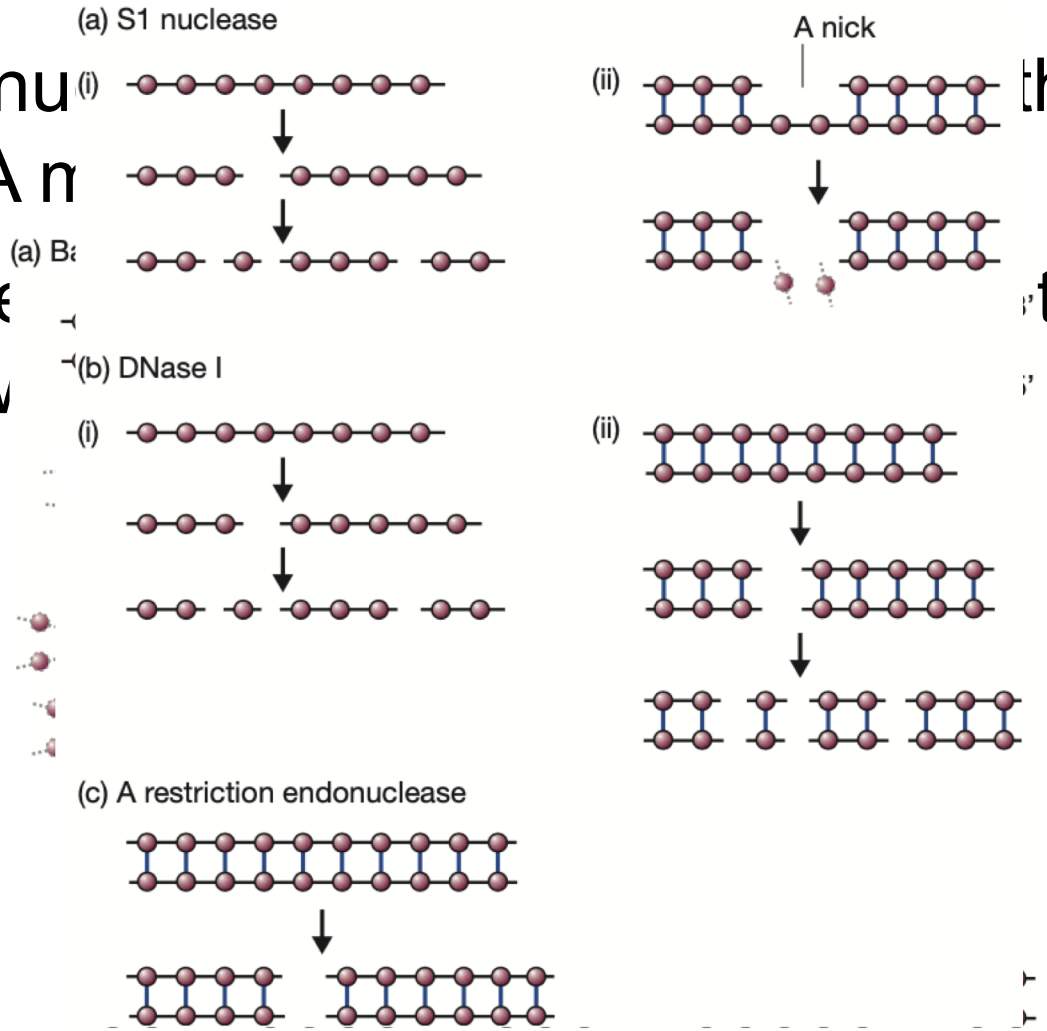
1. The range of DNA manipulative enzymes.
2. Enzymes for cutting DNA—restriction endonucleases.
3. Ligation—joining DNA molecules together.

DNA manipulative enzymes

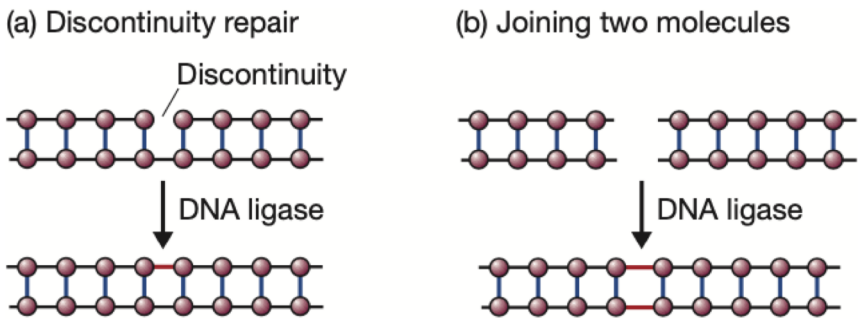
- Nucleases, are enzymes that cut, shorten, or degrade NA molecules.
- Ligases, join NA molecules together.
- Polymerases, makes copies of NA molecules.
- Modifying enzymes, remove/add chemical groups.

Nucleases

- Exonucleases, remove nu the end of a DNA m
- Endonucleases, are able ter bonds v



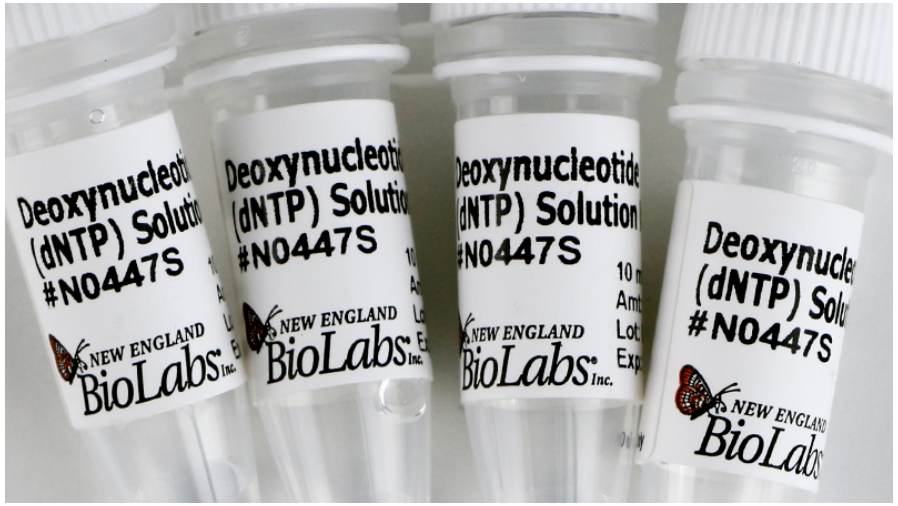
Ligases


(a) Discontinuity repair
Discontinuity
DNA ligase
(b) Joining two molecules
DNA ligase

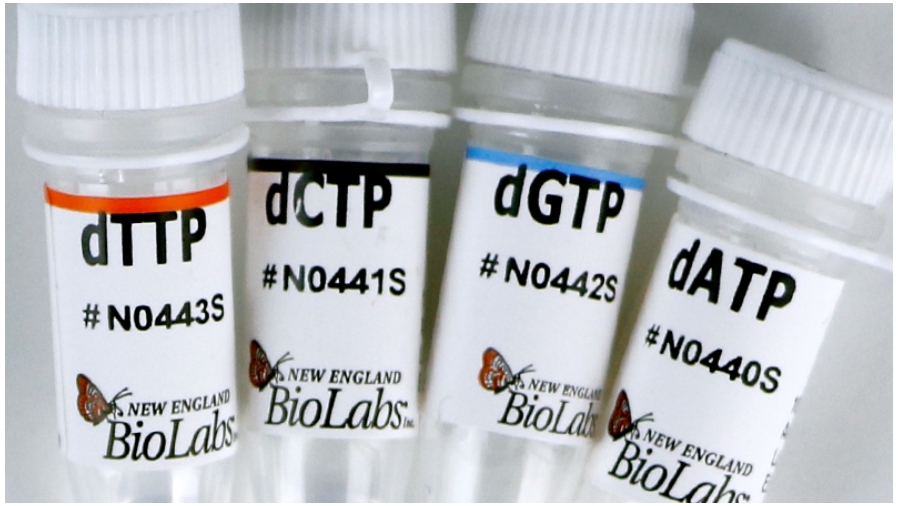

invitrogen
15224-025
Store at: -30 °C
T4 DNA Ligase
Qty: 500 U (1 U/µL)
1296528
Mar2015
For Research Use Only
5791 Van Allen Way Carlsbad, CA 92008
Read SDS
Made in USA
For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.
24-hour Medical or Spill Emergency Response (Chemtrec)
North America +1 866 536 0631 / Other +1 301 431 8585
5X T4 DNA
Lot no. 1253588
T4 DNA Ligase

DNA polymerase

DNA polymerase

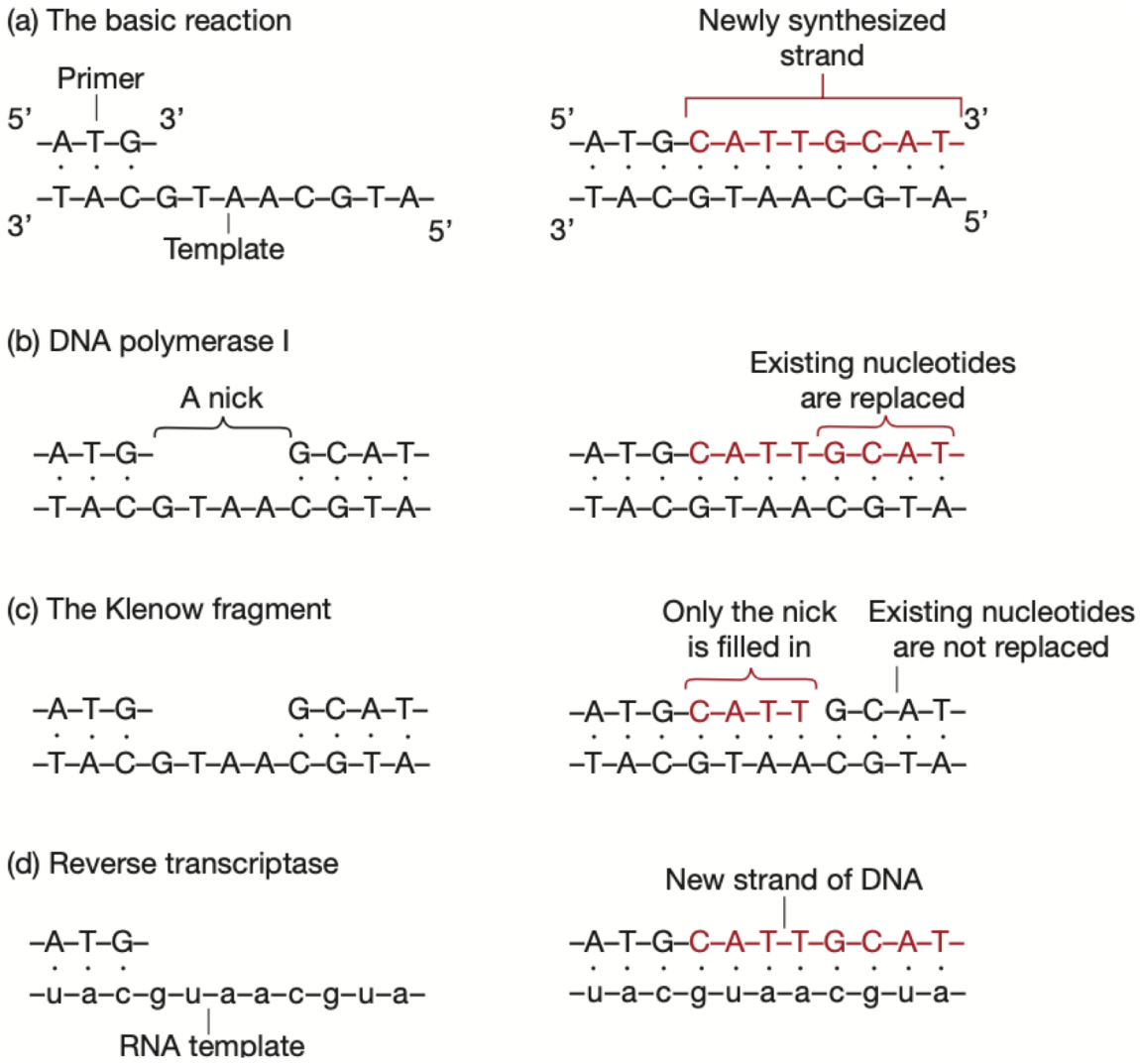


dNTPs



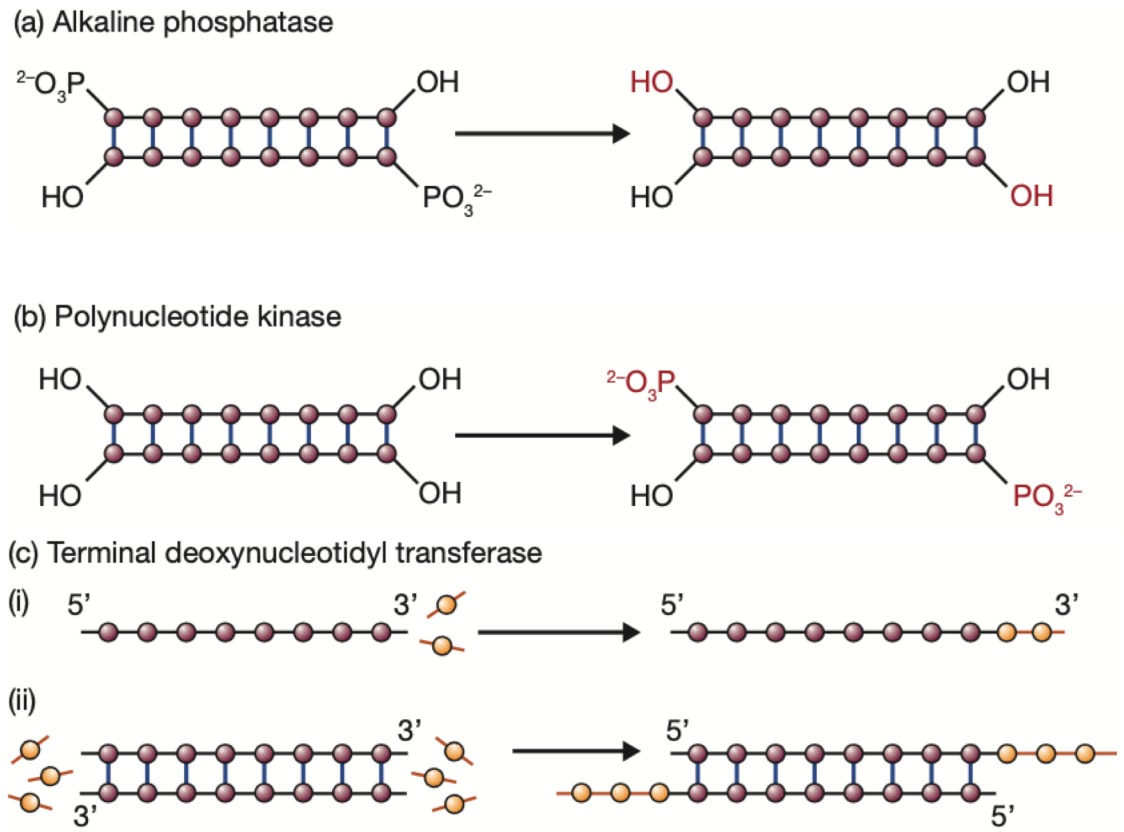
Primers

catalyzed by DNA polymerase

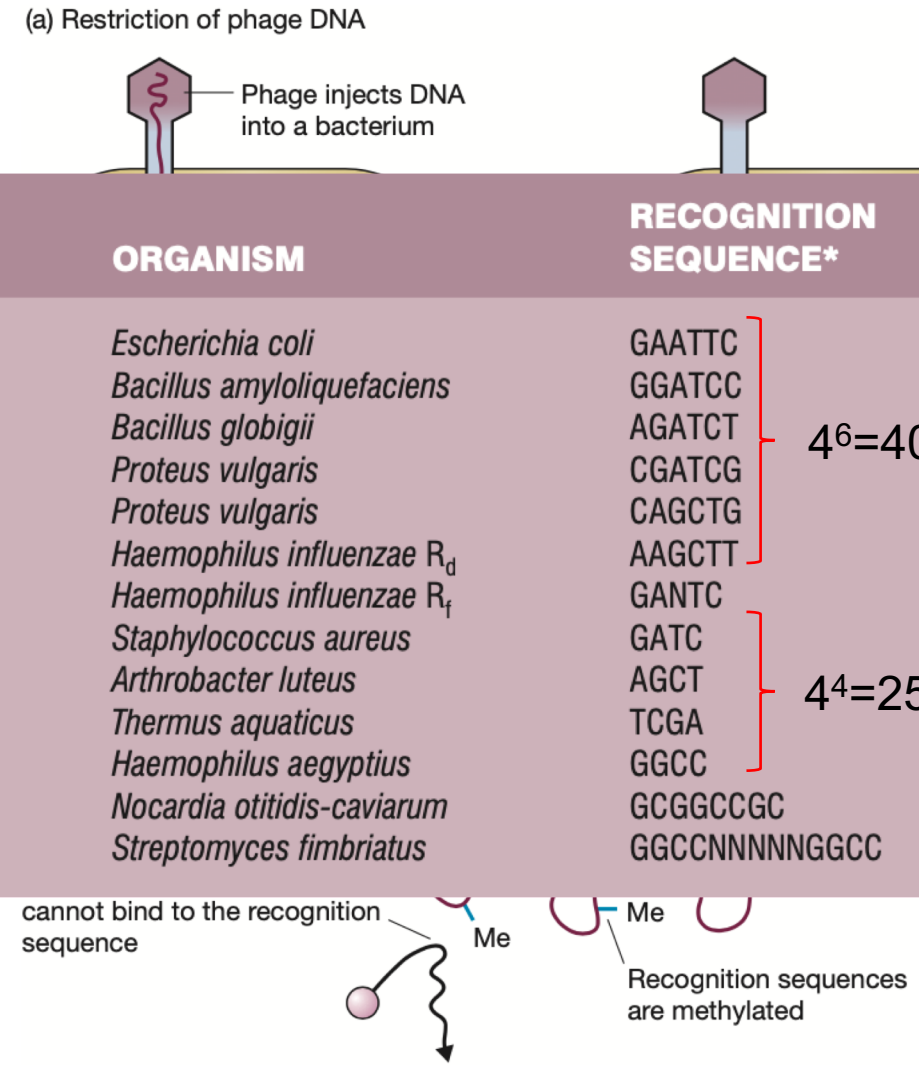


DNA modifying enzymes

- Alkaline phosphatase, removes the phosphate group present at the 5′ terminus of a DNA molecule.
- Polynucleotide kinase adds phosphate groups onto free 5′ termini
- Terminal deoxynucleotidyl transferase adds one or more deoxyribonucleotides onto the 3′ terminus of a DNA molecule.

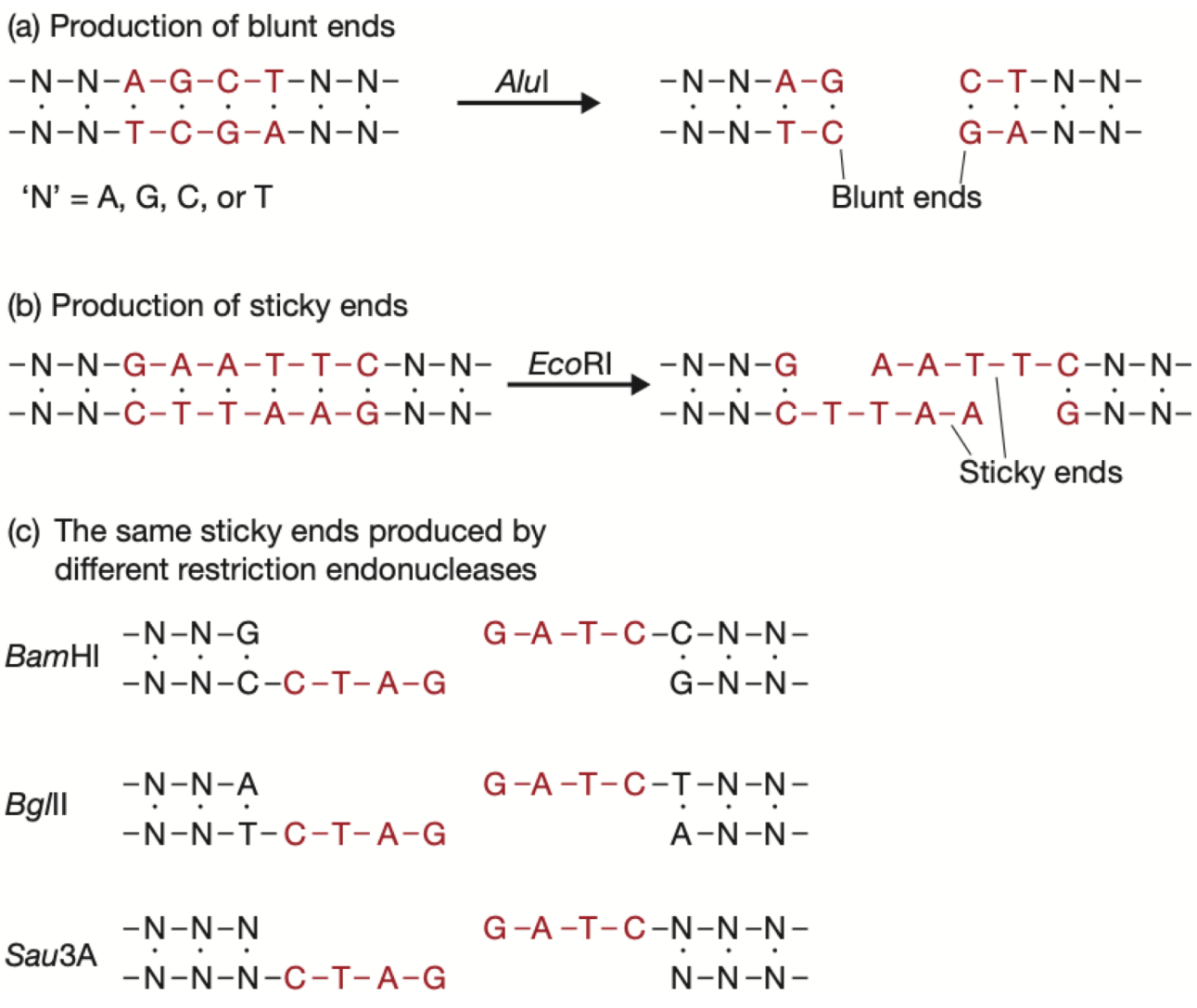


Restriction endonucleases

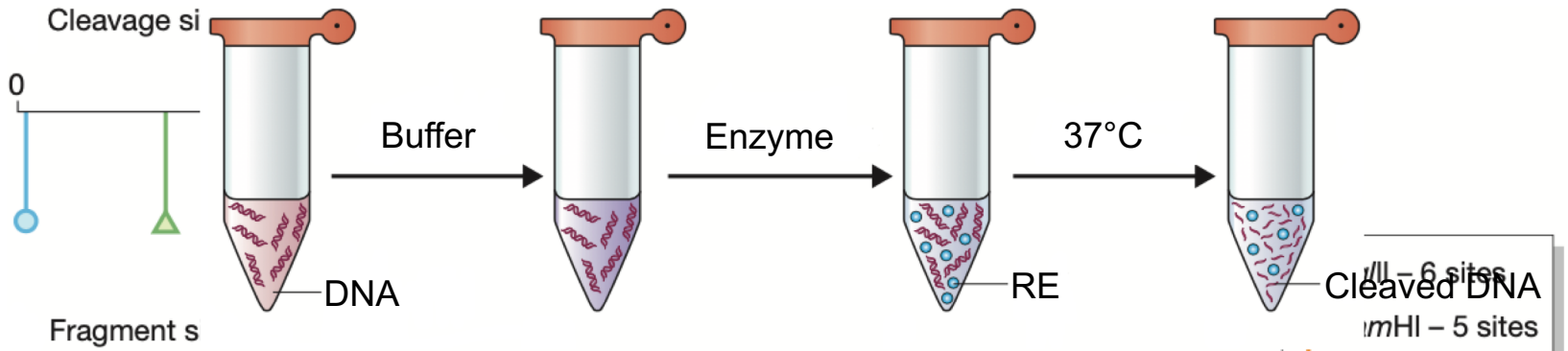


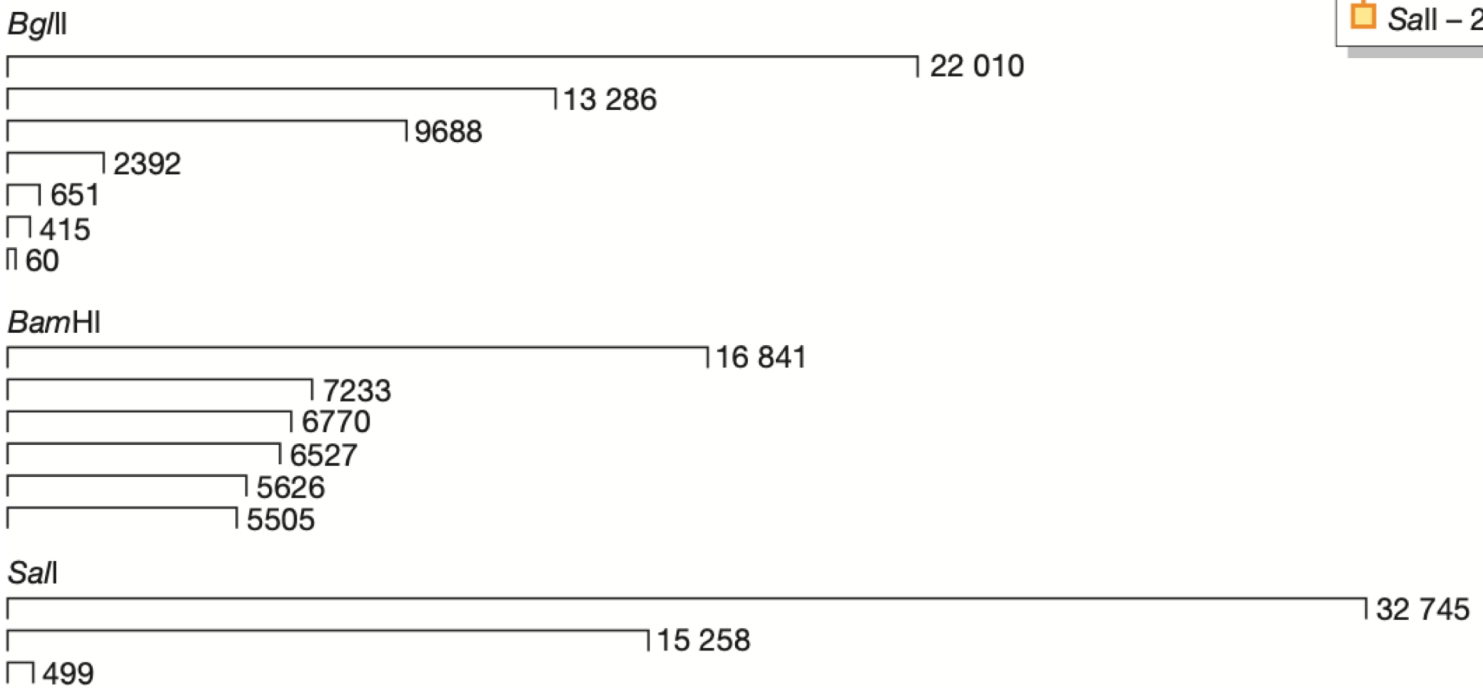
ENZYME	ORGANISM	RECOGNITION SEQUENCE*		BLUNT OR STICKY END
*Eco*RI	*Escherichia coli*	GAATTC		Sticky
*Bam*HI	*Bacillus amyloliquefaciens*	GGATCC		Sticky
*Bgl*II	*Bacillus globigii*	AGATCT	4^6=4096	Sticky
*Pvu*I	*Proteus vulgaris*	CGATCG		Sticky
*Pvu*II	*Proteus vulgaris*	CAGCTG		Blunt
*Hind*III	*Haemophilus influenzae* R_d	AAGCTT		Sticky
*Hin*fI	*Haemophilus influenzae* R_f	GANTC		Sticky
*Sau*3A	*Staphylococcus aureus*	GATC		Sticky
*Alu*I	*Arthrobacter luteus*	AGCT	4^4=256	Blunt
*Taq*I	*Thermus aquaticus*	TCGA		Sticky
*Hae*III	*Haemophilus aegyptius*	GGCC		Blunt
*Not*I	*Nocardia otitidis-caviarum*	GCGGCCGC		Sticky
*Sfi*I	*Streptomyces fimbriatus*	GGCCNNNNNGGCC		Sticky

blunt vs sticky ends

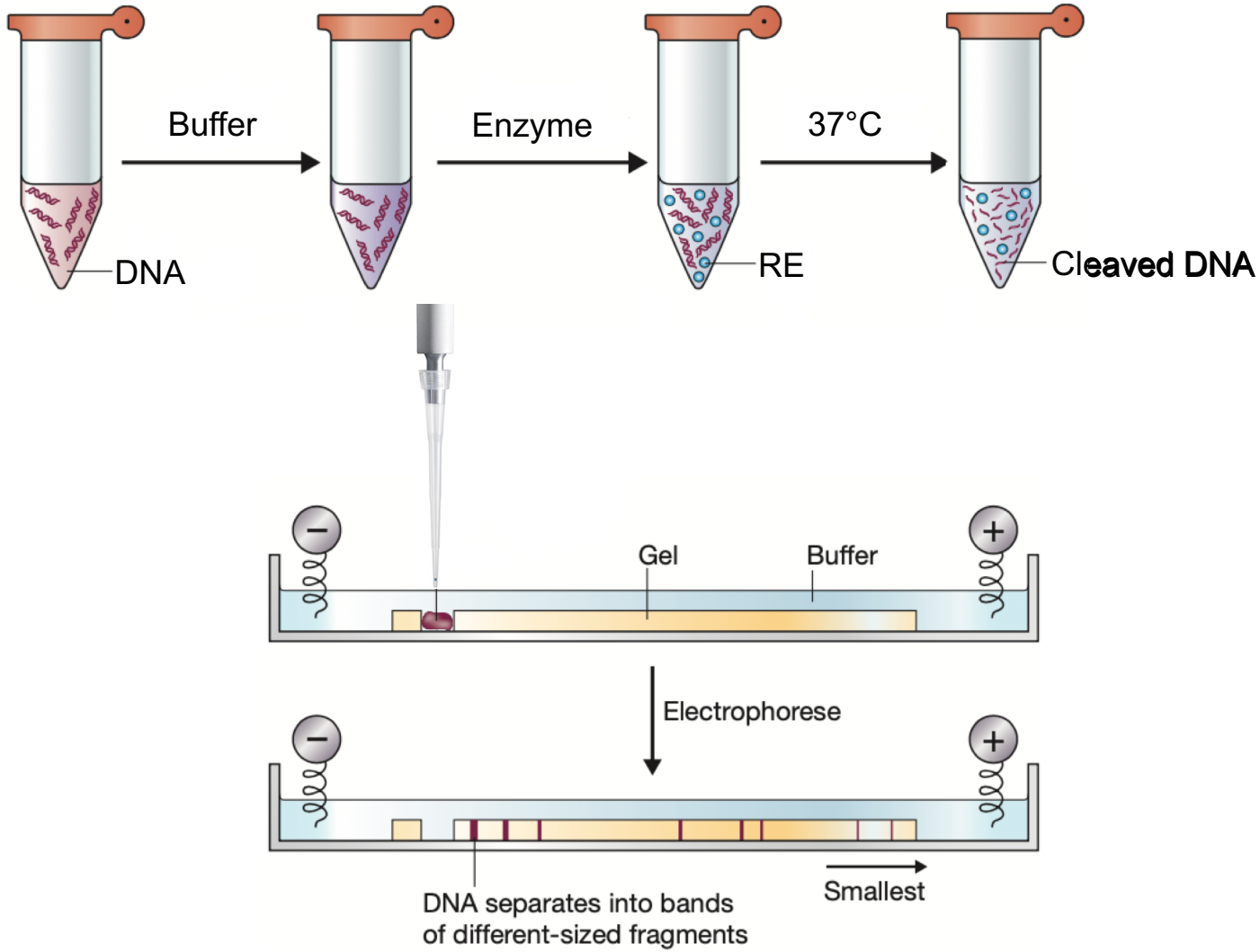


Restriction digestion @lab

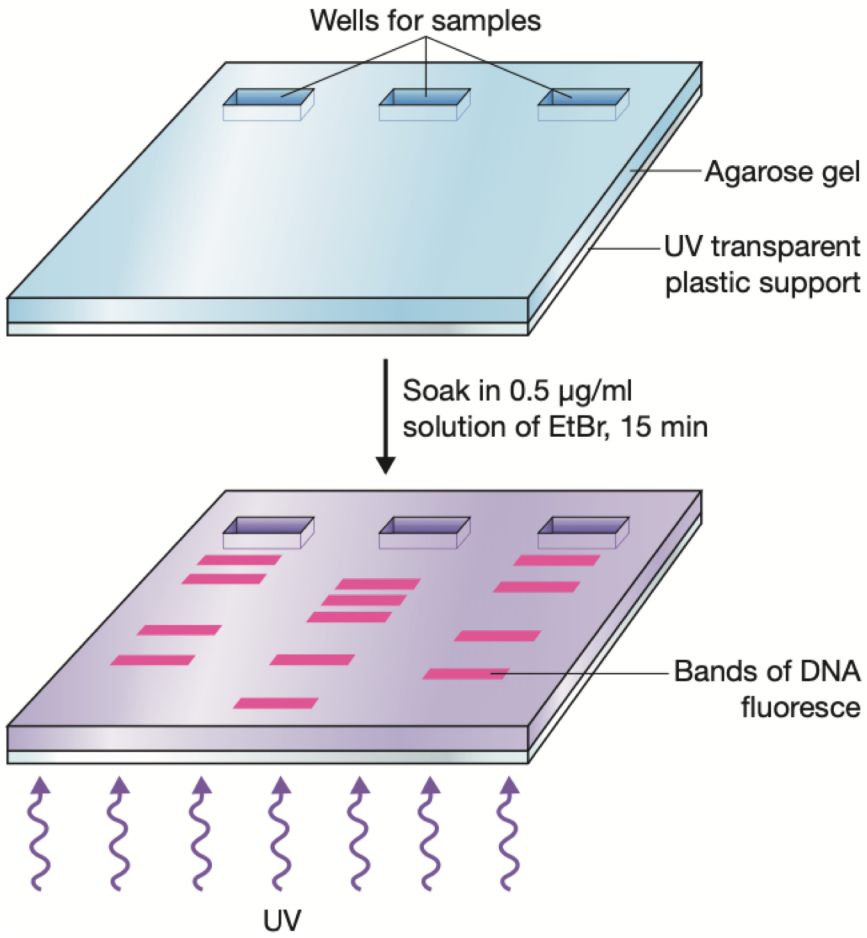


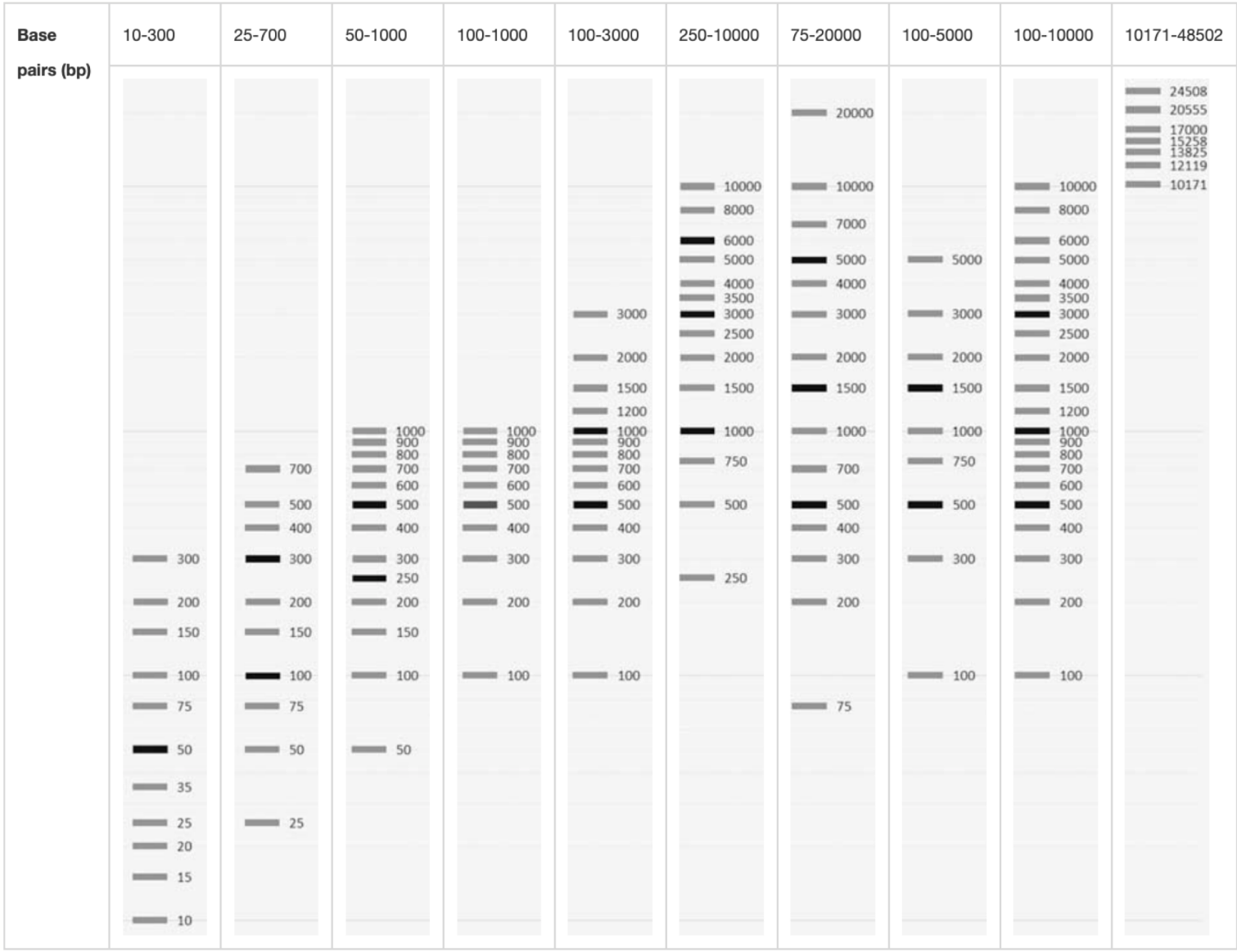


Restriction digestion @lab

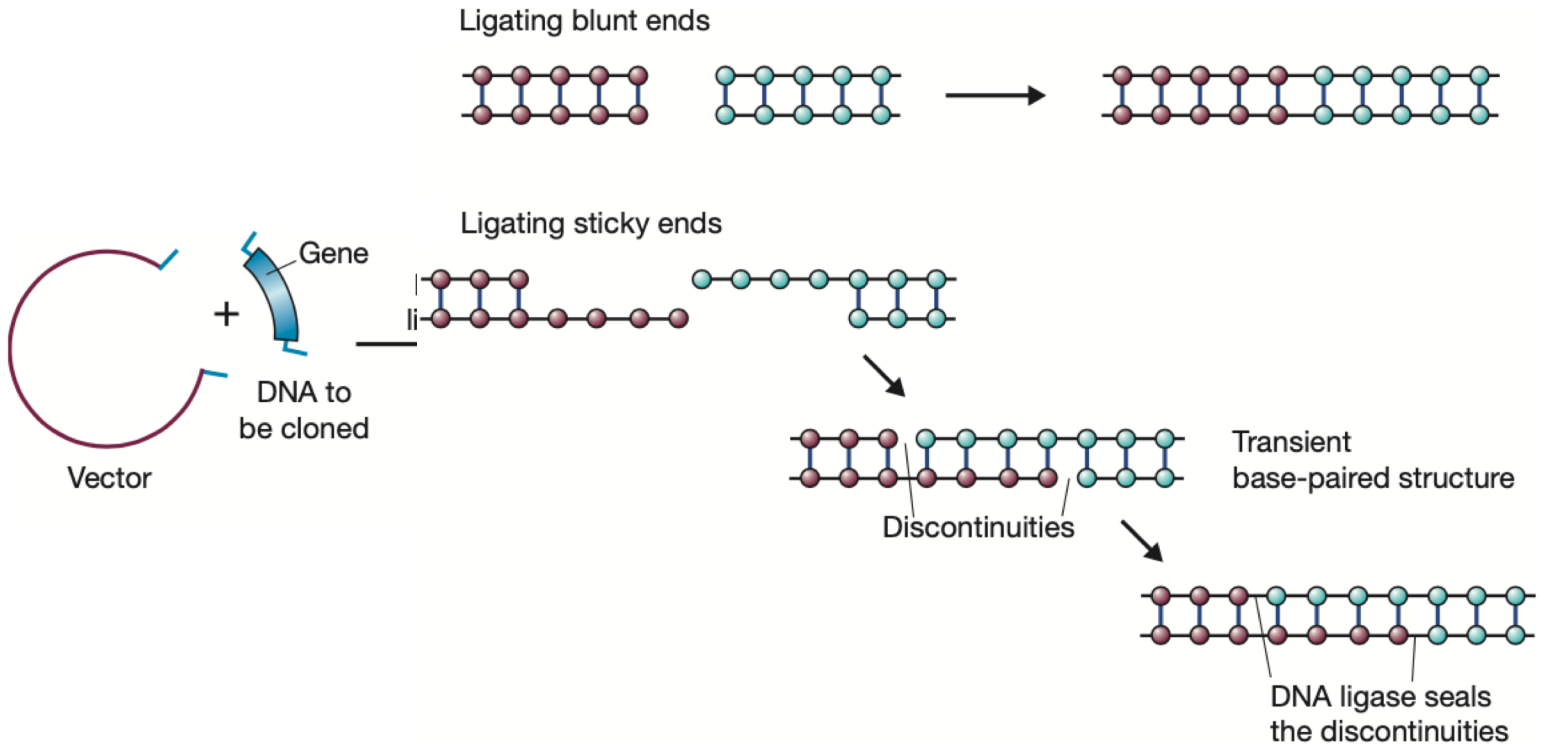


seeing the DNA





ligating the DNA



calculating the DNA to insert ratio

www.insilico.uni-duesseldorf.de/Lig_Input.html

LIGATION CALCULATOR

Please provide the following information:

.....vector size (in bp):

vector amount (in ng):

.....insert size (in bp):

Please enter the molar vector : insert ratio:

(normally a vector to insert ratio of 1 to 3 is used of cohesive end ligations. higher molar ratios can be used for blunt end ligations)

When pressing the "do calculation" button the tool calculates the required amount of insert DNA (in ng) resulting in the given molar ratio

do calculation reset values