université PARIS-SACLAY | FACULTÉ DE PHARMACIE

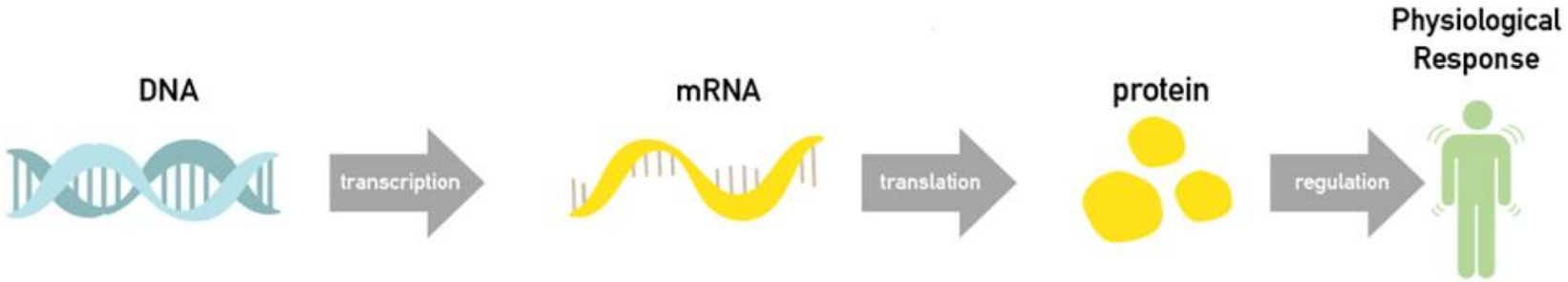
Nucleic acids as diagnostic and therapeutic tools

Dr. Francois Fay
Assistant Professor
Institut Galien Paris-Saclay

Institut Galien PARIS-SACLAY

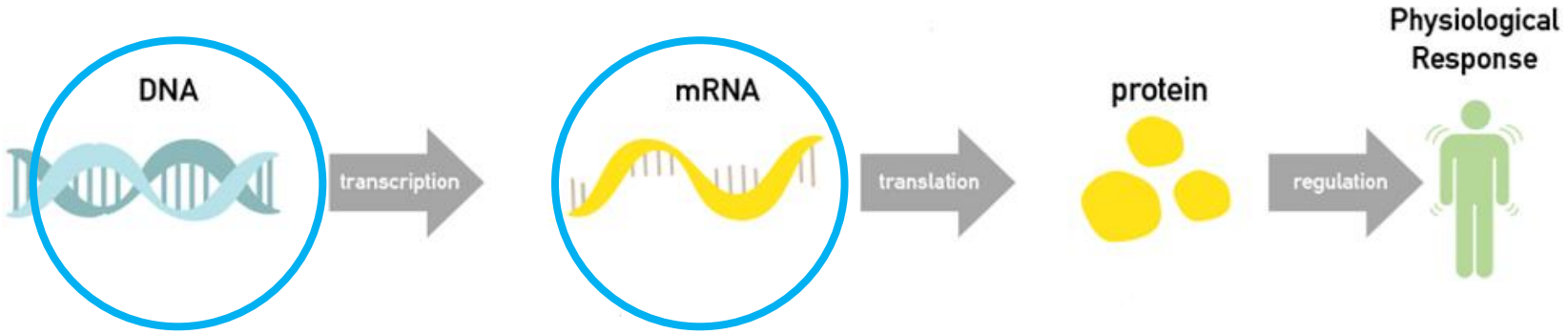
cnrs

Nucleic acids as diagnostic tools and therapeutics



Adapté de http://www.bonac.com/global/en/nucleic/about/

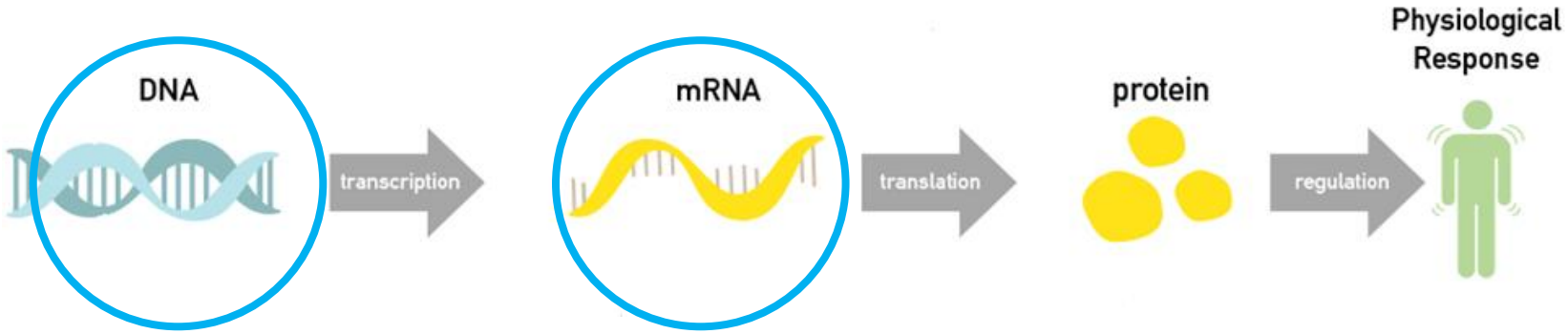
Nucleic acids as diagnostic tools and therapeutics



Adapté de http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as diagnostic tools and therapeutics

Diagnostic Protein production Therapeutics



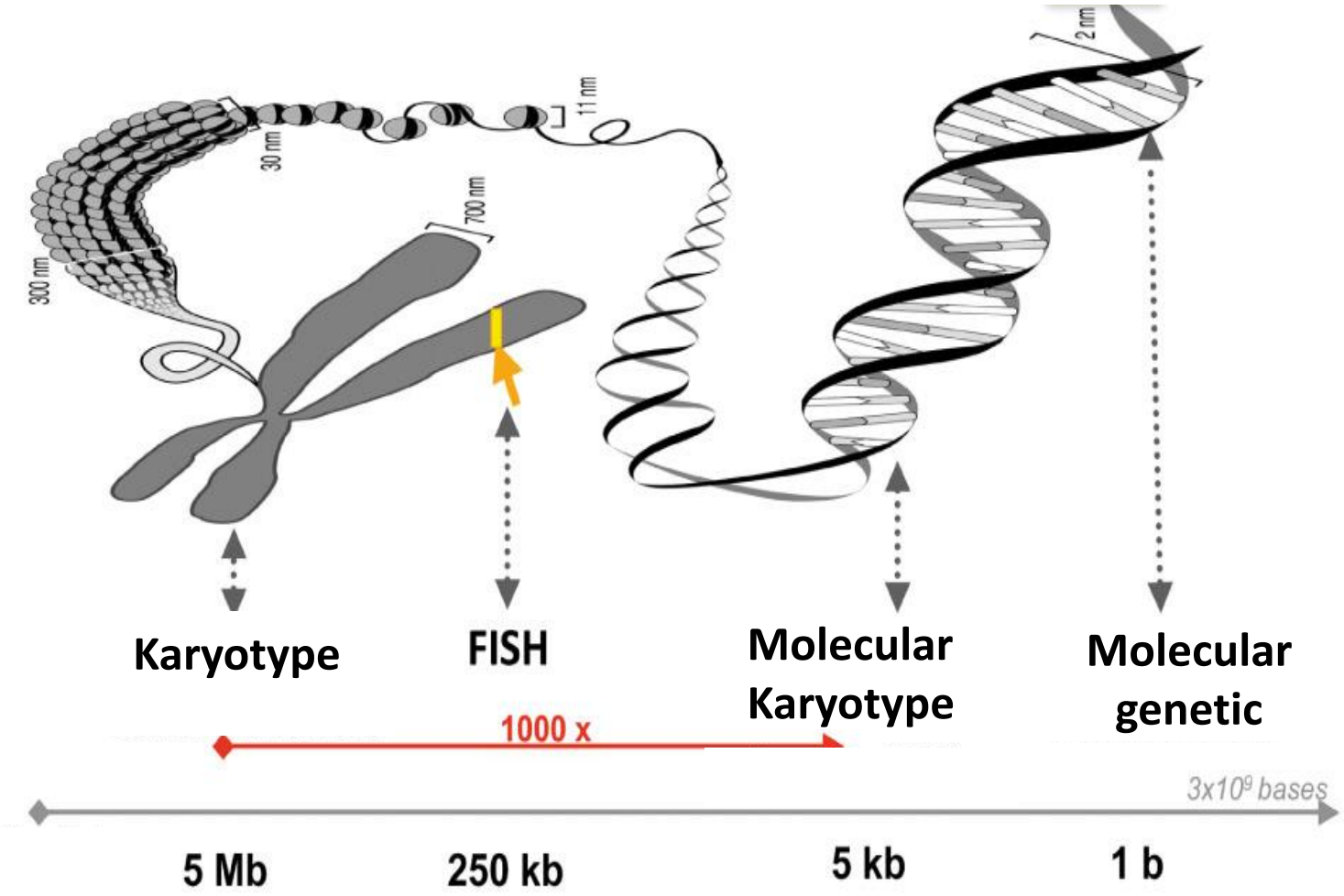
Adapted from http://www.bonac.com/global/en/nucleic/about/

Diagnostic

Ferrarini *et al* Rev Med Suisse 2010

Diagnostic

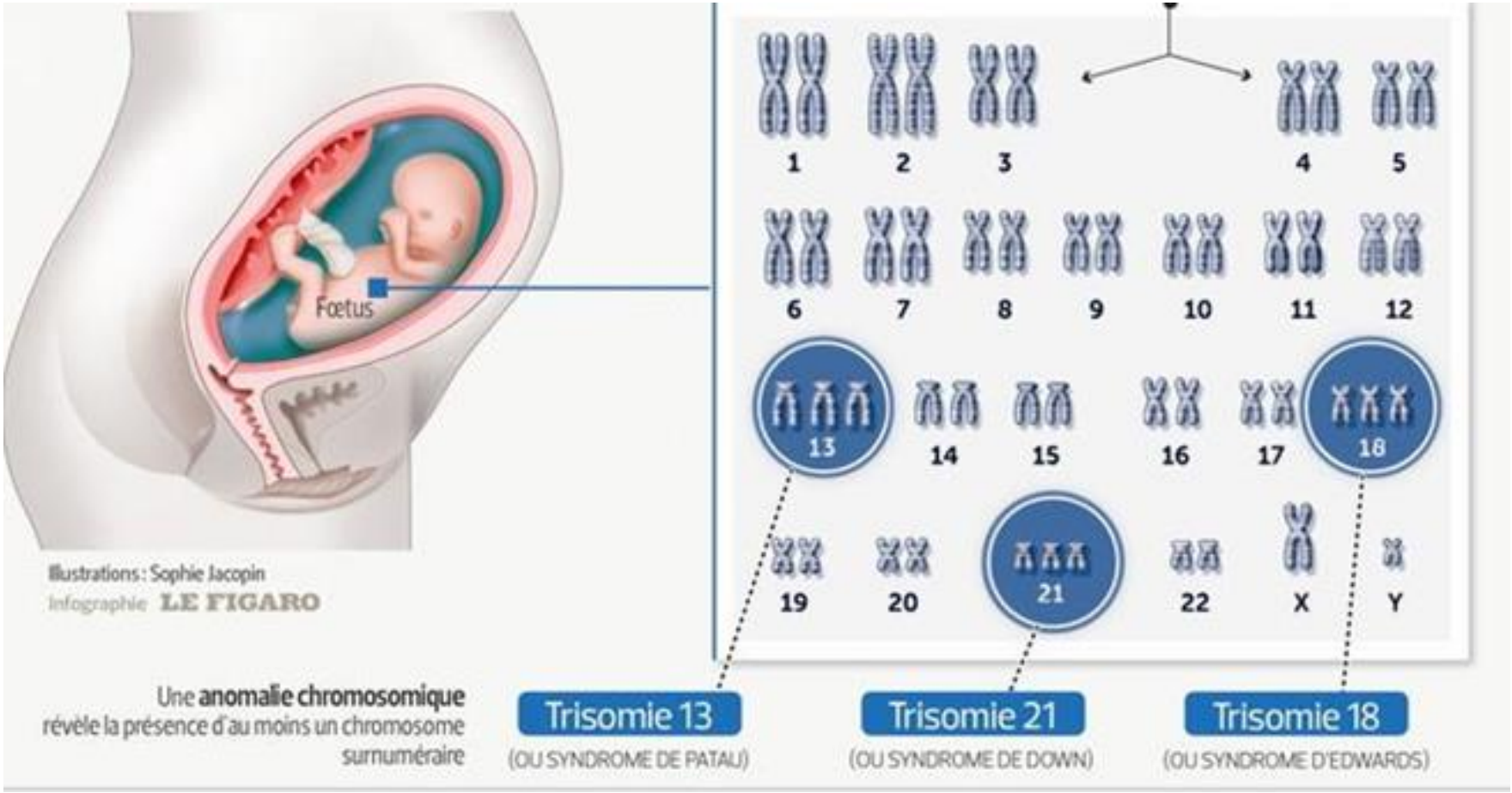
DNA



Ferrarini *et al* Rev Med Suisse 2010

Diagnostic

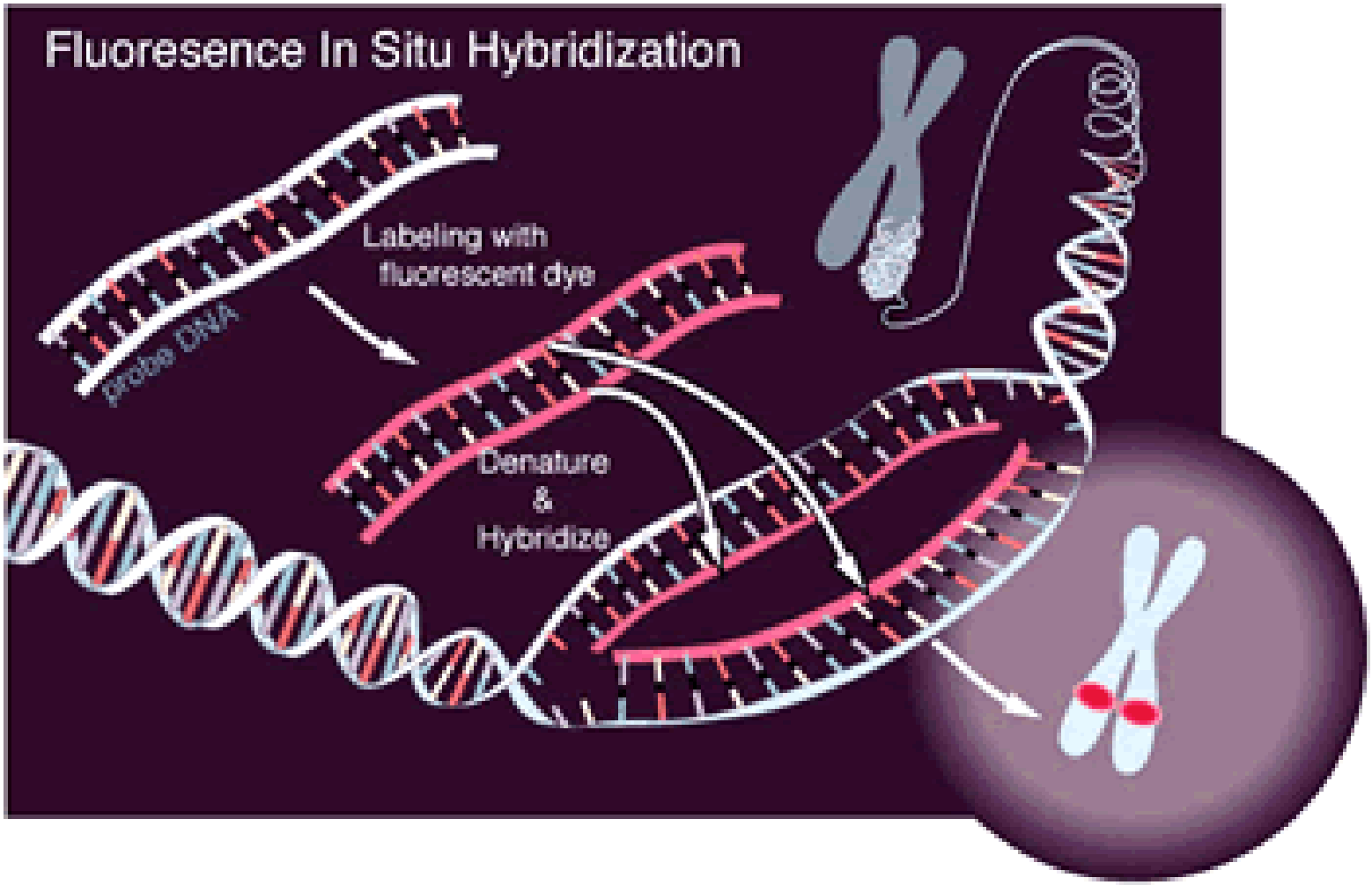
Karyotype



http://sante.lefigaro.fr

Diagnostic

Fluorescence In-Situ Hybridization (FISH)

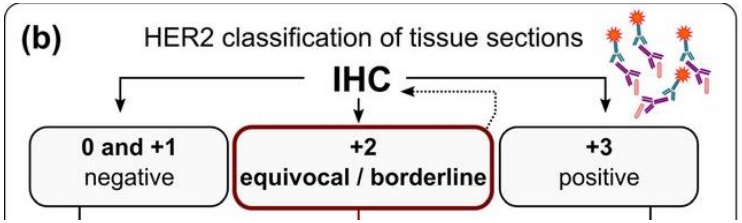


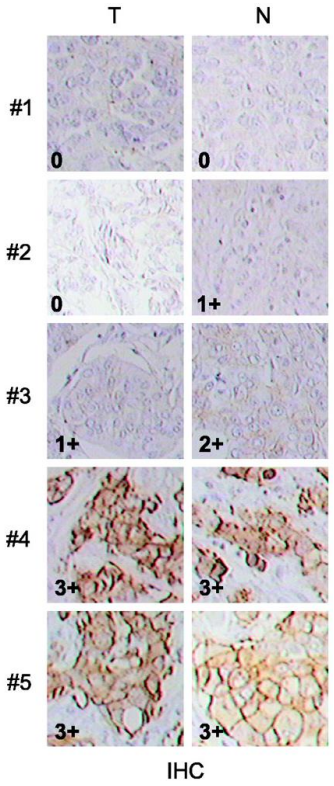
http://pathology.uthscsa.edu/strl/cytogenics/fish.shtml

Diagnostic

Fluorescence In-Situ Hybridization (FISH)

Example: HER2 gene analysis in cancer biopsy





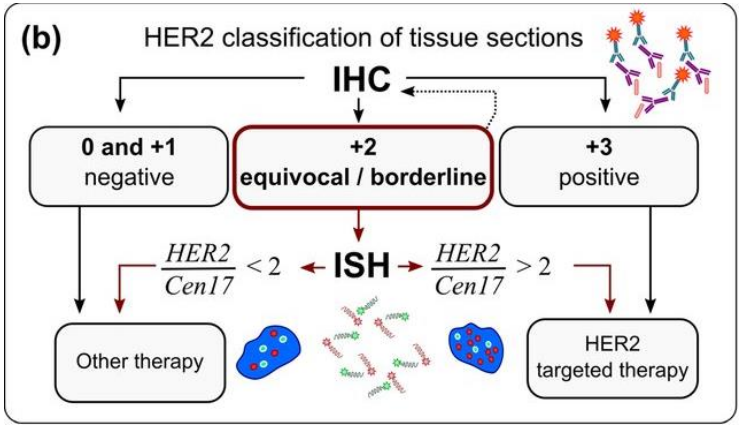
Huber et al Biomicrofluidics 2018

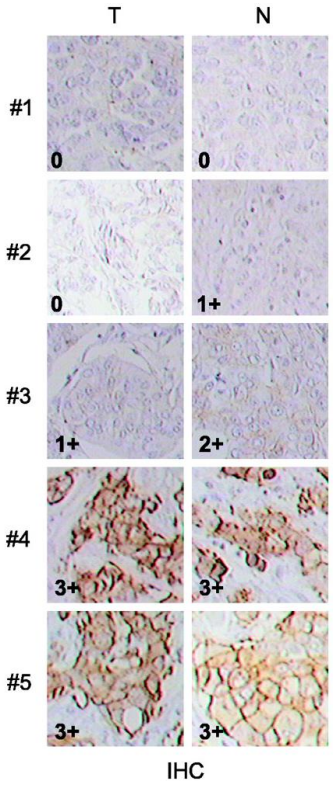
Ursula Vinatzer et al. Clin Cancer Res 2005

Diagnostic

Fluorescence In-Situ Hybridization (FISH)

Example: HER2 gene analysis in cancer biopsy





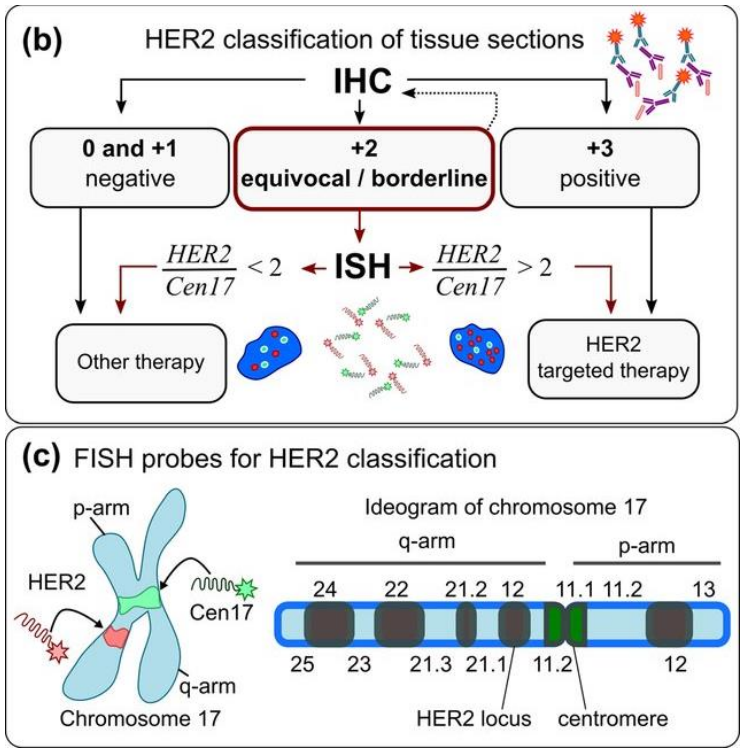
Huber et al Biomicrofluidics 2018

Ursula Vinatzer et al. Clin Cancer Res 2005

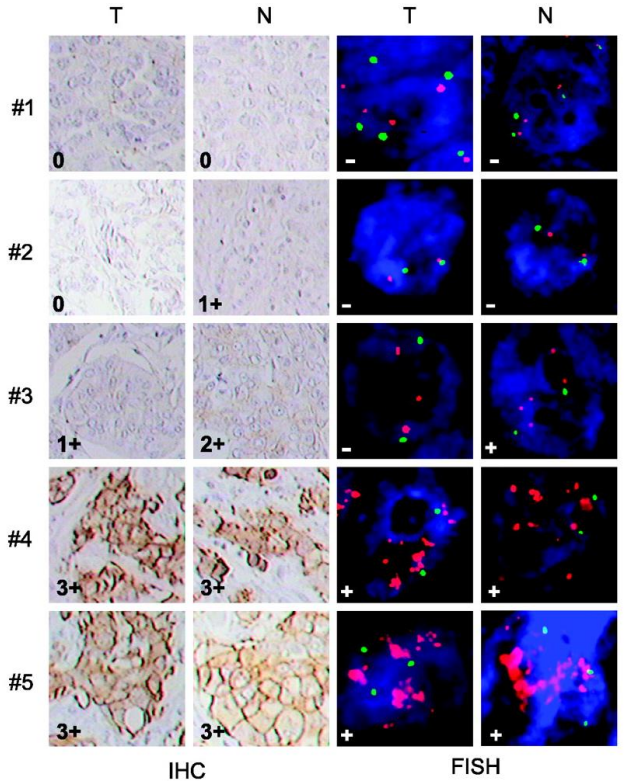
Diagnostic

Fluorescence In-Situ Hybridization (FISH)

Example: HER2 gene analysis in cancer biopsy



Green : control gene / Red : HER2

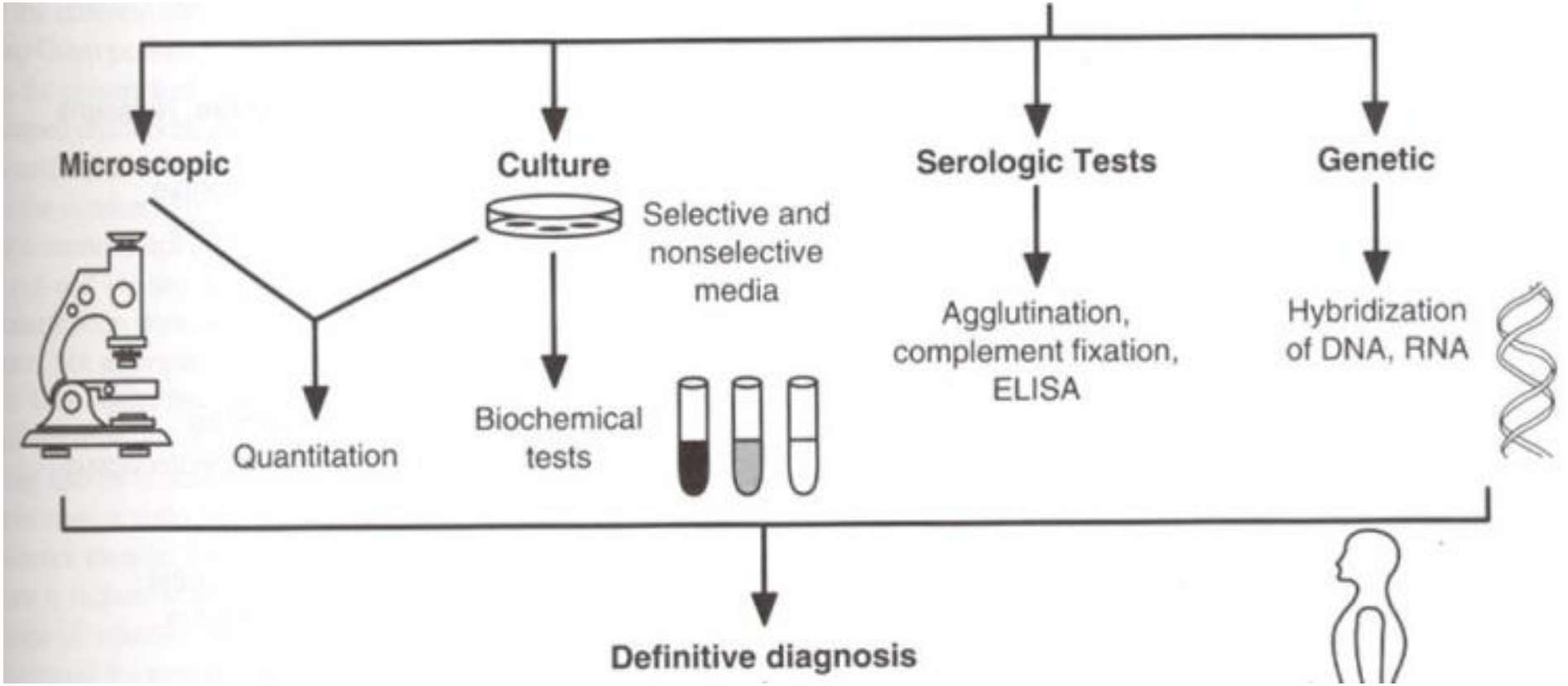


Huber et al Biomicrofluidics 2018

Ursula Vinatzer et al. Clin Cancer Res 2005

Diagnostic

Identification of pathogens

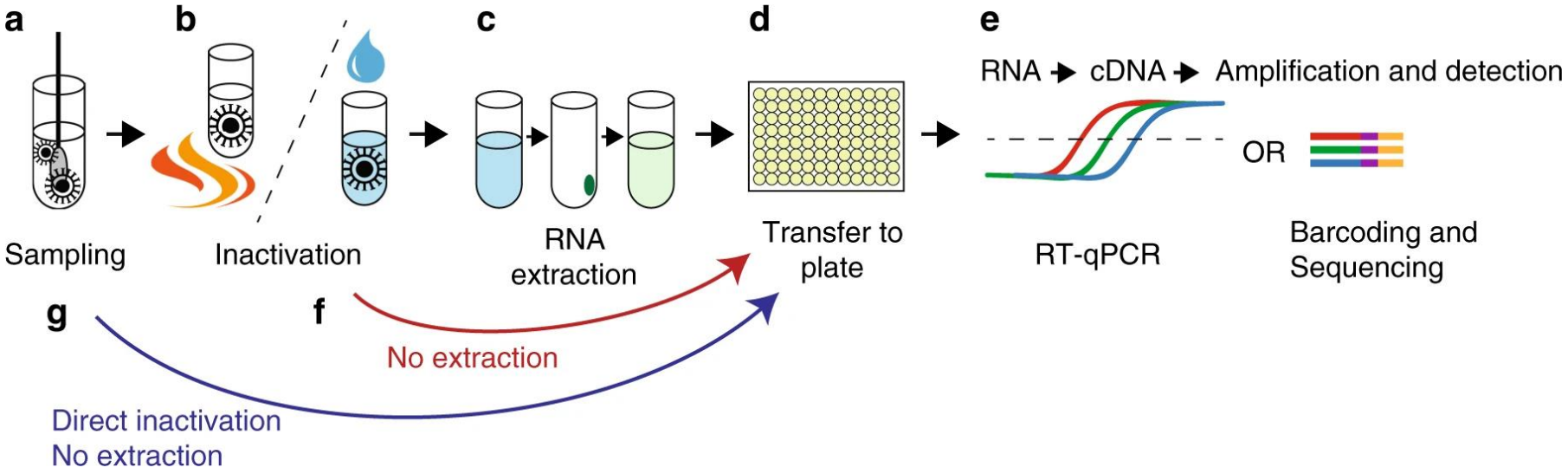


Medical Microbiology. 4th edition. John A. Washington. https://www.ncbi.nlm.nih.gov/books/NBK8014/

Diagnostic

Identification of pathogens DNA/RNA

Schematic overview of SARS-CoV-2 RT-PCR testing procedure.



Smyrlaki et al, Nature Communications 11 4812 (2020)

Diagnostic

Other:

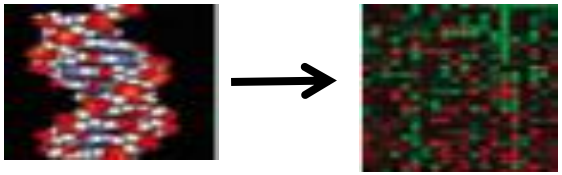
- Mutation : Single nucleotide polymorphisms (SNP)
- Fragmentation DNA
- Methylation DNA

Diagnostic

Transcriptome

Genome **Transcriptome**

DNA RNA



Diagnostic

Transcriptome

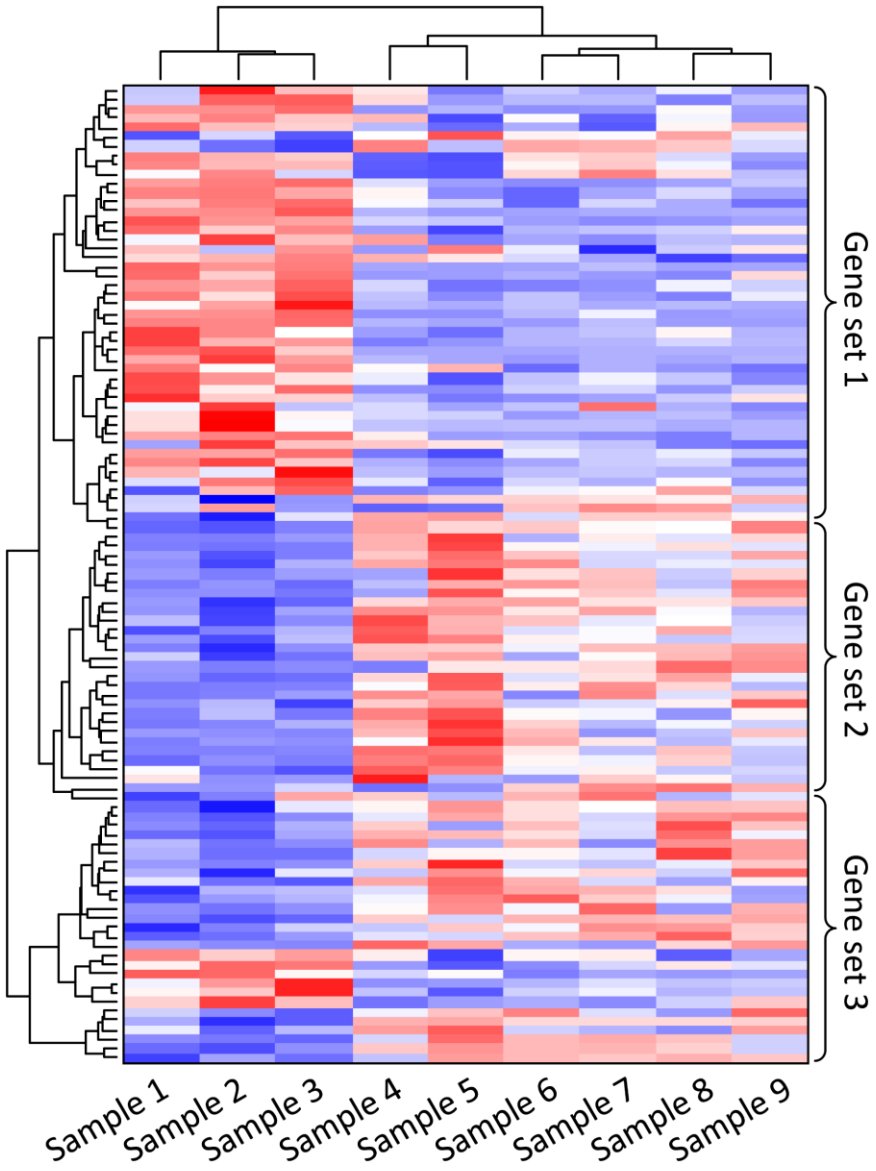
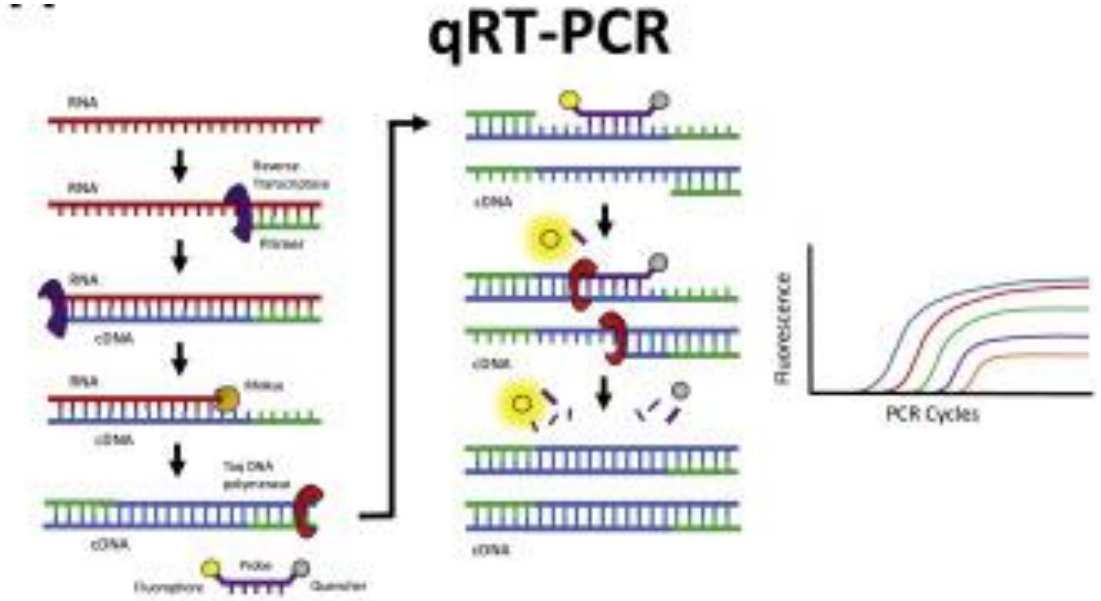


Fig 6. identification of gene co-expression patterns across different samples. Heatmap Each column contains the measurements for gene expression change for a single sample. Relative gene expression is indicated by colour: high-expression (red), median-expression (white) and low-expression (blue). Genes and samples with similar expression profiles can be automatically grouped (left and top trees). Samples may be different individuals, tissues, environments, or health conditions. In this example, expression of gene set 1 is high and expression of gene set 2 is low in samples 1, 2, and 3

Loxe et al PLoS Comput Biol 2017

Diagnostic

Transcriptome



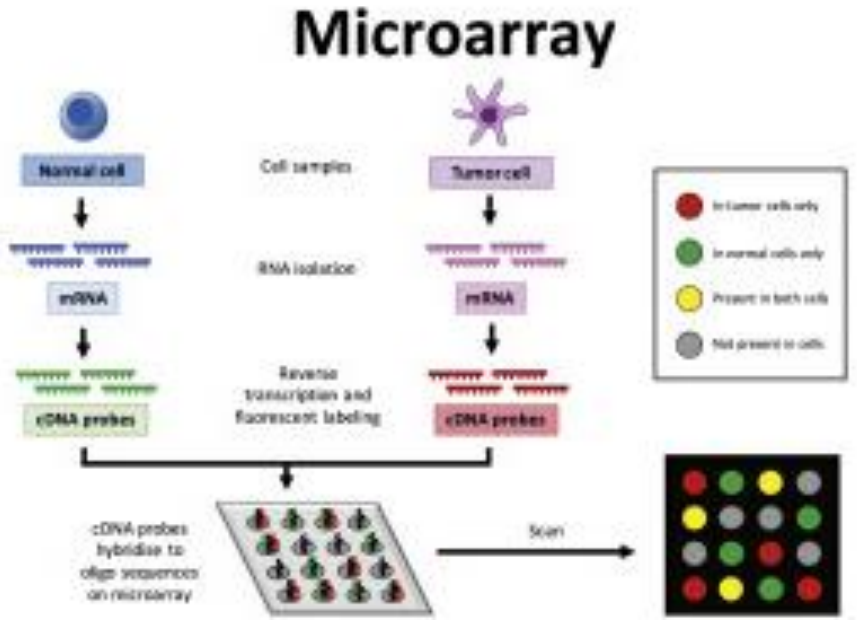
- TaqMan®: Sample RNA is converted into cDNA and amplified by PCR in the presence of a target-specific oligonucleotide bound to a fluorescent probe and fluorescence quencher.
- As DNA polymerase synthesizes the new DNA strand, it cleaves the fluorescent probe off the oligonucleotide, freeing it to fluoresce.
- The fluorescence grows stronger with each PCR cycle as more fluorescent probes are freed.

(other methods than TaqMan exist)

Rogawski DS et al Transl Res. 2017 doi: 10.1016/j.trsl.2017.06.013

Diagnostic

Transcriptome

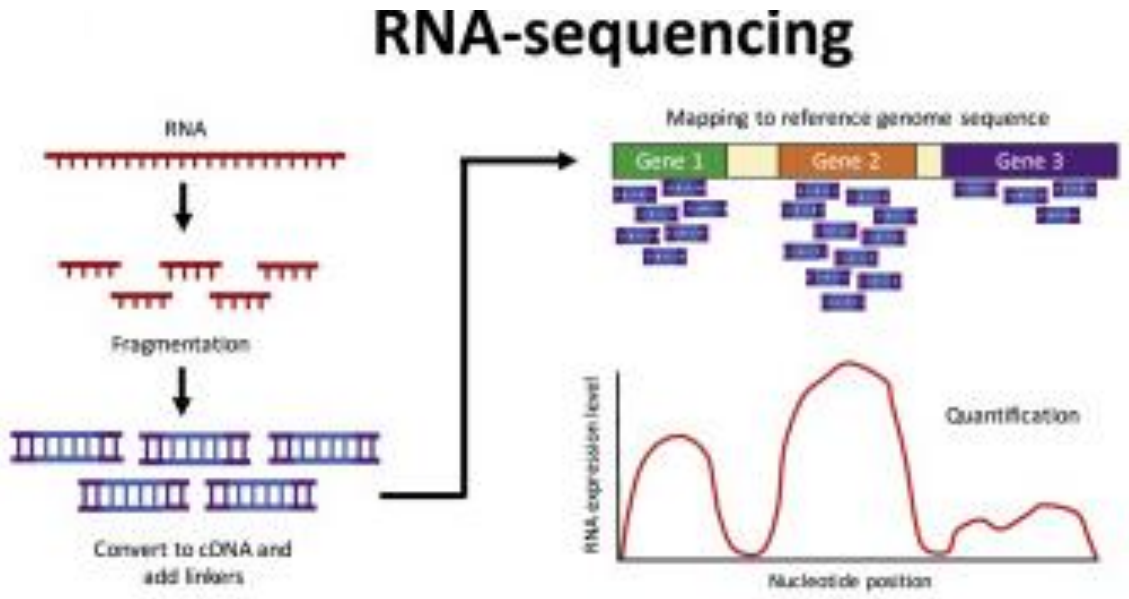


- RNA is extracted from cells, reverse transcribed, and labeled with fluorescent probes (example green for normal cDNA, red for tumor cDNA).
- The cDNAs are applied to a microarray chip, where they bind to complementary sequences from annotated genes.
- The relative amount of green versus red fluorescence corresponds to the relative expression of genes in normal versus tumor cells.

Rogawski DS et al Transl Res. 2017 doi: 10.1016/j.trsl.2017.06.013

Diagnostic

Transcriptome

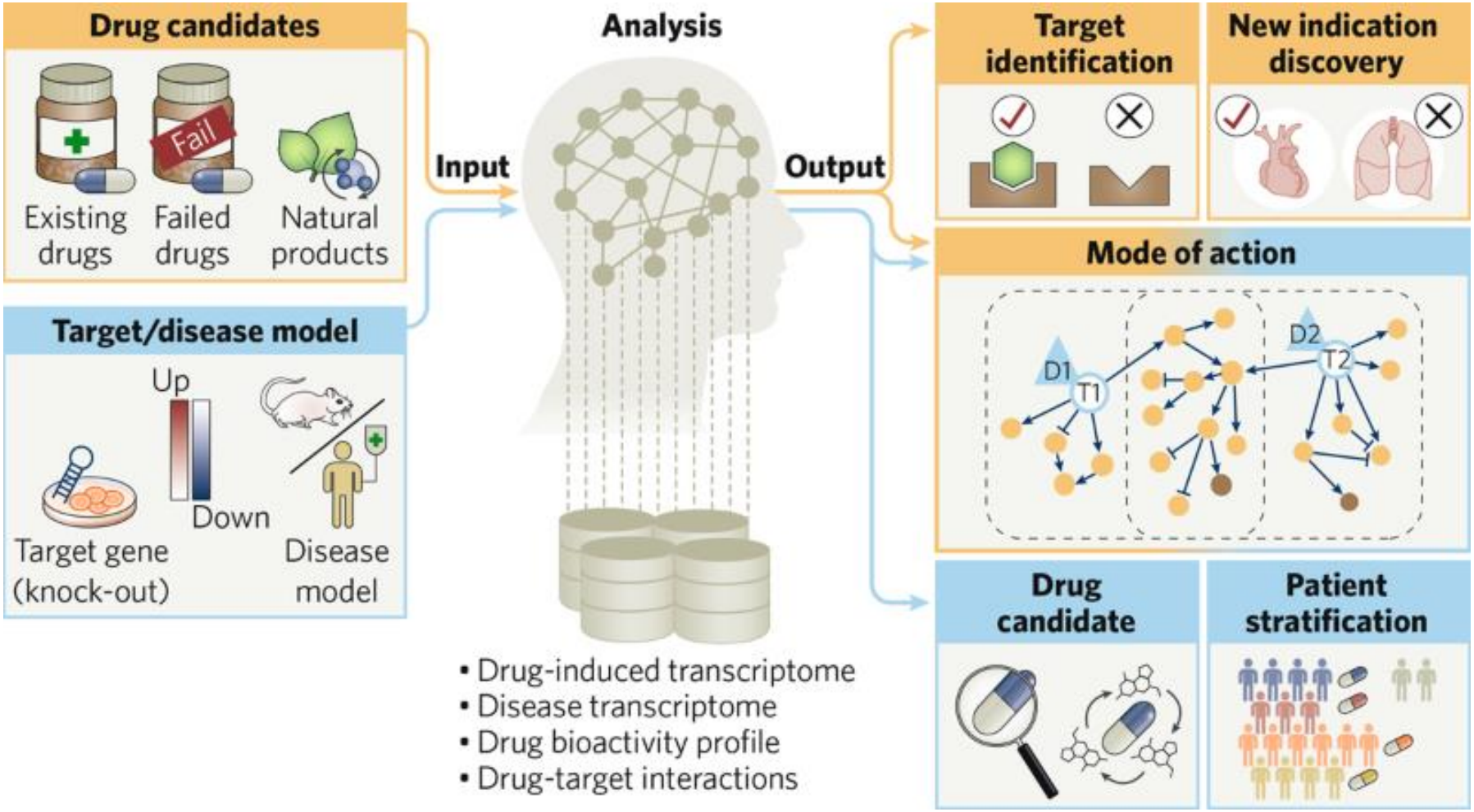


- Extracted RNA is fragmented, reverse-transcribed, and modified with linkers to aid sequencing.
- The cDNA is sequenced, and the resulting sequences are aligned against a reference genome to reveal the expression levels of various genes in the

Rogawski DS et al Transl Res. 2017 doi: 10.1016/j.trsl.2017.06.013

Diagnostic

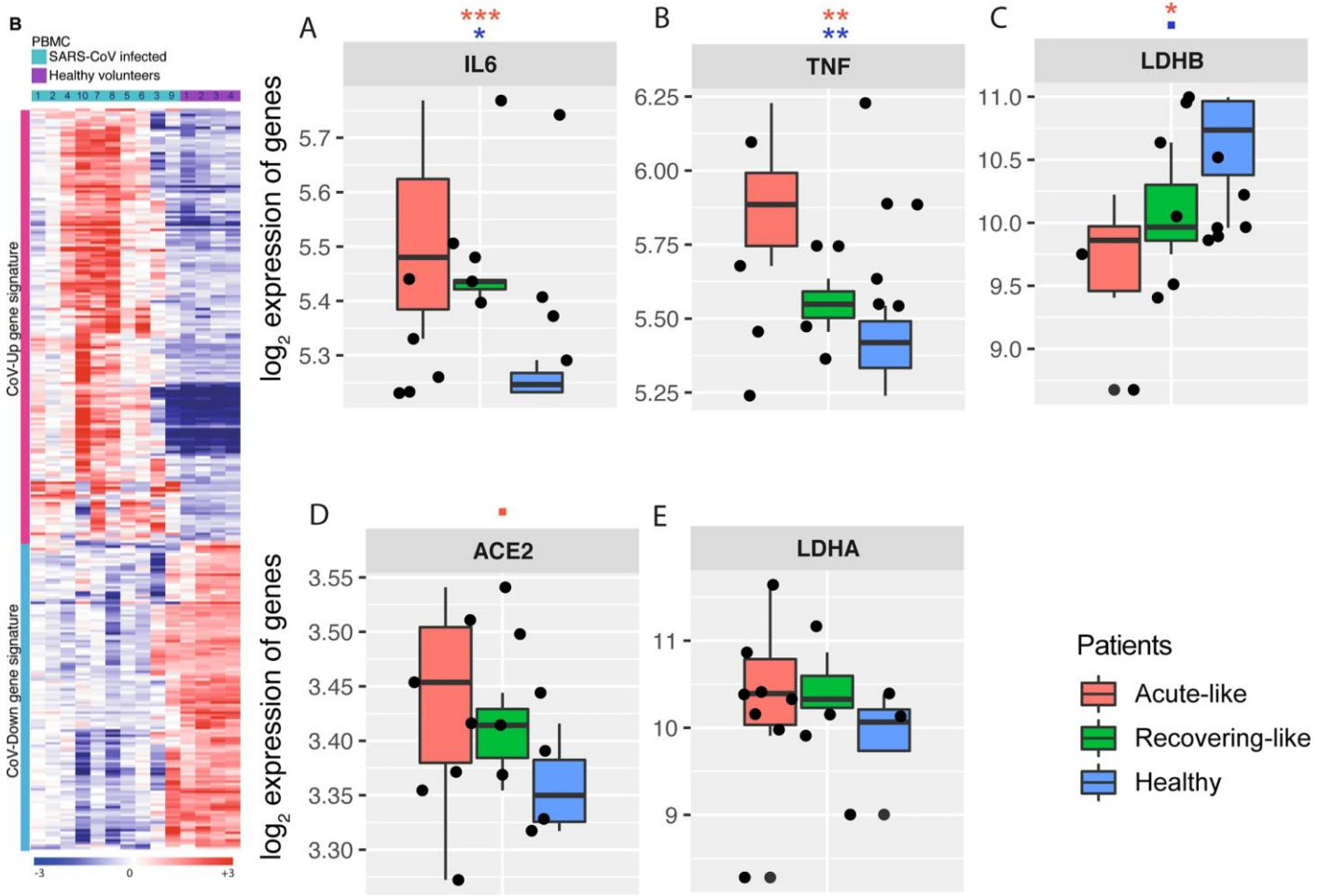
Transcriptome



Adaptée https://www.nature.com/articles/d43747-021-00035-9

Diagnostic

Transcriptome : Covid-engine



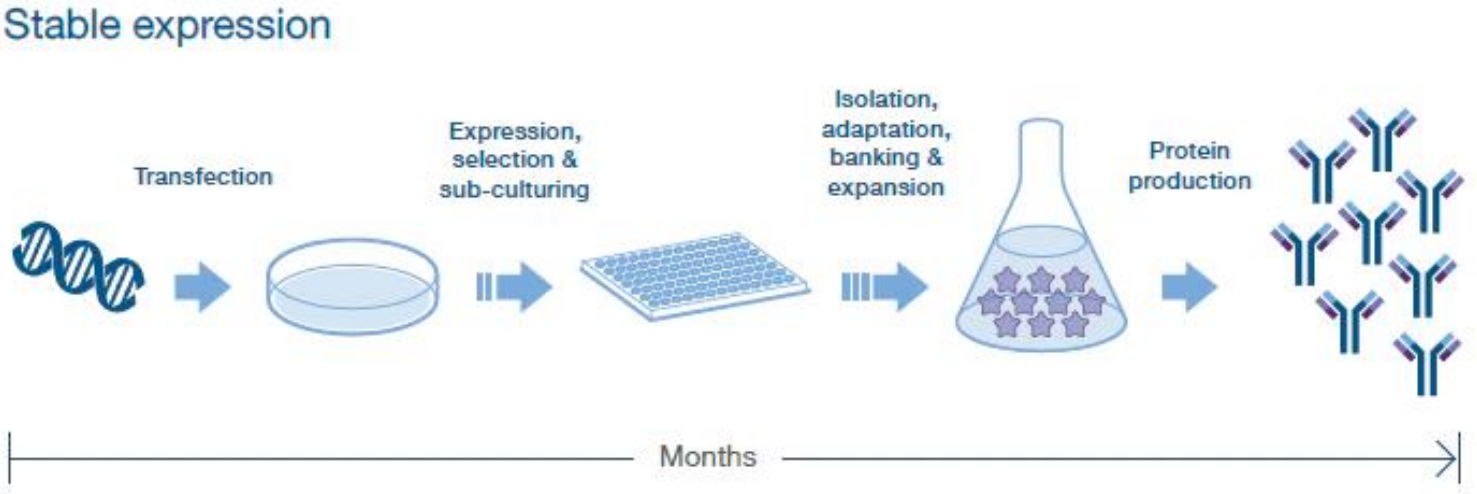
(Angiotensin-Converting Enzyme 2)
(Lactate Dehydrogenase A/B)

Adaptée de Sadanandam, et al Cell Death Discovery 6 : 141 (2020)

Nucleic acids as diagnostic tools and therapeutics

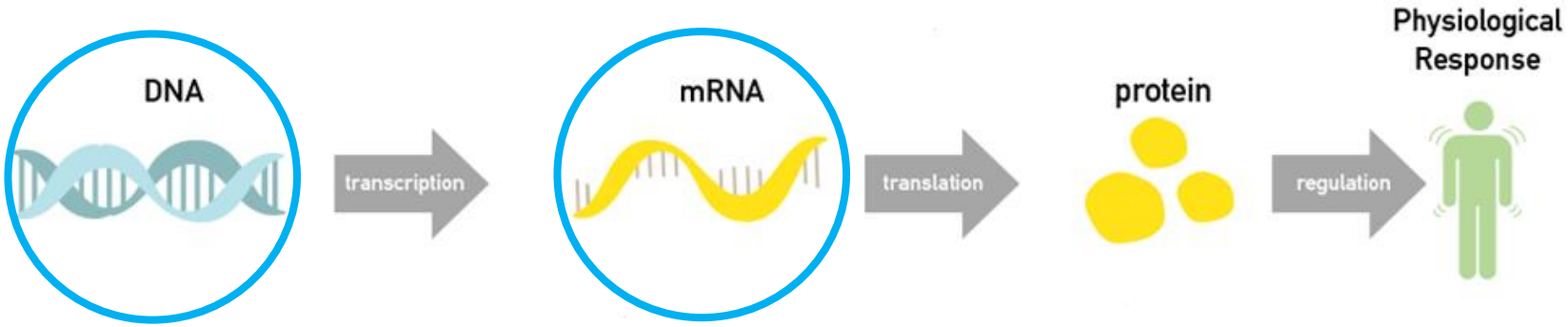
Protein production

Protein production



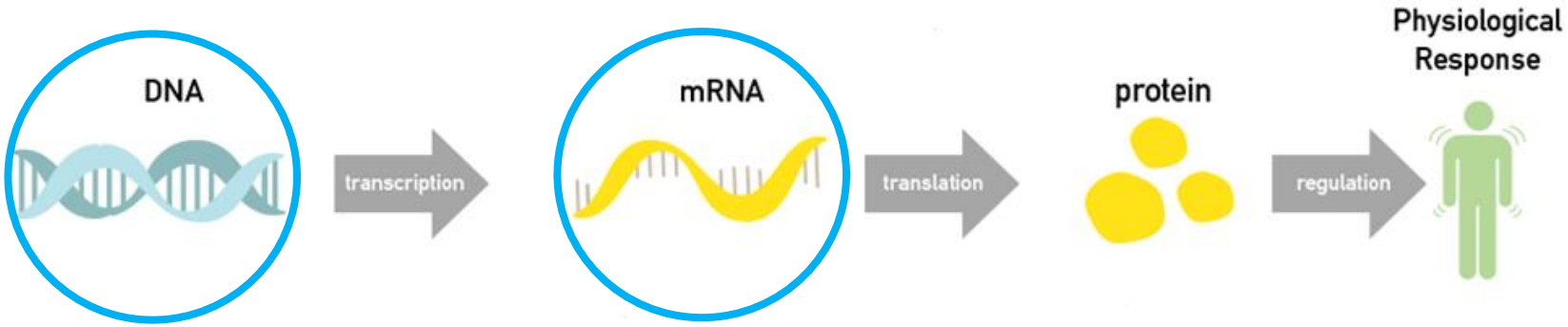
Nucleic acids as diagnostic tools and therapeutics

Therapeutics



http://www.bonac.com/global/en/nucleic/about/

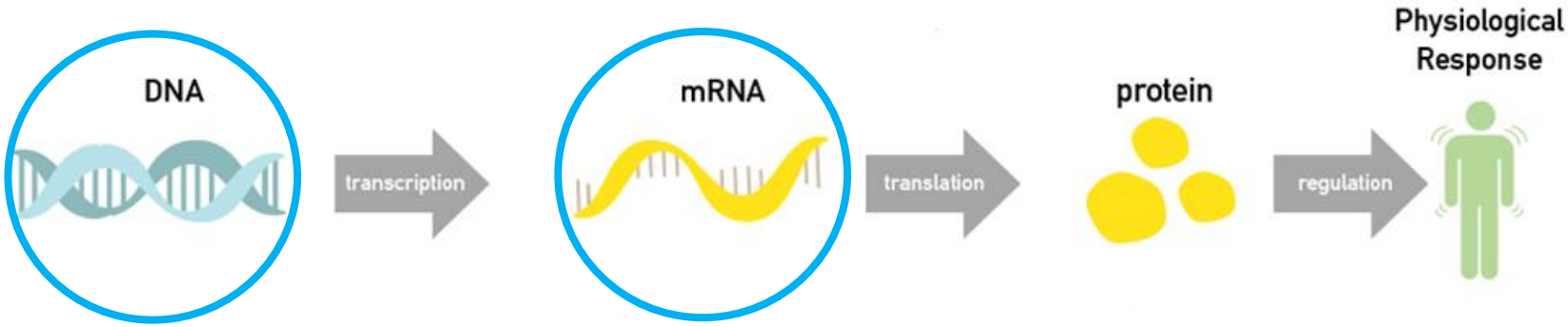
Nucleic acids as Therapeutics



Tiré de http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as Therapeutics

- Add DNA
- Add RNAs
- Other



- Delete DNA
- Modify DNA

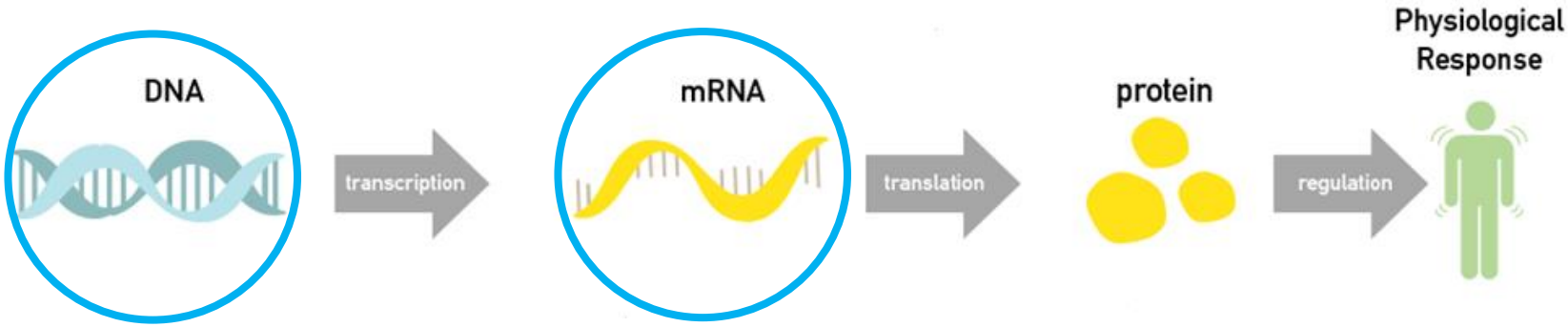
- Delete RNAs
- Modify translation

http://www.bonac.com/global/en/nucleic/about/

exercice

Nucleic acids as Therapeutics

- Add DNA
- Add RNAs
- Other



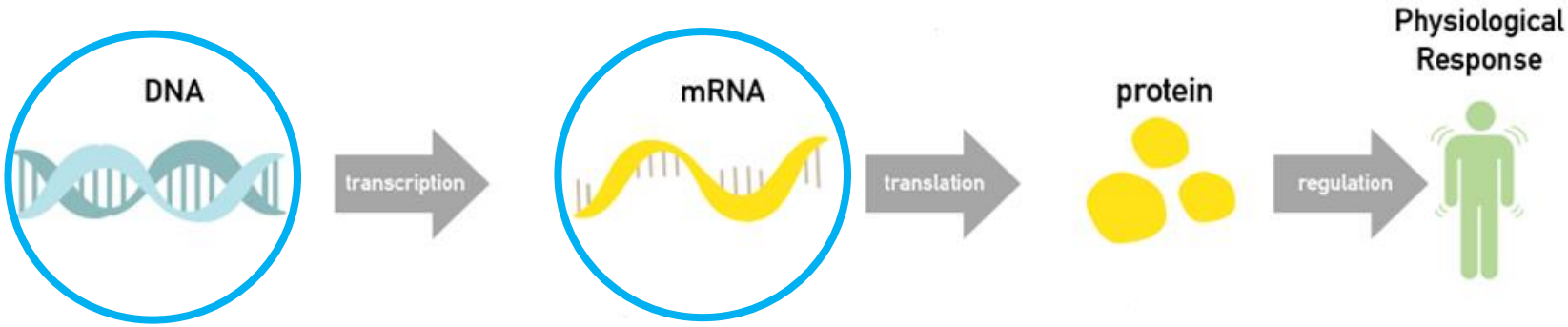
- Delete DNA
- Modify DNA

- Delete RNA
- Modify translation

http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as Therapeutics

- Plasmids
- mRNA
- Aptamers

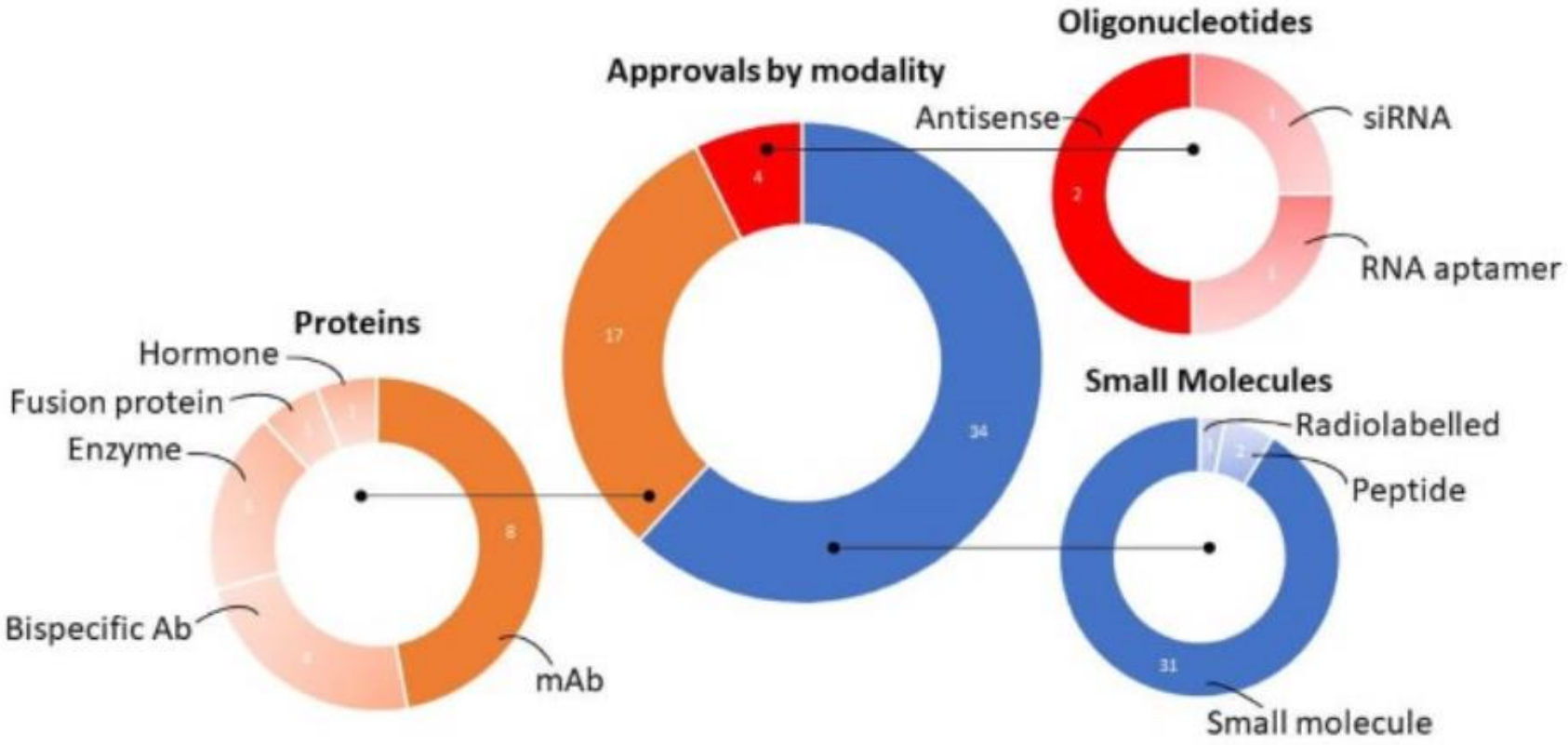


- *CRISPR/cas9*
- miRNAs / anti-miRNAs
- siRNAs
- shRNAs
- ASOs

http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as Therapeutics

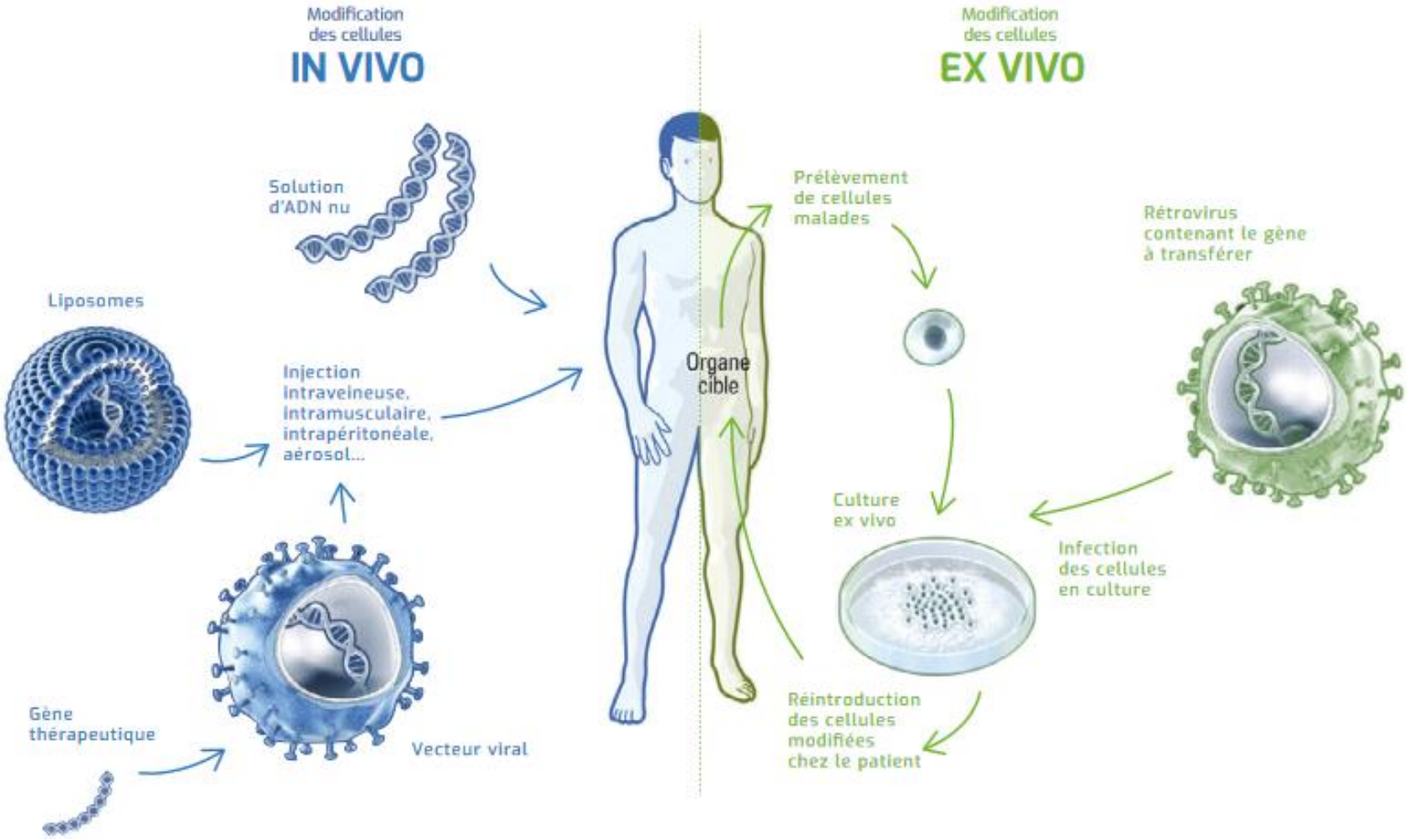
FDA 2018 - 2019



Adapted from Muller A, 2023 FDA approvals Nature Reviews in Drug Discovery, Jan 2024.

Nucleic acids as Therapeutics

Therapeutic Strategies



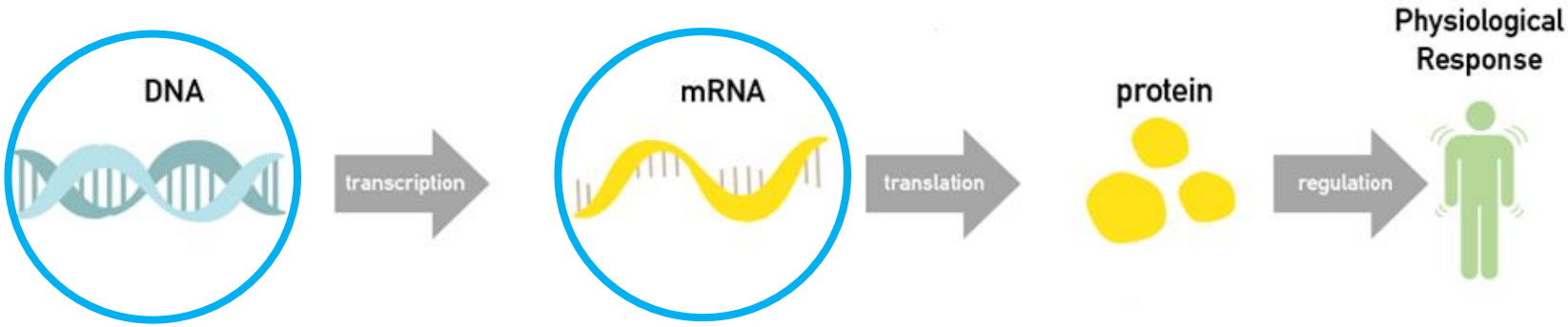
https://www.leem.org/la-therapie-genique

Nucleic acids as Therapeutics

- Plasmids
- Virus

- mRNA

- Aptamers



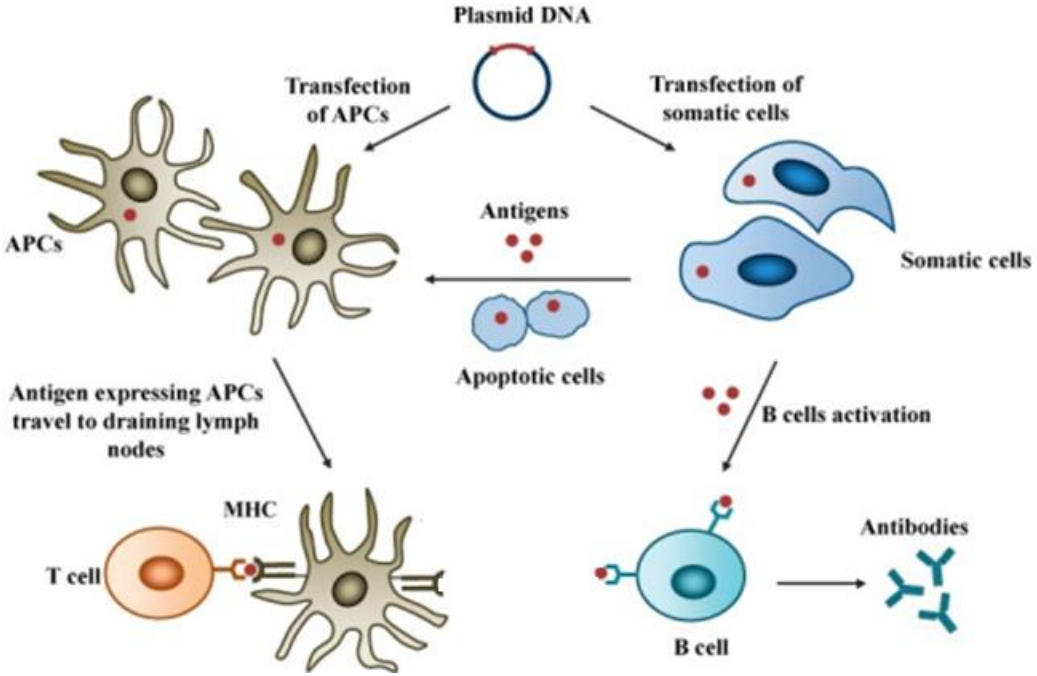
- *CRISPR/cas9*

- miRNAs / anti-miRNAs
- siRNAs
- shRNAs
- ASOs

http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as Therapeutics

Plasmid Vaccines



Plasmid with genes coding for proteins / peptids that are specific to the pathogens.

In the immune cells
DNA transcribed → peptids are recognized as non-self
→ peptids are presented as antigens (CMH I)
→ **Specific Immune Response**

Xu Y, Yuen PW, Lam JK - Pharmaceutics (2014)

Nucleic acids as Therapeutics

Plasmid Vaccines

Table 4. DNA vaccines approved for veterinary use.

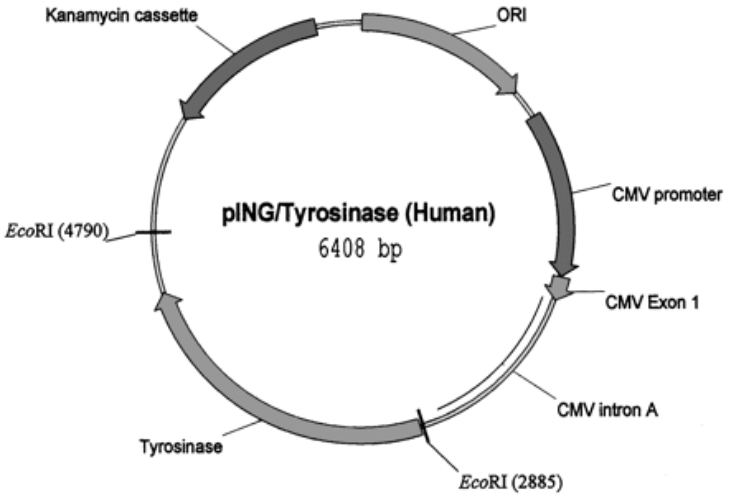
Type	Species	Target	Product/Company	License date/ country	Route of administration	Benefits
Prophylactic vaccine	Horses	West Nile Virus	West Nile-Innovator®/ Fort Dodge Animal Health	2005 USA	IM[1]	Production of protective antibodies
Prophylactic vaccine	Salmon	Infectious haematopoietic necrosis virus (IHNV)	Apex-IHN®/Novartis Animal Health	2005 Canada	IM	Stimulation of innate and adaptive immune responses improving the welfare and product yield
Immunotherapy of cancer	Dogs	Melanoma	Oncept™/Merial	2010 USA	ID[2] needle-free.	Production of antibodies capable of preventing the progress of the disease and prolonging the animal's life

Pereira et all World Journal of Vaccines 2014

Nucleic acids as Therapeutics

Plasmid Vaccines: Oncept (USDA, ~~EMA~~)

Oncept Melanoma contains a plasmid DNA expressing the gene coding for the human tyrosinase, pINGhT, in an aqueous solution. The principle of xenogeneic DNA vaccination was applied with Oncept Melanoma to overcome immunological tolerance to canine tyrosinase in dogs with melanoma. The xenogeneic (human) tyrosinase protein is foreseen to stimulate in dogs an immune response, cross-reacting with canine tyrosinase protein and canine melanoma cells expressing the canine tyrosinase.



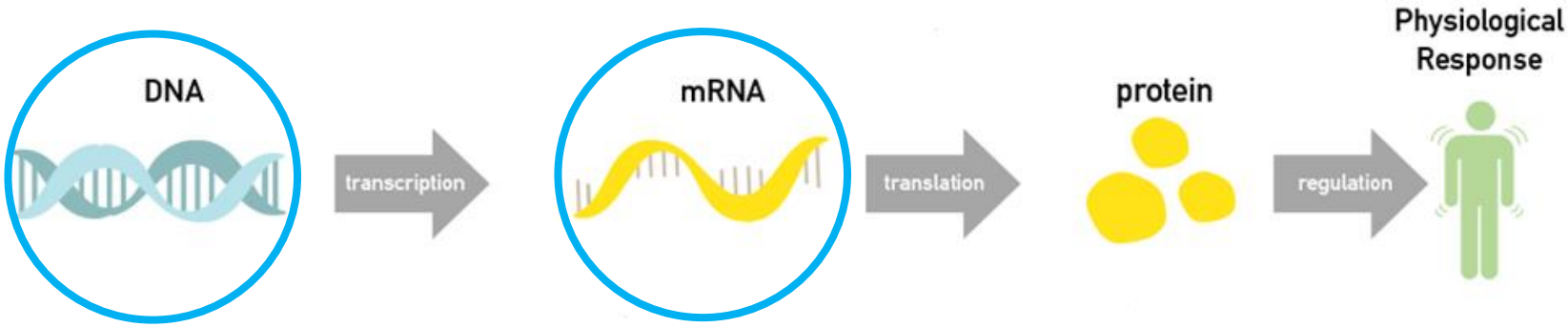
*"The bacterial and plasmid DNA itself contains immunostimulatory sequences that **may** act as a potent immunological adjuvant in the immune response"*

"DNA is relatively inexpensive and simple to purify in large quantity."

Bergman et al Clin Cancer Res. 2003 Apr;9(4):1284-90

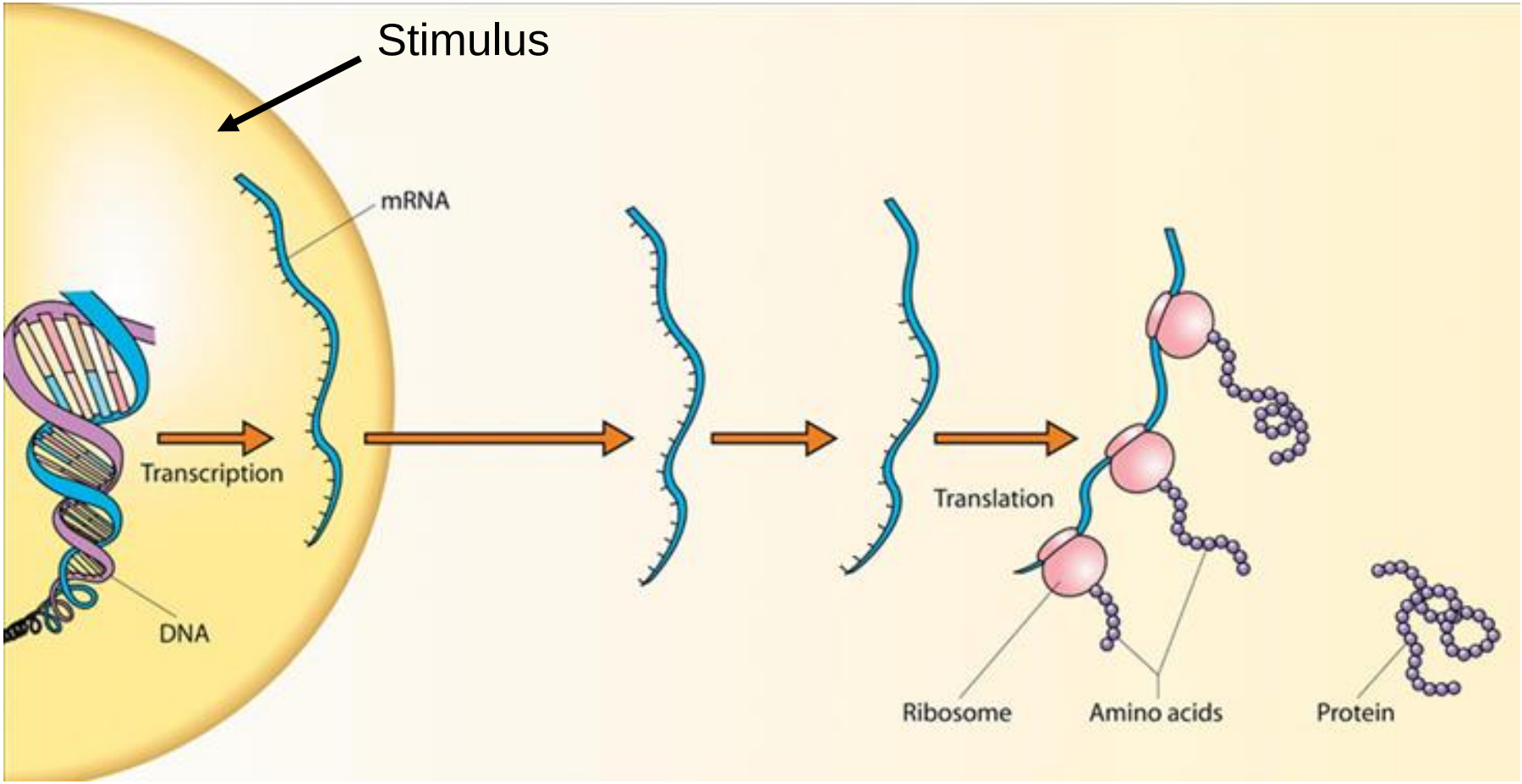
Nucleic acids as Therapeutics

- Plasmids
- mRNA vaccines
- Aptamers



- *CRISPR/cas9*
- miRNAs / anti-miRNAs
- siRNAs
- shRNAs
- ASOs (antisense oligonucleotide)

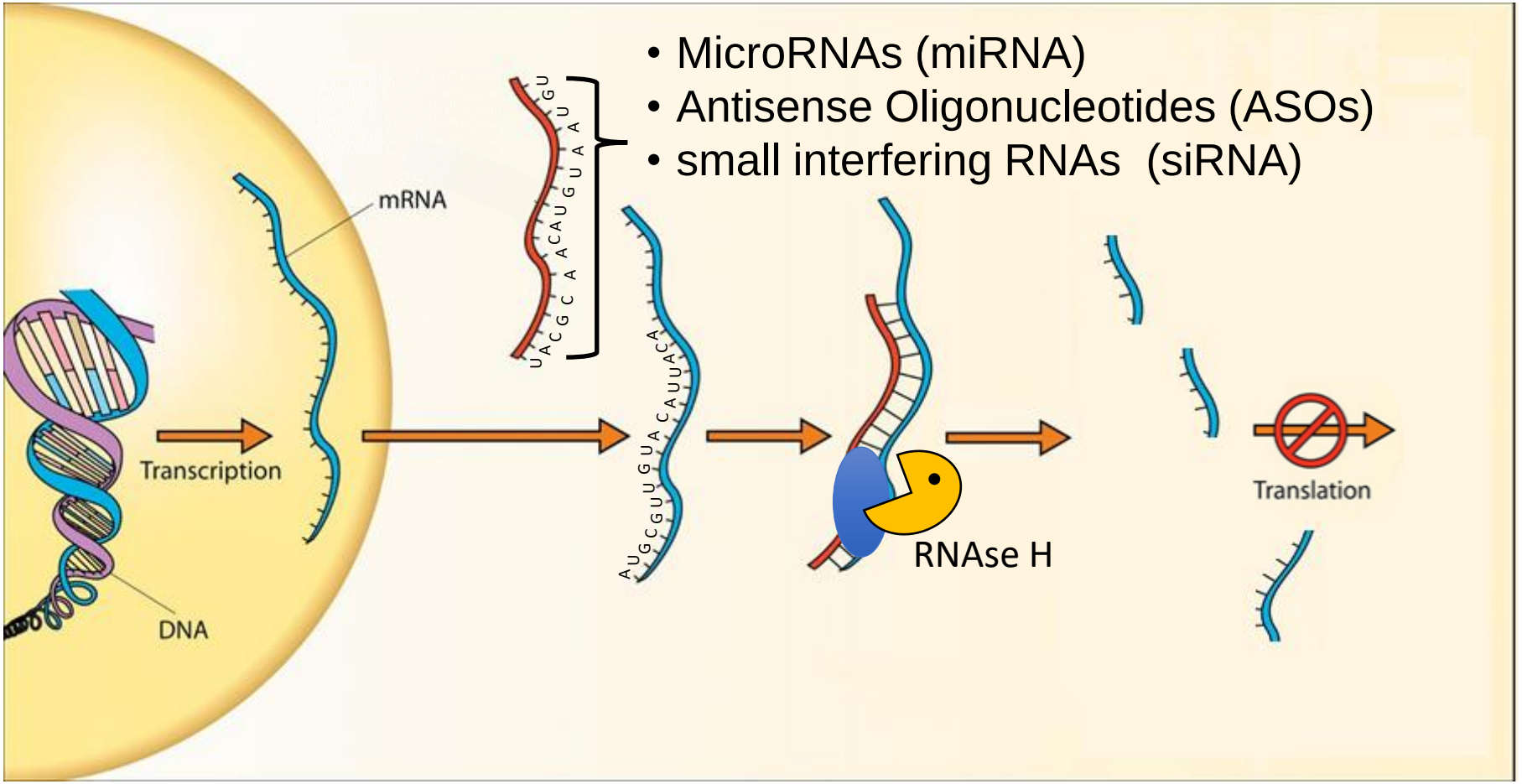
http://www.bonac.com/global/en/nucleic/about/



Adapted from R Robinson PLoS Biol 2(1): e28 2004

Nucleic acids as Therapeutics

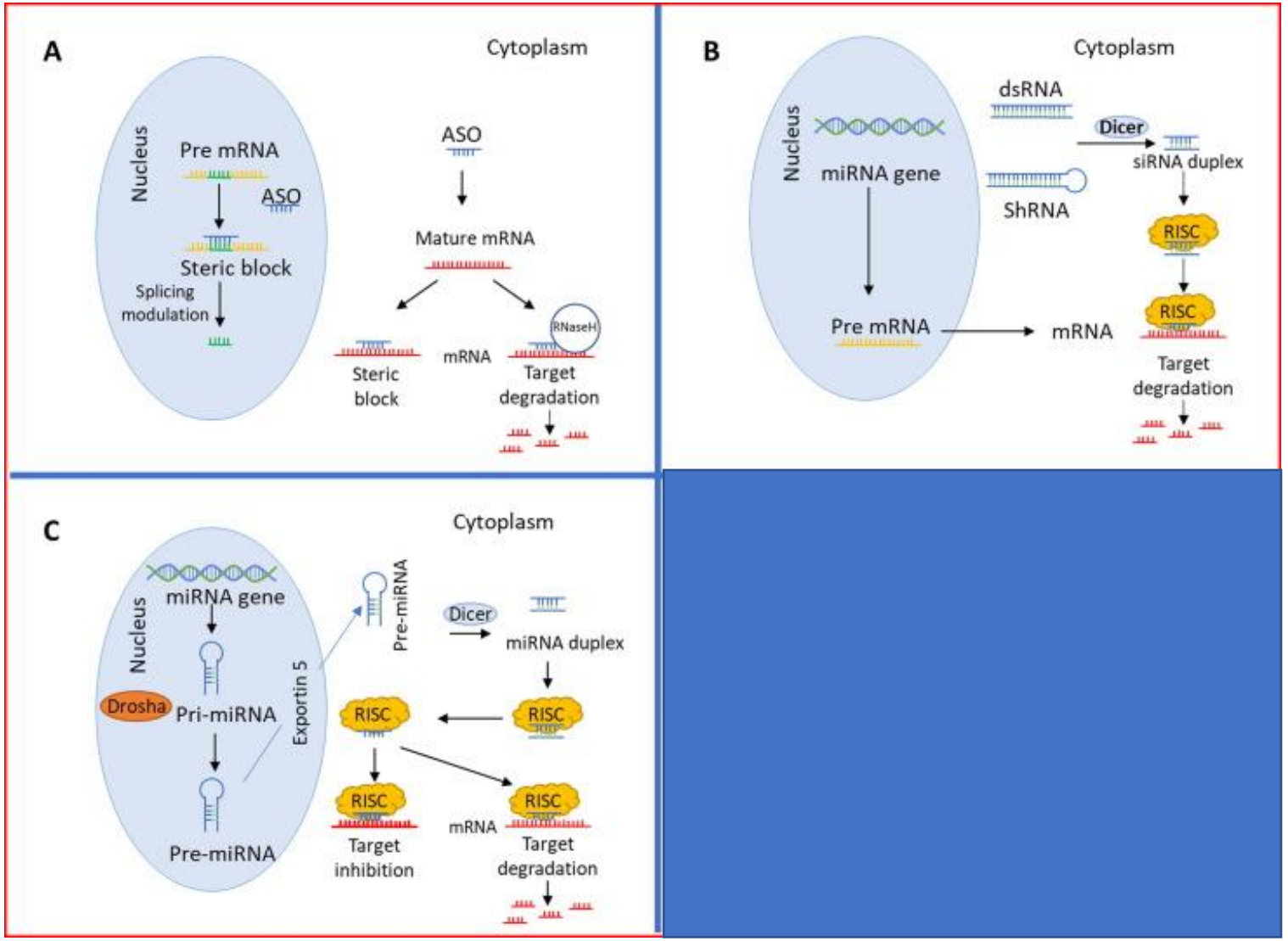
RNA interference



Adapted from R Robinson PLoS Biol 2(1): e28 2004

Nucleic acids as Therapeutics

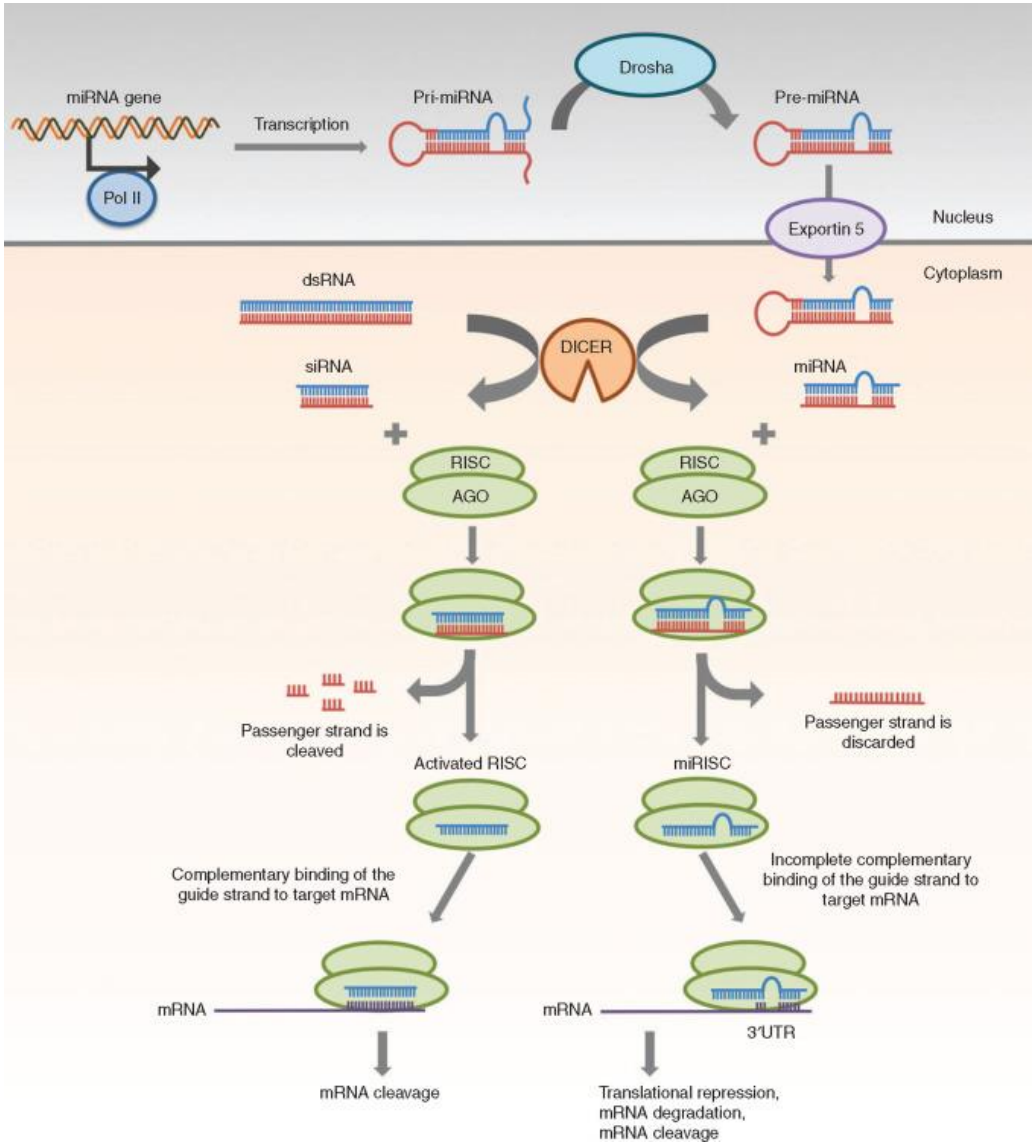
RNA interference



Fattal and Fay Advanced Drug Delivery Reviews 2021

Nucleic acids as Therapeutics

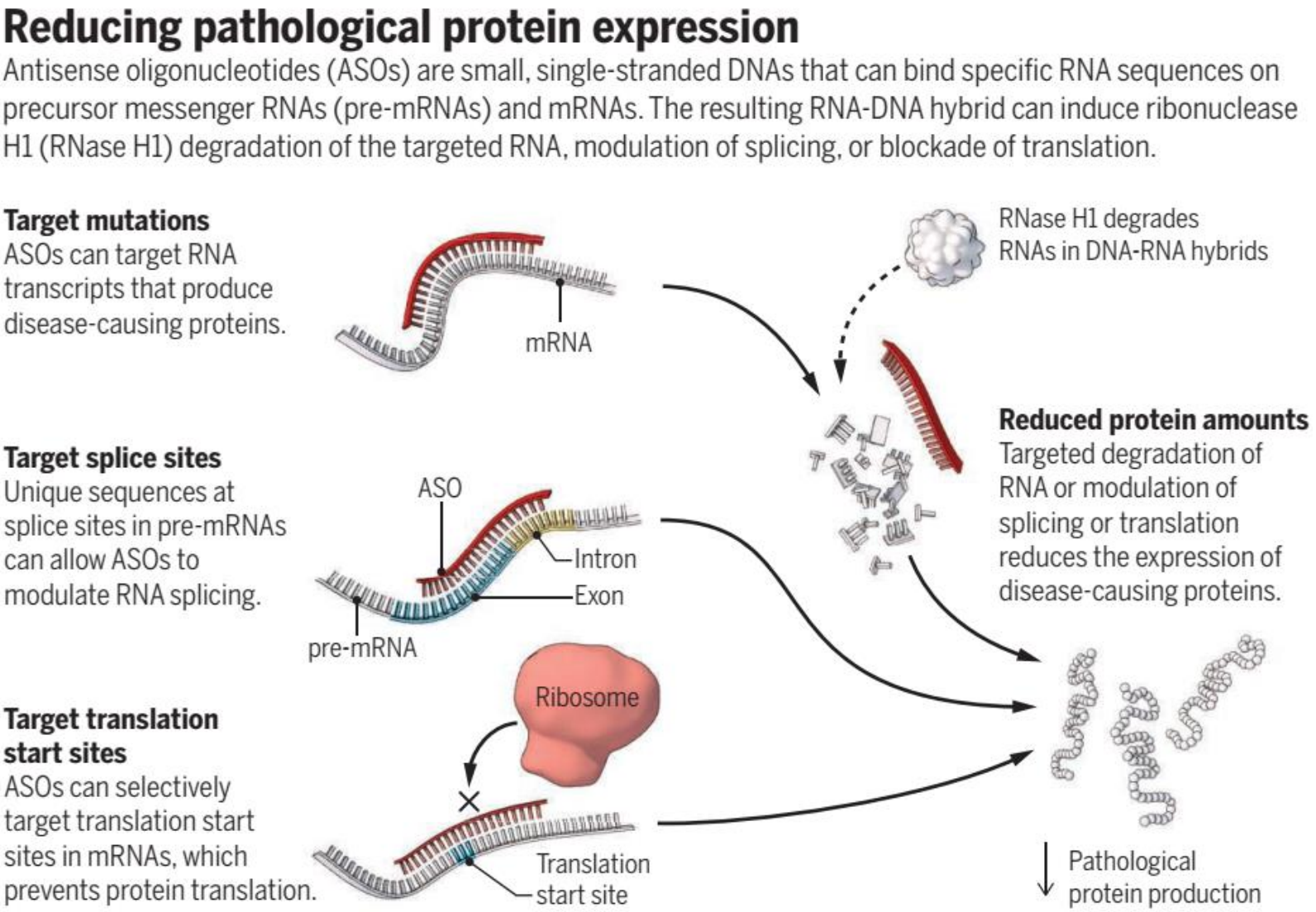
RNA interference



Lam et al Molecular Therapy—Nucleic Acids (2015) 4, e252;

Nucleic acids as Therapeutics

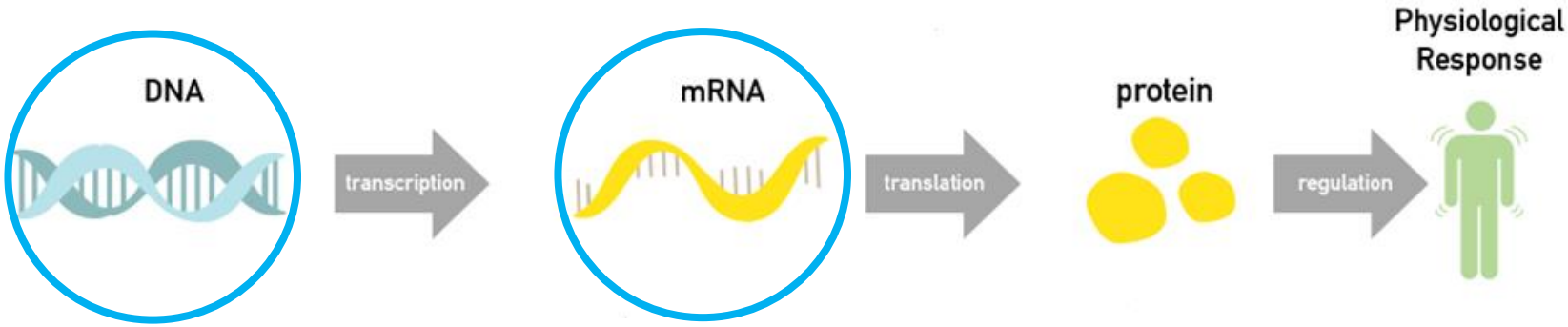
ASOs



Leavitt et al Science 2020

Nucleic acids as Therapeutics

- Plasmids
- mRNA
- Aptamers



- *CRISPR/cas9*
- miRNAs / anti-miRNAs
- siRNAs
- shRNAs

http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as Therapeutics

mRNA Vaccines

Pfizer/BioNTech vaccine announcement is cause for cautious celebration

Interim trial results are encouraging as scientists welcome news

- Coronavirus - latest updates
- See all our coronavirus coverage

▲ The Pfizer/BioNTech trial will continue into December. Photograph: Carlo Allegri/Reuters

It is not yet the end of the pandemic, but the announcement by Pfizer/BioNTech that their vaccine has been 90% successful in the vital large-scale trials has got even the soberest of scientists excited.

These are interim results and the trial will continue into December to collect

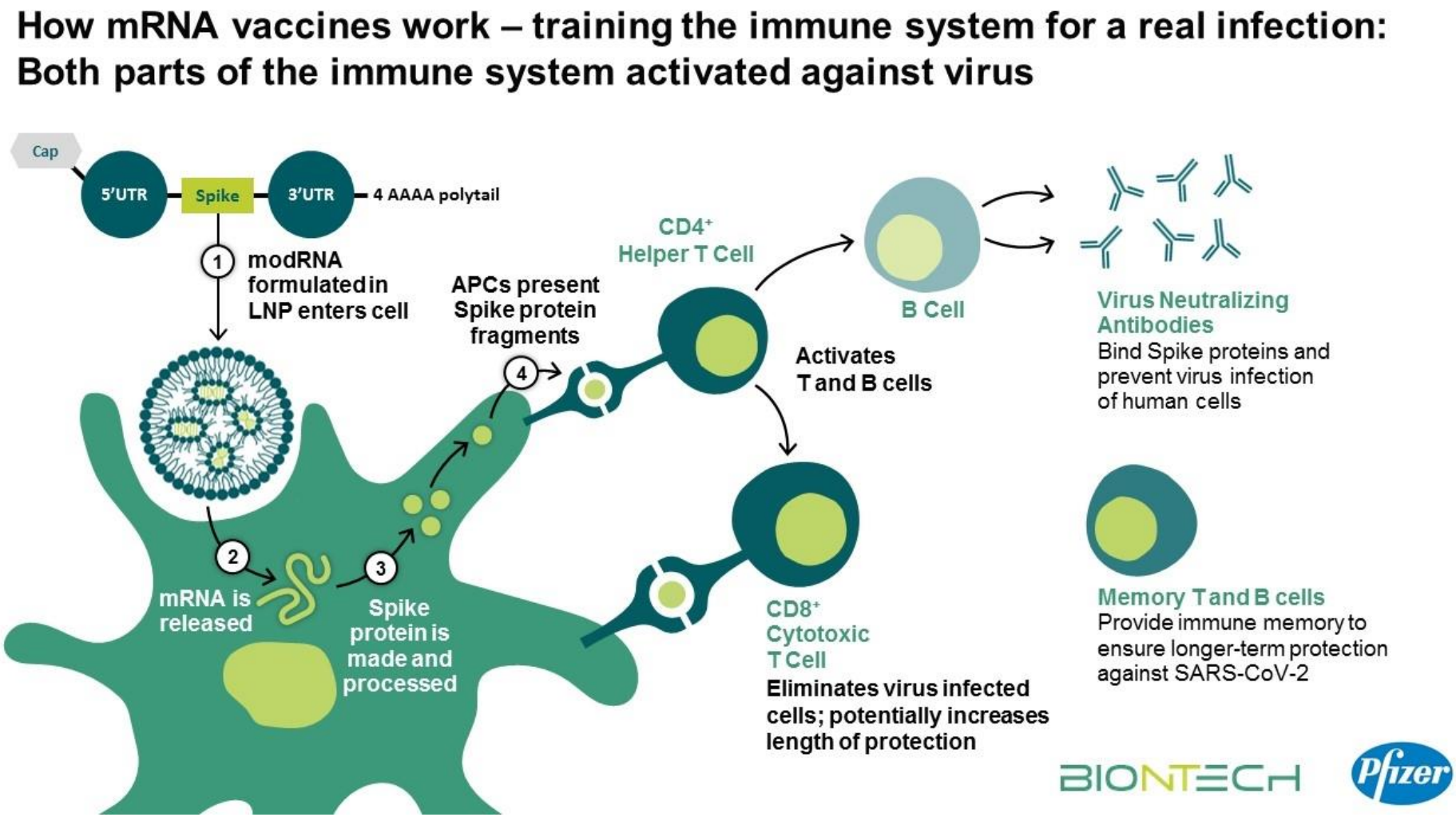
Moderna's COVID-19 vaccine candidate moves into late-stage trial

There are more than 150 coronavirus vaccine candidates worldwide, with Moderna's candidate among the most developed.

More than 150 coronavirus vaccine candidates are in various stages of development, with 23 prospects in human trials across the globe and Moderna's candidate among the farthest along in development [Brian Snyder/Reuters]

Nucleic acids as Therapeutics

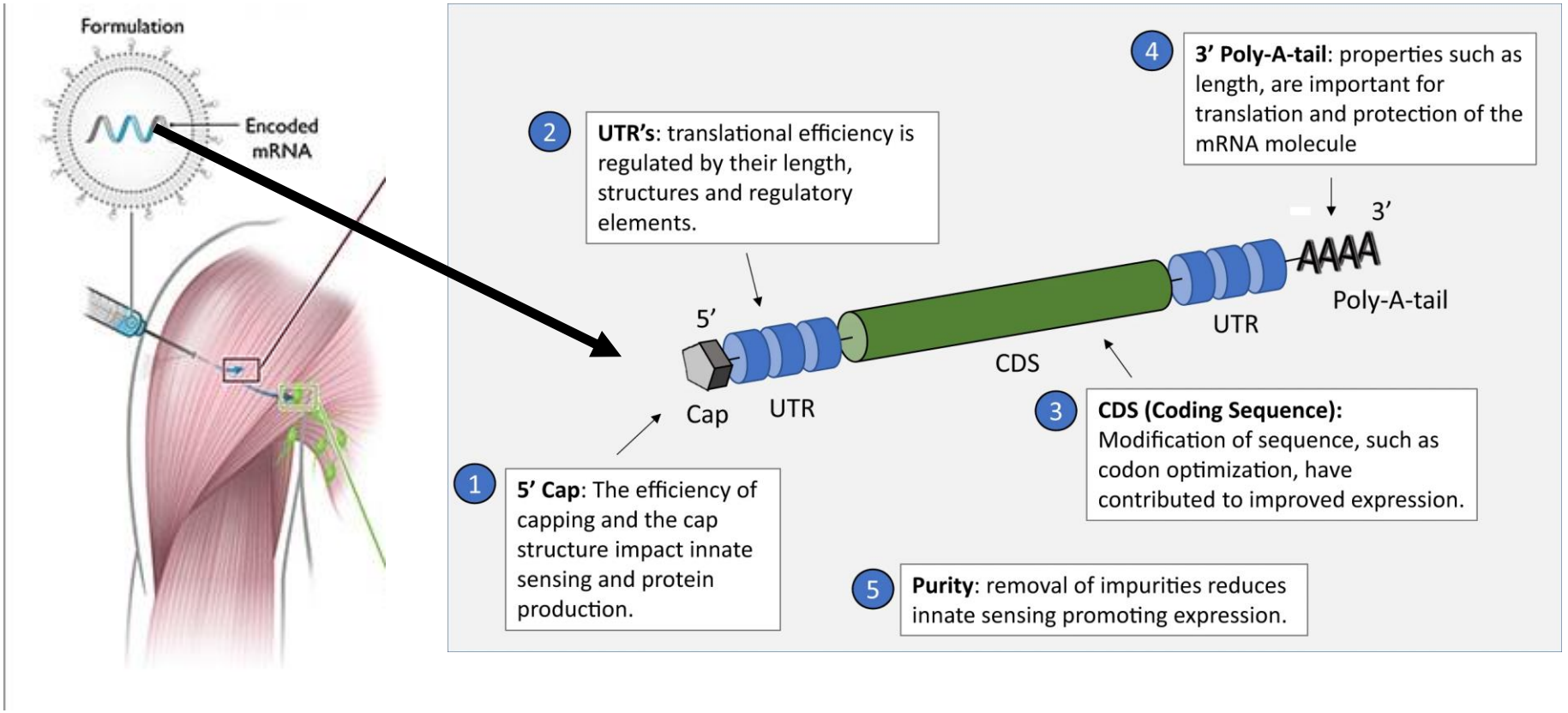
mRNA Vaccines



biontech.de

Nucleic acids as Therapeutics

mRNA Vaccines



Moderna

Nicholas A. C. Jackson et al, npj Vaccines 5 (2020)

Nucleic acids as Therapeutics

mRNA Vaccines

Table 3 | **Clinical trials with mRNA vaccines against cancer** (As in2017)

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
Antwerp University Hospital	DC EP with TAA mRNA (i.d. or NA)	AML	• NCT00834002 (I) • NCT01686334 (II)	• Completed[206,207] • Recruiting
	TAA tumor-associated antigen	AML, CML, multiple myeloma	NCT00965224 (II)	Unknown
		Multiple solid tumours	NCT01291420 (I/II)	Unknown[208]
		Mesothelioma	NCT02649829 (I/II)	Recruiting
		Glioblastoma	NCT02649582 (I/II)	Recruiting
Argos Therapeutics	DC EP with autologous tumour mRNA with or without CD40L mRNA (i.d. or NA)	Renal cell carcinoma	• NCT01482949 (II) • NCT00678119 (II) • NCT00272649 (I/II) • NCT01582672 (III) • NCT00087984 (I/II)	• Ongoing • Completed[209] • Completed; results NA • Ongoing • Completed; results NA
		Pancreatic cancer	NCT00664482 (NA)	Completed; results NA
Asterias Biotherapeutics	DC loaded with TAA mRNA (NA)	AML	NCT00510133 (II)	Completed[210]
BioNTech RNA Pharmaceuticals GmbH	Naked TAA or neo-Ag mRNA (i.nod.)	Melanoma	• NCT01684241 (I) • NCT02035956 (I)	• Completed; results NA • Ongoing
	Liposome-complexed TAA mRNA (i.v.)	Melanoma	NCT02410733 (I)	Recruiting[59]
	Liposome-formulated TAA and neo-Ag mRNA (i.v.)	Breast cancer	NCT02316457 (I)	Recruiting
CureVac AG	RNActive TAA mRNA (i.d.)	Non-small-cell lung cancer	• NCT00923312 (I/II) • NCT01915524 (I)	• Completed[211] • Terminated[200]
		Prostate cancer	• NCT02140138 (II) • NCT00831467 (I/II) • NCT01817738 (I/II)	• Terminated • Completed[151] • Terminated[212]

Nucleic acids as Therapeutics

mRNA Therapies

Table 1 | Summary of different classes of potential mRNA therapeutics

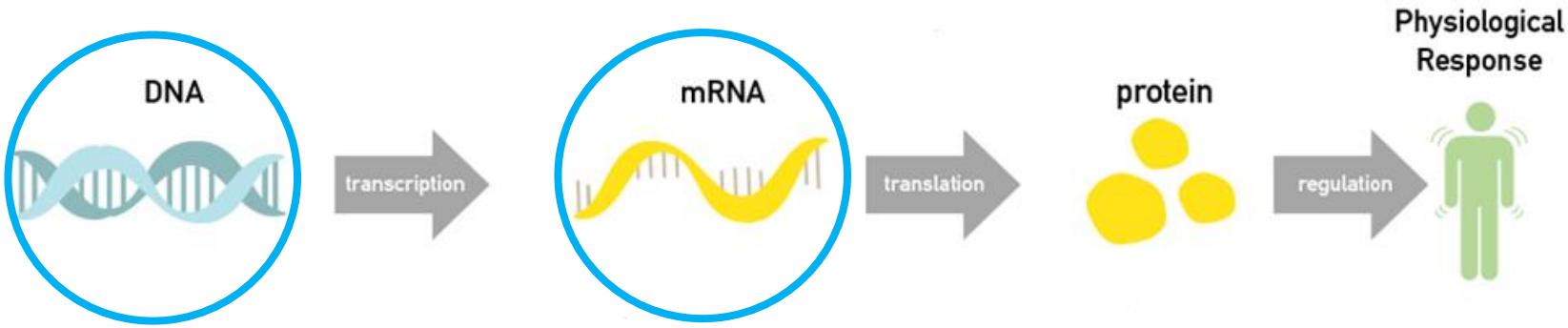
Organ of interest	Type of therapy	Disease	mRNA cargo	Delivery frequency
Liver	ERT (endocrine)	Hemophilia A[a]	Factor VIII	Chronic injection
		Hemophilia B[a]	Factor IX	
		Crigler–Najjar syndrome[a]	UGT1A1	
	ERT (intracrine)	Methylmalonic acidemia[a,b]	MMUT	Chronic injection
		Propionic acidemia[a,b]	PCCA/PCCB	
		OTC deficiency[b]	OTC	
		Gycogen storage disease type 1A[b]	Glucose-6 phosphatase	
	Gene editing (intracrine)	Transthyretin amyloidosis[a,b]	Cas9/TTR	Single injection
		Heriditary angioedema[b]	Cas9/KLKB1	
Lungs	ERT (intracrine)	Cystic fibrosis[a,b]	CFTR	Chronic injection
Heart	Regenerative (paracrine)	Heart failure[a,b]	VEGF-A	Single injection
	Antibody (intracrine)	Heart failure[a]	Anti-PLN intrabody	Single injection
Cancer	Immuno-oncology (intra- or endocrine)	Solid tumors[b]	IL-23, IL-36, OX40L IFN-2b, GM-CSF, IL-15 IL-7, CLDN6, IL-12, IL-2	Single injection
	Antibody (endocrine)	Breast cancer[b]	Anti-HER2, CD40L, CD70, caTLR4	Chronic injection
		Solid tumors[b]	Anti-CLDN18.2	
Autoimmunity	Immune tolerization (endocrine)	Autoimmune disorders[b]	HSA-IL2m	Single injection
		Autoimmune encephalomyelitis[a]	MOG_{35-55}	Chronic injection
Multiple tissues	ERT (intra- or endocrine)	Fabry disease[a]	α-Gal A	Chronic injection
	Gene editing (intracrine)	Hereditary angioedema[b]	Cas9/KLKB1	Single injection

ERT, enzyme replacement therapy; UGT1A1, UDP glucuronosyltransferase family 1 member A1; MMUT, methylmalonyl-CoA mutase; PCCA, propionyl-CoA carboxylase-α; PCCB, propionyl-CoA carboxylase-β; OTC, ornithine transcarbamylase; TTR, transthyretin; KLKB1, kallikrein B1; CFTR, cystic fibrosis transmembrane conductance regulator; VEGF, vascular endothelial growth factor; PLN, phospholamban; IL, interleukin; OX40L, tumor necrosis factor superfamily member 4 (TNFSF4); IFN, interferon; GM-CSF, granulocyte-macrophage colony stimulating factor; CLDN, claudin; HER2, ERB-B2 receptor tyrosine kinase 2; caTLR4, constitutively active Toll-like receptor 4; HSA, human serum albumin; IL2m, interleukin-2-mutein fusion protein; MOG_{35-55}, myelin oligodendrocyte glycoprotein peptide; α-Gal A, α-galactosidase-A. [a]See Supplementary Table 1 for further information. [b]See Supplementary Table 2 for further information.

Rohner, E. *Nat Biotechnol* (2022). https://doi.org/10.1038/s41587-022-01491-

Nucleic acids as Therapeutics

- Plasmids
- mRNA vaccines
- Aptamers

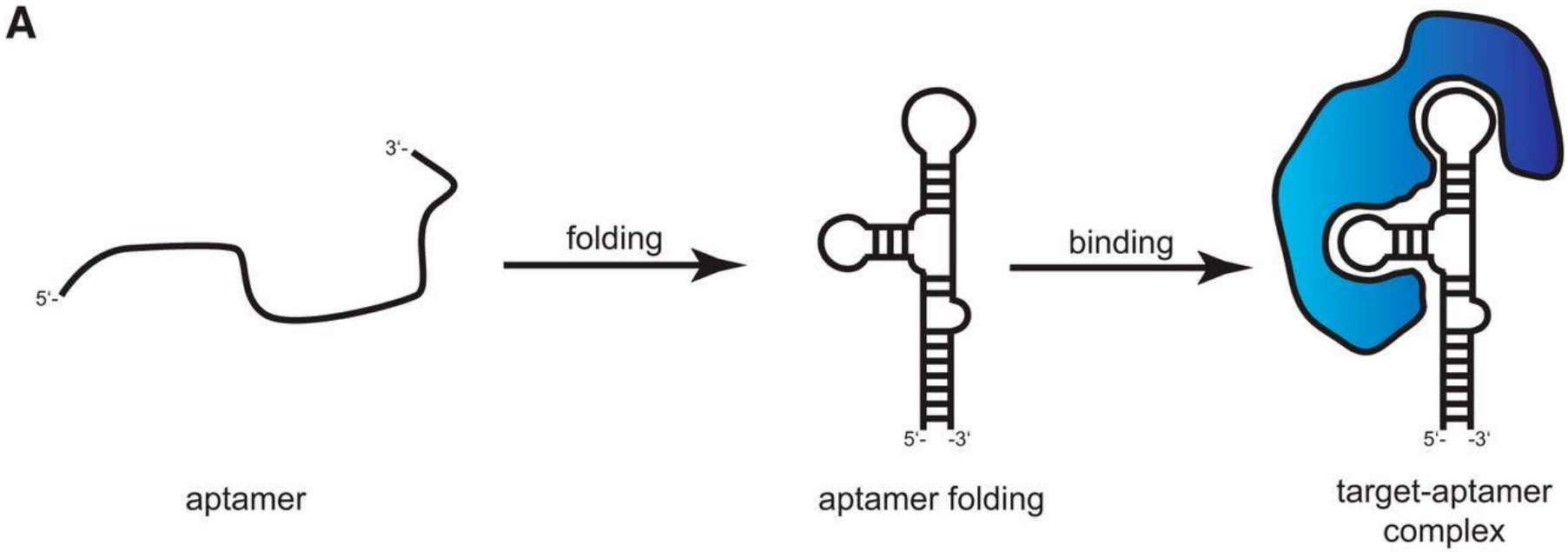


- *CRISPR/cas9*
- miRNAs / anti-miRNAs
- siRNAs
- shRNAs

http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as Therapeutics

Aptamers



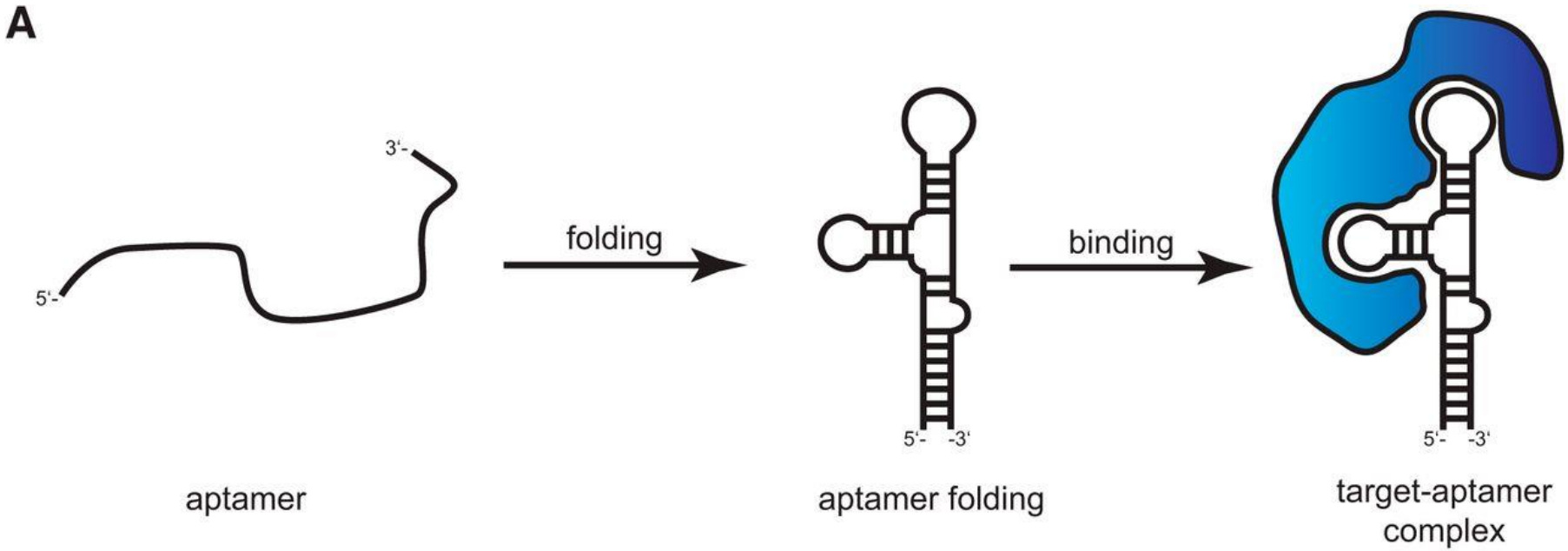
- **DNA or RNA oligonucleotids, single stranded, 15-60 b with 3D high affinity to their ligands**

- **Ligands :** nucleic acids, proteins, receptors, organic molecules, organisms (virus, bactéria)…

Adapted from de C Delomenie
Wolter et al Journal of Neuroscience 8 March 2017

Nucleic acids as Therapeutics

Aptamères



Pros compare to antibodies:

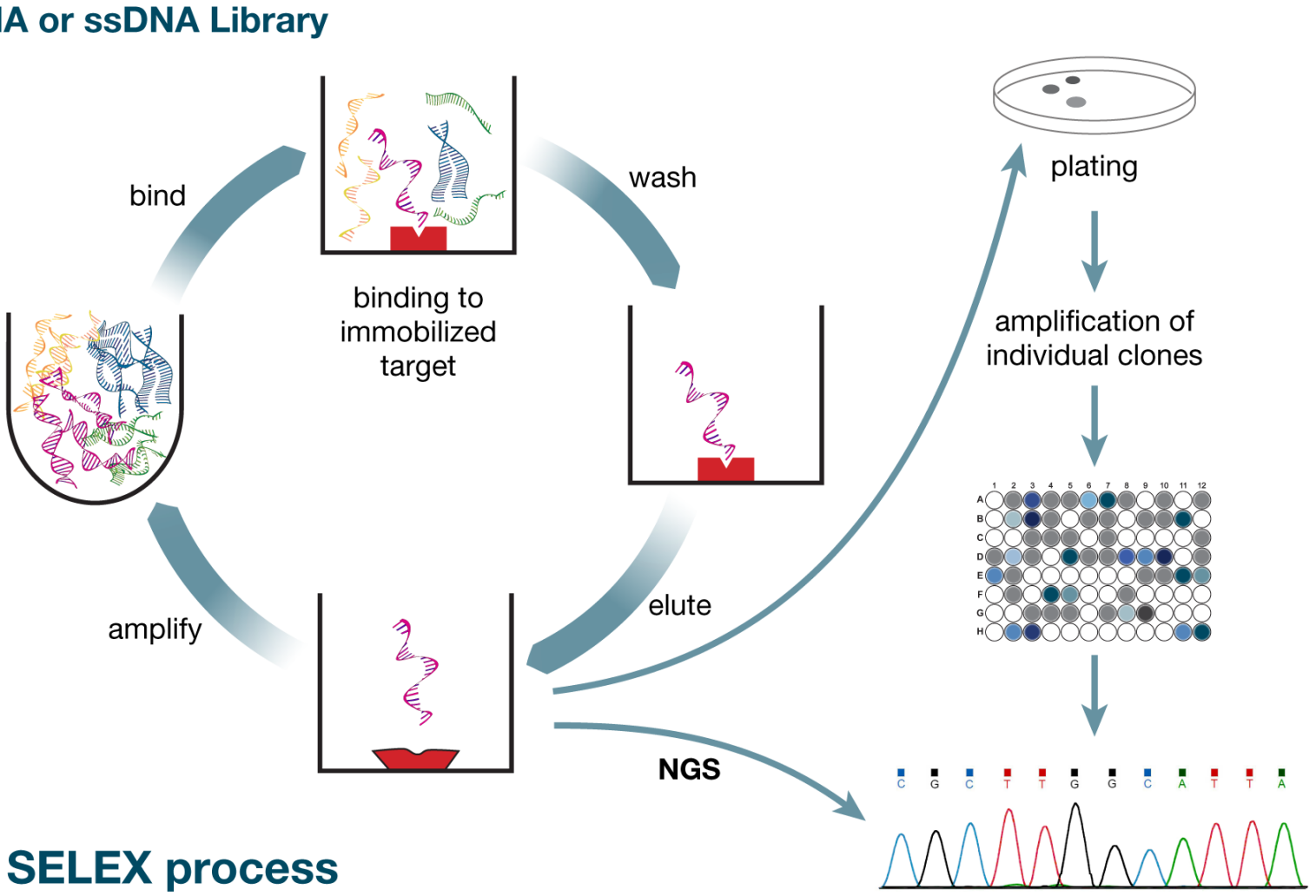
- **High Affinity**
- **Stables, easy to handle**
- **Less immunogenic**
- **Lower production cost**

Adapted from C Delomenie
Wolter et al Journal of Neuroscience 8 March 2017

Nucleic acids as Therapeutics

Aptamers

- *in vitro* repetitive selection process : **SELEX**



Adapted from de C Delomenie https://ngsdataanalysis.com

Nucleic acids as Therapeutics

Aptamers : Macugen

NDA 21-756/S-018
Page 8

11 DESCRIPTION

Pegaptanib sodium is a covalent conjugate of an oligonucleotide of twenty-eight nucleotides in length that terminates in a pentylamino linker, to which two 20-kilodalton monomethoxy polyethylene glycol (PEG) units are covalently attached via the two amino groups on a lysine residue.

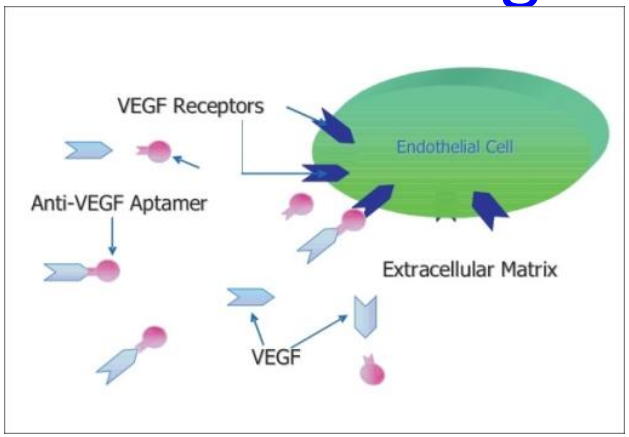
Pegaptanib sodium is represented by the following structural formula:

FDA approved 2004, EMA 2005

Shukla et al Indian Journal of Ophthalmology 2007

Nucleic acids as Therapeutics

Aptamers : Macugen



12.1 Mechanism of Action

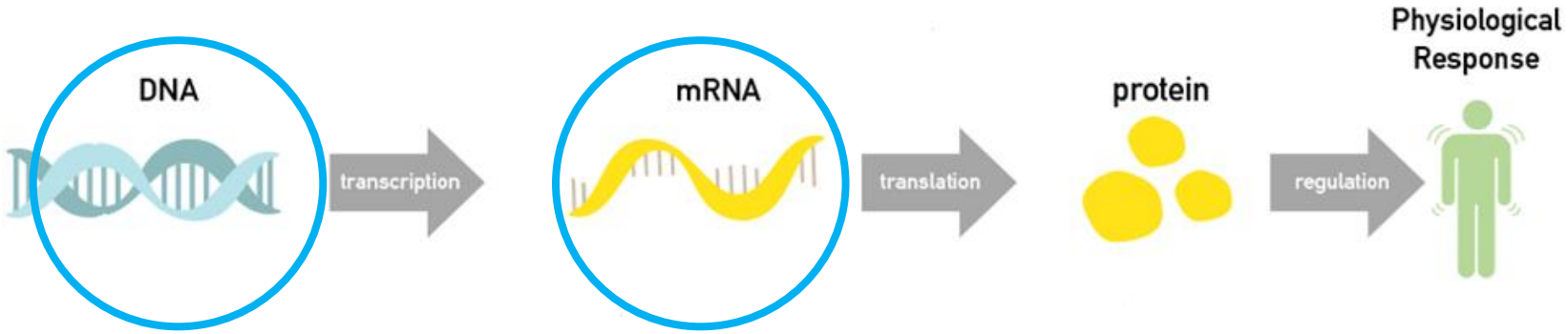
Pegaptanib is a selective vascular endothelial growth factor (VEGF) antagonist. VEGF is a secreted protein that selectively binds and activates its receptors located primarily on the surface of vascular endothelial cells. VEGF induces angiogenesis, and increases vascular permeability and inflammation, all of which are thought to contribute to the progression of the neovascular (wet) form of age-related macular degeneration (AMD), a leading cause of blindness. VEGF has been implicated in blood retinal barrier breakdown and pathological ocular neovascularization.

Pegaptanib is an aptamer, a pegylated modified oligonucleotide, which adopts a three-dimensional conformation that enables it to bind to extracellular VEGF. Under in vitro testing conditions, pegaptanib binds to the major pathological VEGF isoform, extracellular $VEGF_{165}$, thereby inhibiting $VEGF_{165}$ binding to its VEGF receptors. The inhibition of $VEGF_{164}$, the rodent counterpart of human $VEGF_{165}$, was effective at suppressing pathological neovascularization.

Macugen NDA approval FDA
Kim R - Indian J Ophthalmol 2007

Nucleic acids as diagnostic tools and therapeutics

Diagnostic Protein production Therapeutics



Adapted from http://www.bonac.com/global/en/nucleic/about/

Nucleic acids as diagnostic tools and therapeutics

Table 2 A comparison between small molecules, protein-based drugs (including monoclonal antibodies) and siRNA/miRNA-based drugs

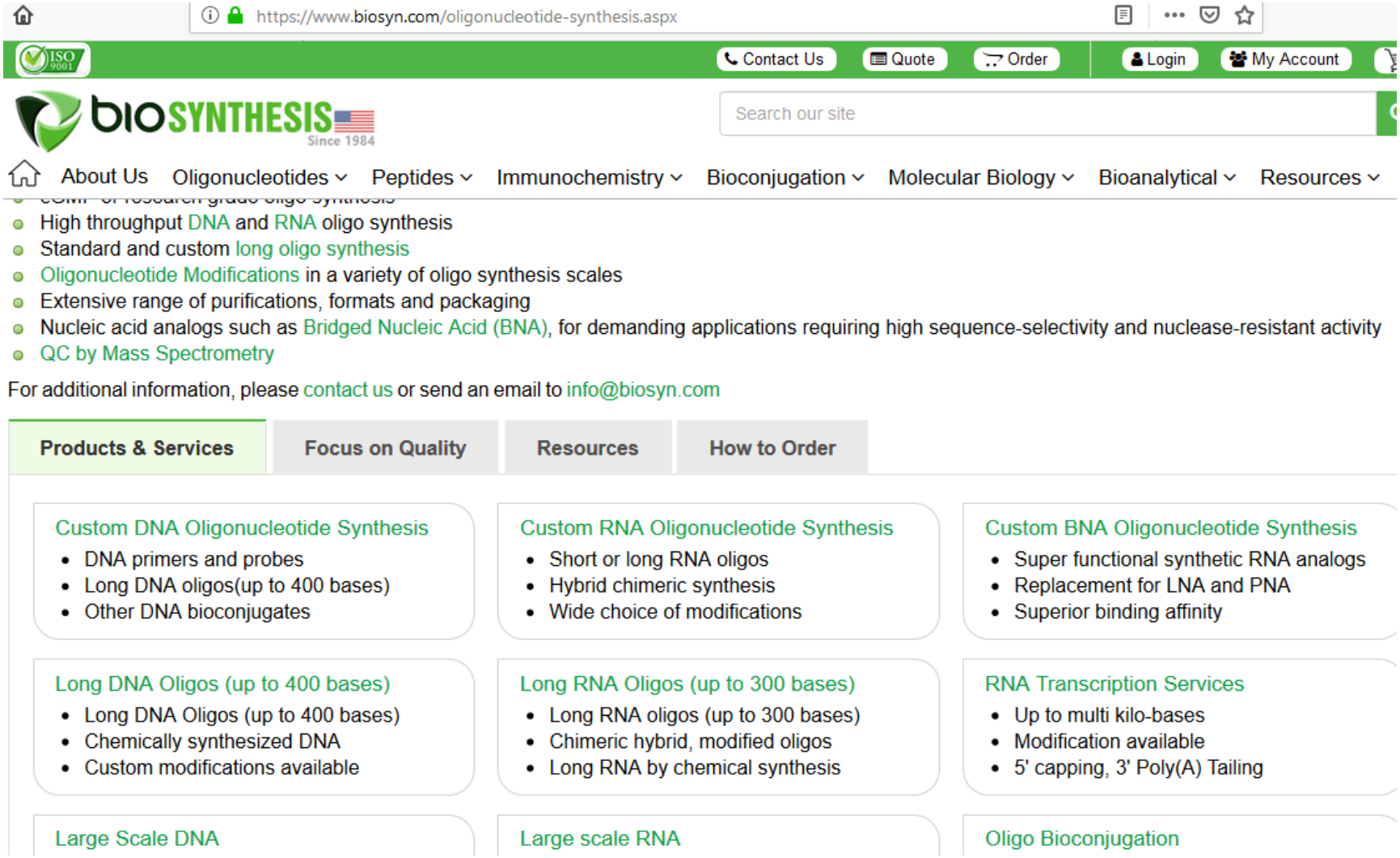
Properties	Small molecules	Protein-based drugs	siRNA/miRNA-based drugs
Nature of action	Activation or inhibition of targets	Activation or inhibition of targets	Inhibition of targets
Site of target proteins	Extracellular and Intracellular	Mainly extracellular	Virtually any sites
Selectivity and potency	Variable (depending on binding-site and ligand specificity, their affinity and efficacy etc.)	Highly specific and potent	Highly specific and potent
Lead optimization	Slow	Slow	Rapid
Manufacture	Easy	Difficult	Easy
Stability	Stable	Unstable	Unstable
Delivery	Easy	Difficult	Difficult

Data taken from ref. 201.

Lam et al DOI:https://doi.org/10.1038/mtna.2015.23

Nucleic acids production

Synthetic production



Nucleic acids production

Synthetic production

https://www.biosyn.com/dna-synthesis.aspx

ISO 9001 | Contact Us | Quote | Order | Login | My Account

bioSYNTHESIS Since 1984

Search our site

About Us | Oligonucleotides | Peptides | Immunochemistry | Bioconjugation | Molecular Biology | Bioanalytical | Resources

2' Deoxy DNA Oligonucleotide Synthesis

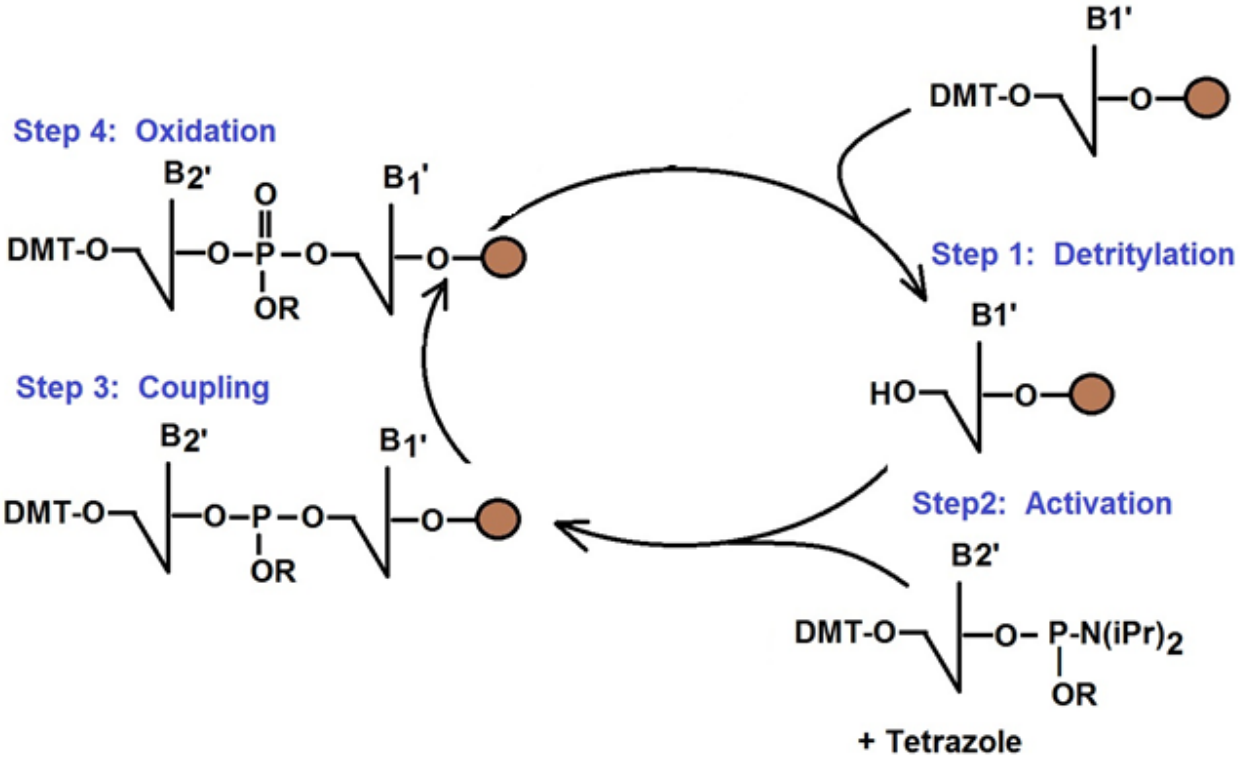
Linkages	Scale	Estimate Yields	Price	Restrictions
Phosphodiester (PO) $10 Minimum (25 to 50 nmole) $20 Minimum (100 to 250 nmole) $60 Minimum (1 to 15 µmole)	50 nmole	3-5 ODs	$0.42/base	15-60 bases
	100 nmole	5-10 ODs	$0.85/base	10-90 bases
	250 nmole	15-20 ODs	$1.00/base	5-100 bases
	1 µmole	20-60 ODs	$1.85 /base	5-100 bases
	2 µmole	40-120 ODs	$2.95 /base	5-100 bases
	5 µmole	100-300 ODs	$9.00/base	5-100 bases
	10 µmole	200-600 ODs	$12.00/base	5-100 bases
	15 µmole	300-750 ODs	$15.00/base	5-100 bases
Phosphorothioate (PS) $40 Minimum	250 nmole	15 ODs	$3.50/bond	10-25 base
	1 µmole	20-60 ODs	$5.50/bond	10-25 base
	5 µmole	100-300 ODs	$20.00/bond	10-25 base
	10 µmole	200-600 ODs	$35.00/bond	10-25 base
	15 µmole	300-750 ODs	Inquire	10-25 base

Nucleic acids production

Synthetic production

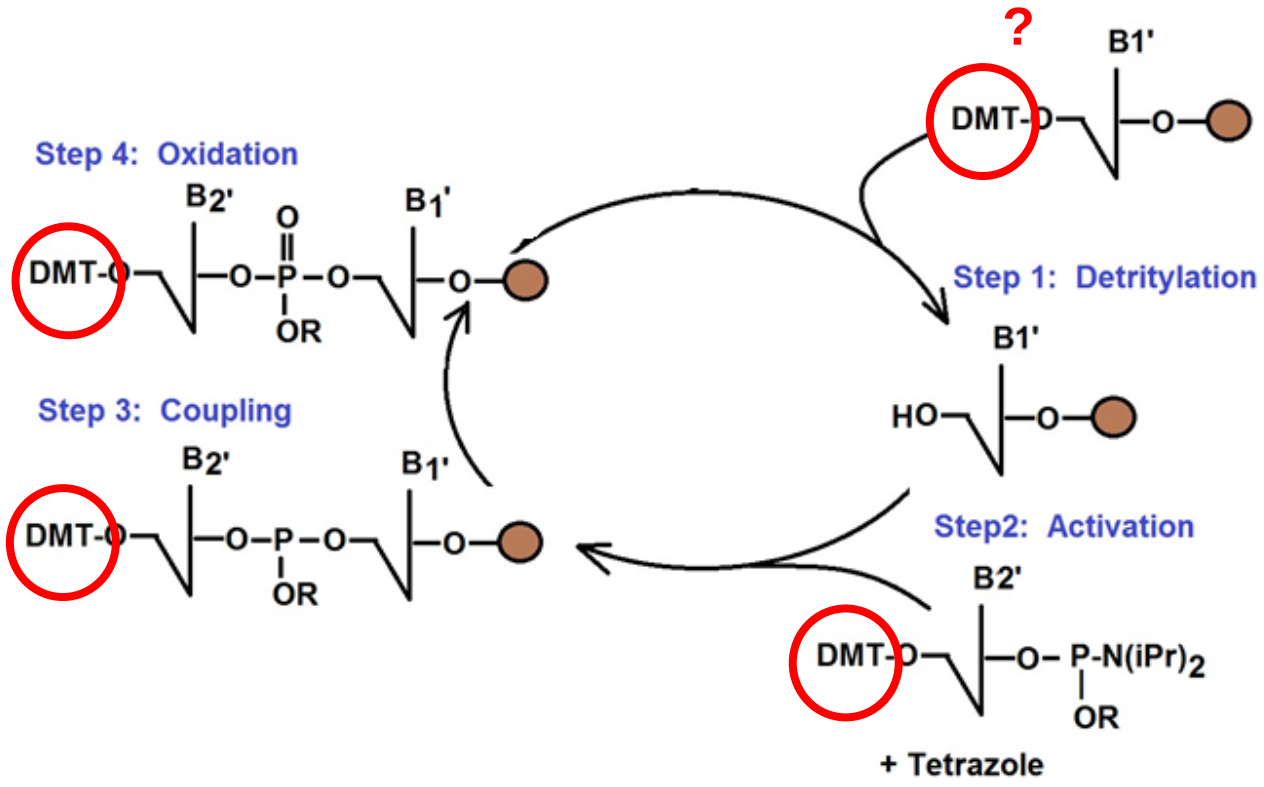
Nucleic acids production

Synthetic production



Nucleic acids production

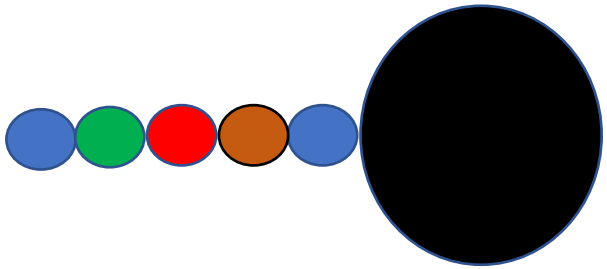
Synthetic production



DMT: Dimethoxytrityl

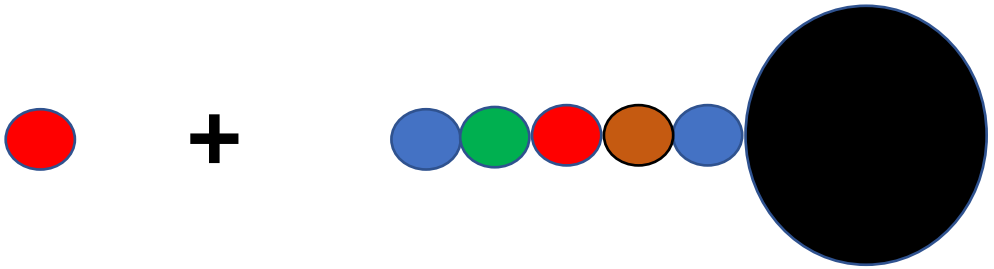
Nucleic acids production

Synthetic production



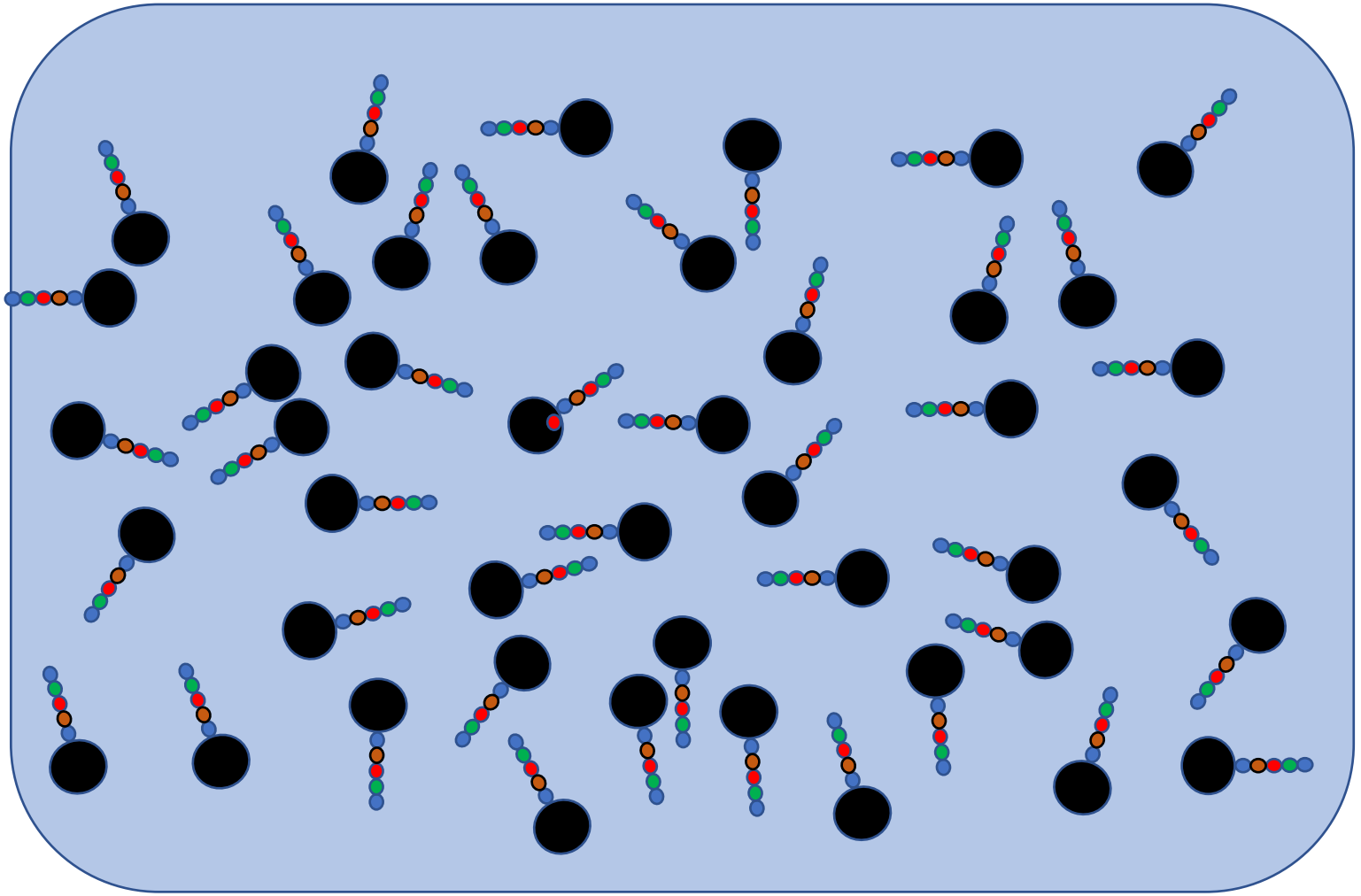
Nucleic acids production

Synthetic production



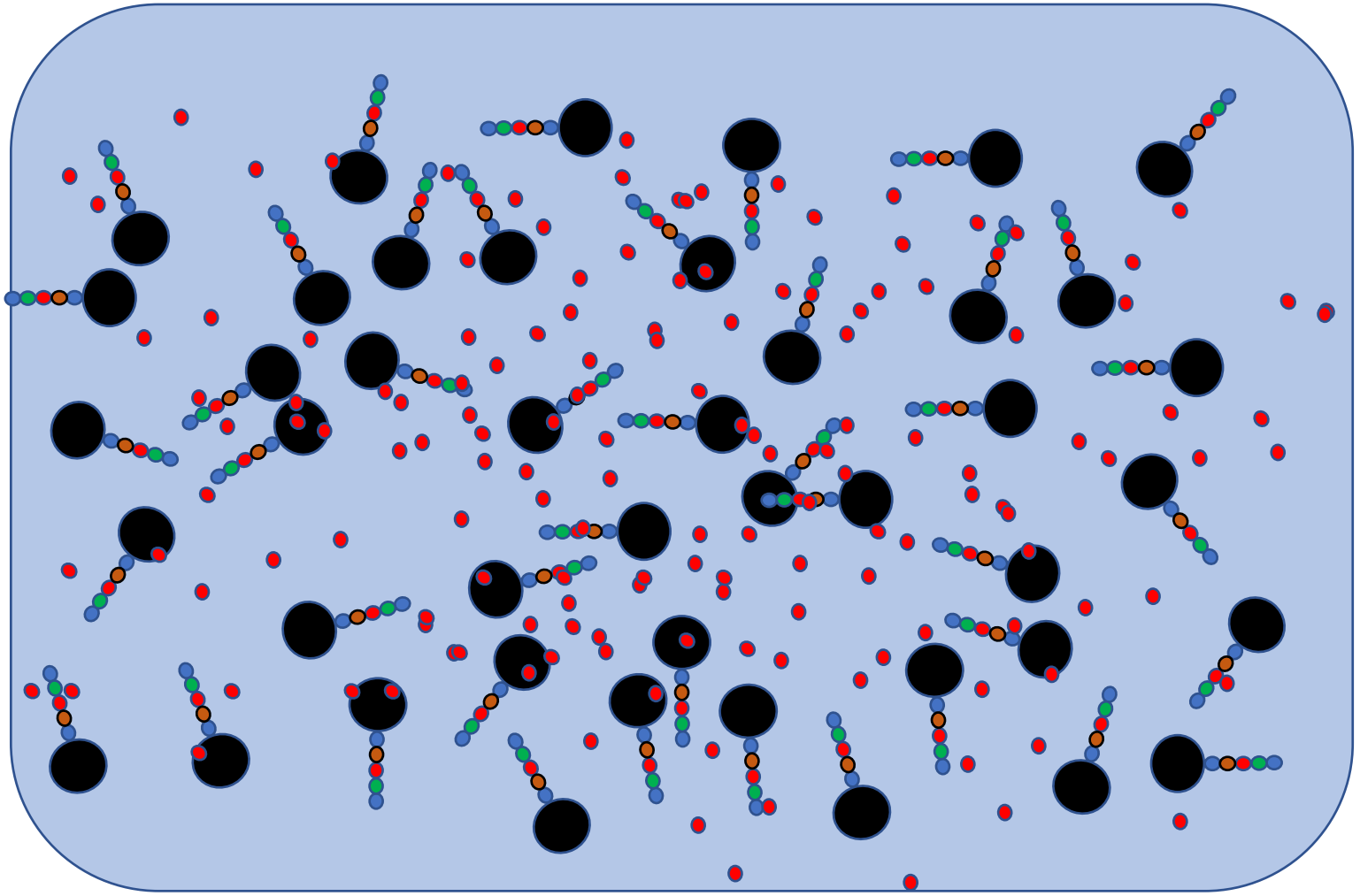
Nucleic acids production

Synthetic production



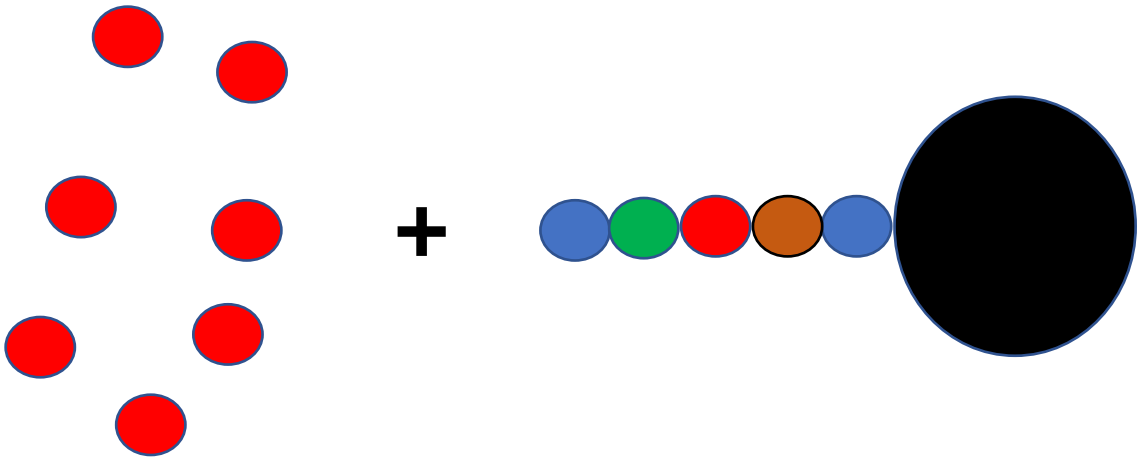
Nucleic acids production

Synthetic production



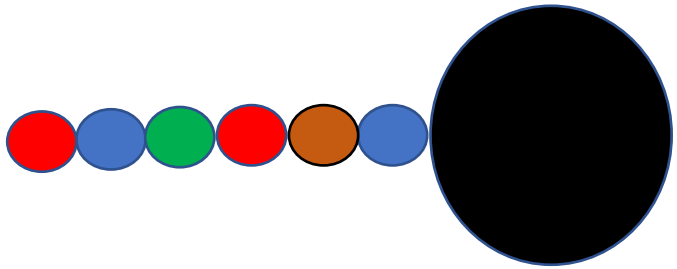
Nucleic acids production

Synthetic production



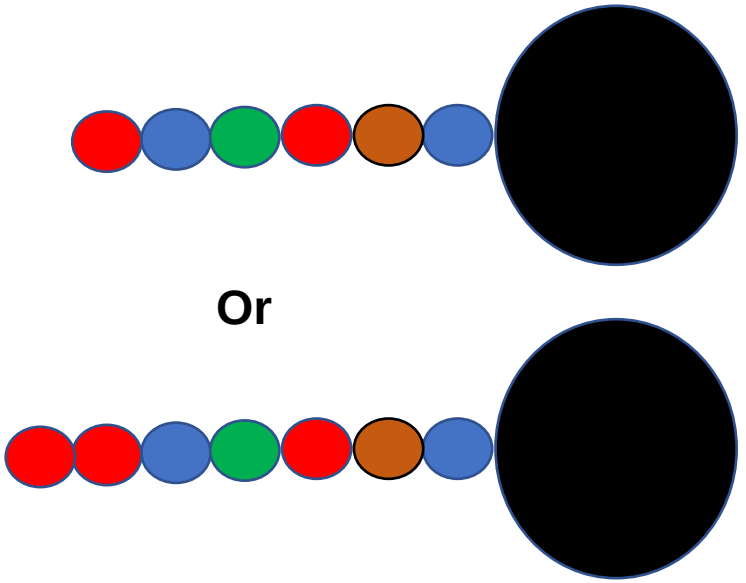
Nucleic acids production

Synthetic production



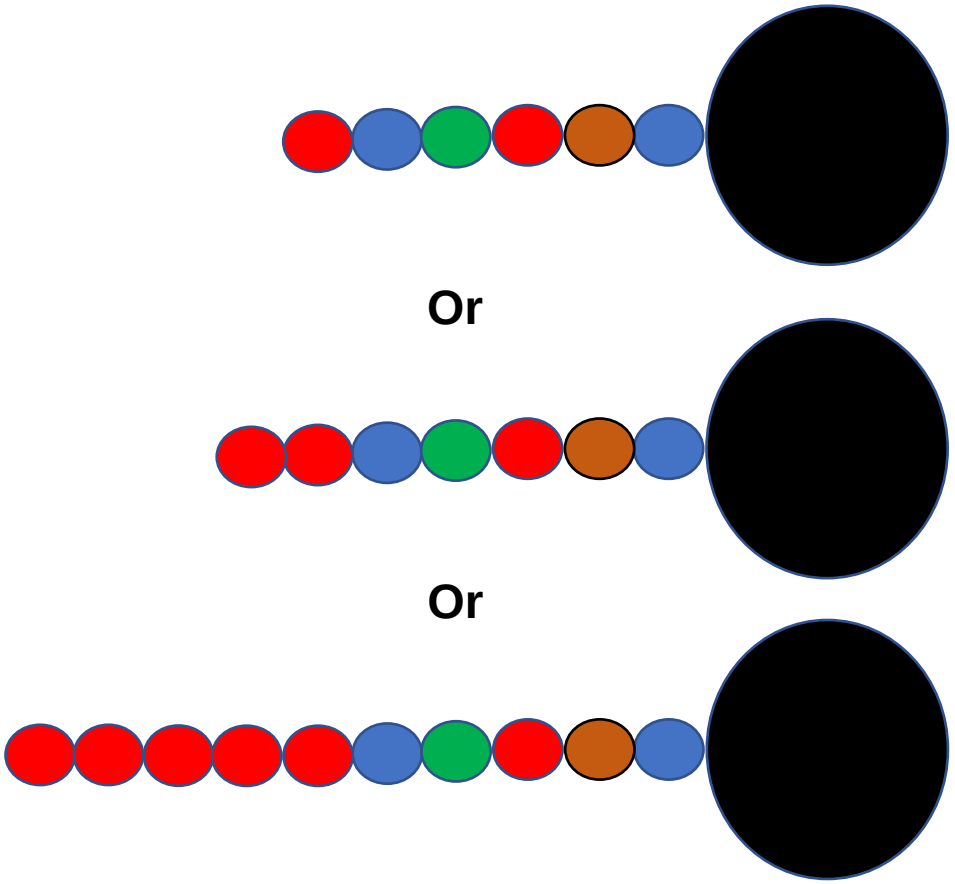
Nucleic acids production

Synthetic production



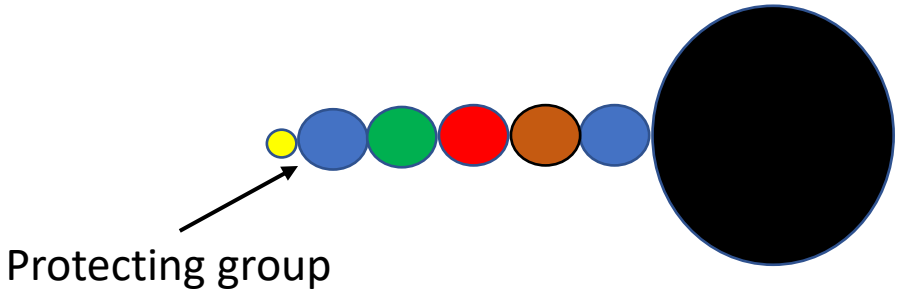
Nucleic acids production

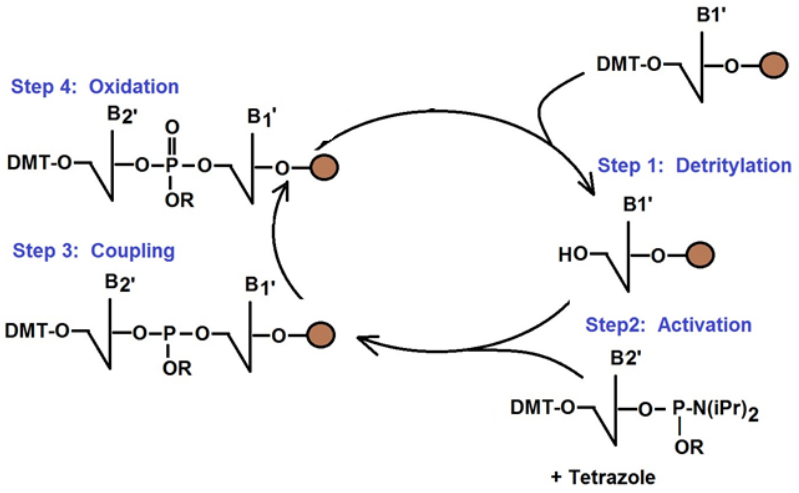
Synthetic production



Nucleic acids production

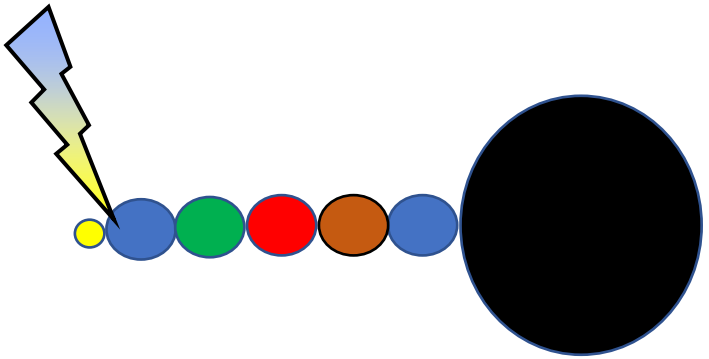
Synthetic production

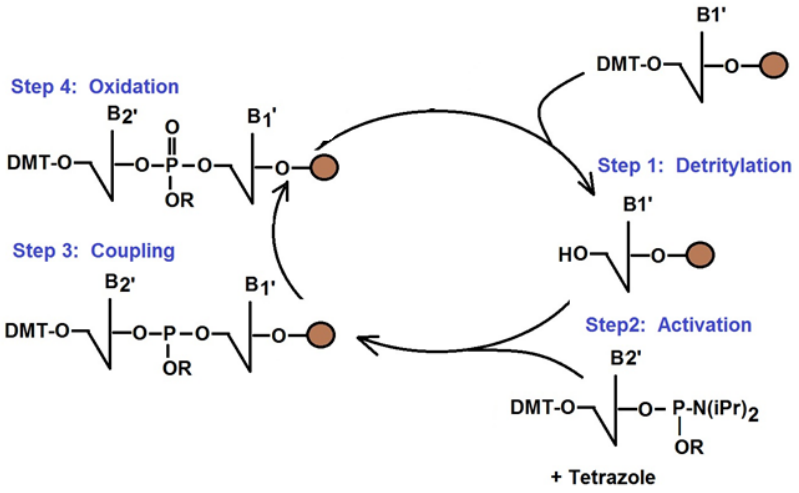




Nucleic acids production

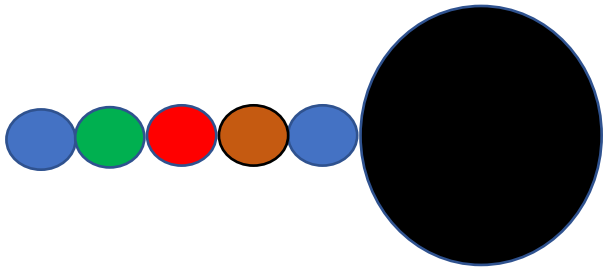
Synthetic production





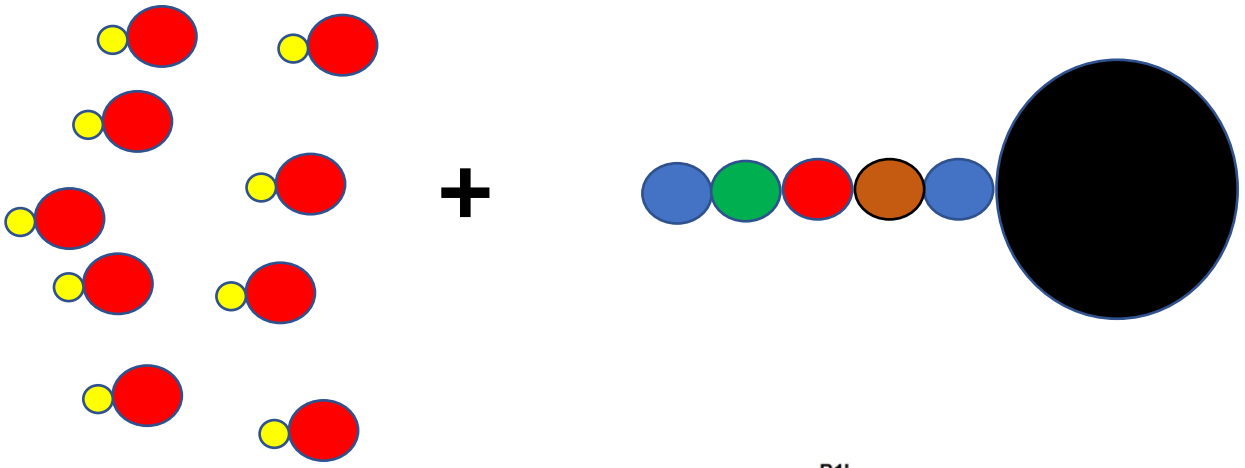
Production des acides nucléiques

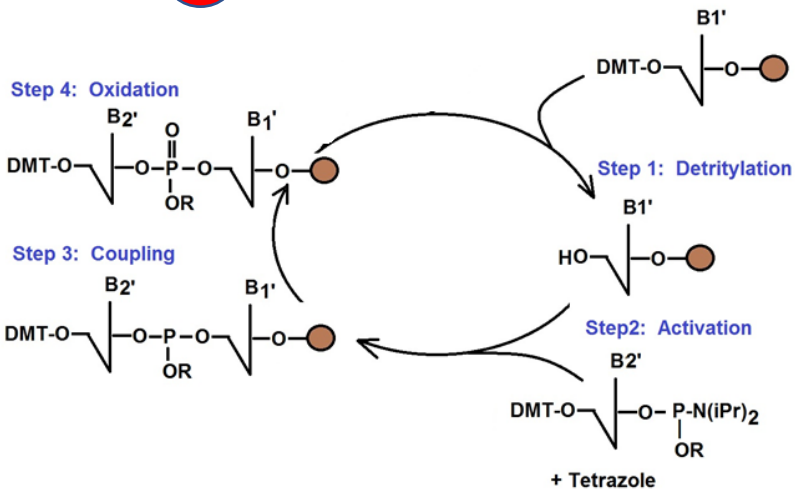
Production synthétique



Nucleic acids production

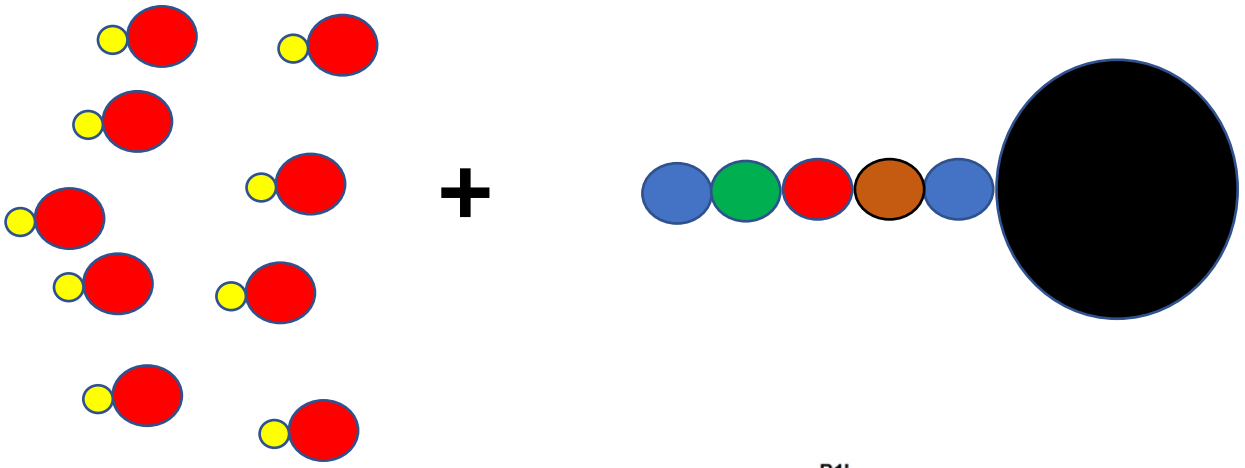
Synthetic production

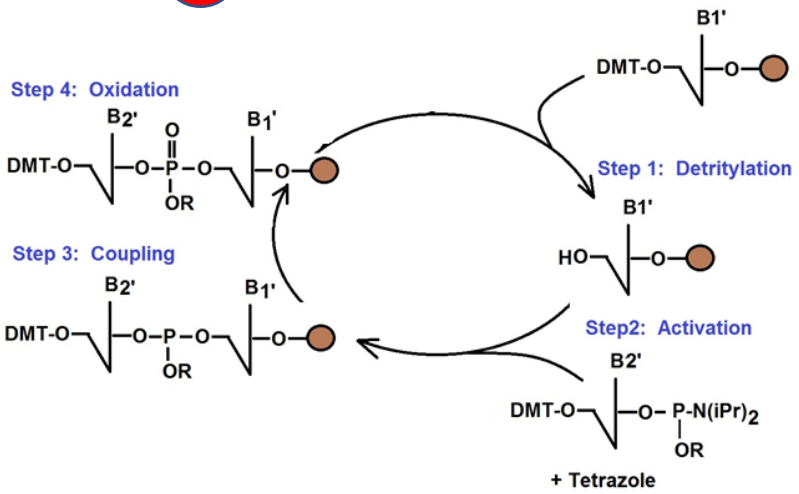




Nucleic acids production

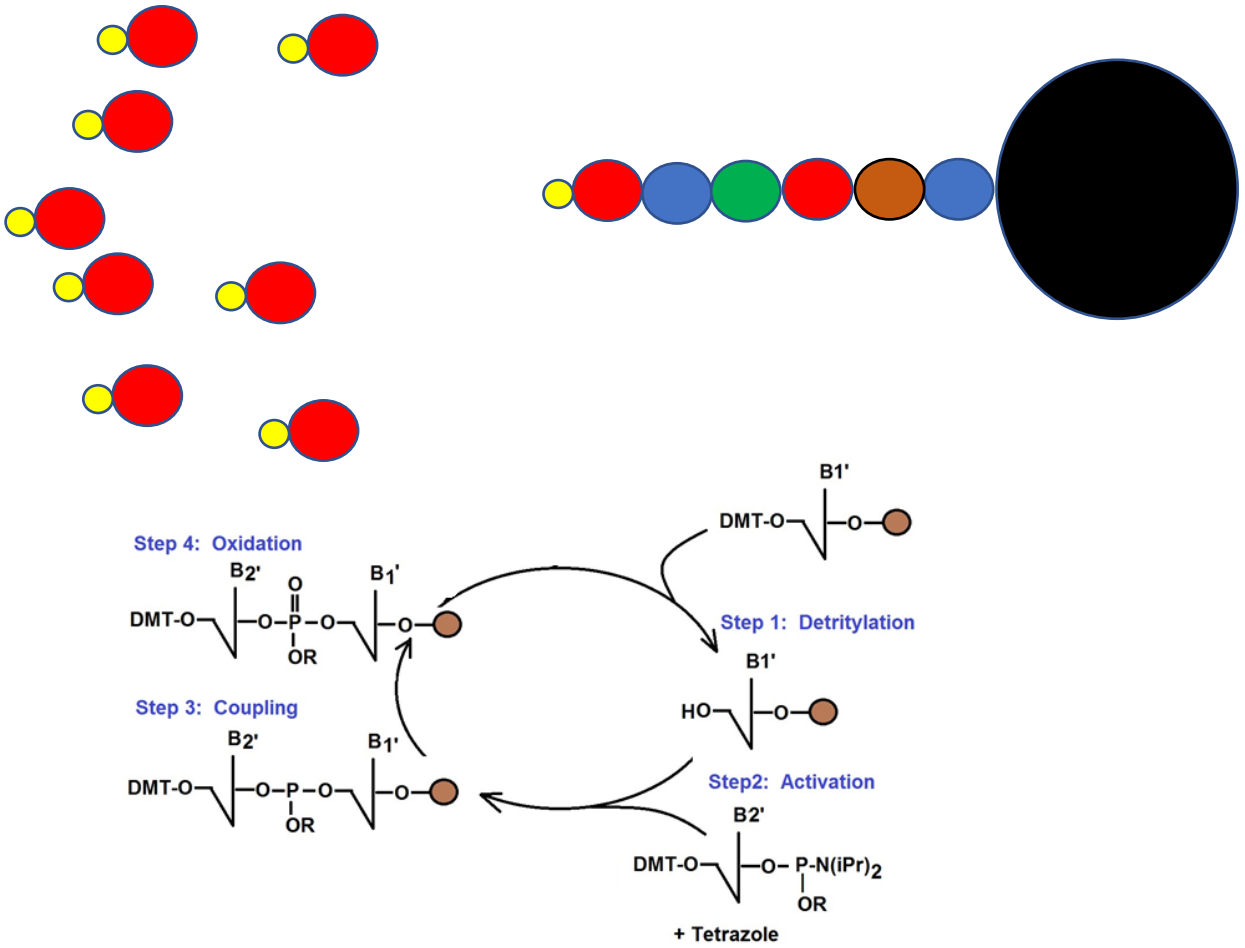
Synthetic production





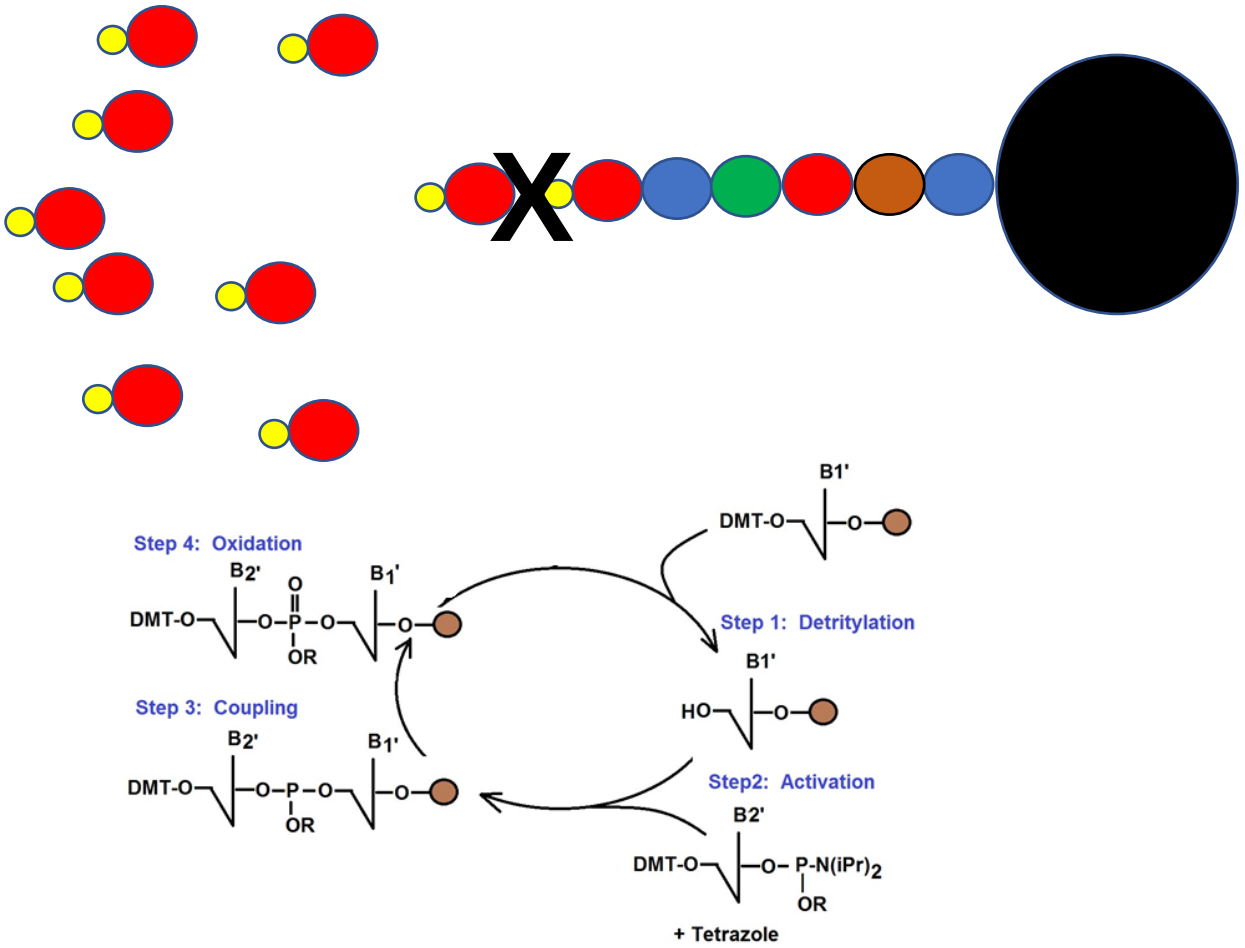
Nucleic acids production

Synthetic production



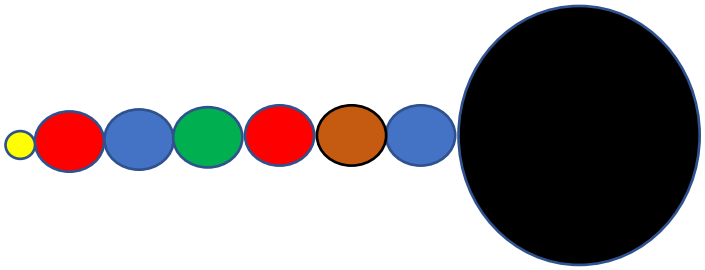
Nucleic acids production

Synthetic production



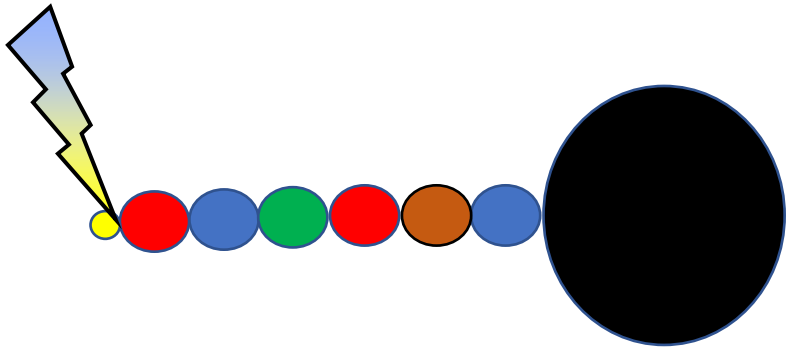
Nucleic acids production

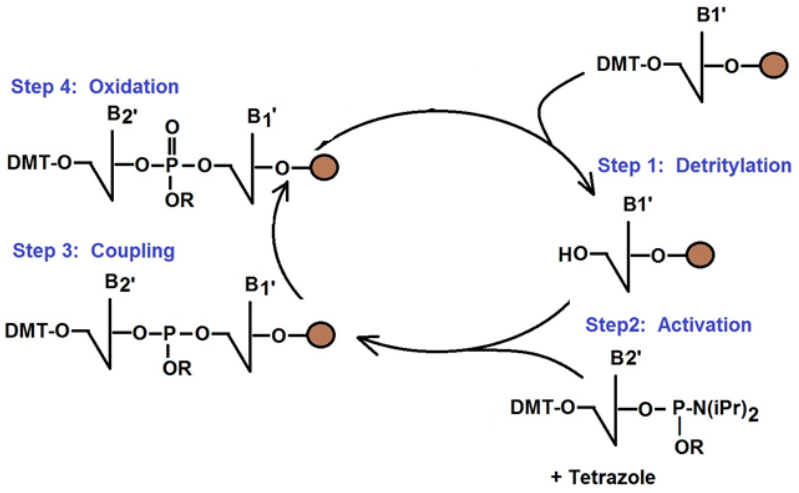
Synthetic production



Nucleic acids production

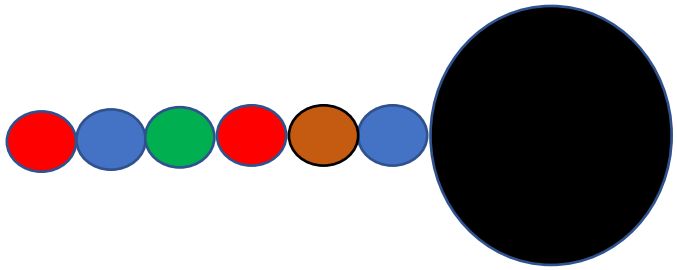
Synthetic production

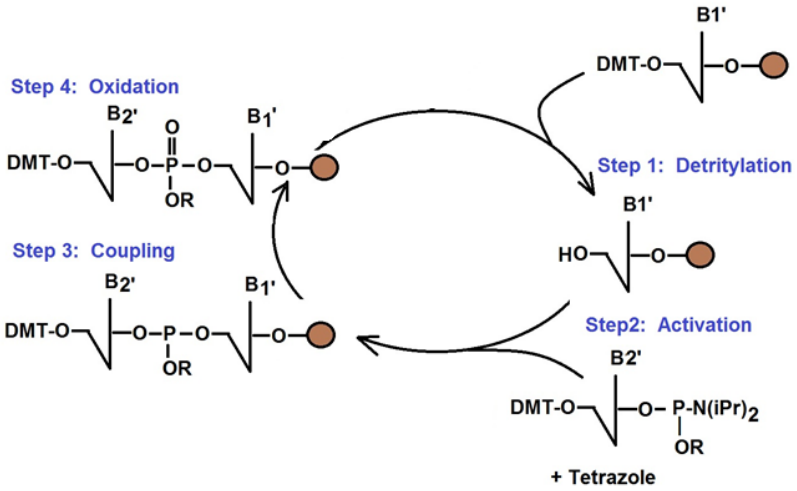




Nucleic acids production

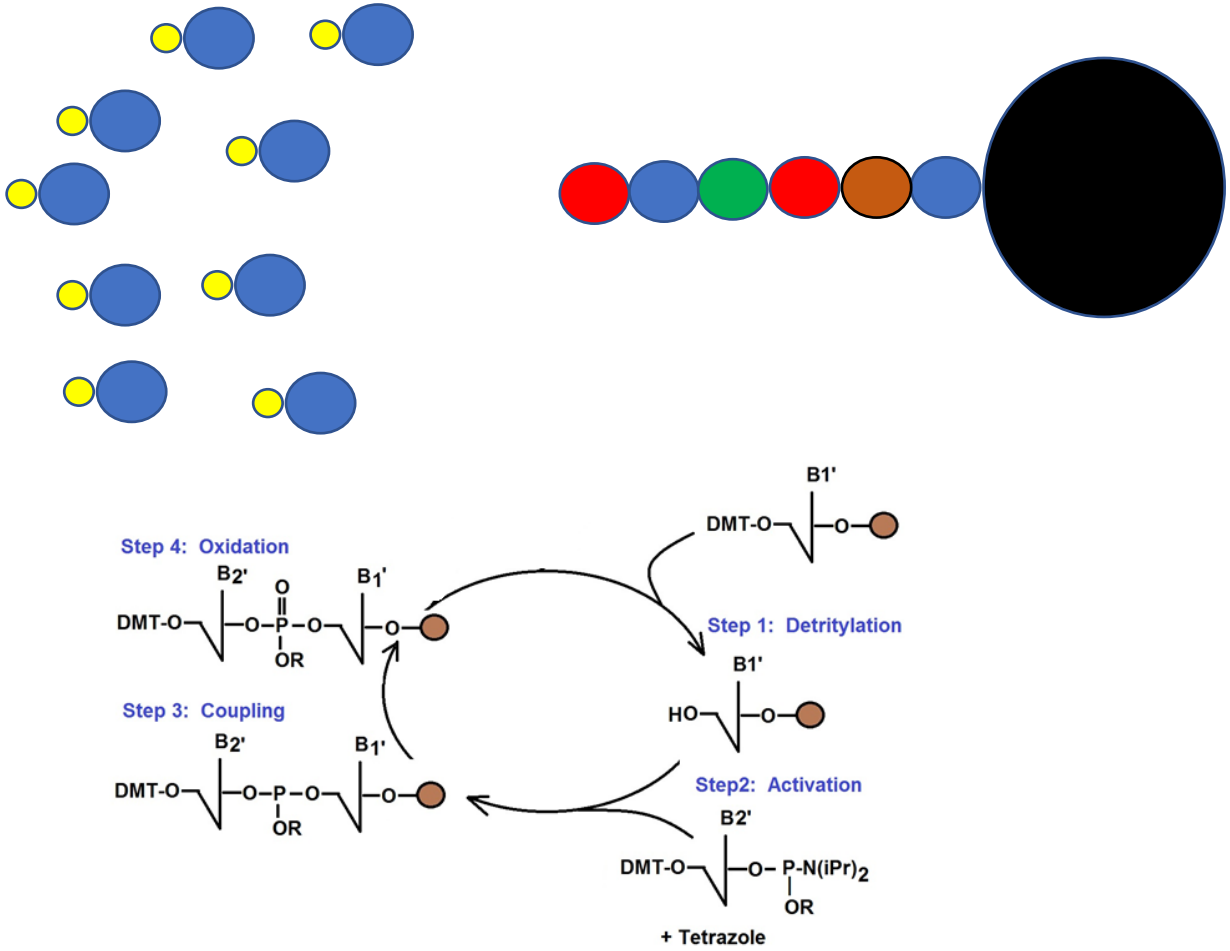
Synthetic production





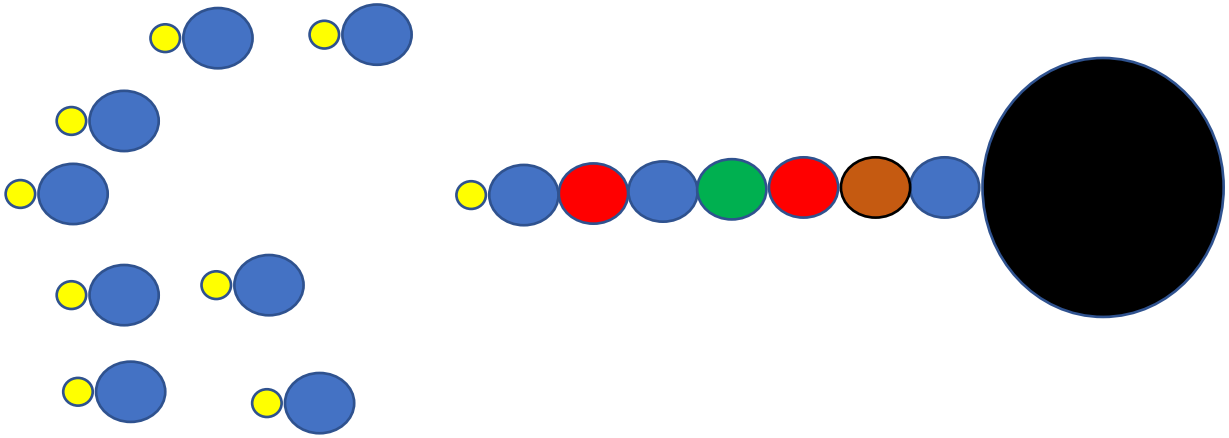
Nucleic acids production

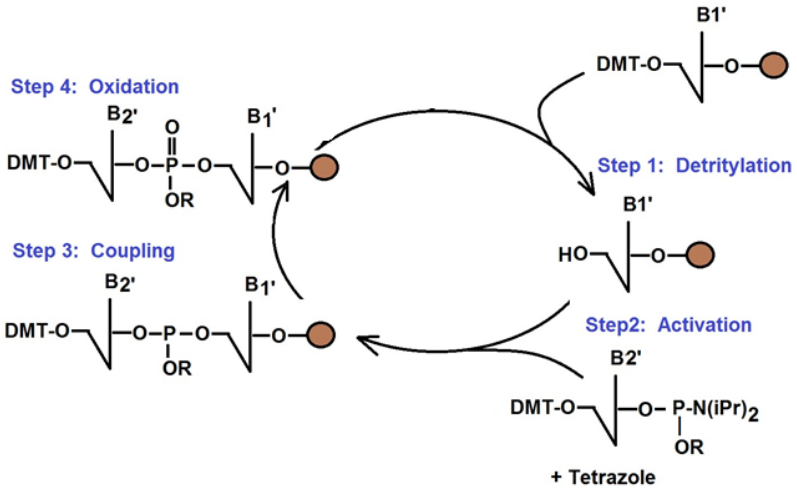
Synthetic production



Nucleic acids production

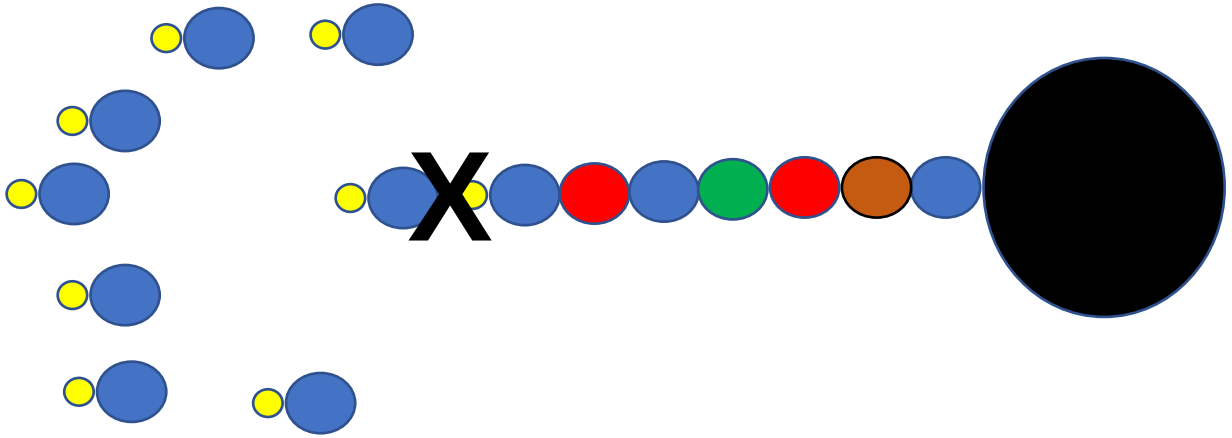
Synthetic production

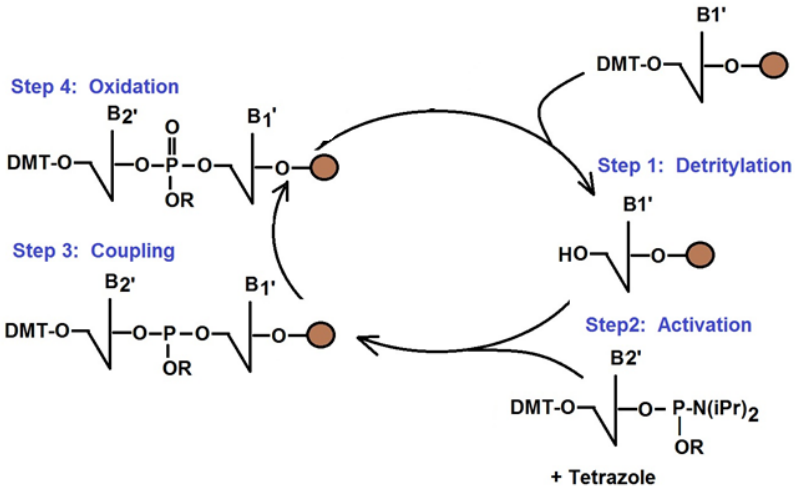




Nucleic acids production

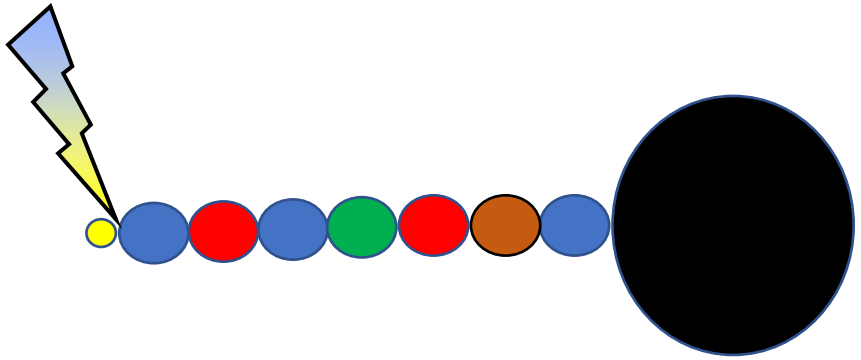
Synthetic production

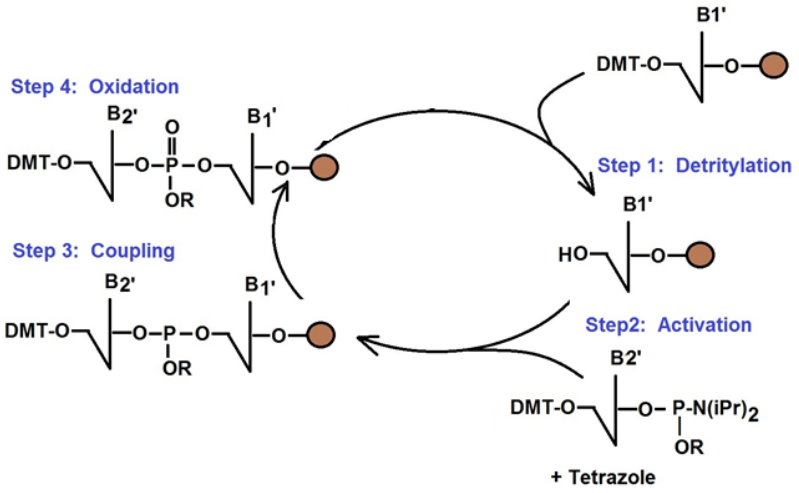




Nucleic acids production

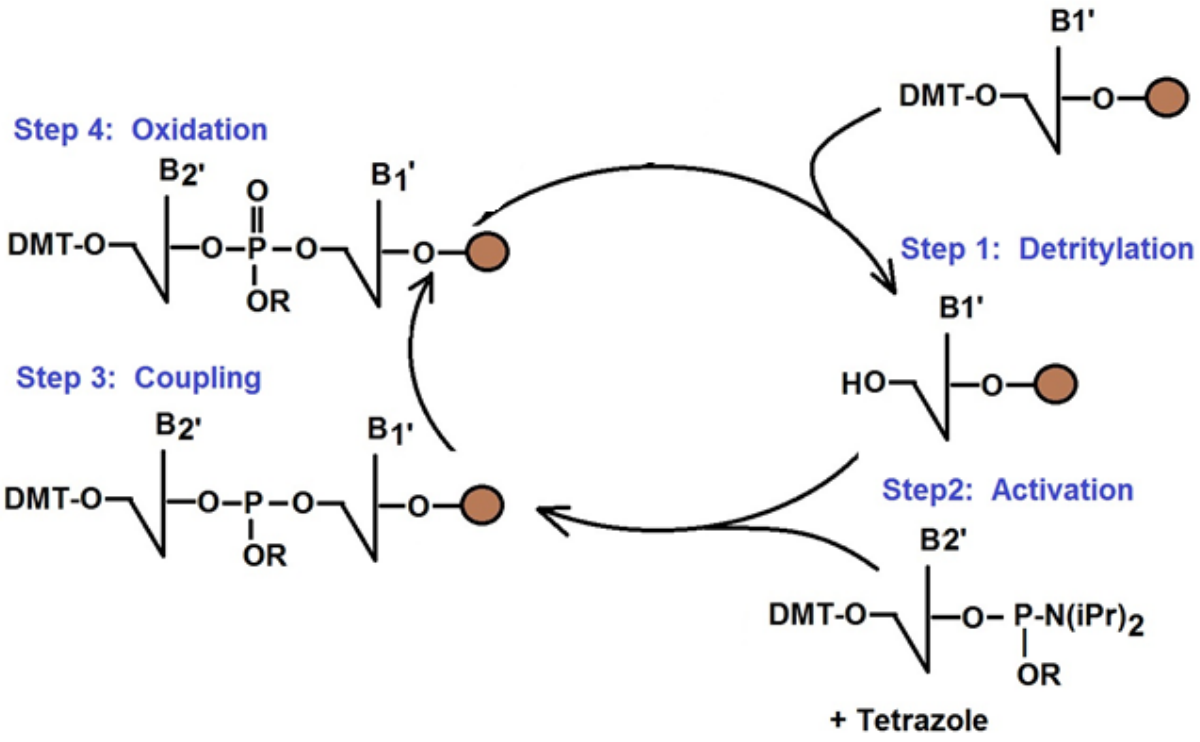
Synthetic production





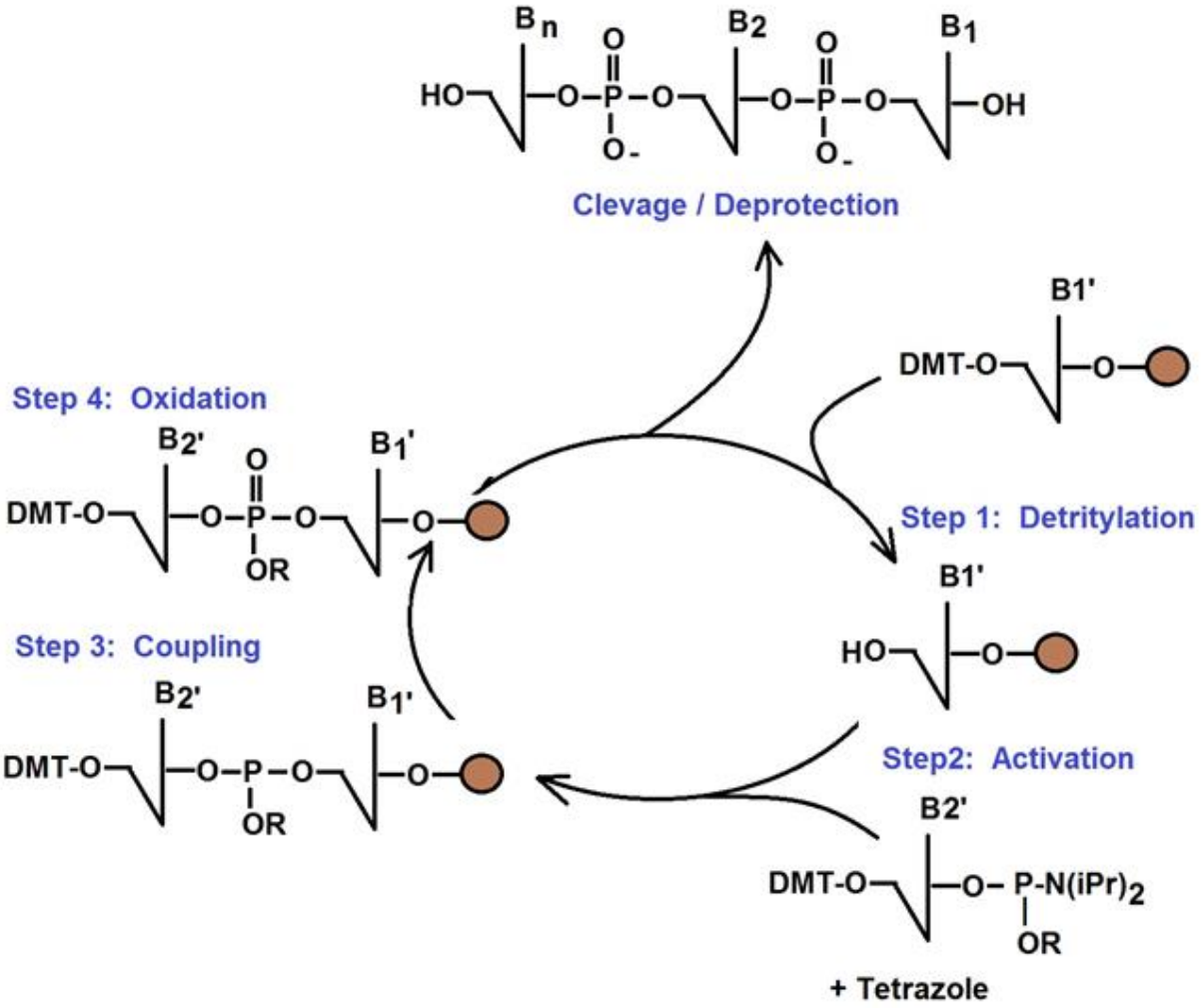
Nucleic acids production

Synthetic production



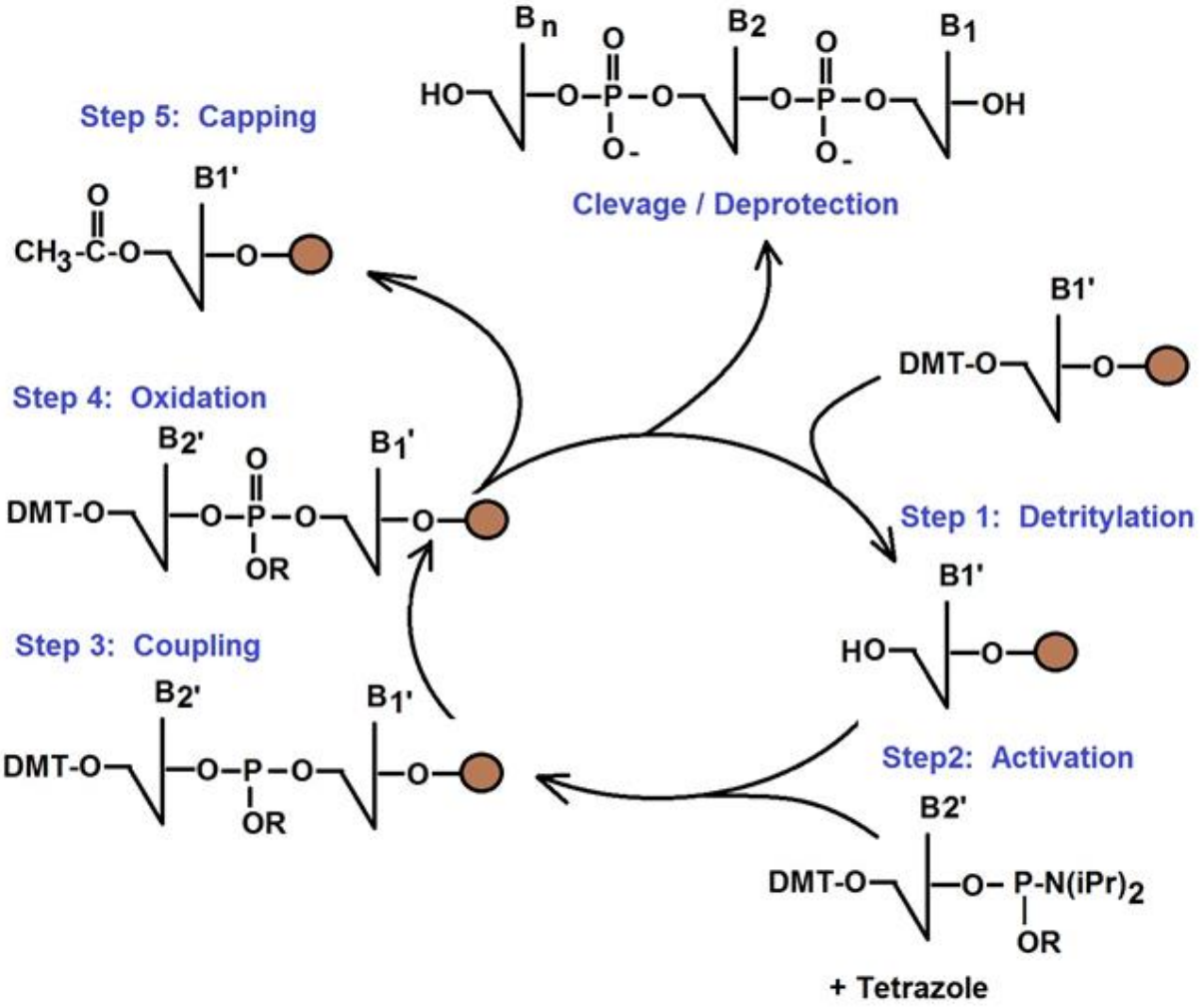
Nucleic acids production

Synthetic production



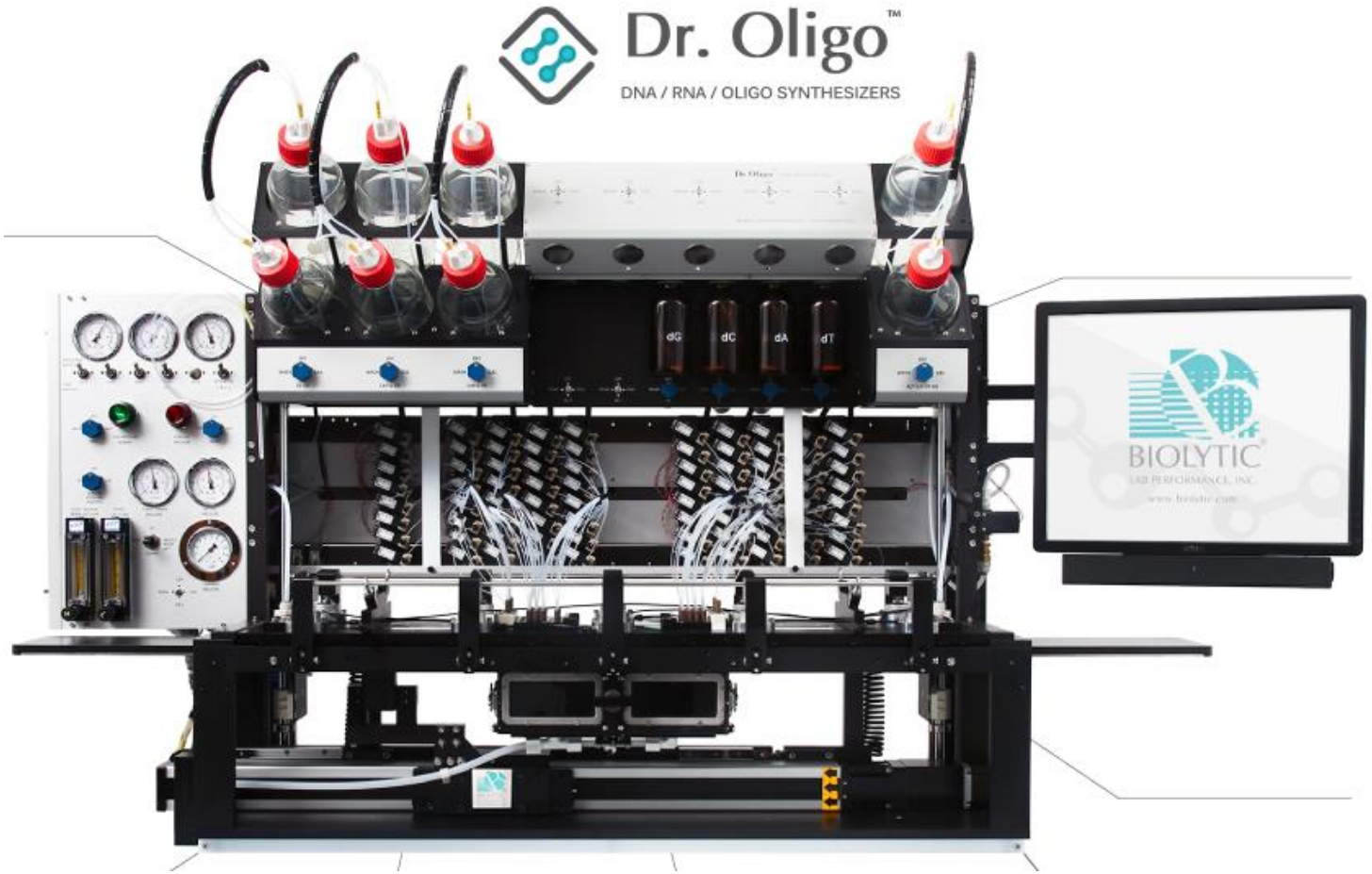
Nucleic acids production

Synthetic production



Nucleic acids production

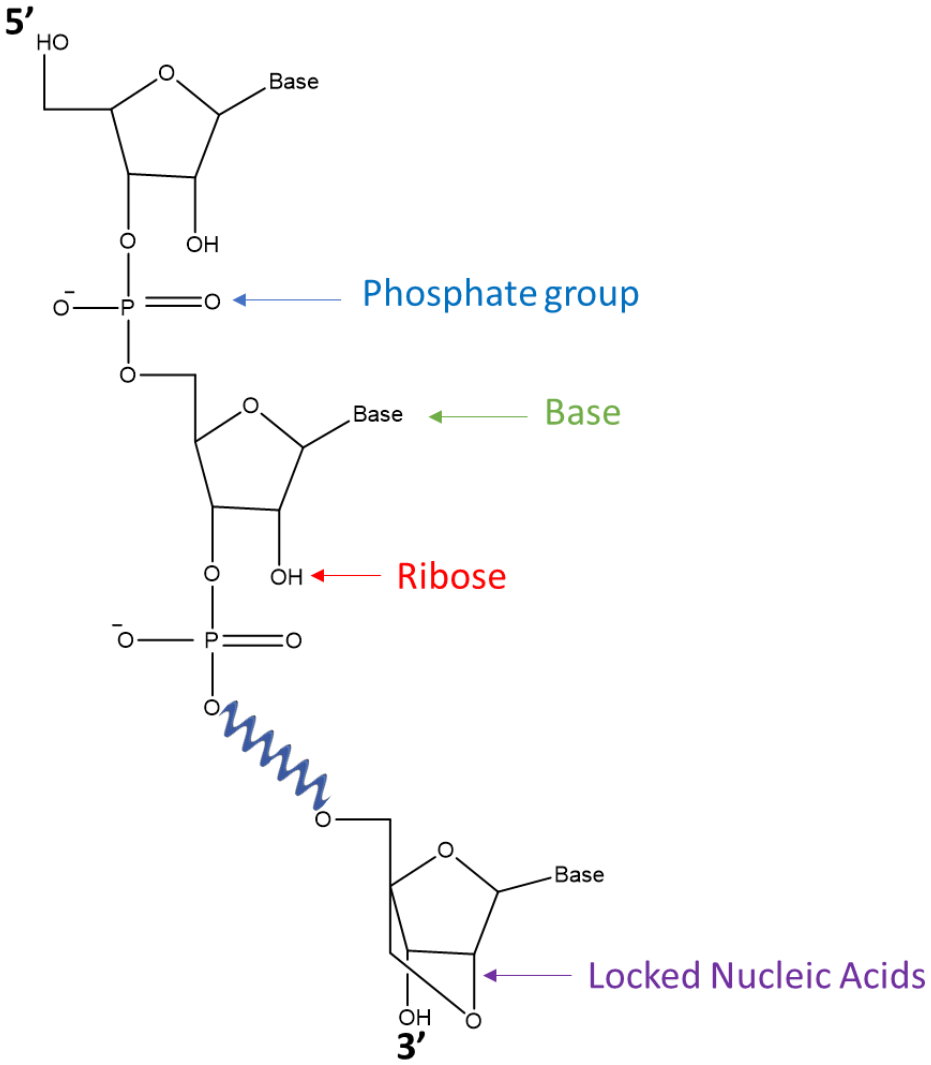
Synthetic production



https://www.biolytic.com

Nucleic acids as Therapeutics

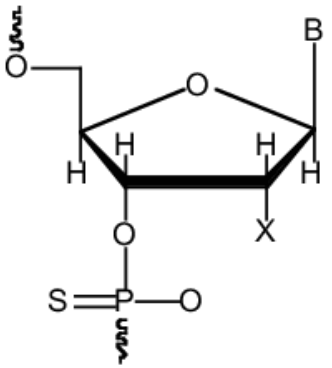
Possible modifications



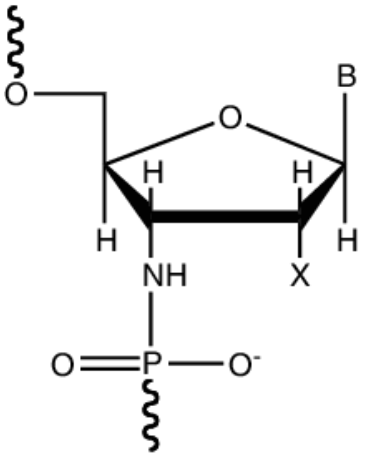
Nucleic acids as Therapeutics

Possible modifications

BACKBONE STRUCTURE

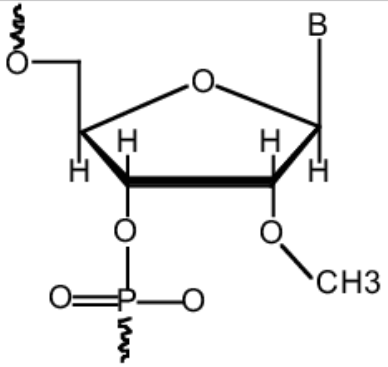


Phosphorothioate (PS)

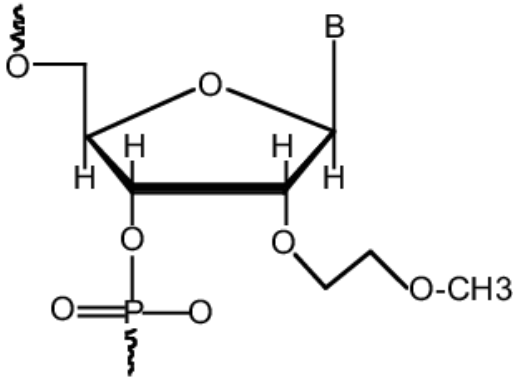


N'3 Phosphoramidate (NP)

SUGAR RING



2′*O*-Methyl (2′*O*-Me)



2′*O*-Metoxyethyl (MOE)

Duarte Moreno DOI:10.3389/fchem.2014.00087

Nucleic acids production

Synthetic production

Impureties:

Nucleic acids production

Synthetic production

Impureties:

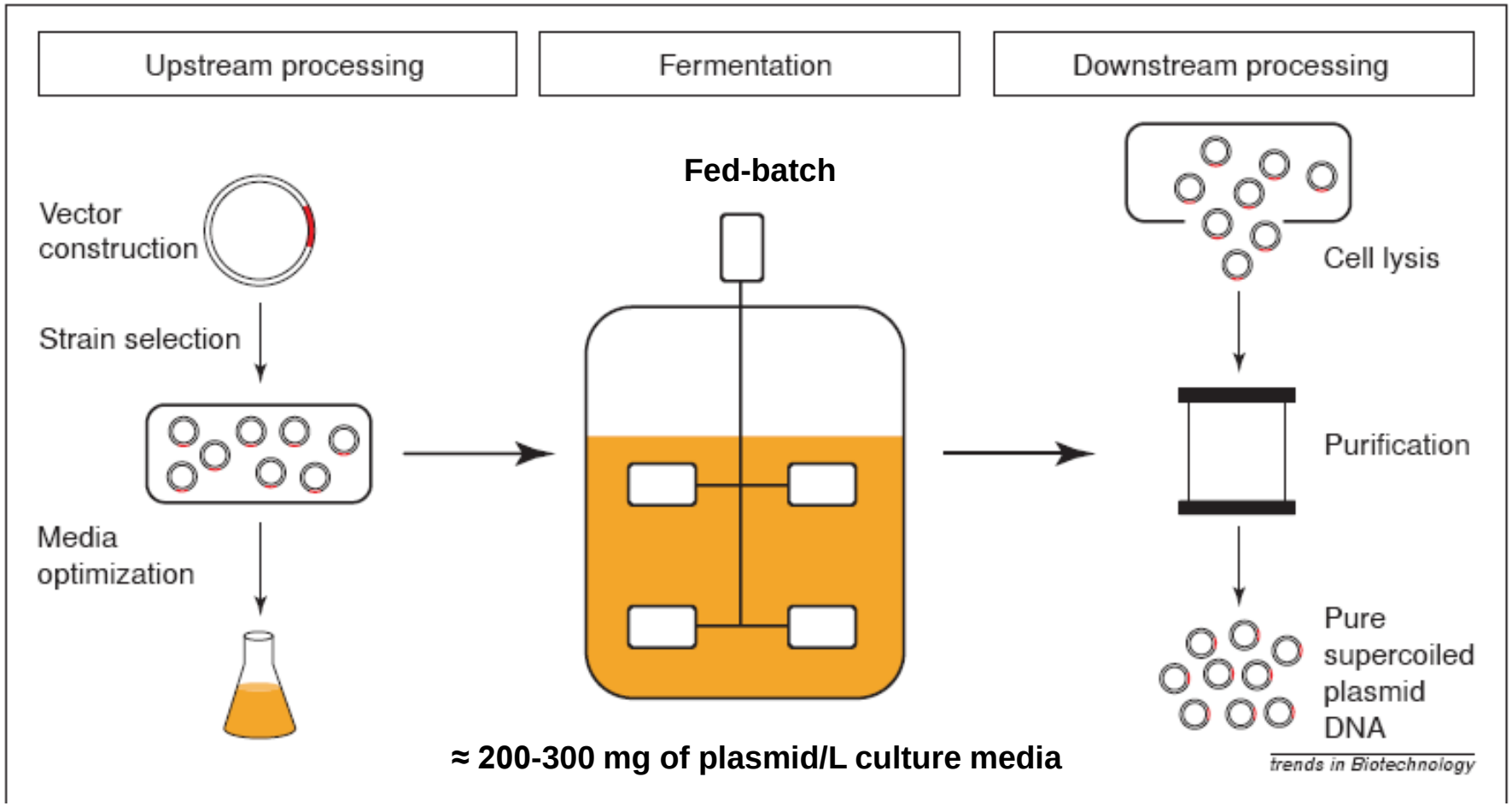
- Bad sequences (n+1, n+2...) ou (n-1, n+2…).
  - HPLC
  - Capillary electrophoresis
- Organic solvents
  - Mass spectrometry
- Inorganic molecules: metals, salts, catalyzers….
  - Chromatography with flame ionization detector
  - Mass spectrometry

Nucleic acids production

Biological production

Nucleic acids production

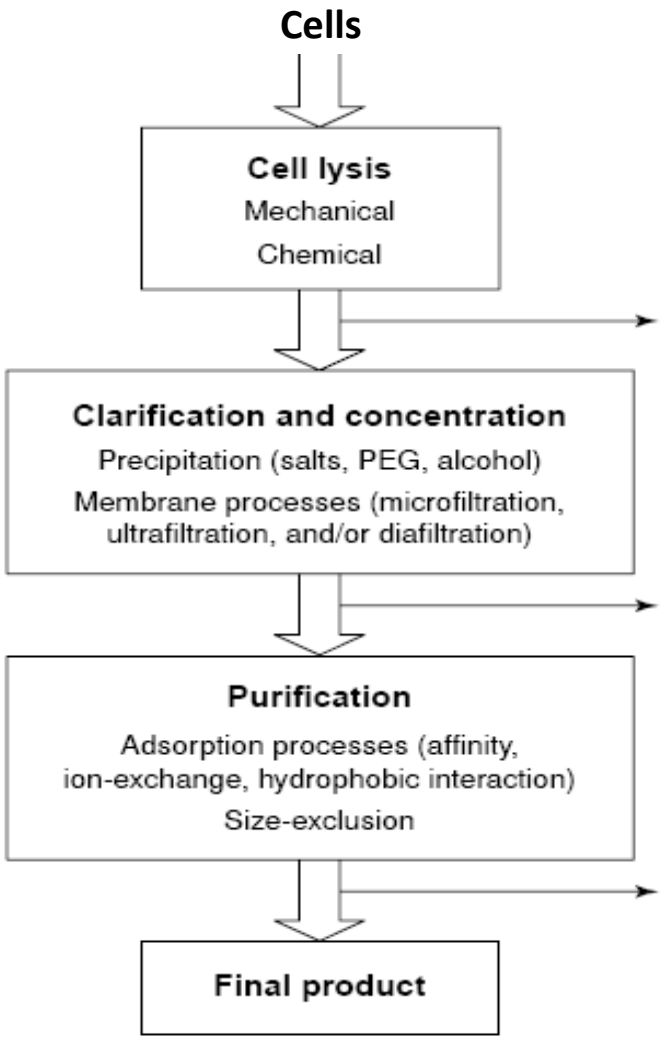
Biological production
(almost exclusively plasmids)



Adapted from C Delomenie

Nucleic acids production

Biological production

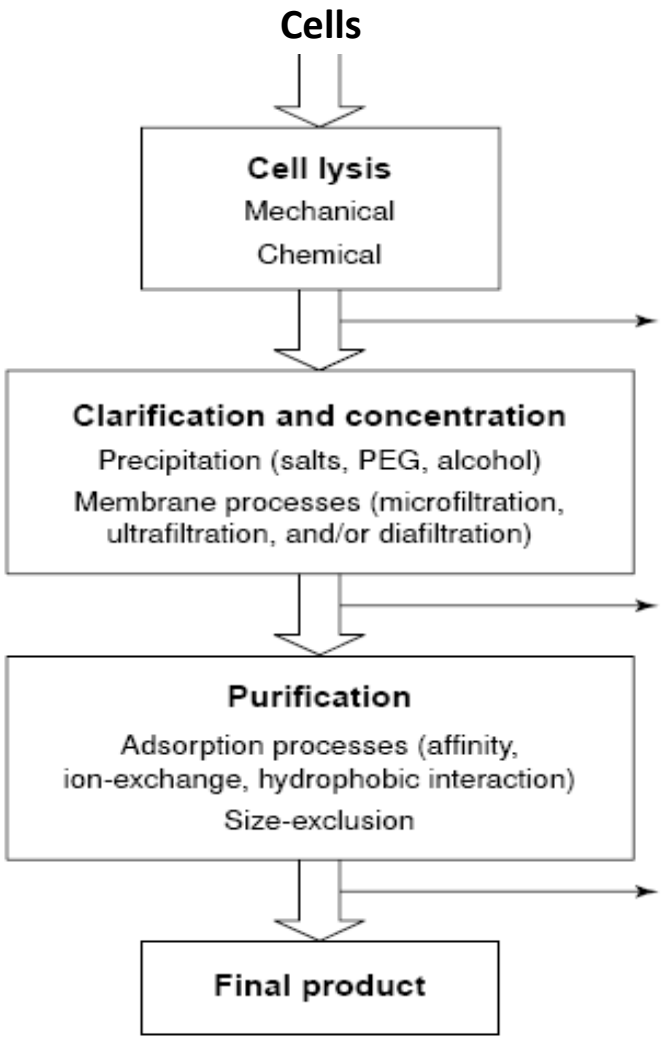


Adapted from C Delomenie

Nucleic acids production

Biological production

Impurities ?

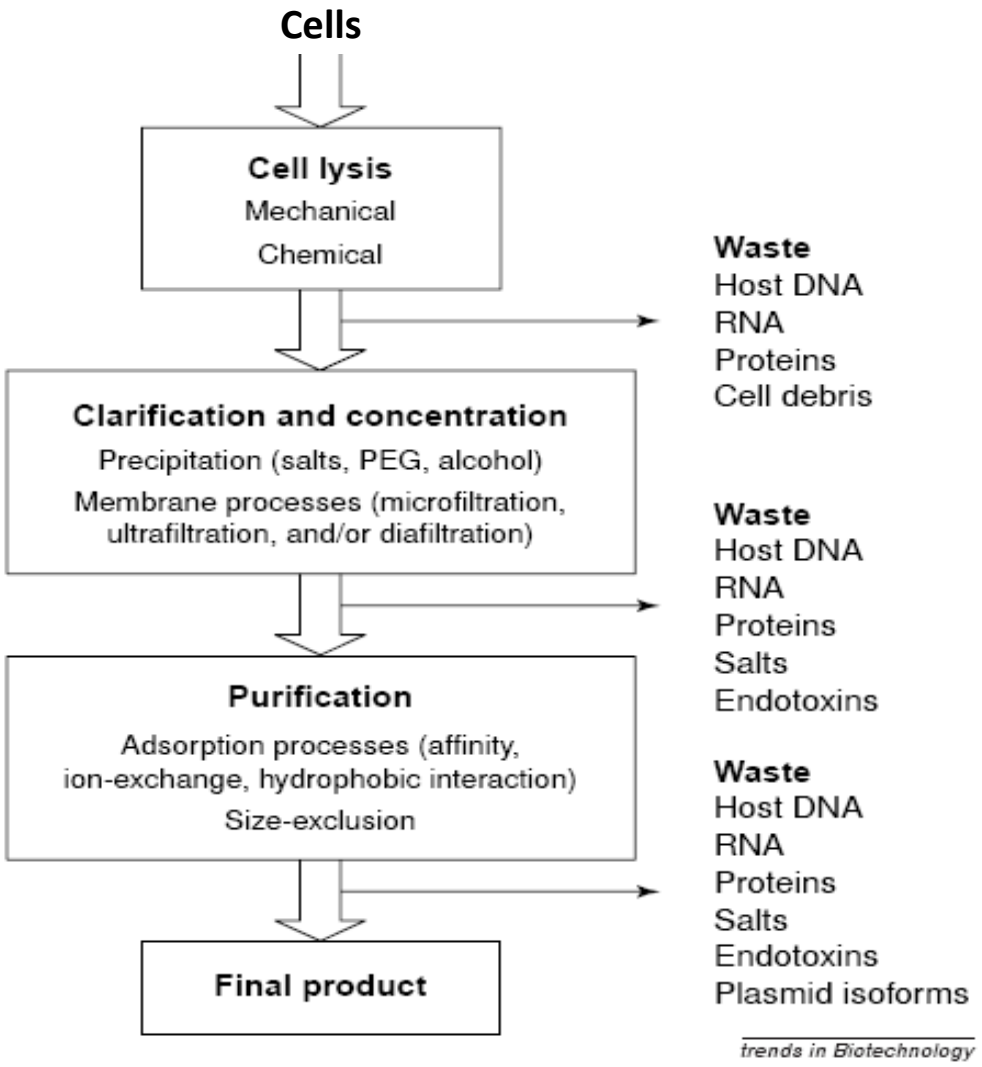


Adapted from C Delomenie

Nucleic acids production

Biological production

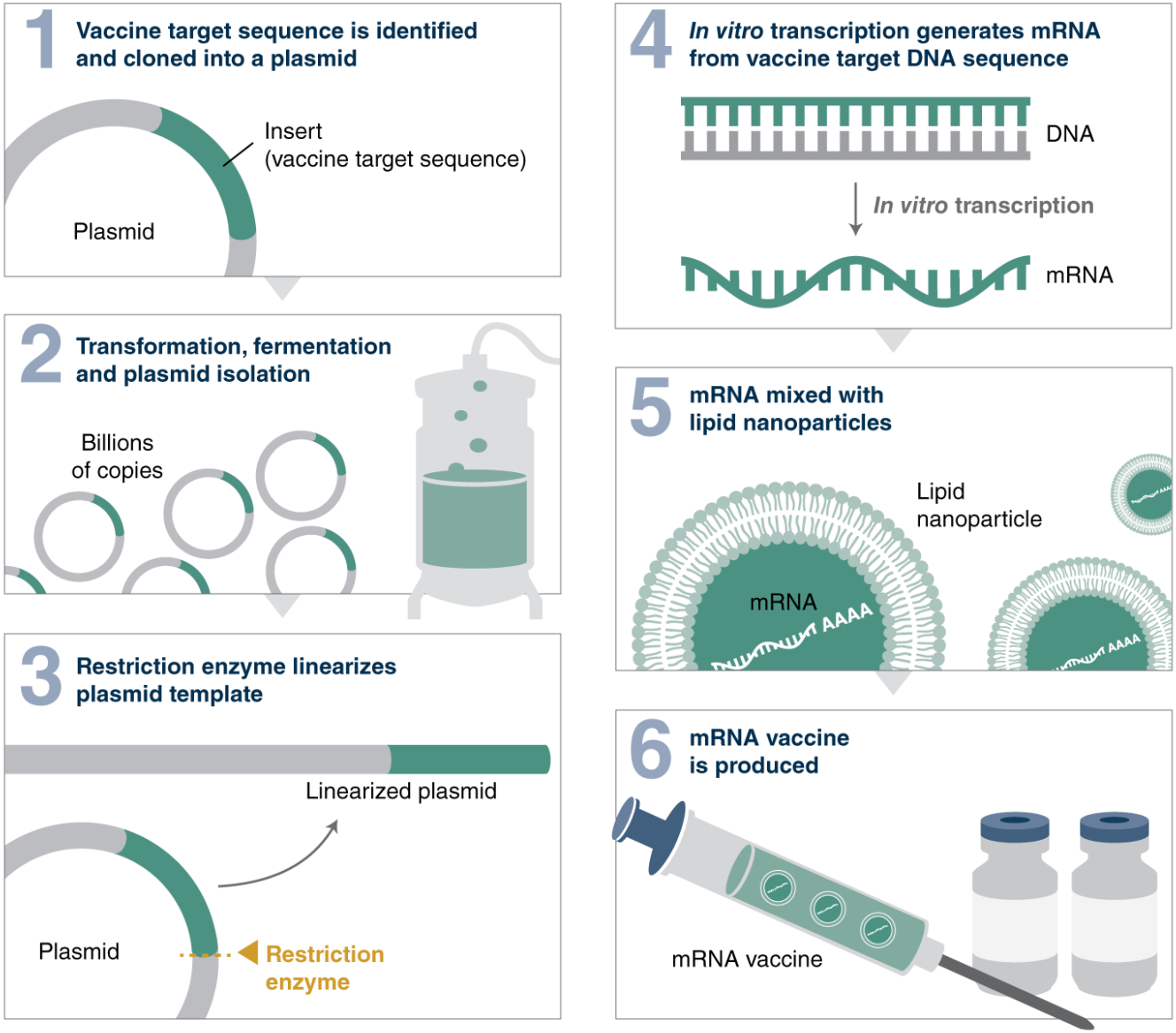
Impurities



Adapted from C Delomenie

Nucleic acids production

Recent advance in mRNA production



Nucleic acids production

Quality control

Impurities	Recommended Test	Expected result
Bacterial DNA	Agarose gel Southern Blot PCR	undetectable <0.01µg/ µg plasmid
RNA	Agarose gel	undetectable
Plasmid isoform	Agarose gel	<5%
Proteins	BCA SDS-PAGE	undetectable
Endotoxins	Test LAL	<0.1 U/ µg plasmide
Sterility		No bacteria,yeast, virus
DNA characterization	Restriction map	same reference map
	Sequencing	

Adapted from C Delomenie

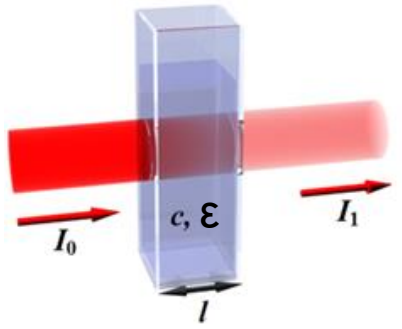
Quality control

UV Spectrometry

Beer-Lambert-Bouguer law:

$$OD = -\log_{10}(I_t/I_0) = \varepsilon \cdot C \cdot L \rightarrow C = OD \times (1 / \varepsilon)$$

OD : optical density, absorbance
ε : molar absorptivity
C : concentration (g/l)
L : length of light path (cuvette dimension)



L = 1 cm = 10 mm

Nucleic bases absorb roughly all at **260 nm**

Quality control

UV Spectrometry

→ Pure nucleic acids

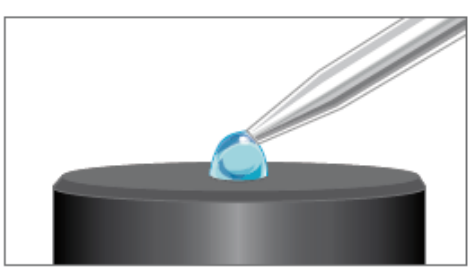
		$DO_{260\ nm} = 1$	
Type	**$\varepsilon_{260\ nm}$ ($l.g^{-1}.cm^{-1}$)**	**C (g/l)**	**C (µg/ml ; ng/µl)**
dsDNA	**20**	**0,05**	**50**
ssRNA	**25**	**0,04**	**40**
ssDNA	**27**		**37 (ADN longs)** **20 to 37 (depending on size and sequence)**

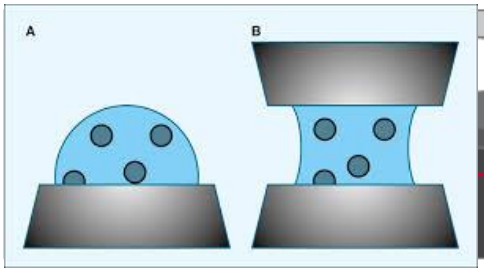
→ Detection limits (dsDNA) depend of the instrument :

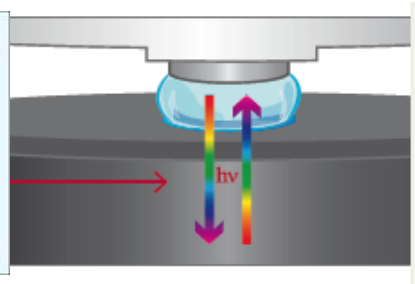
DO=0,001 ⇔ 50 µg/ml / DO=0,005 ⇔ 250 ng/ml

Adaptée de C Delomenie

Microvolumes



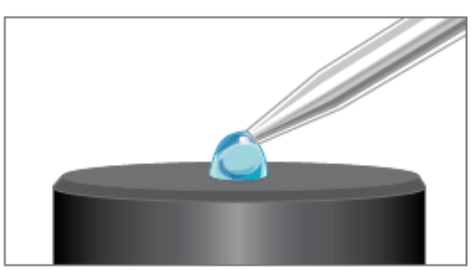


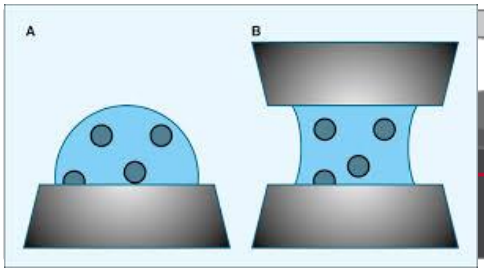


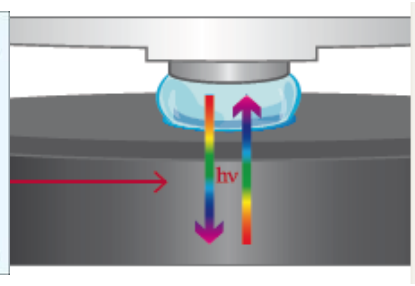
- **Measurements on a droplet** (1 to 5 µl)

www.thermofisher.com
Adaptée de C Delomenie

Microvolumes







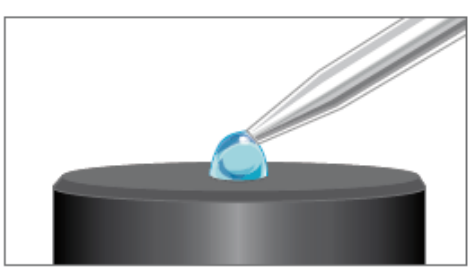
- **Measurements on a droplet** (1 to 5 µl)

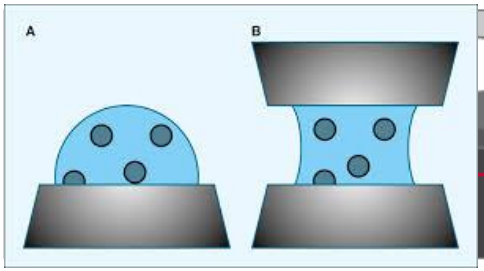
- **Shorter light path** (10 → 0.2 to 1 mm) → Wider range

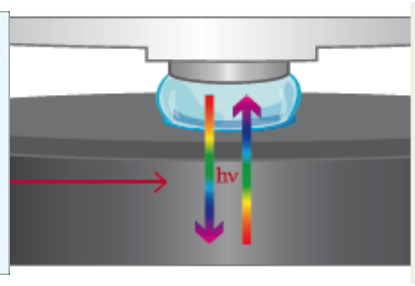
Shorter light path ⇔ dilution	$DO_{260\ nm}$	C_{dsDNA} (µg/ml ; ng/µl)
0,2 mm ⇔ 50	1	2500
10 mm ⇔ 1	1	50

www.thermofisher.com
Adaptée de C Delomenie

Microvolumes







- **Measurements on a droplet** (1 to 5 µl)

- **Shorter light path** (10 → 0.2 to 1 mm) → Wider range

Shorter light path ⇔ dilution	$DO_{260\ nm}$	C_{dsDNA} (µg/ml ; ng/µl)
0,2 mm ⇔ 50	1	2500
10 mm ⇔ 1	1	50

- Quick
- Less sample needed
- Can be used higher concentration: less dilution

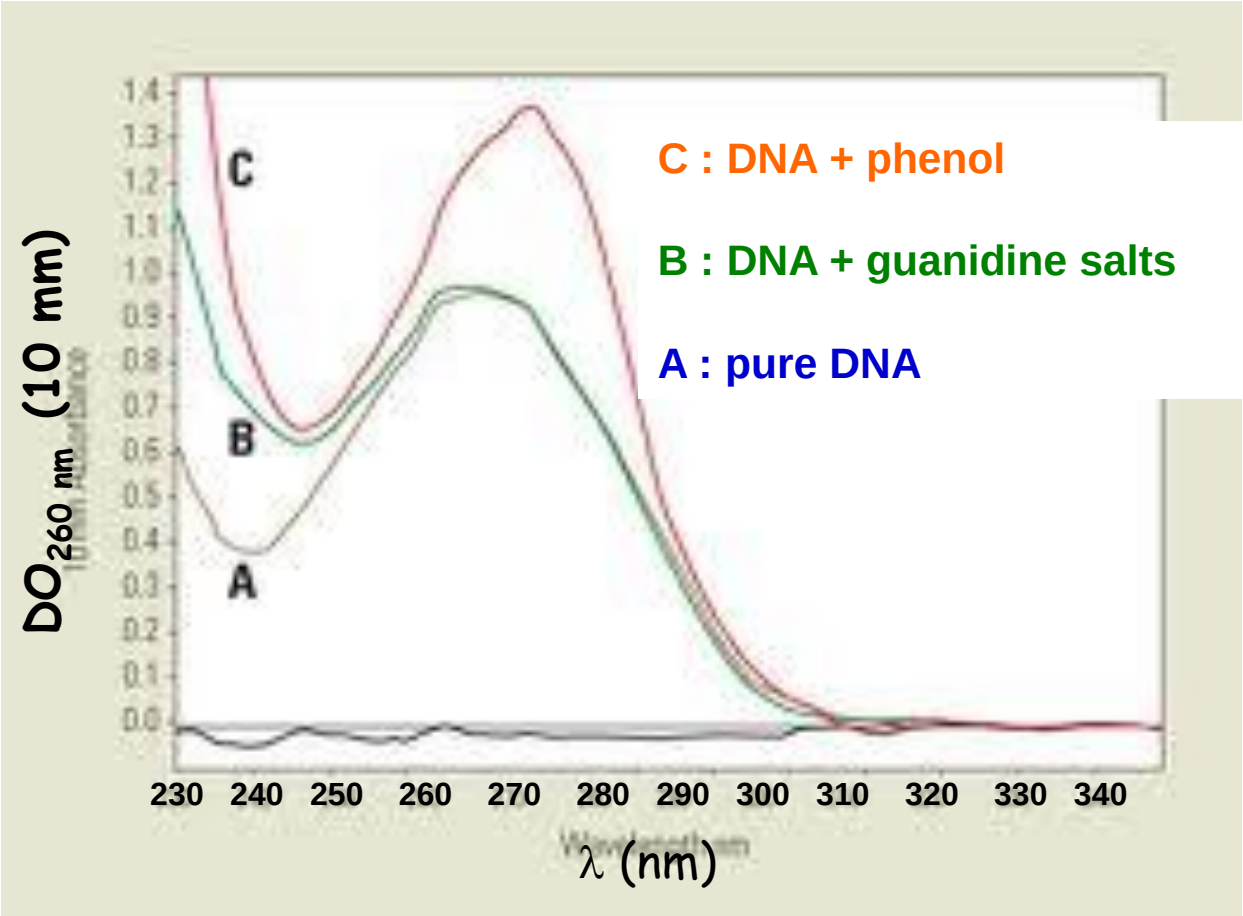
- Sensible to manipulation: measures need to be repeated
- Possible contamination between samples: clean

www.thermofisher.com
Adaptée de C Delomenie

Quality control

Spectrometry : contaminants

UV spectra give us info on purity



Adaptée de C Delomenie

Quality control

Spectrometry : contaminants

Contaminants and Impurities	**→ Risks**	**l (nm)**	**Ratio → Normes**
Organic compounds (sugars, lipids), salts (buffer), solvents	Inhibition des enzymes (RT, Taq, T7 pol…)	**230**	**A260/A230 ≥ 1.7**
ARN, ADN	-	**260**	**-**
Phenol	overestimation [DNA, RNA], Enzyme inhibition (RT, Taq, T7 pol…)	**270**	**A260/A270 ≥ 1.2**
Proteins, phenol	overestimation [DNA, RNA], Enzyme inhibition (RT, Taq, T7 pol…)	**280**	**ADN : 1.8<A260/A280<2** **ARN : A260/A280>2**
Particles (fibre, dust, bubbles)	Diffraction => wrong values	**320**	**A320 → 0**

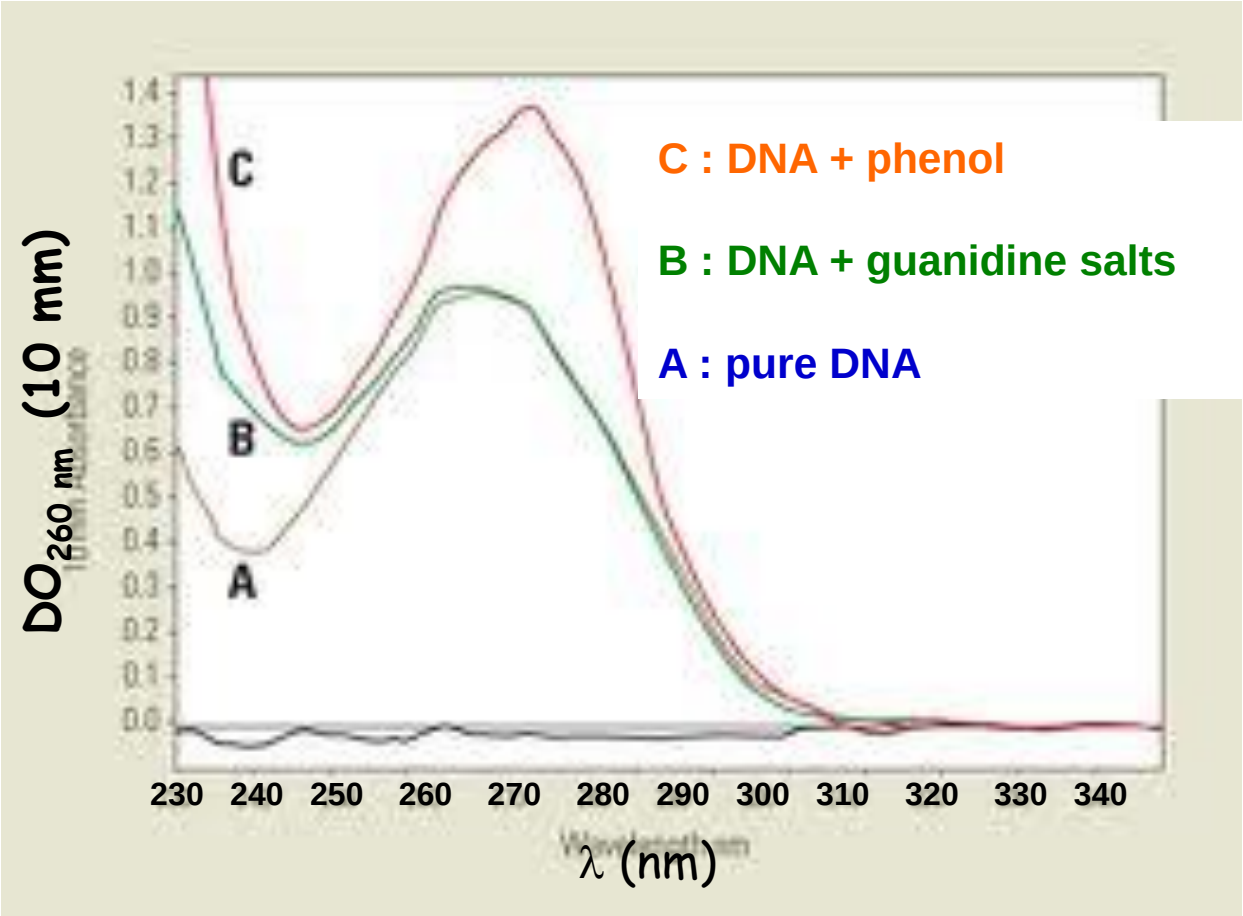
-contaminants → further purification - particles → centrifugation

Adaptée de C Delomenie

Quality control

Spectrometry : contaminants

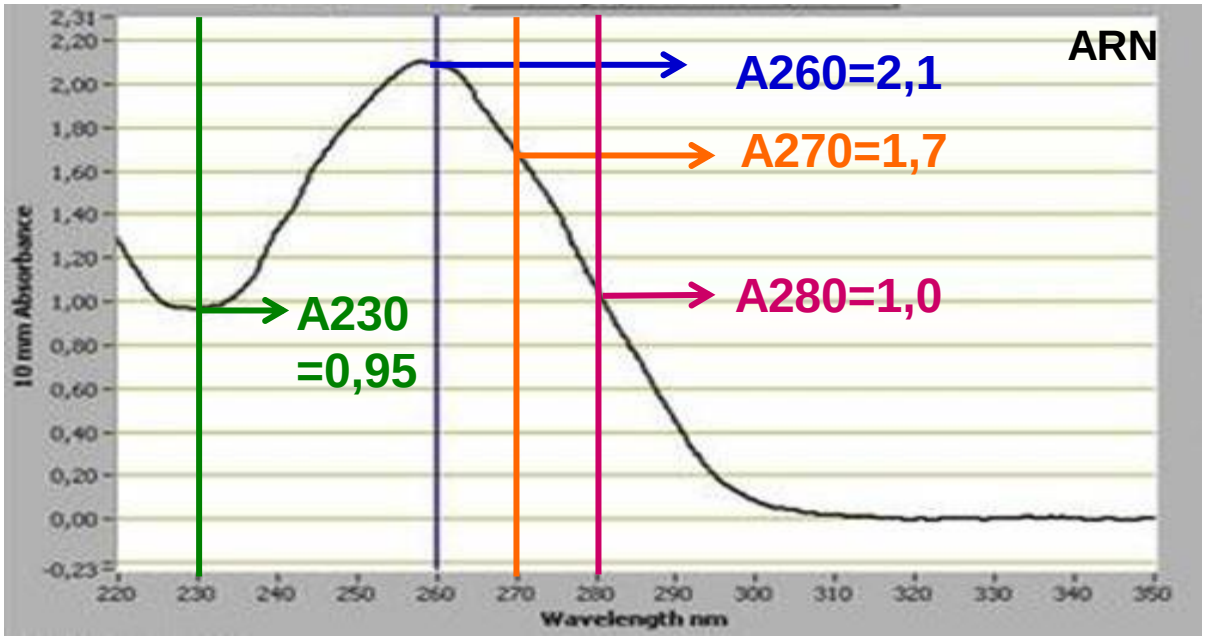
UV spectra give us info on purity



Adaptée de C Delomenie

Quality control

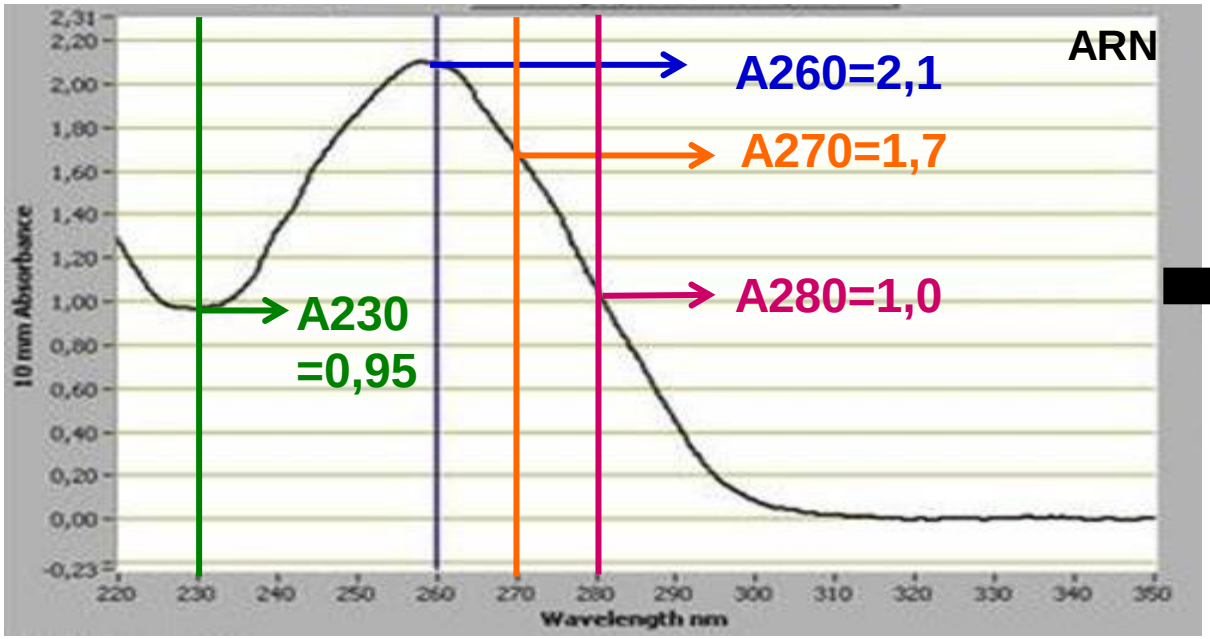
Spectrometry : contaminants



Adaptée de C Delomenie

Quality control

Spectrometry : contaminants



A260/A280=2,10

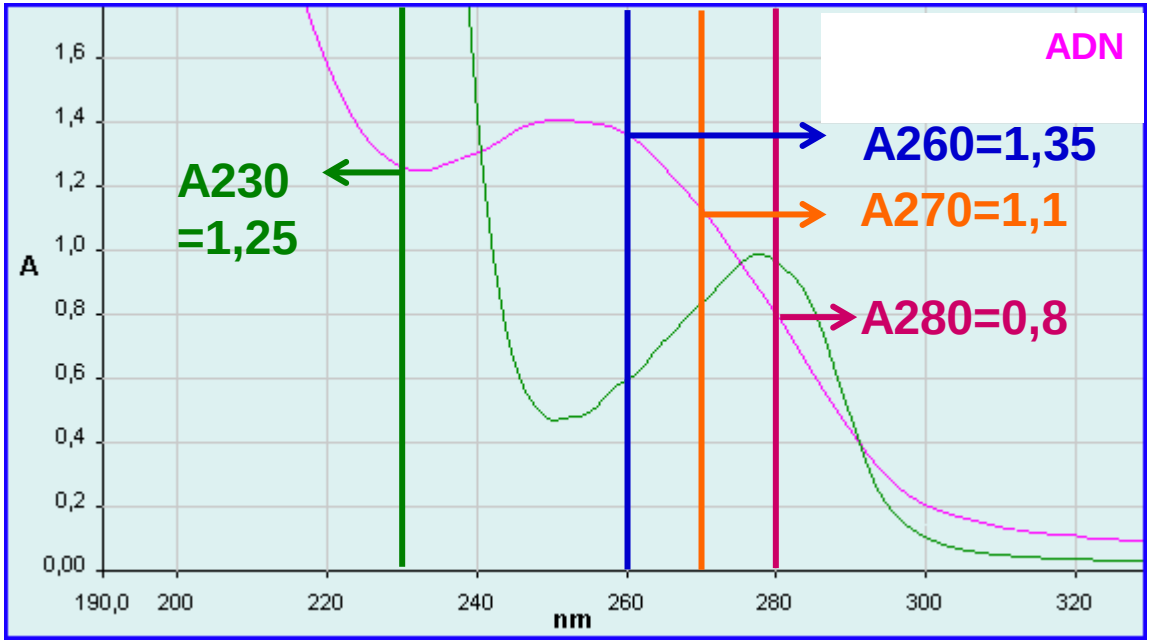
A260/A230=2,21

A260/A270=1,24

Adaptée de C Delomenie

Quality control

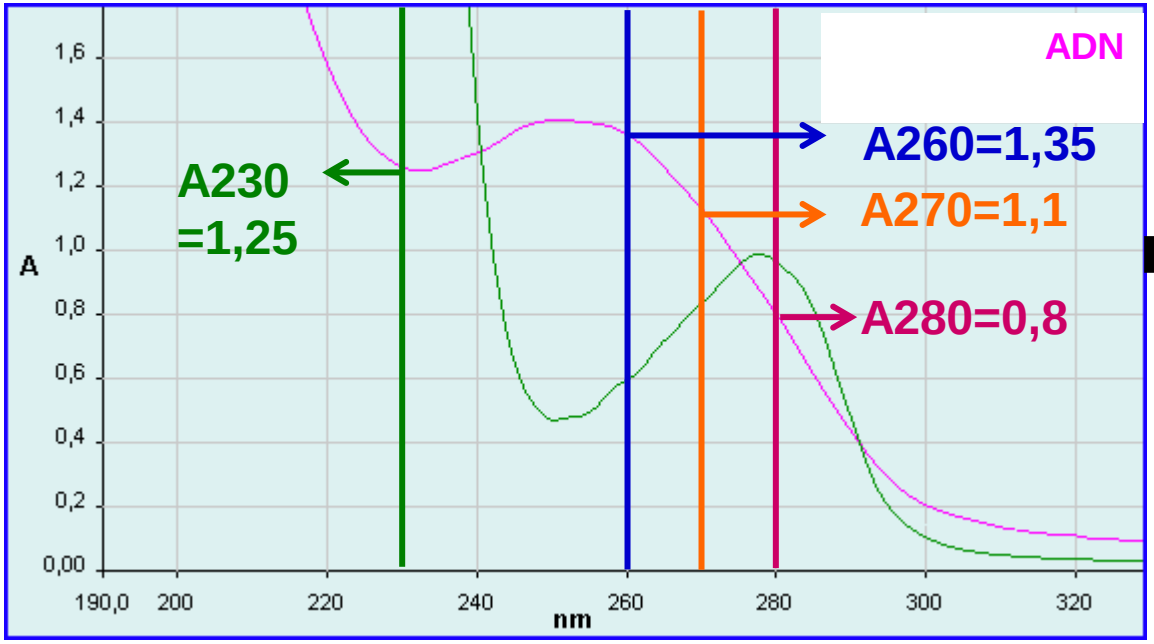
Spectrometry : contaminants



Adaptée de C Delomenie

Quality control

Spectrometry : contaminants



A260/A280=1,69

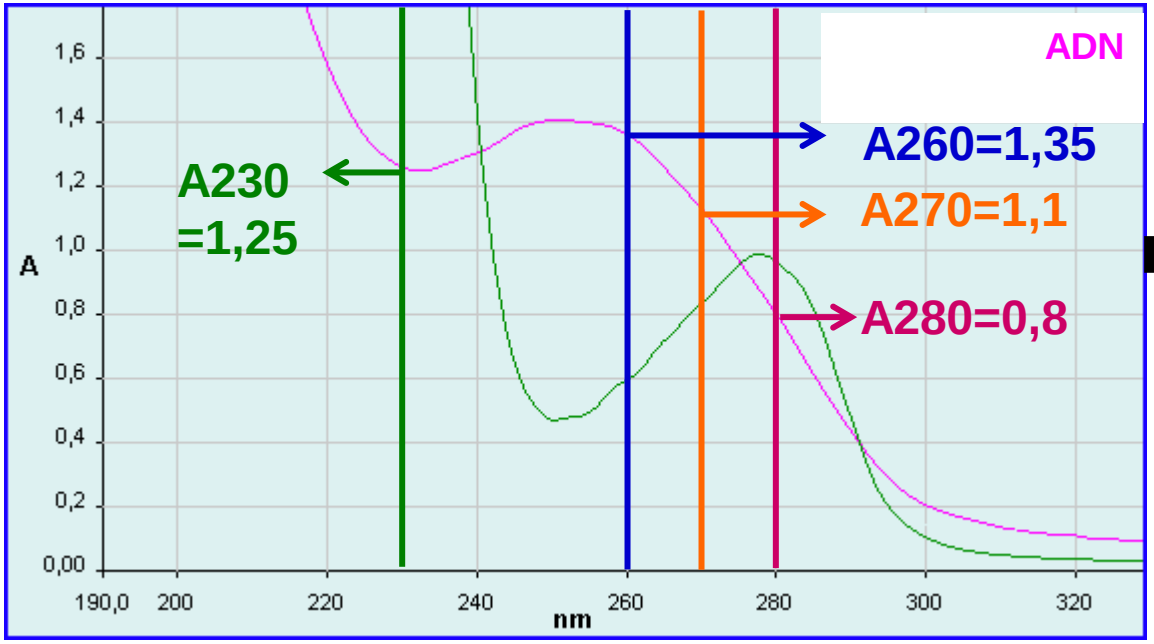
A260/A230=1,08

A260/A270=1,23

Adaptée de C Delomenie

Quality control

Spectrometry : contaminants



A260/A280=1,69

A260/A230=1,08

A260/A270=1,23

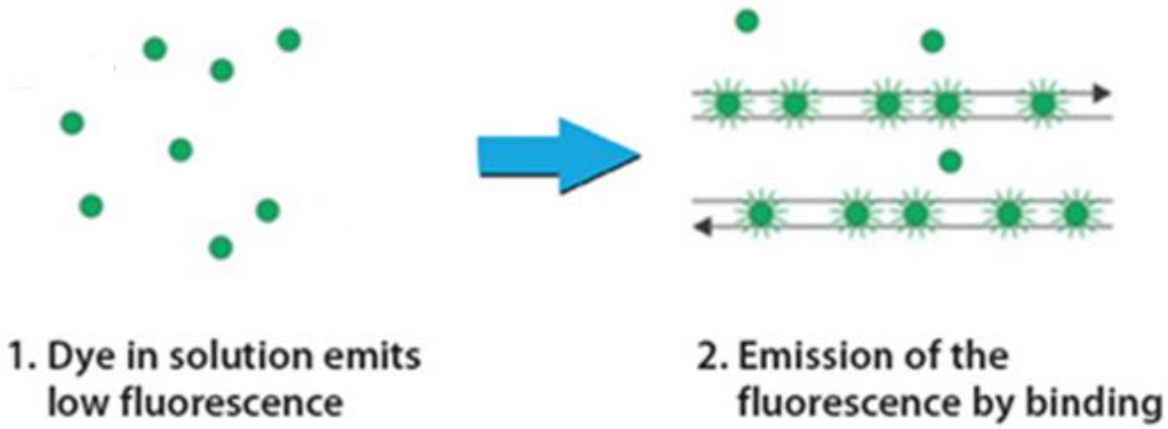
Adaptée de C Delomenie

Quantification & Contrôles qualités

Fluorogenic intercalating dyes

Use of a fluorescent dye capable of interacting specifically with nucleic acids (specific to DNA, RNA...) and whose fluorescence is strongly increased after binding to the nucleic acids (example of DNA: BET X20; SYBR Green X1000)

→ **Signal emitted is proportional to the quantity of nucleic acids present**



All intercalating agents are potentially mutagenic!

Adaptée de C Delomenie

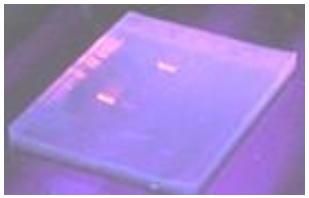
Fluorogenic intercalating dyes

Dye	Specificity	l ex	l em	Sensitivity	Usage
Ethidium Bromide	dsDNA, (ssDNA, ssRNA)	302 493	605	**1 µg / ml** 5 ng / bande	Gel staining
Propidium iodine (PI)	dsDNA ssRNA	536	617	-	Necrosis et apoptosis measure (flow cytometry)
SYBR Green I	dsDNA	490	520	**40 ng/ ml** 200 pg /bande	-Gel staining -DNA quantification (qPCR)
RiboGreen	RNA, DNA	500	525	**~ 1 ng/ml**	Dosing small quantity
OliGreen	ssDNA (cDNA, oligos >10 b)	500	523	**100 pg/ml**	
PicoGreen	dsDNA (ssDNA, ssRNA)	502	523	**25 pg/ml**	
Hoescht 33258	DNA,	365	450	**10 ng/ ml**	Nuclei staining (imaging)
DAPI	DNA	344	466	**~ 1 ng/ml**	

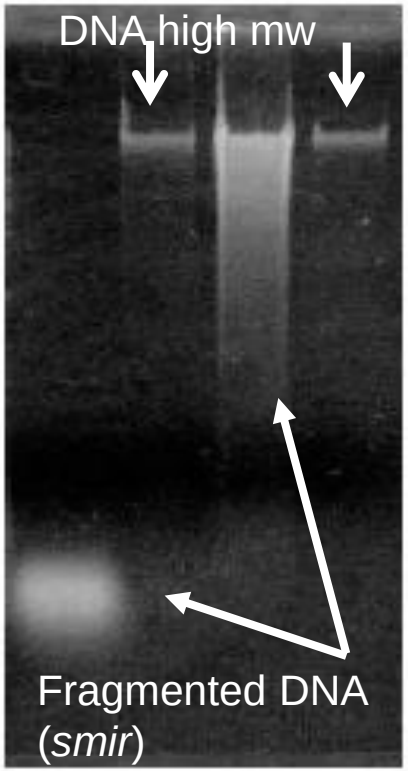
- SYBR Green 25x more sensitive than Ethidium Bromide
- Pico Green 40000x more sensitive than Ethidium Bromide

Fluorogenic intercalating dyes

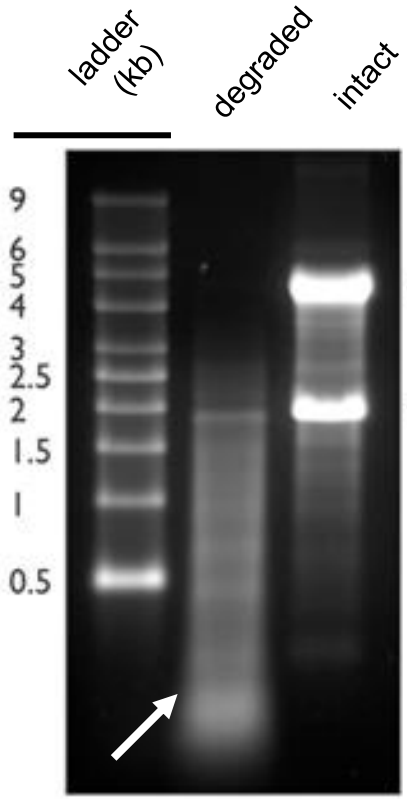
Ethidium bromide



- Genomic DNA



- Total RNA total eucaryote



Intact RNA :

(28S / 18S ~ 2)

Degradation :

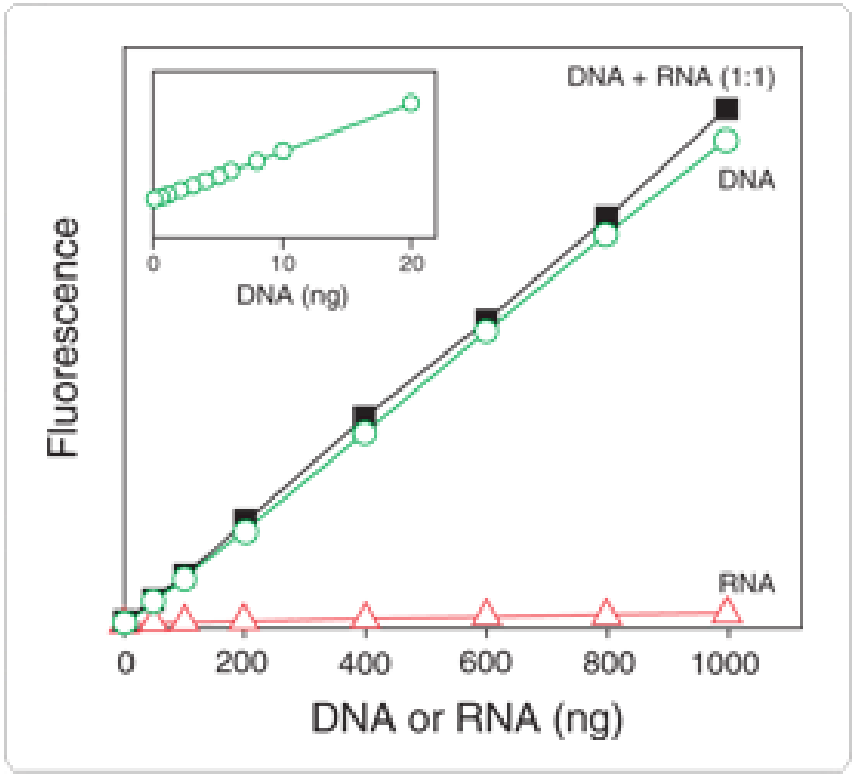
- fragmentation
- Loss of 28S
- ↘ 28S/18S ratio

Adaptée de C Delomenie

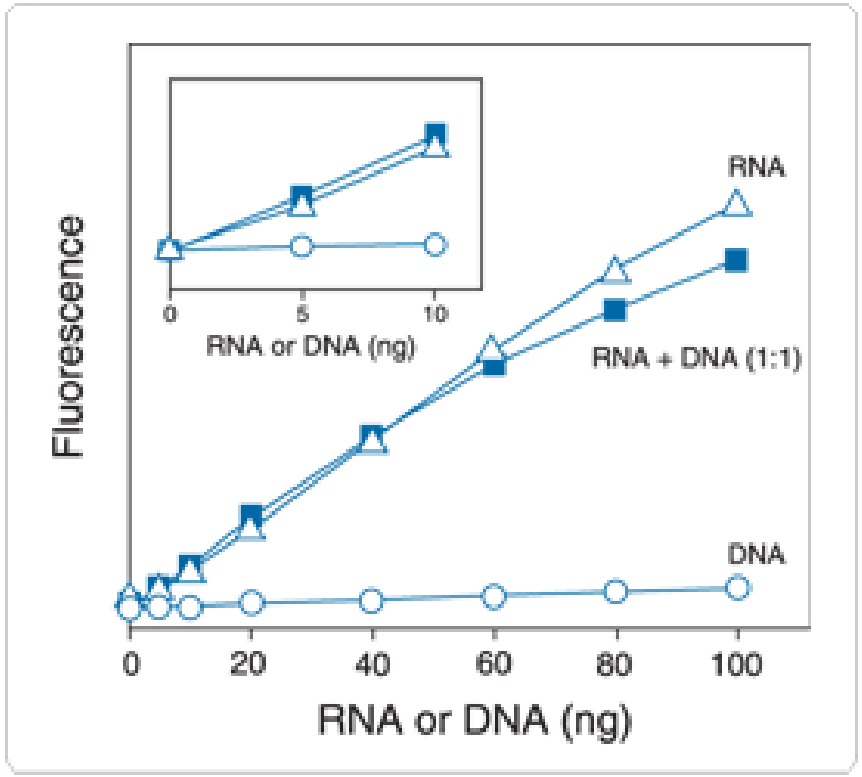
Kit using fluorogenic intercalating dyes

Kits:

E. coli DNA



E. coli rRNA



Triplicate 10 µL samples –
Fluorescence measured at 485-530

Triplicate 10 µL samples –
Fluorescence measured at 630/680

Method with the best sensibility and specificity

www.thermofisher.com
Adaptée de C Delomenie

UV absorbance vs fluorimetry to measure RNAs

	Advantages	Drawbacks
ABS	• Simple et cheap • Contaminant detection → Concentration estimation → Control of Purity	• Do not discriminate between DNA and RNA if not known. • No information on **degradation** • Possible overestimation if there are contaminants
FLUO	• **Sensibility +++** • **Specific to** poly-nucleotide chains • Dyes are specific of each type of nucleic acid → Specific **quantification**	• Expensive : dyes / instrument • No detection of contaminants (nucleotides, oligos, proteins, salt…)

→ **Complementary approaches**

Adapted from C Delomenie