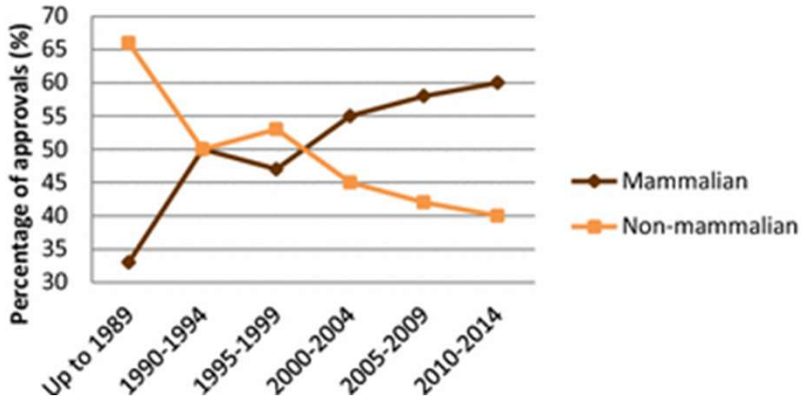
12. Molecular Biotechnology in Medicine II

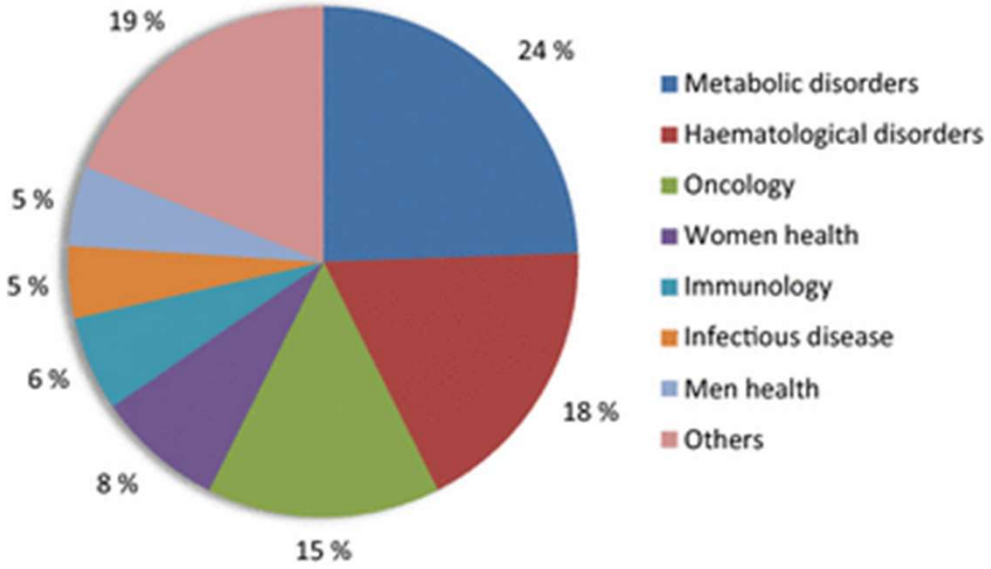
Outline

- Protein therapeutics
- Recombinant proteins
  - Monoclonal antibodies
- Gene therapy
  - Antigen and antisense oligonucleotides
  - Ribozymes / deoxyribozymes
  - Chimeraplasts
  - Triplex Forming Oligonucleotides
  - Human Artificial Chromosomes
- Clinical Trials

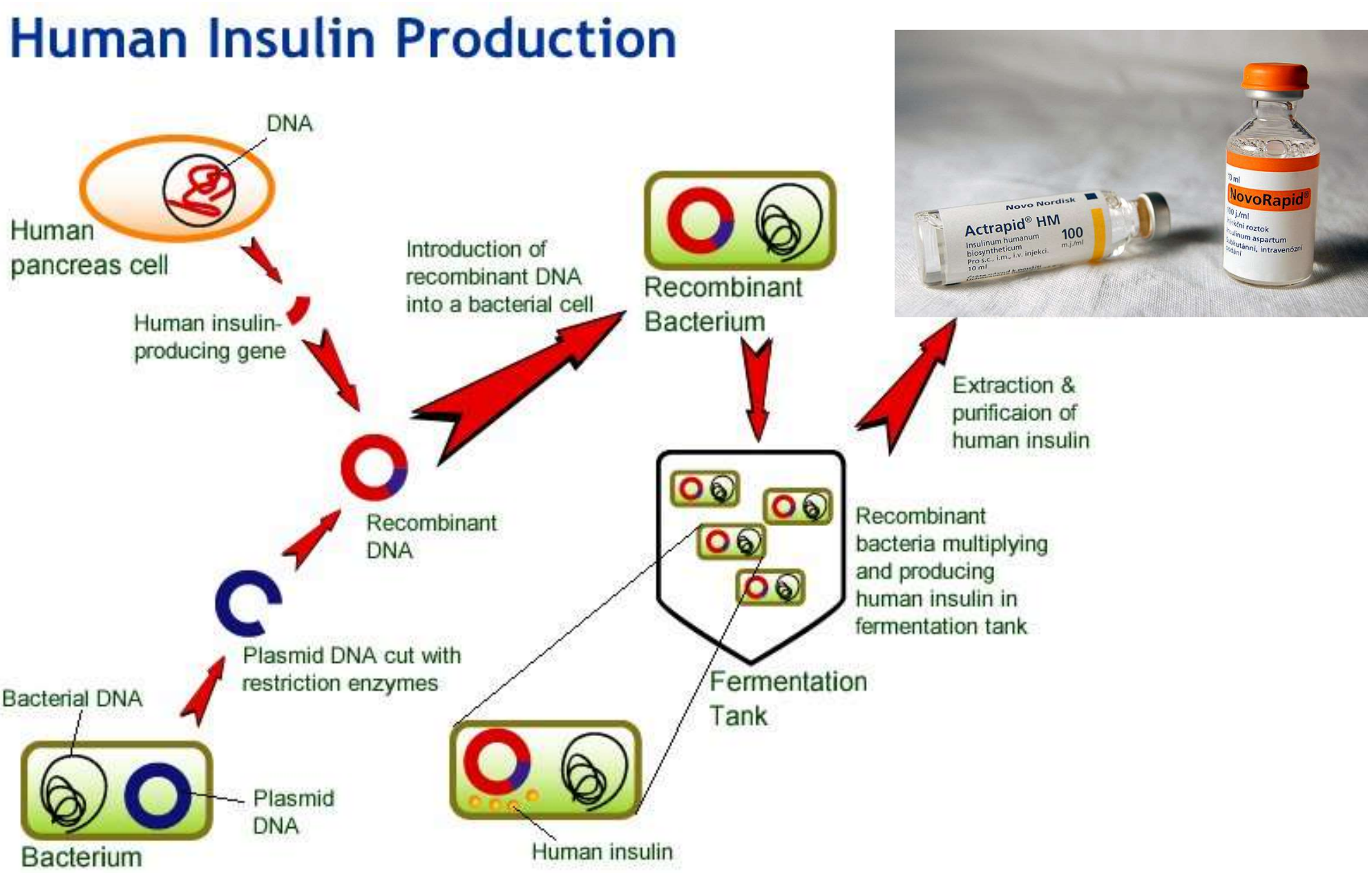
Recombinant proteins

- **Interferons**
- **Human Growth Hormone**
- **Enzymes**
  - DNase I
  - Alginate Lyase
  - Phenylalanine Ammonia Lyase
  - α_1-Antitrypsin
  - Glycosidases
- **Alginate Lyase**
- **Antibodies**
- Etc.





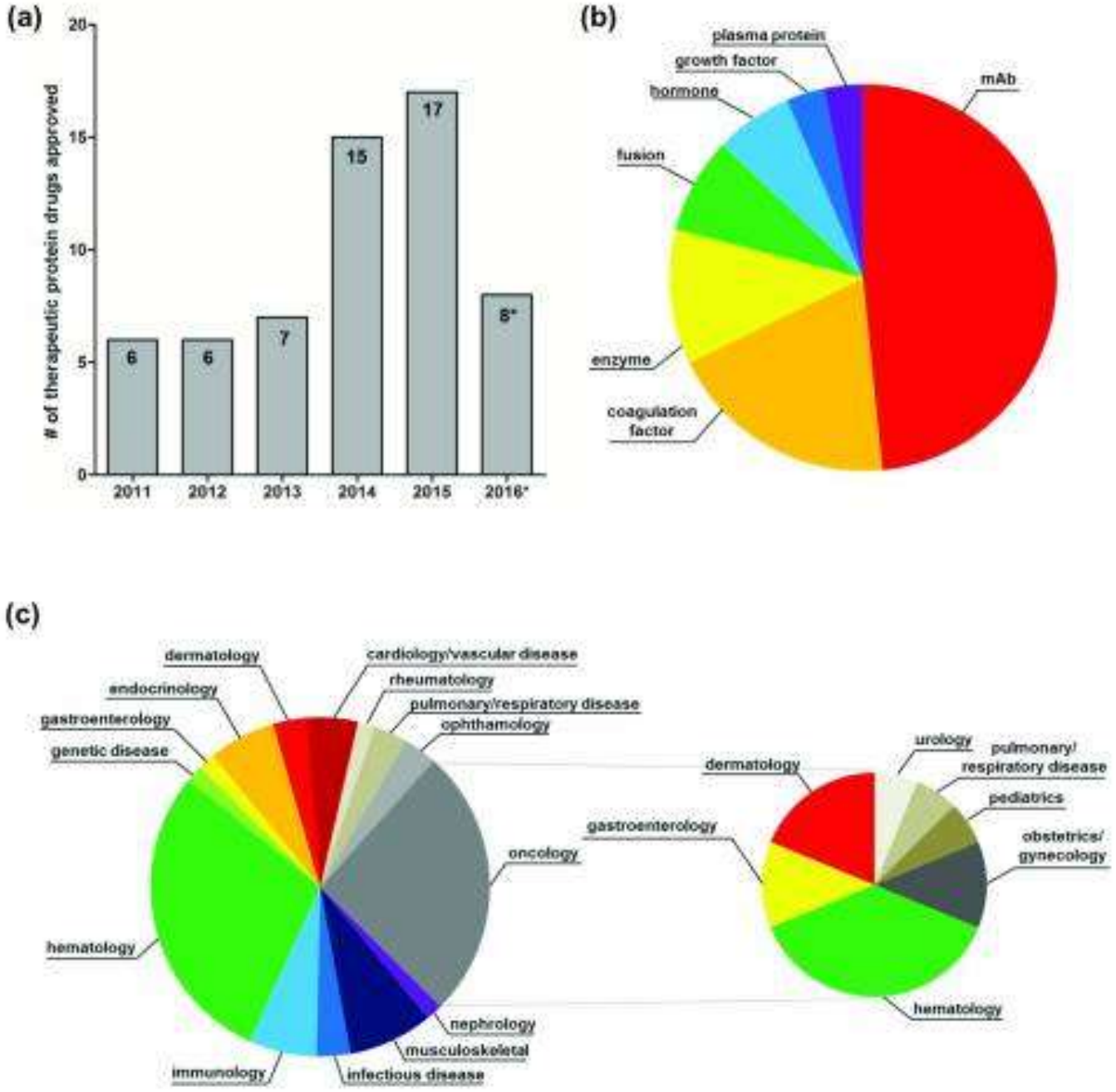
Recombinant proteins - Insulin



U.S. FDA-approved protein therapeutics (2016)

40 11/30/2015	elotuzumab [Empliciti; Bristol Myers Squibb]	mAb [humanized anti-CD319(SLAMF7)]	oncology [cancer (multiple myeloma)]
41 12/8/2015	sebelipase alfa [Kanuma; Alexion Pharmaceuticals]	enzyme [lysosomal acid lipase]	cardiology/vascular diseases/genetic disease [lysosomal acid lipase deficiency]
42 3/18/2016	obiltoxaximab [Anthim; Elusys Therapeutics]	mAb [mouse/human chimeric anti-*Bacillus anthracis*]	infections and infectious disease [infectious disease (inhalational anthrax)]
43 3/22/2016	ixekizumab [Taltz; Eli Lilly and Company]	mAb [humanized anti-IL-17a]	dermatology/immunology [autoimmunity (plaque psoriasis)]
44 3/23/2016	reslizumab [Cinqair; Teva Respiratory]	mAb [humanized anti-IL-5]	pulmonary/respiratory disease [asthma]
45 4/5/2016	infliximab-dyyb [Inflectra; Celltrion]	mAb [mouse/human chimeric anti-TNFα]	musculoskeletal/rheumatology [inflammatory (Crohn's disease/ulcerative colitis/rheumatoid arthritis/ankylosing spondylitis/psoriatic arthritis/plaque psoriasis)]

U.S. FDA-approved protein therapeutics (2011-2016*)



THPdb

A database of FDA approved therapeutic peptides and proteins

HOME | SEARCH | BROWSE | TOOLS | INFORMATION | DOWNLOADS | DEVELOPERS | HELP

Basic Search in THPdb

This page is designed to facilitate user to search THPdb database using query. This search module allows user to perform search on any or all fields of THPdb. It also allows to DISPLAY all fields or user selected fields. For more information see HELP page.

Query Submission Form

Please paste/insert/type your query to be searched:

Enter Keyword ....

Select Fields to be SEARCHED:

- [x] Name [Lepirudin]
- [ ] Chemical Formula [CO_2]
- [ ] Category[Hormone]
- [ ] Company[Eli Lilly]
- [ ] Functional Classification [IIb]
- [x] Indication[cystic fibrosis]
- [x] Sequence[PHWSYLLR]
- [ ] Isoelectric Point [4.58]
- [ ] Patent number [US7276477]
- [ ] Physcial Apperance [Lyophilized]
- [x] Brand [Enbrel]
- [x] Route[Intravenous]

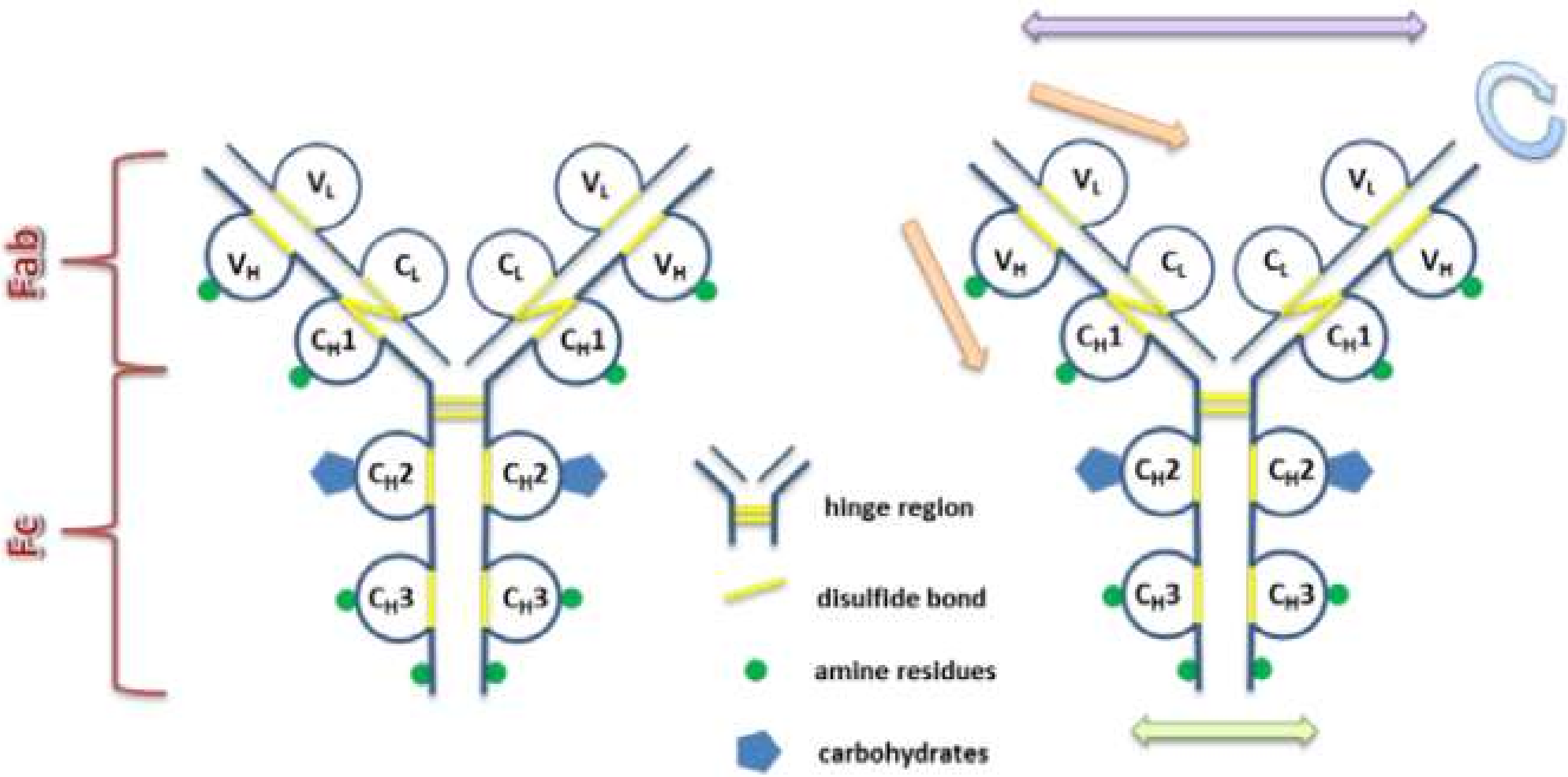
Please select field you wish to DISPLAY: (Max seven field be can selected once)

- [x] Therapeutic peptide Name
- [ ] Chemical Formula
- [x] Half life
- [ ] Pharmacodynamics
- [ ] Absorbtion
- [ ] Patent Number
- [ ] Target
- [ ] Prescribed for
- [ ] Route of administration
- [x] Sequence
- [ ] Isoelectric point
- [x] 3-d Structure
- [ ] Mechanism
- [ ] Volume of Distribution
- [ ] Date of Issue
- [x] Brand
- [ ] Chemical name
- [ ] Recommended Dosage
- [ ] Functional Classification
- [ ] Hydrophobicity
- [x] Description
- [ ] Toxicity
- [ ] Clearance
- [ ] Date of Expiry
- [ ] Company
- [ ] Formulation
- [ ] Contraindication
- [ ] Molecular Weight
- [ ] Melting Point
- [ ] Indication
- [ ] Metabolism
- [ ] Category
- [ ] Drug Interaction
- [ ] Brand Description
- [ ] Physical Appearance
- [ ] Side effect
- [ ] Useful link
- [ ] PubMed ID

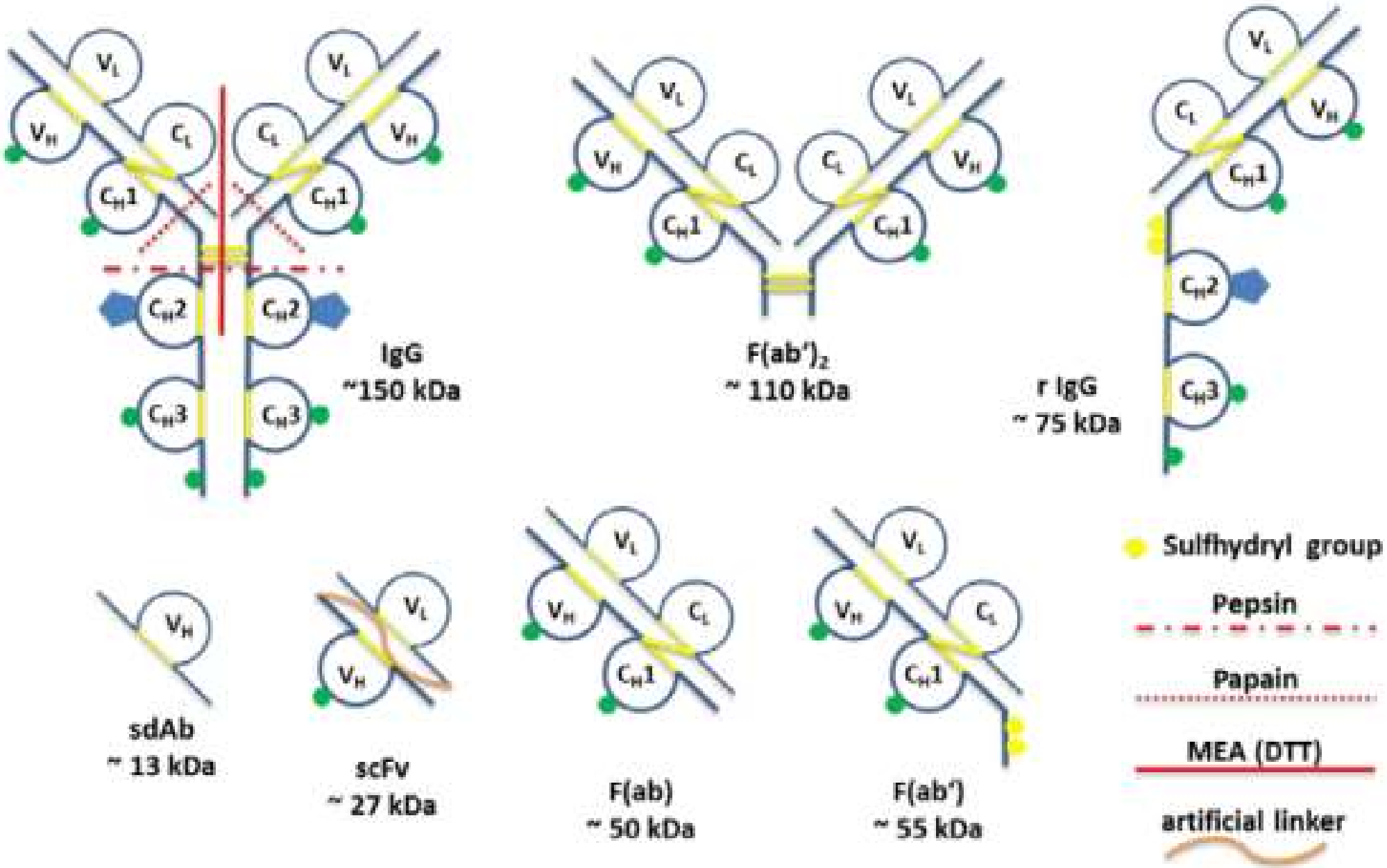
Clear or Reset | Search

https://webs.iiitd.edu.in/raghava/thpdb/index.html

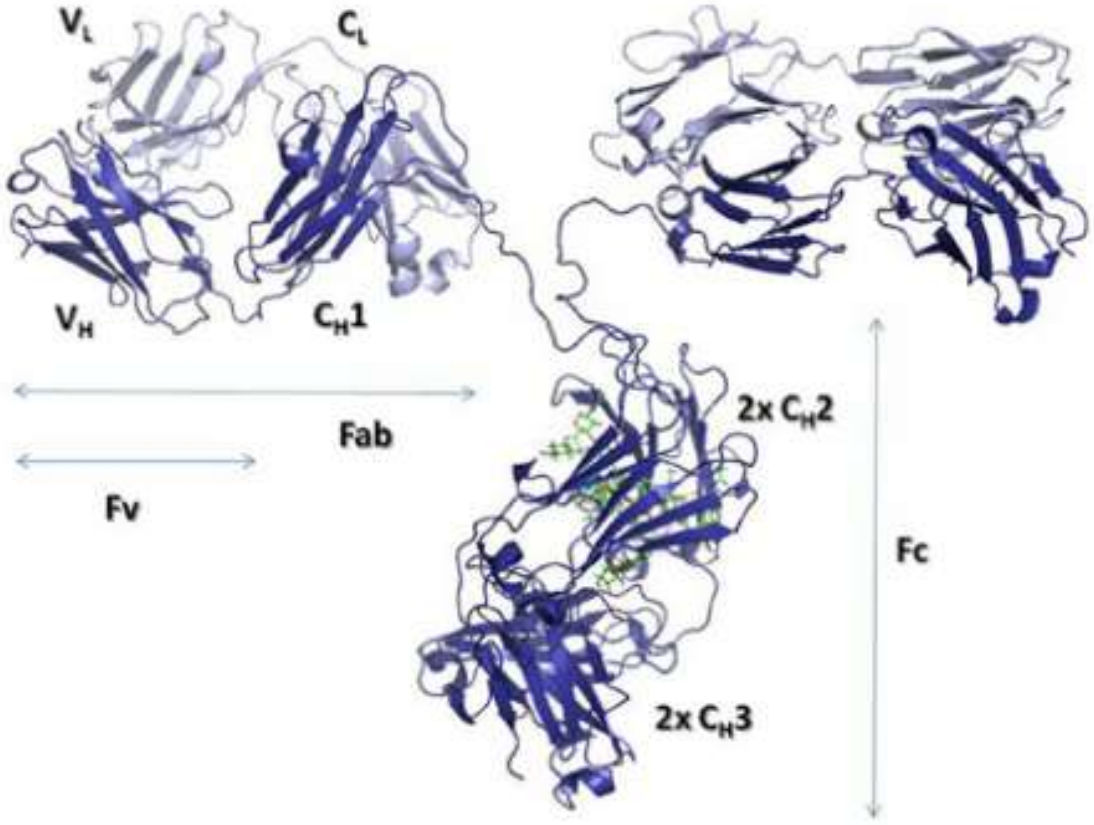
Antibodies – Basic Knowledge

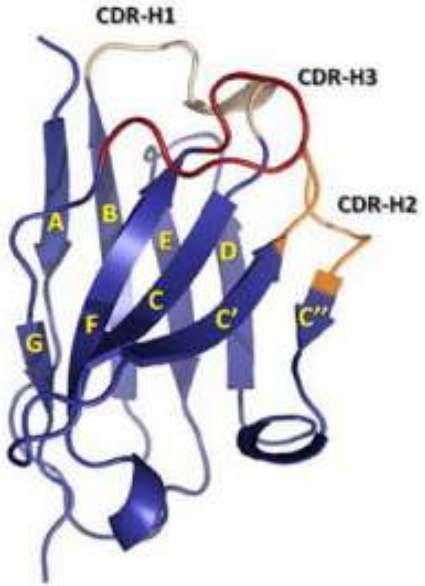


Antibodies – Basic Knowledge



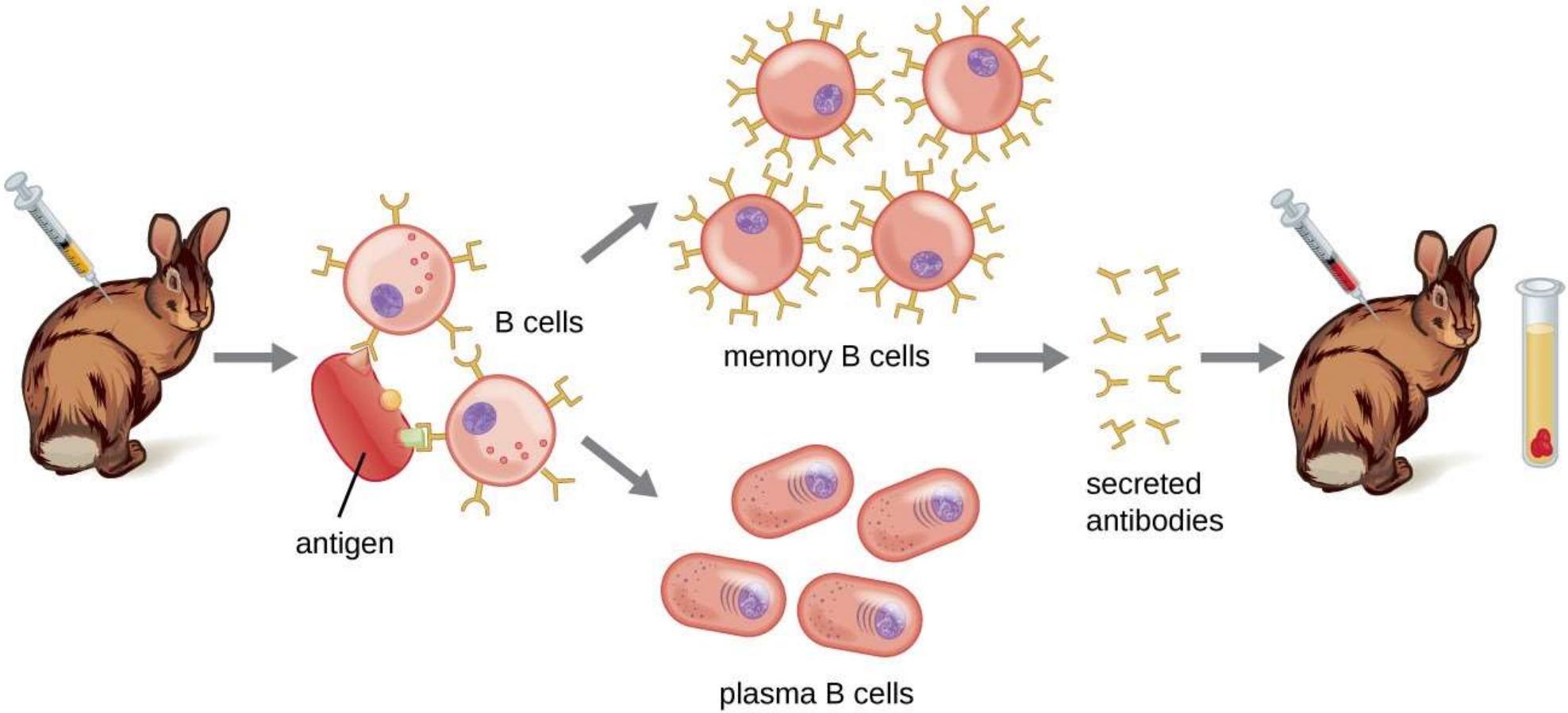
Antibodies – Basic Knowledge



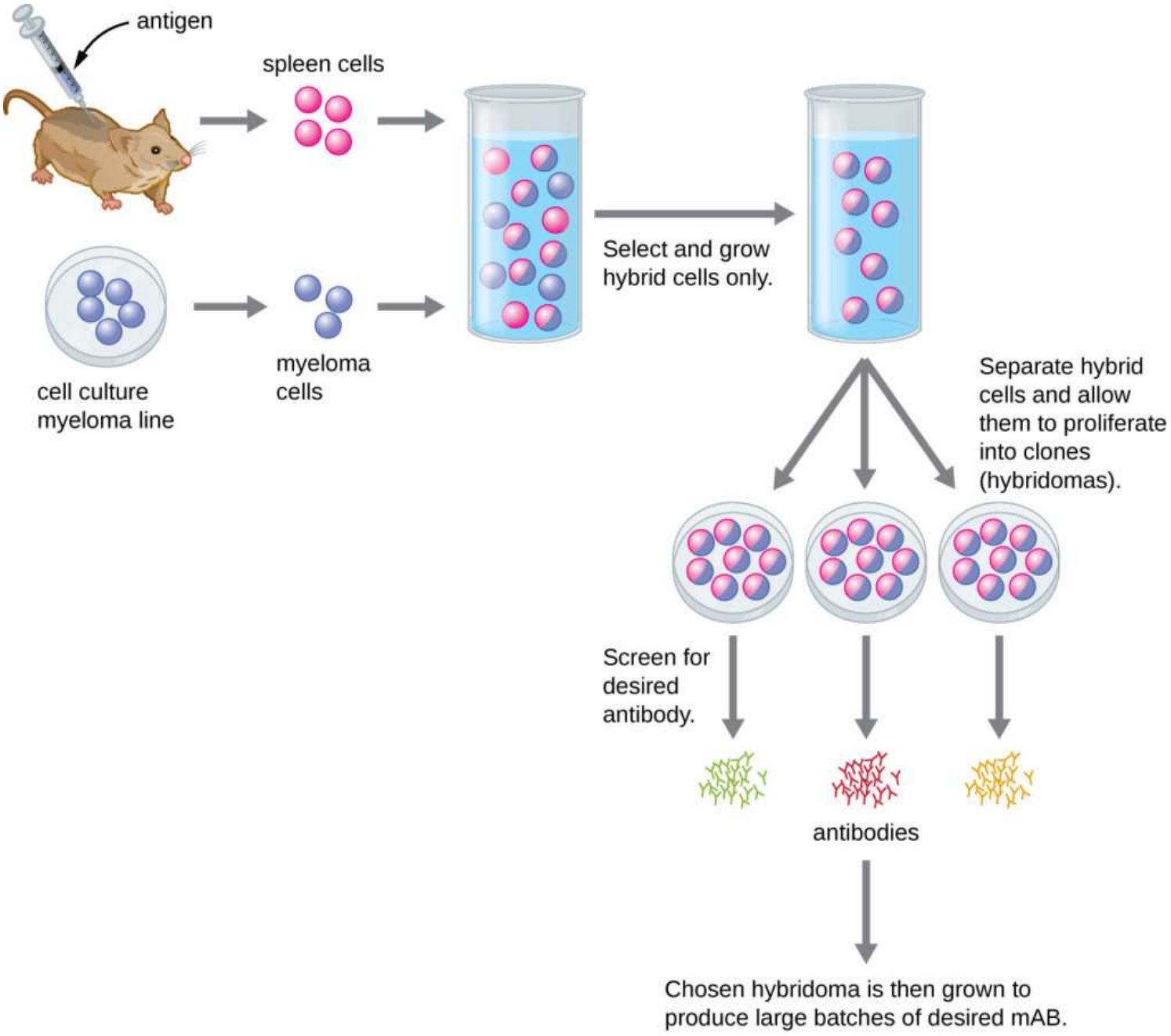


Polyclonal Antibody Production

1. Inject antigen into rabbit.
2. Antigen activates B cells.
3. Plasma B cells produce polyclonal antibodies.
4. Obtain antiserum from rabbit containing polyclonal antibodies.



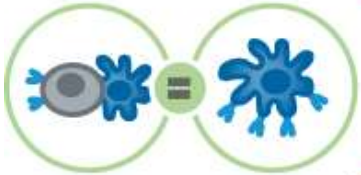
Monoclonal Antibody Production



Monoclonal Antibody Production

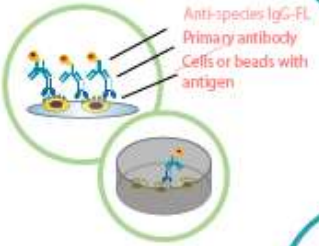
Immunization of mice & isolation of splenocytes
Mice are immunized with an antigen and later their blood is screened for antibody production. The antibody-producing splenocytes are then isolated for *in vitro* hybridoma production.

Preparation of myeloma cells
Myeloma cells are immortalized cells that, once fused with spleen cells, can result in a hybridoma capable of unlimited growth. Myeloma cells are prepared for fusion.



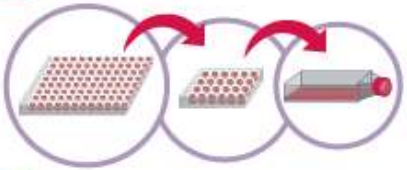
Fusion
Myeloma cells and isolated splenocytes are fused together to form hybridomas in the presence of polyethylene glycol (PEG), which causes cell membranes to fuse.

Clone screening and picking
Clones are screened and selected on the basis of antigen specificity and immunoglobulin class.



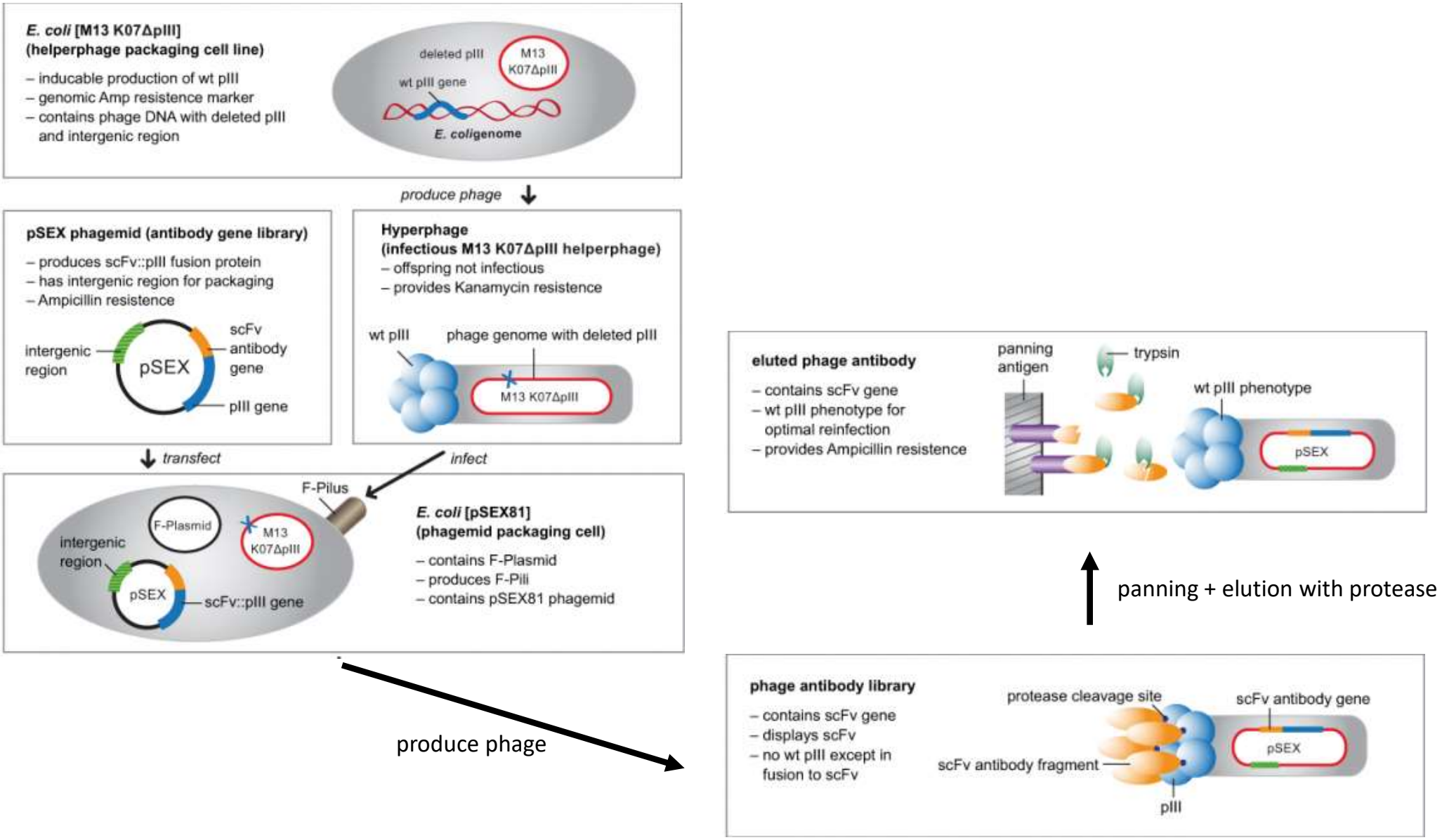
Functional characterization
Confirm, validate, and characterize (e.g. ELISA) each potentially high-producing colony.

Scale up and wean
Scale up clones producing desired antibodies and wean off selection agent(s).



Expansion
Expand clones producing desired antibodies (e.g. bioreactors or large flasks).

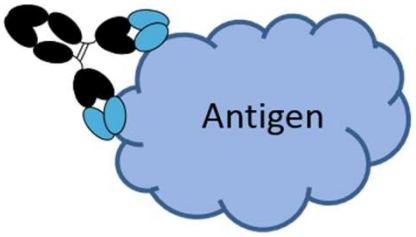
Phage Display Technology



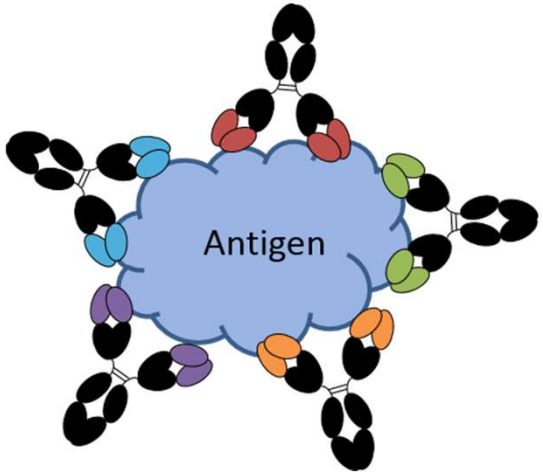
Monoclonal vs. Polyclonal Antibodies

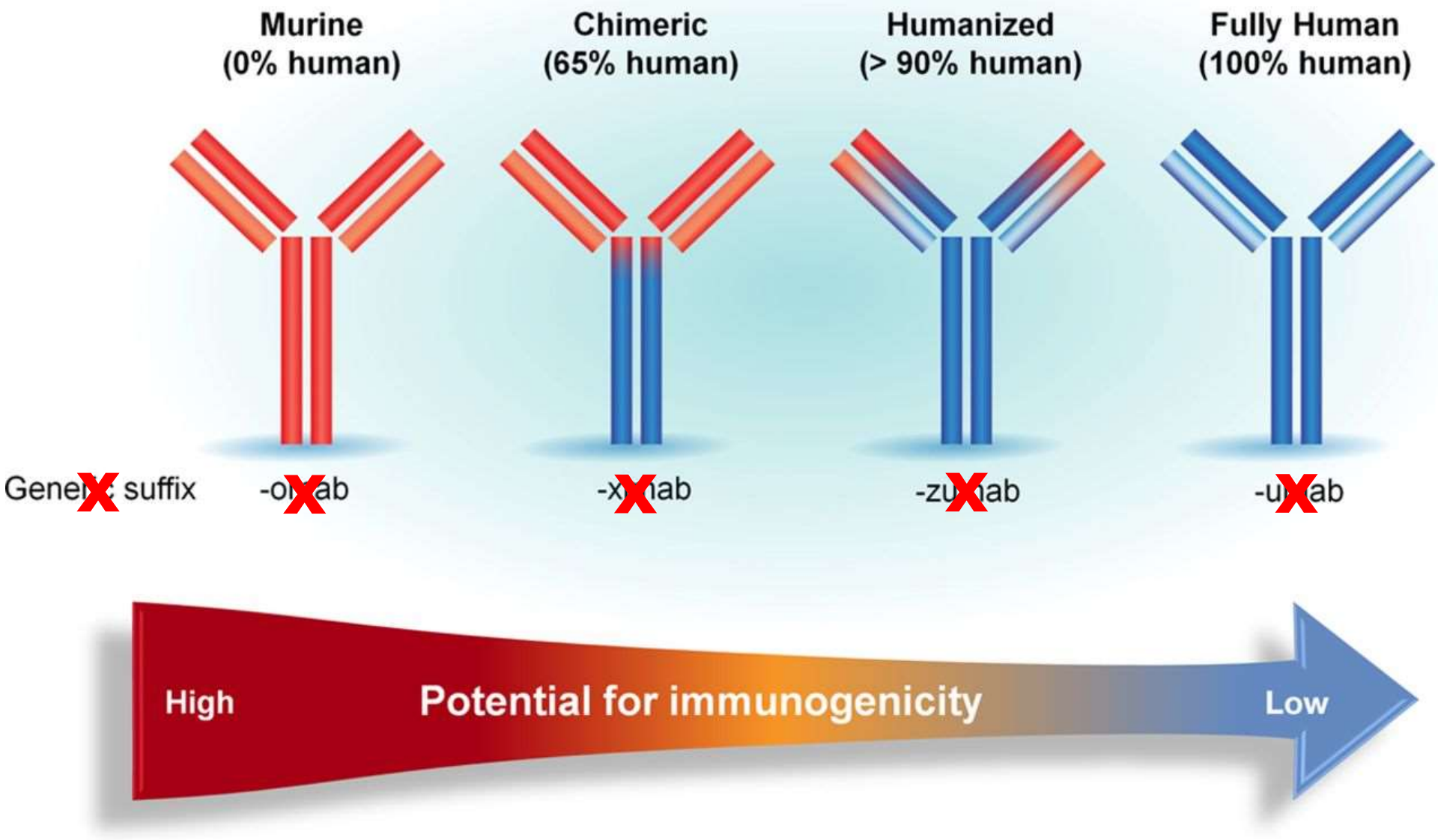
Monoclonal Antibodies	Polyclonal Antibodies
Expensive production	Inexpensive production
Long production time	Rapid production
Large quantities of specific antibodies	Large quantities of nonspecific antibodies
Recognize a single epitope on an antigen	Recognize multiple epitopes on an antigen
Production is continuous and uniform once the hybridoma is made	Different batches vary in composition

Monoclonal antibody



Polyclonal antibody




Murine
(0% human)
Chimeric
(65% human)
Humanized
(> 90% human)
Fully Human
(100% human)
Generic suffix
-omab
-ximab
-zumab
-umab
High
Potential for immunogenicity
Low

Monoclonal antibody (mAb) nomenclature scheme

- **WHO - Geneva, 26 May 2017**

Table 1: Previous mAb nomenclature scheme.

Prefix:	Substem A: target class	Substem B: the species	Stem:
random	*-b(a)-* bacterial	*-a-* rat	*-mab*
	-am(i)- serum amyloid protein (SAP)/amyloidosis *(pre-substem)*	*-axo-* rat-mouse *(pre-substem)*	
	-c(i)- cardiovascular	*-e-* hamster	
	-f(u)- fungal	*-i-* primate	
	-gr(o)- skeletal muscle mass related growth factors and receptors *(pre-substem)*	*-o-* mouse	
	-k(i)- interleukin	*-u-* human	
	-l(i)- immunomodulating	*-vet-* veterinary use *(pre-substem)*	
	-n(e)- neural	*-xi-* chimeric	
	-s(o)- bone	*-xizu-* chimeric-humanized	
	-tox(a)- toxin	*-zu-* humanized	
	-t(u)- tumour		
	-v(i)- viral		

International Nonproprietary Name, INN

World Health Organization

Table 2: New mAb nomenclature scheme.

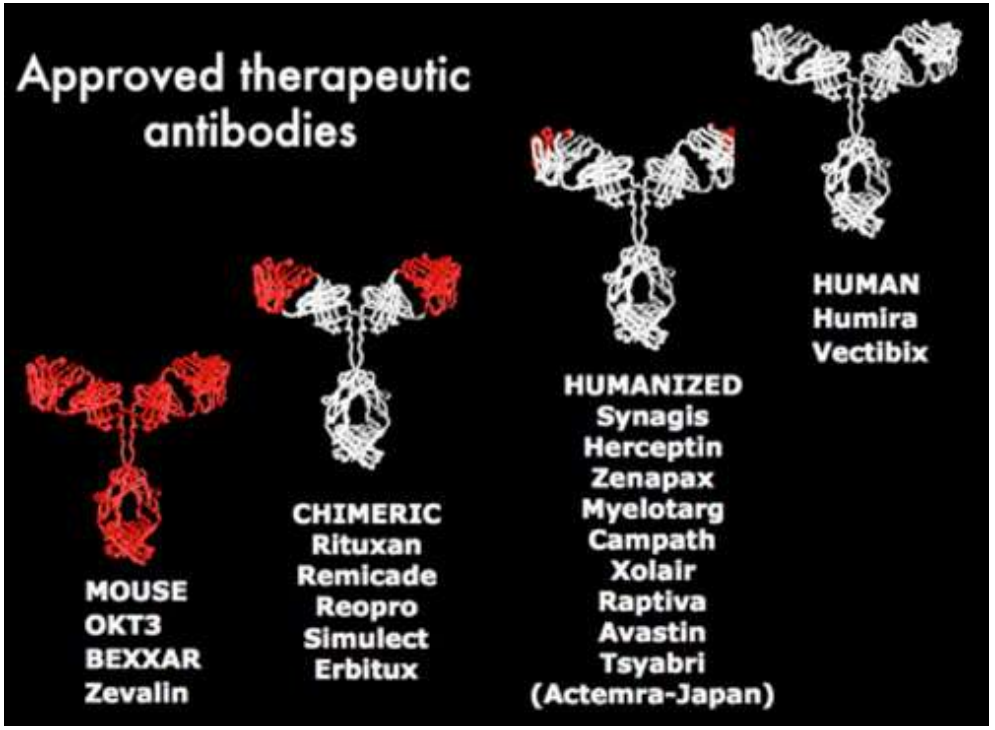
Prefix:	Substem A*: target class	Stem:
random	*-ba-* bacterial	*-mab*
	-ami- serum amyloid protein (SAP)/amyloidosis *(pre-substem)*	
	-ci- cardiovascular	
	-fung- fungal	
	-gros- skeletal muscle mass related growth factors and receptors *(pre-substem)*	
	-ki- interleukin	
	-li- immunomodulating	
	-ne- neural	
	-os- bone	
	-toxa- toxin	
	-ta- tumour	
	-vet- veterinary use *(pre-stem)*	
	-vi- viral	

* The substem A is currently under revision.

doi: 10.3233/HAB-180347

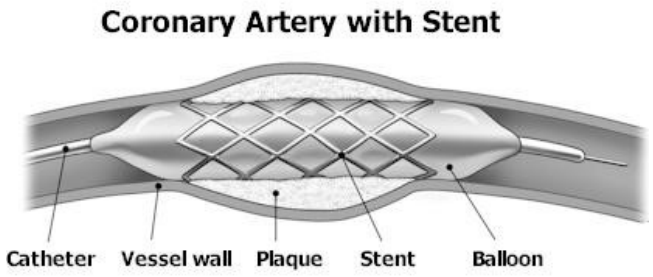
Therapeutic mAb

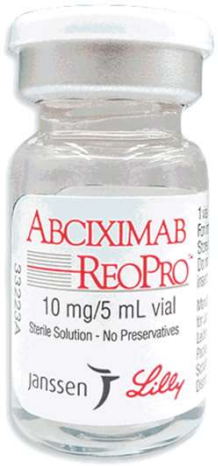
- **Function**
  - activate, repress, or alter endogenous immune responses to specific cells or molecules
- **Treatment of**
  - cancer, inflammatory and autoimmune disease, and many other types of disease



Chimeric and Humanized Therapeutic mAb

- **1st chimeric mAb**
  - Abciximab, for percutaneous coronary intervention
  - platelet aggregation inhibitor
  - since 1994





- **1st humanized mAb**
  - Daclizumab, to prevent rejection in organ transplantation
  - binds to CD25, the alpha subunit of the IL-2 receptor of T-cells
  - since 1997

Fully Human Therapeutic mAb

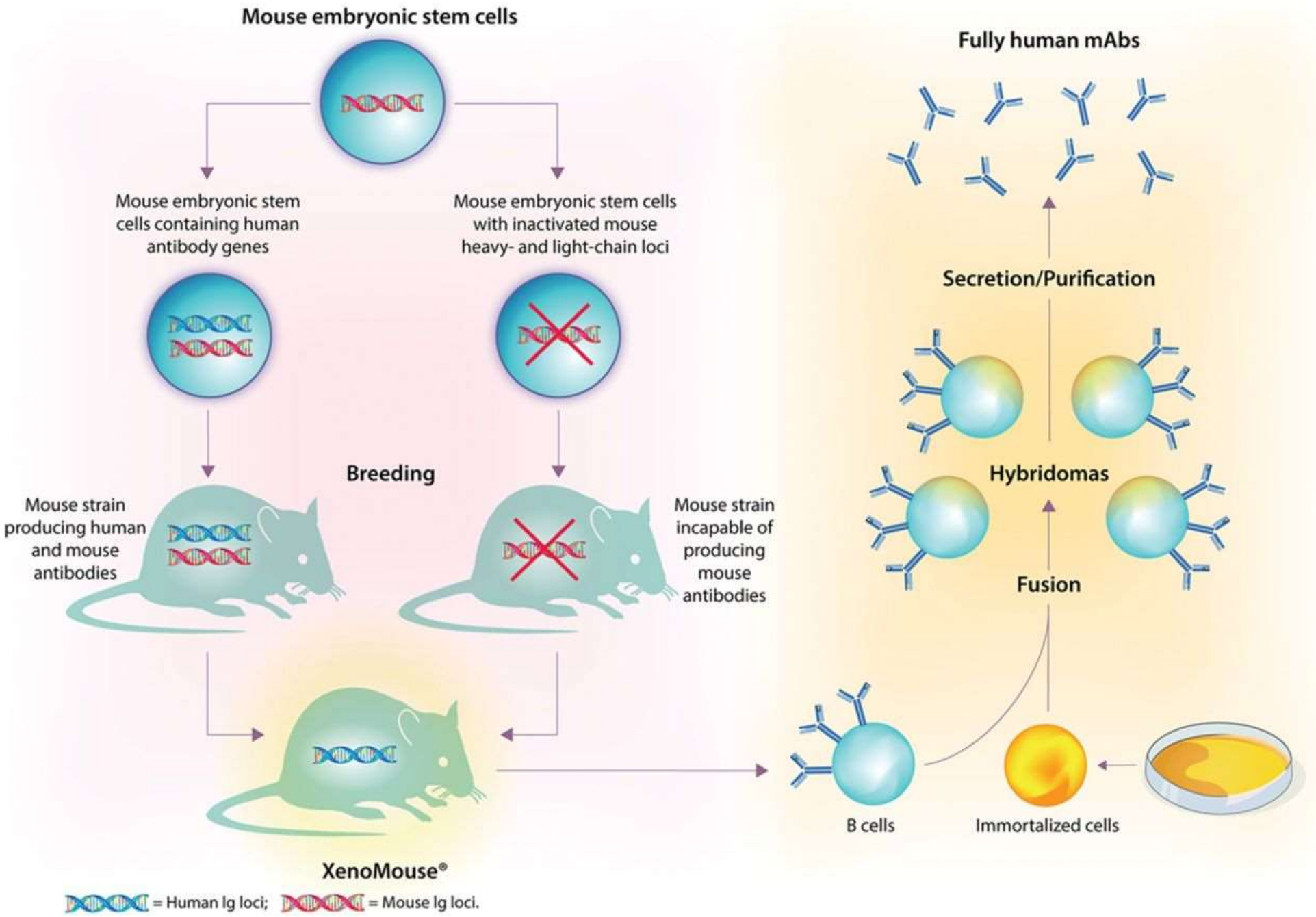
- **phage-display platforms**
- **transgenic mouse platforms**

- **1st human mAb**
  - Adalimumab (Humira)
  - rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, chronic psoriasis, hidradenitis suppurativa, and juvenile idiopathic arthritis.
  - binds to TNFα receptors
  - since 2005

SARS-CoV-2 „treatment“

- **Bamlanivimab (plus Etesevimab), Casirivimab Plus Imdevimab**
- **Sotrovimab**
- **Granted by FDA and EMA**
- Emergency Use Authorization (EUA)

XenoMouse Hybridoma Technology



International Immunopharmacology 85 (2020) 106639

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives

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ARTICLE INFO

Keywords:
Hybridoma
Clinical trials
Monoclonal antibodies
Biosimilar
Therapeutics
Antibody engineering

ABSTRACT

The advancements in technology and manufacturing processes have allowed the development of new derivatives, biosimilar or advanced improved versions for approved antibodies each year for treatment regimen. There are more than 700 antibody-based molecules that are in different stages of phase I/II/ III clinical trials targeting new unique targets. To date, approximately more than 80 monoclonal antibodies (mAbs) have been approved. A total of 7 novel antibody therapeutics had been granted the first approval either in the United States or European Union in the year 2019, representing approximately 20% of the total number of approved drugs. Most of these licenced mAbs or their derivatives are either of hybridoma origin or their improvised engineered versions. Even with the recent development of high throughput mAb generation technologies, hybridoma is the most favoured method due to its indigenous nature to preserve natural cognate antibody pairing information and preserves innate functions of immune cells. The recent advent of antibody engineering technology has superseded the species level barriers and has shown success in isolation of hybridoma across phylogenetically distinct species. This has led to the isolation of monoclonal antibodies against human targets that are conserved and non-immunogenic in the rodent. In this review, we have discussed in detail about hybridoma technology, its expansion towards different animal species, the importance of antibodies isolated from different animal sources that are useful in biological applications, advantages, and limitations. This review also summarizes the challenges and recent progress associated with hybridoma development, and how it has been overcome in these years to provide new insights for the isolation of mAbs.

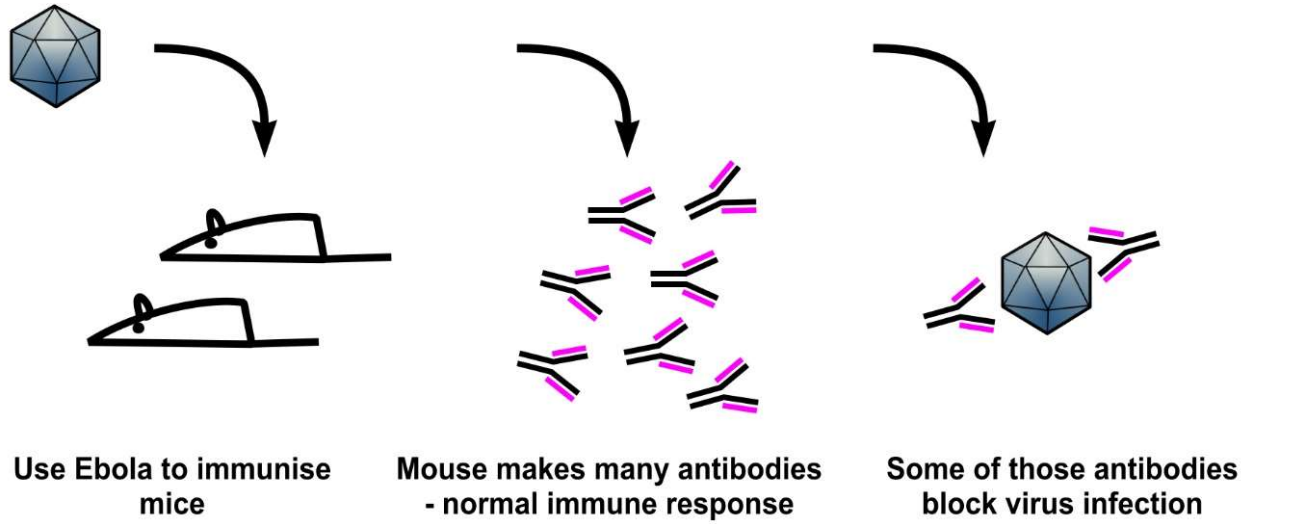
frontiers in Immunology

REVIEW
published: 28 August 2020
doi: 10.3389/fimmu.2020.01986

Phage Display Derived Monoclonal Antibodies: From Bench to Bedside

*Mohamed A. Alfaleh[1,2], Hashem O. Alsaab[3], Ahmad Bakur Mahmoud[4], Almohanad A. Alkayyal[5], Martina L. Jones[6,7], Stephen M. Mahler[6,7] and Anwar M. Hashem[2,8]**

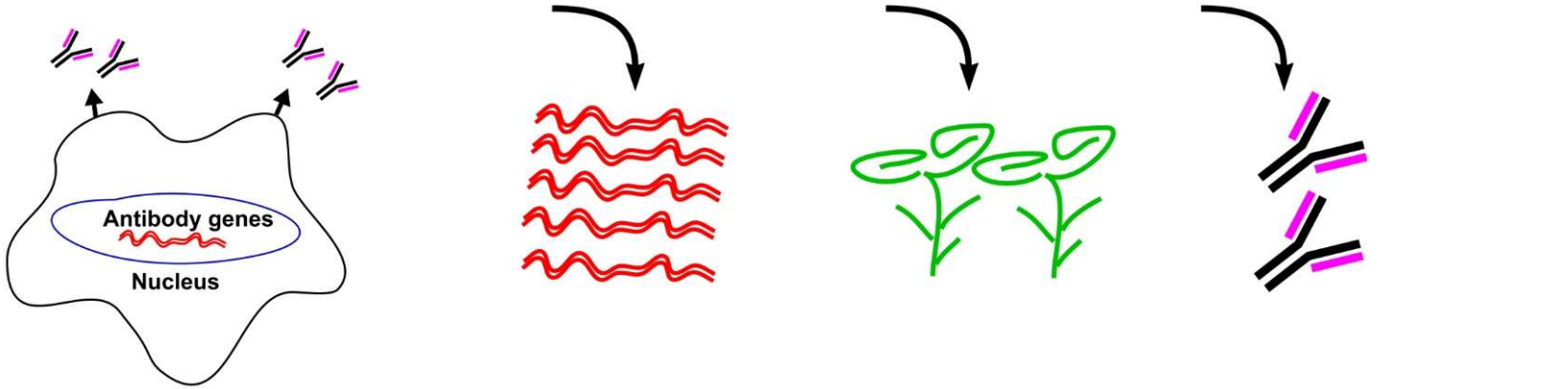
Therapeutic antibody grown in plants



Use Ebola to immunise mice

Mouse makes many antibodies - normal immune response

Some of those antibodies block virus infection



Plasma cell secreting key Ebola blocking antibodies

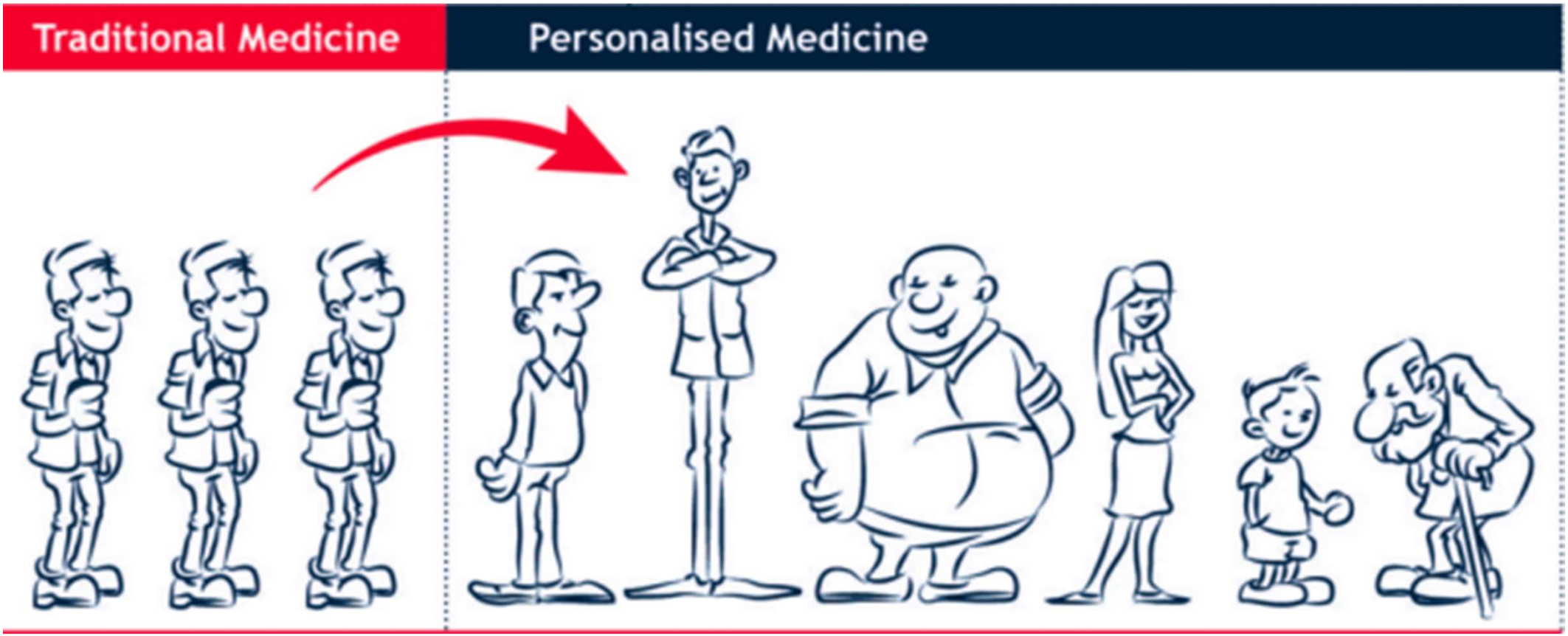
Isolate genes for key Ebola blocking antibodies

Introduce antibody genes into plants

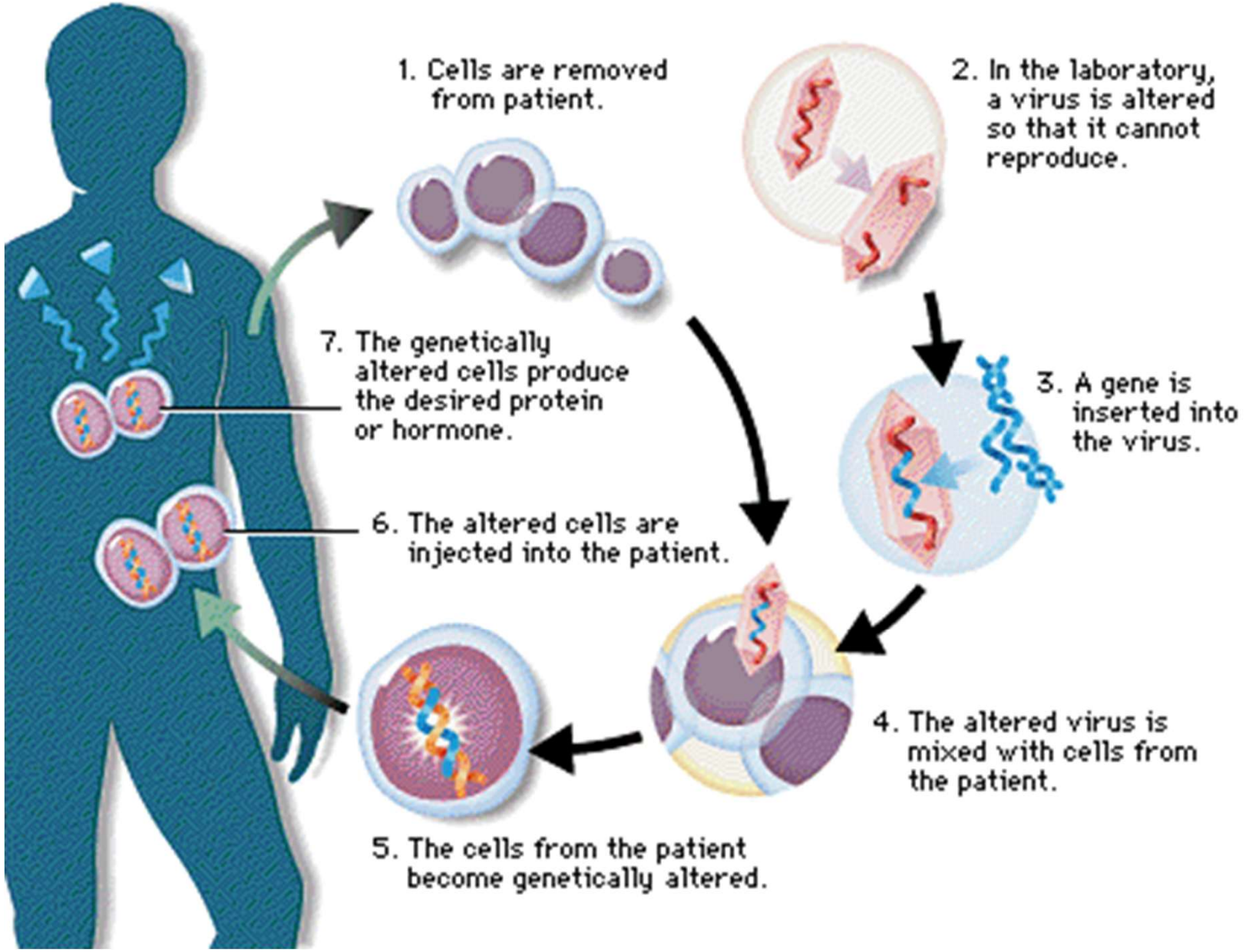
Grow and extract antibodies from plants. Same antibodies, same Ebola blocking but grown in a field

Ref. 10.1073/pnas.1213709109 **and** 10.1126/scitranslmed.3006608

Break 5 min

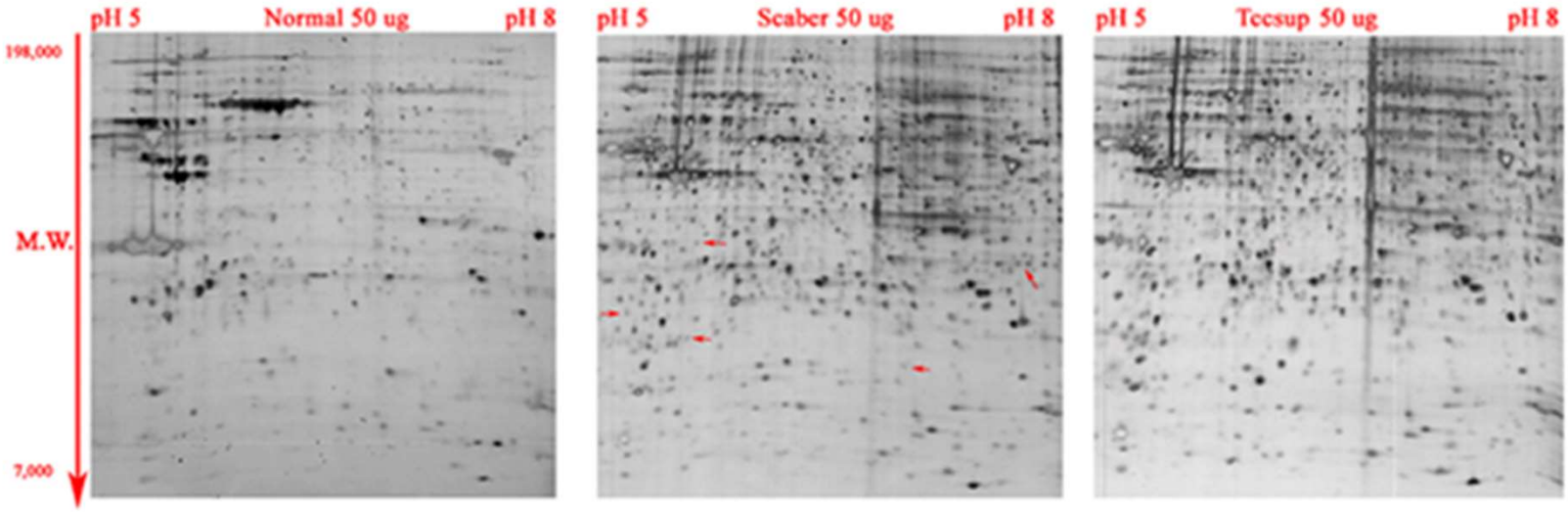

Traditional Medicine
Personalised Medicine

Gene Therapy

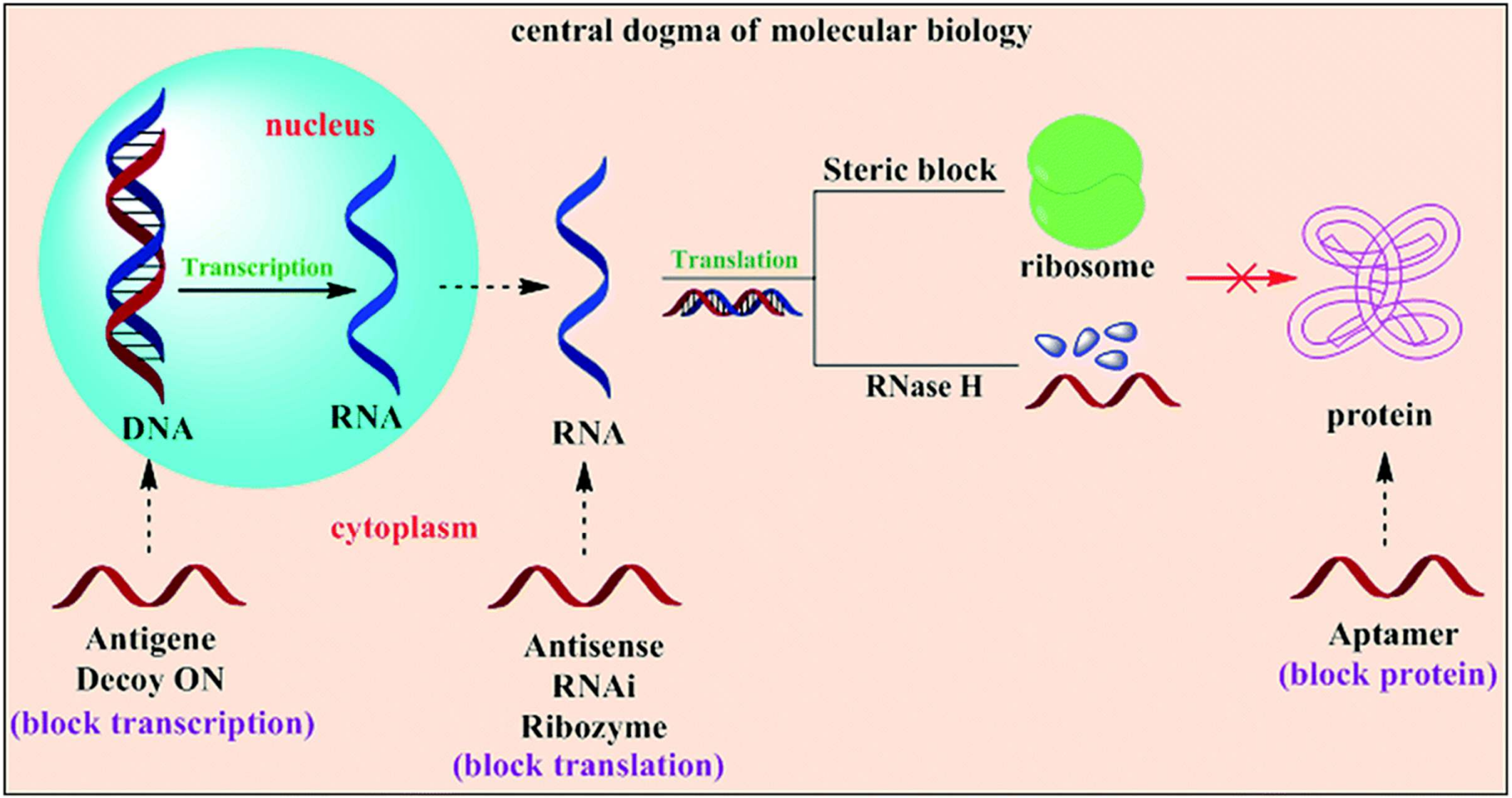


Nucleic Acids as Therapeutics

- Human disorders result in the overexpression of a normal protein
- Treatment approach
  - Lowering of transcription or translation
- Antigen oligonucleotide – binds to the gene and block the transcription
- Antisense oligonucleotide – base pairs with a specific mRNA

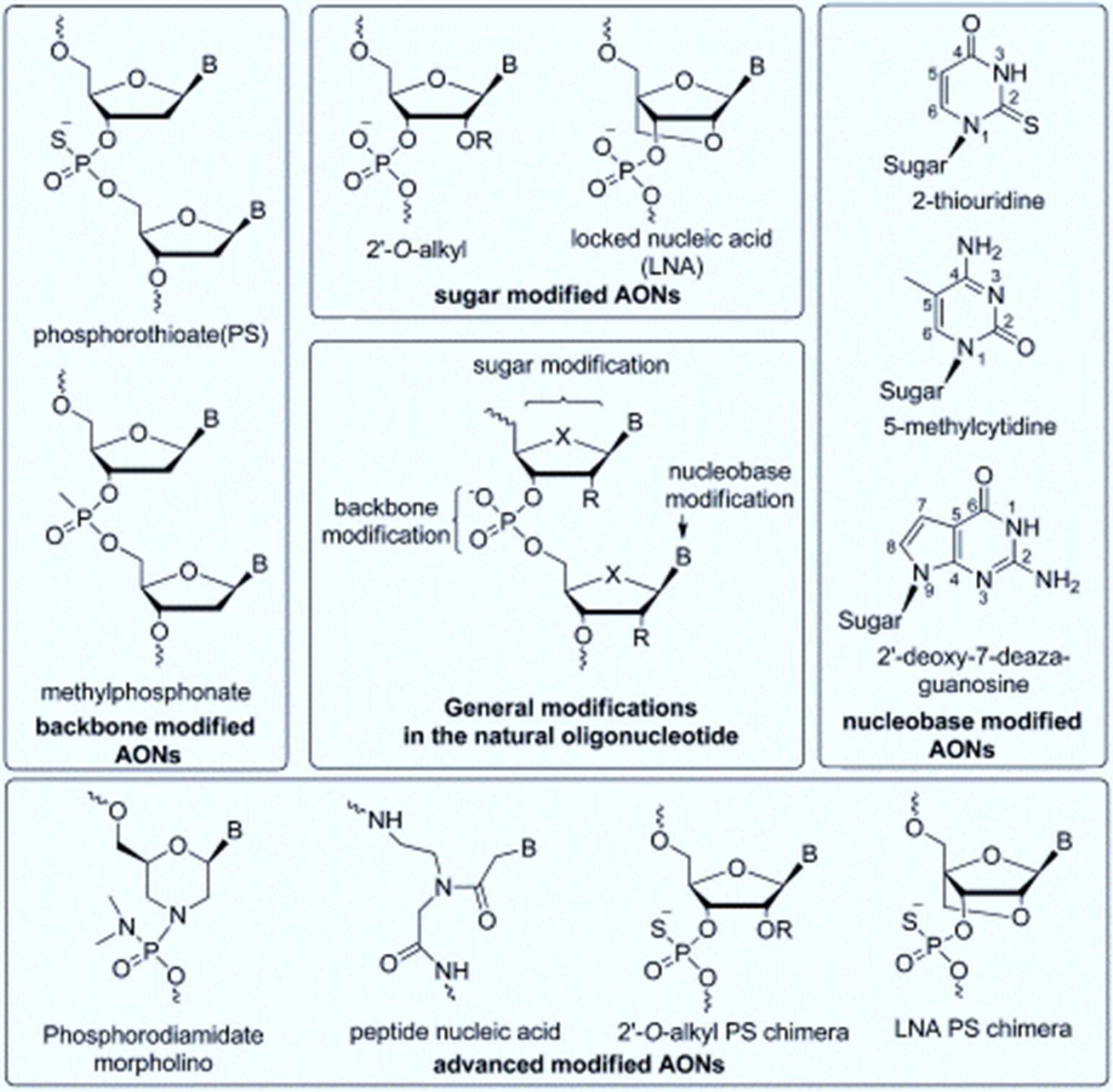


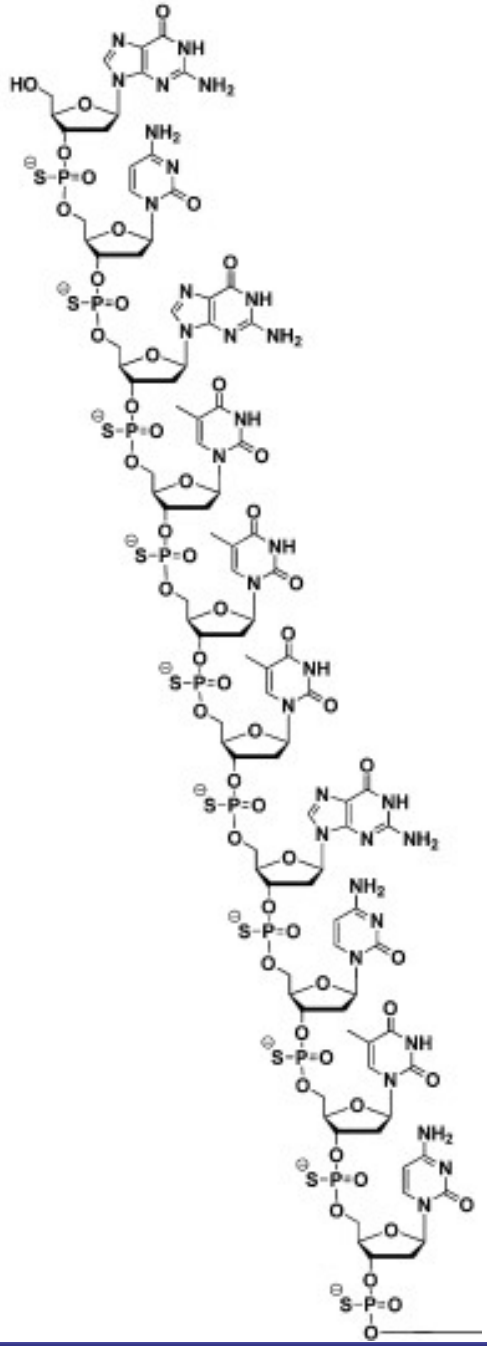
Antisense DNA



- **Susceptibility to degradation by intracellular nucleases!**

Synthetic Antisense Oligonucleotides





Ribozymes

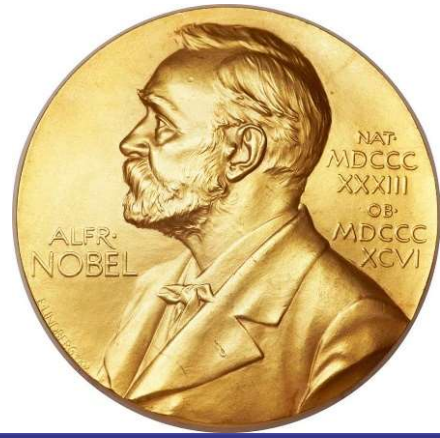
- Catalytic RNAs that cut things, make things, and do odd and useful jobs
- RNA metalloenzymes ~40 to 50 nucleotides in length
- can be engineered to specifically cleave any mRNA sequence
- separate catalytic and substrate-binding domains

The Nobel Prize in Chemistry 1989

Sidney Altman
Prize share: 1/2

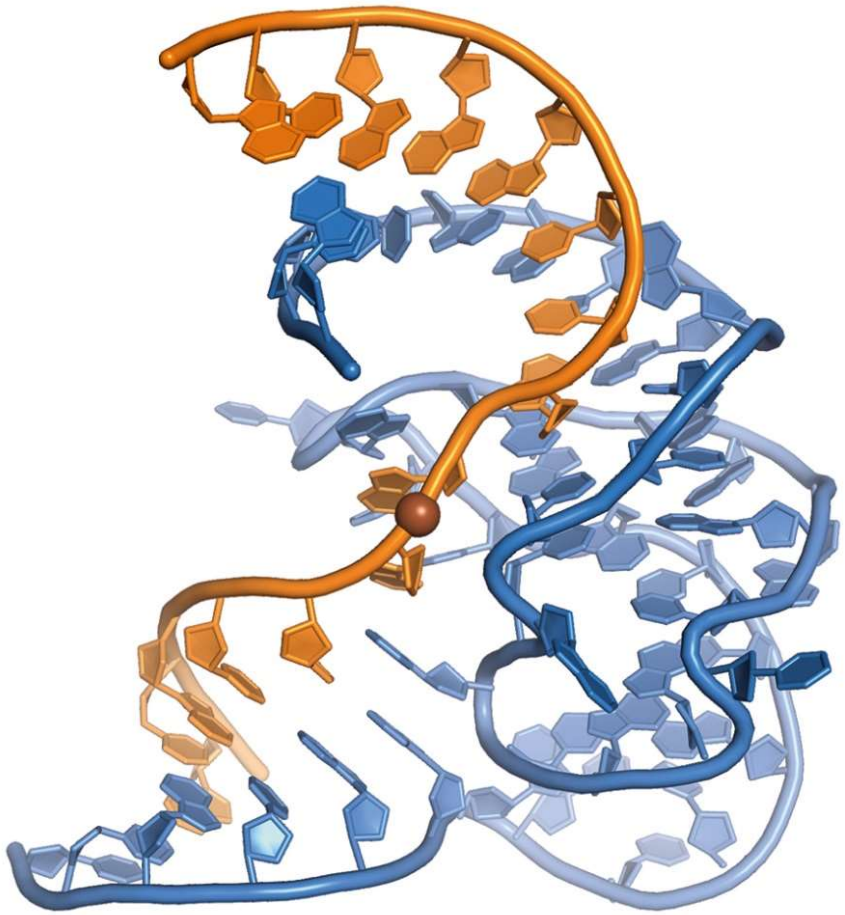
Thomas R. Cech
Prize share: 1/2

"for their discovery of catalytic properties of RNA"



Deoxyribozymes

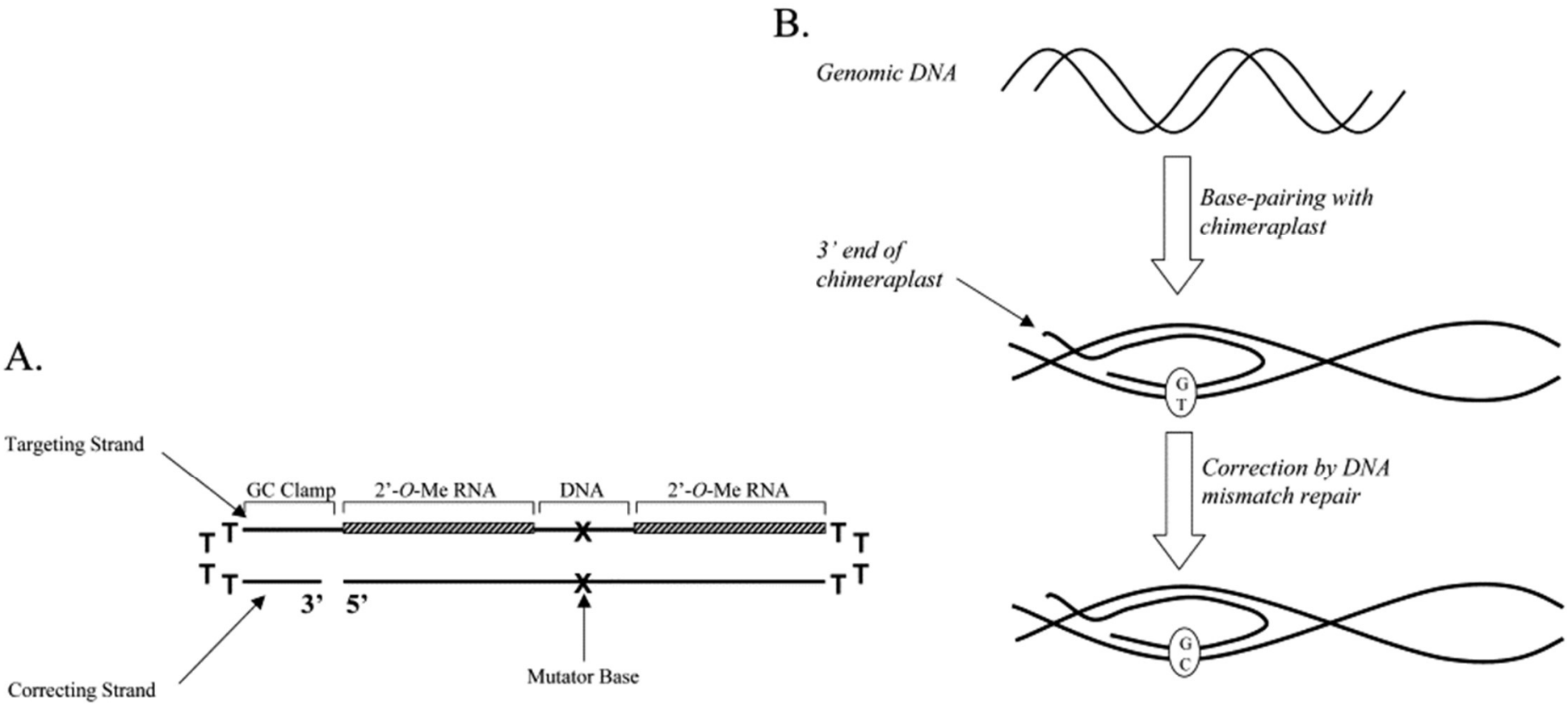
- **No naturally occurring**
- **Artificially synthesized**
- **1,000-fold more stable against hydrolytic destruction than protein**
- **100,000-fold more stable than RNA**



Structure of deoxyribozyme 9DB1, where we can see the synthetic strand of DNA (in blue) once it has catalysed the ligation of two RNA strands (in orange), joined at the point which is represented by a sphere.

Chimeric RNA-DNA molecules - chimeraplasts

- **site-specific point mutations within that sequence**



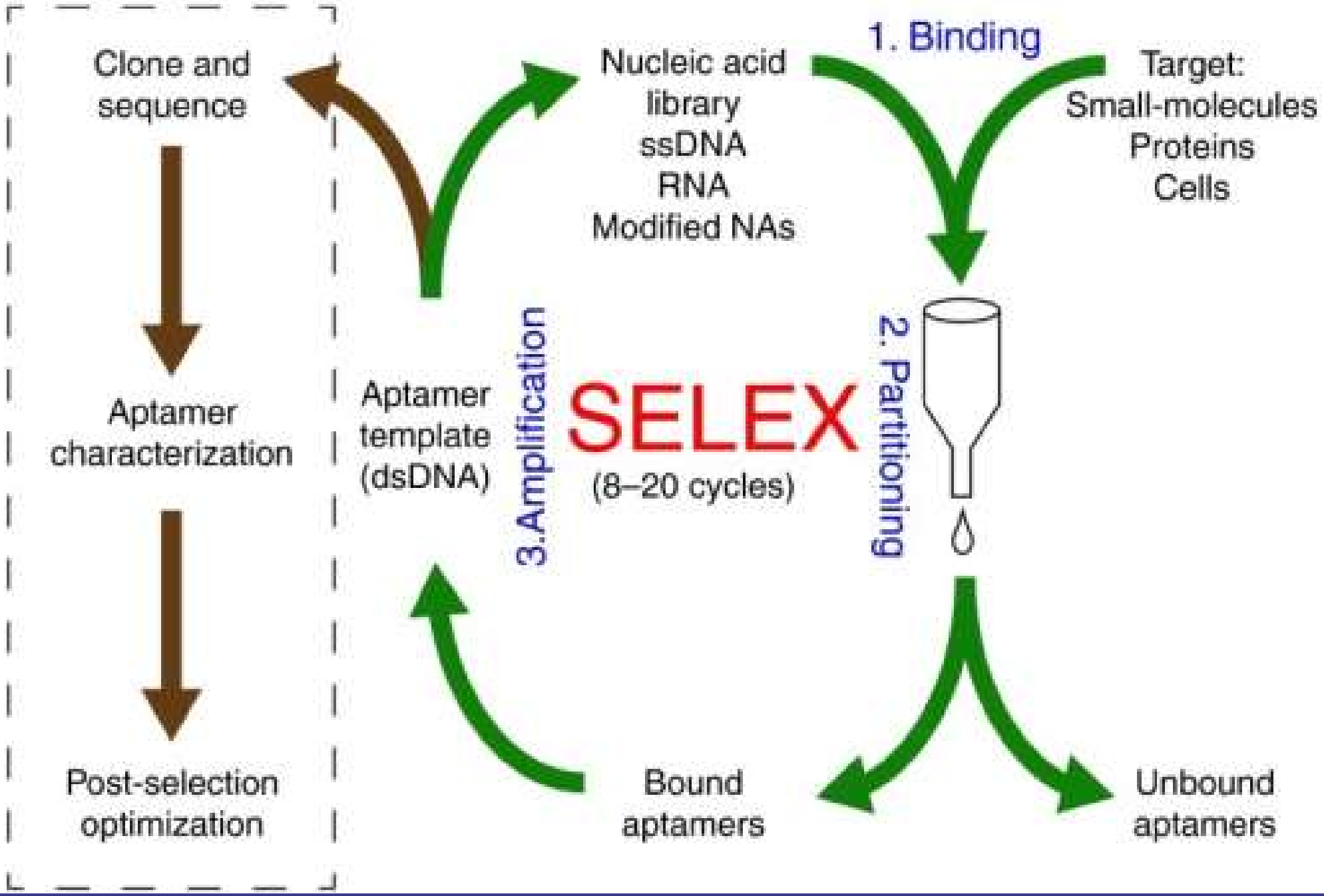
Gene Replacement Therapy vs. Corrective Gene Therapy

Gene Replacement Therapy (Gene Augmentation)	**Corrective Gene Therapy**
Random insertion of healthy counterpart of defective gene somewhere in genome so that its product could be available.	Directing insertion of healthy gene at specific site to displace defective gene is required.
Suitable for recessive disorders and for single gene mutations.	Possible for dominant disorders.
No recombinant event required and non specific insertion will work so long as appropriate regulatory controls are provided for expression.	Insertion at specific site would require some form of induced recombinational event.
Approach is not useful for dominant nature disorders or where errant(defective) gene gives destructive or interfering substance.	This approach would be ideal where errant gene produces destructive or interfering substance.
This approach is feasible today and has effect similar to transplantation approach only thing it bring done still at root level of the defect.	Extensive study is still required to direct gene at correct position in the genome.

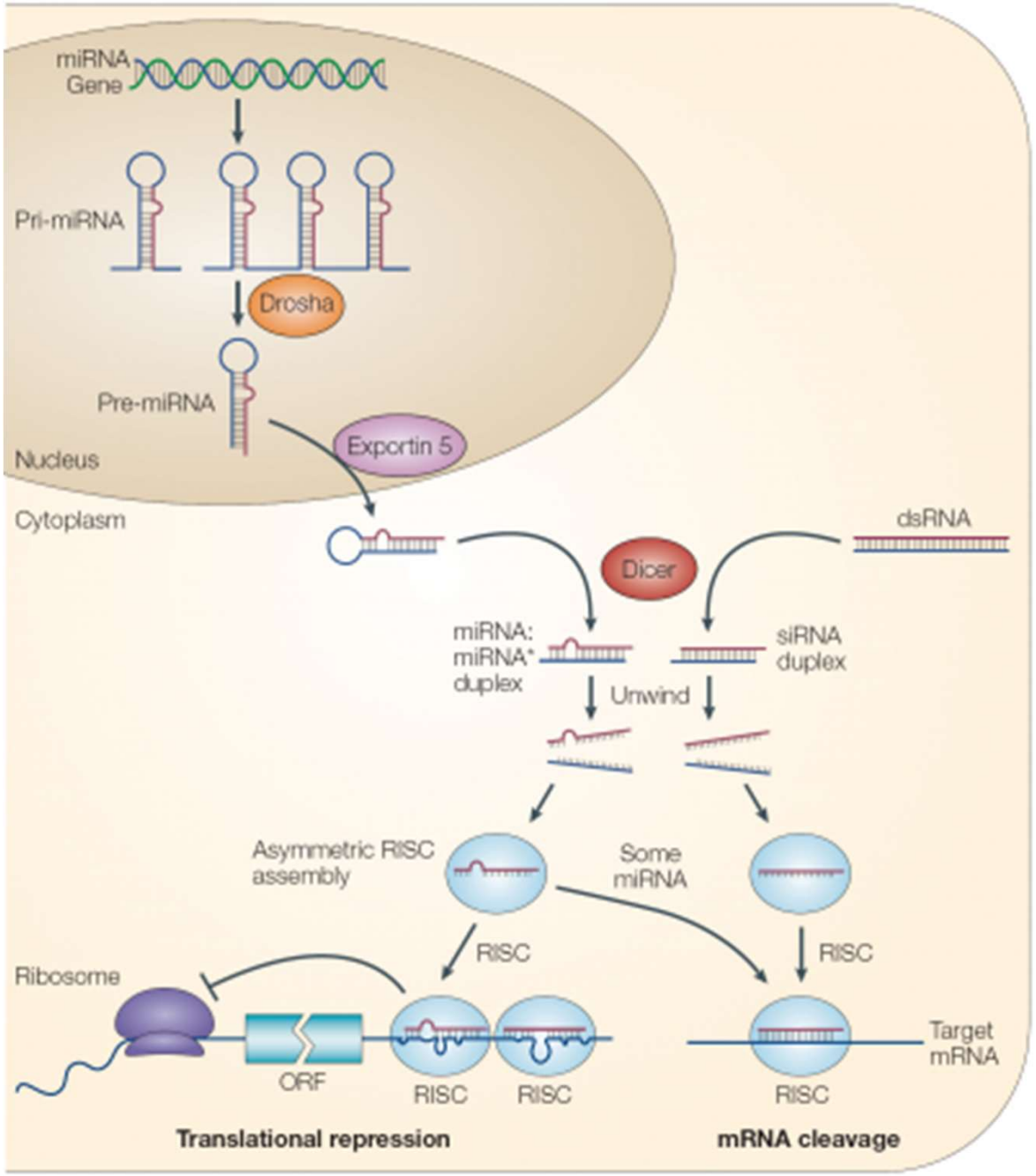
Aptamers

- sequences of NAs that are capable of recognizing and binding to a specific target
  - including metal ions, small molecules, peptides and proteins
  - high affinity and specificity
- Systematic Evolution of Ligands by EXponential enrichment (SELEX)
- DNA (are more stable) or RNA

SELEX protocol



siRNA (miRNA) silencing pathway

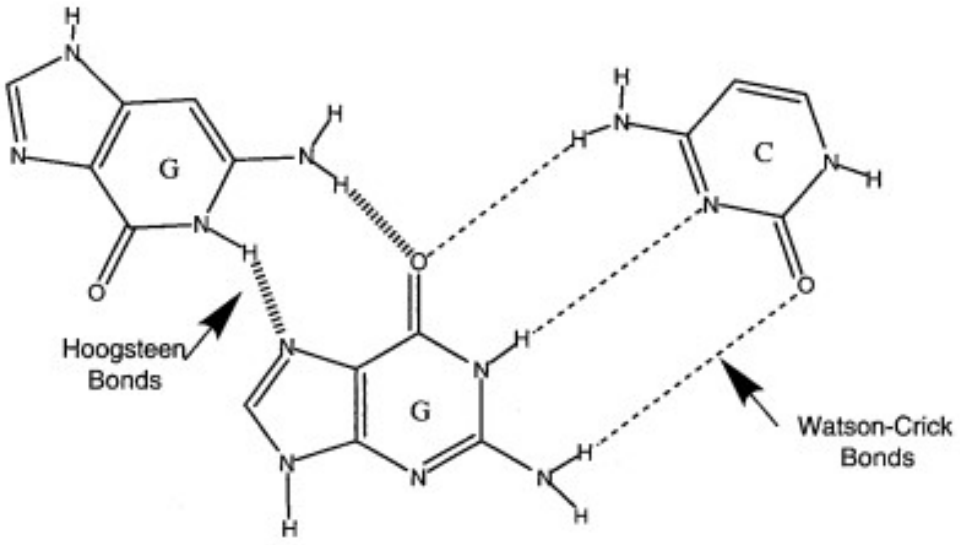


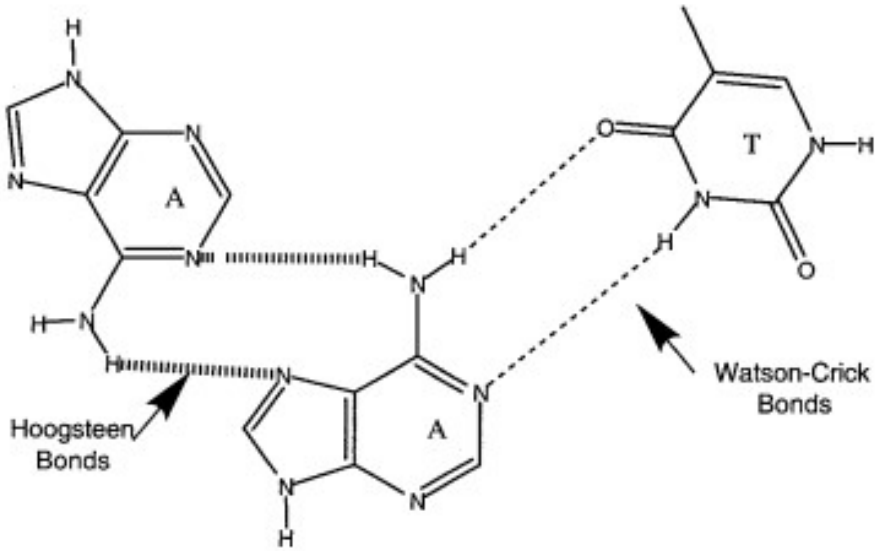
Triplex Forming Oligonucleotides

- Formation of triple helices discovered in 1957 (Felsenfeld *et al.*)
- Morgan (1968) demonstrated ability of a bound RNA third strand to inhibit transcription
- Sequence-specific tools for gene targeting (purine-rich strand)
  - Established binding code
- TFOs bind to a major groove of duplex DNA
  - High specificity and affinity ☺
  - Stabilized by divalent cations
- Homing devices for genetic manipulation *in vivo*
- Potential toll for gene knock out in mammalian cells
- Includes natural and modified DNA, PNAs, polyamides
- Typically 20-30 nt in length

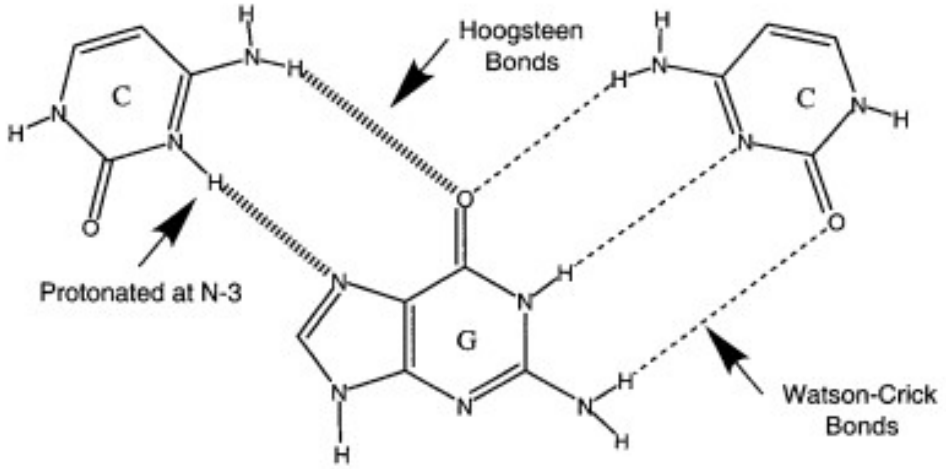
Binding code for TFOs

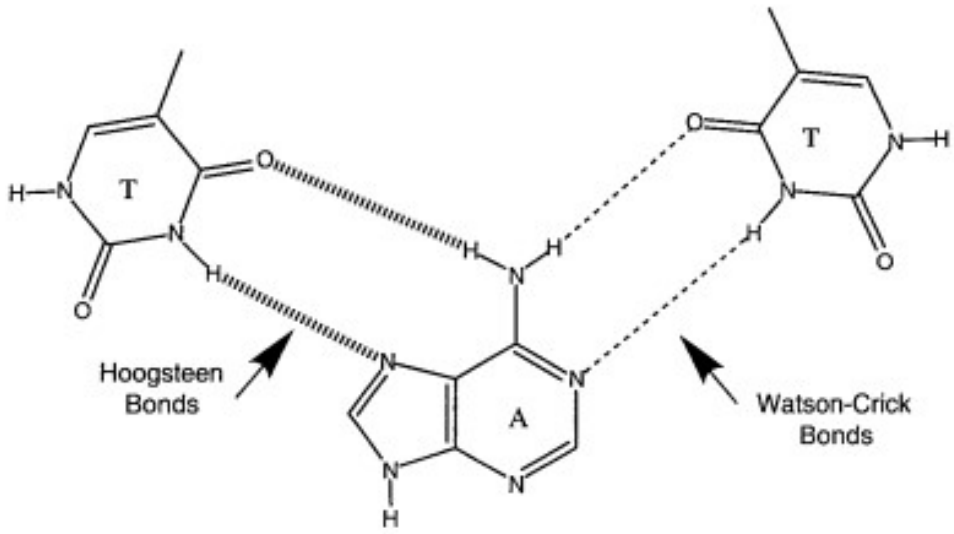
Triple Helix Triplets: Purine Motif



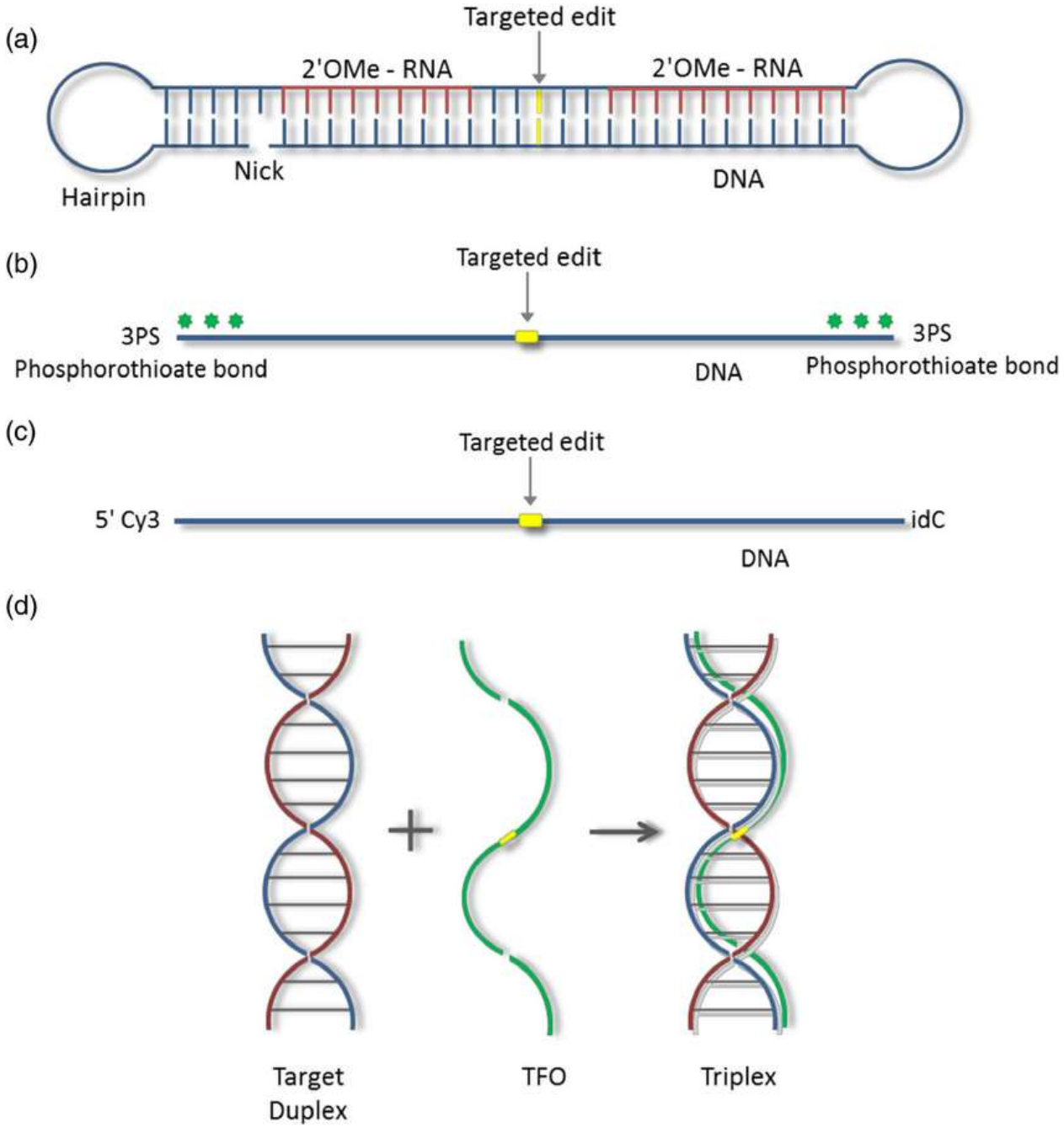


Triple Helix Triplets: Pyrimidine Motif

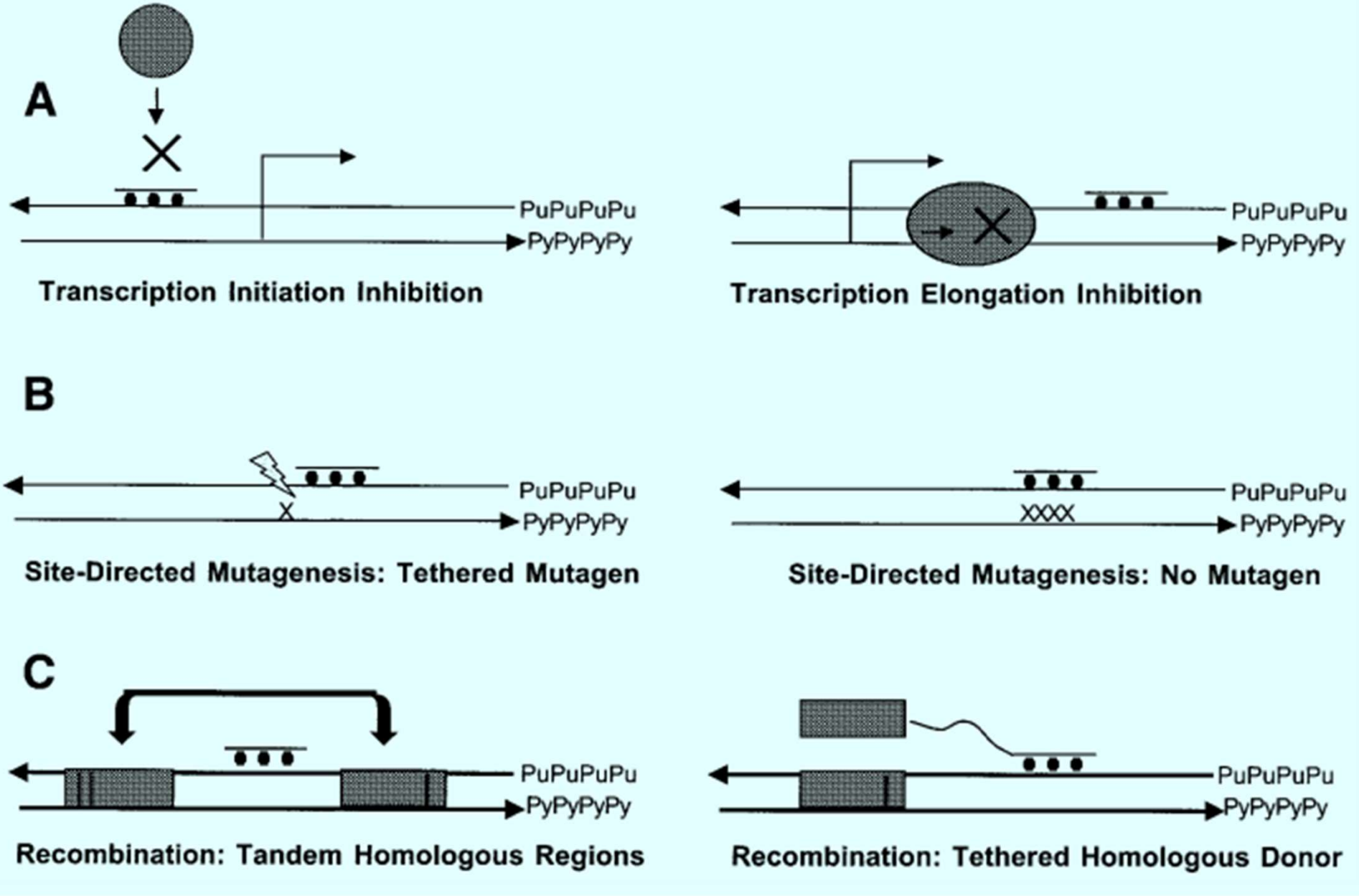




Triplex Forming Oligonucleotides

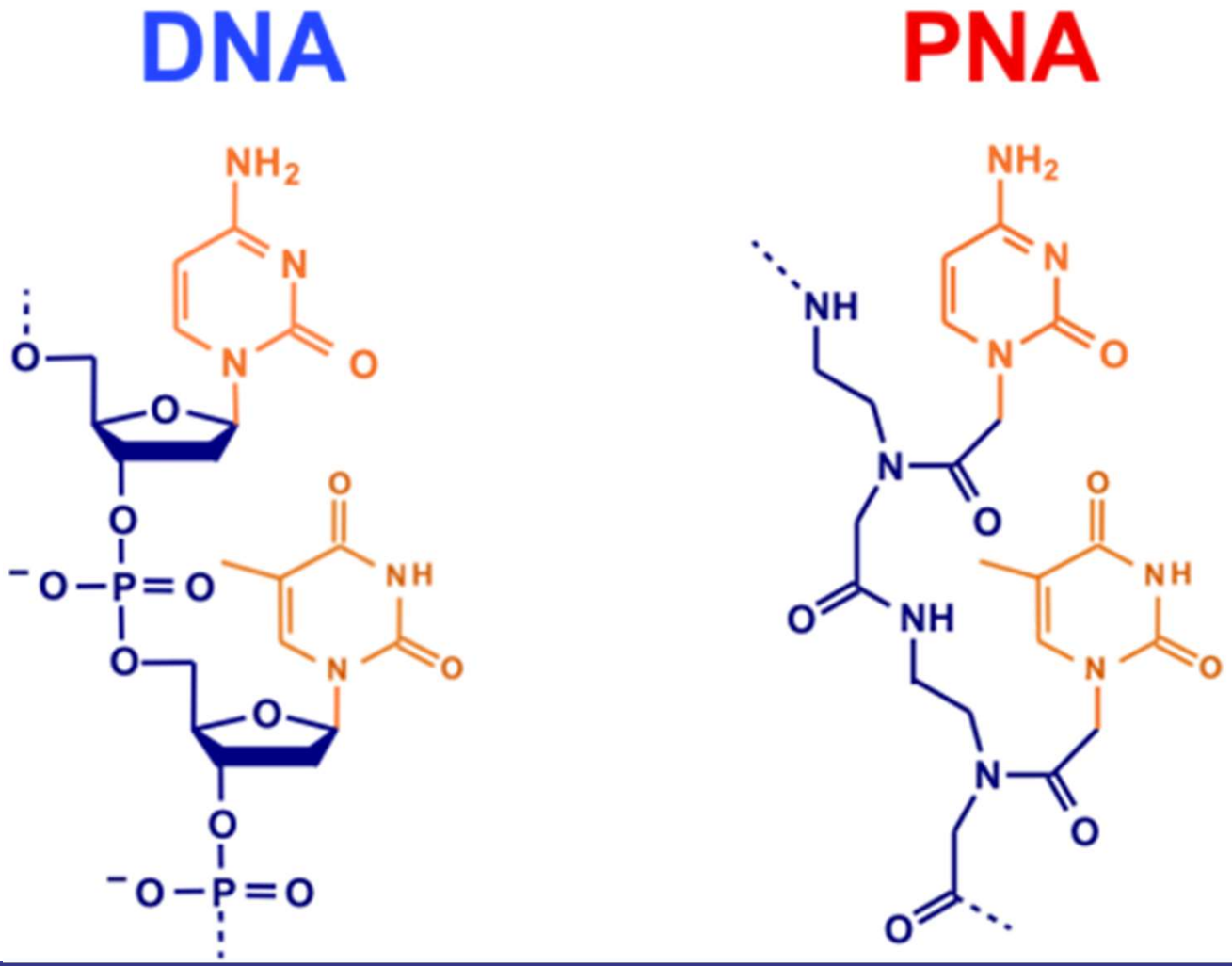


TFOs – gene alteration



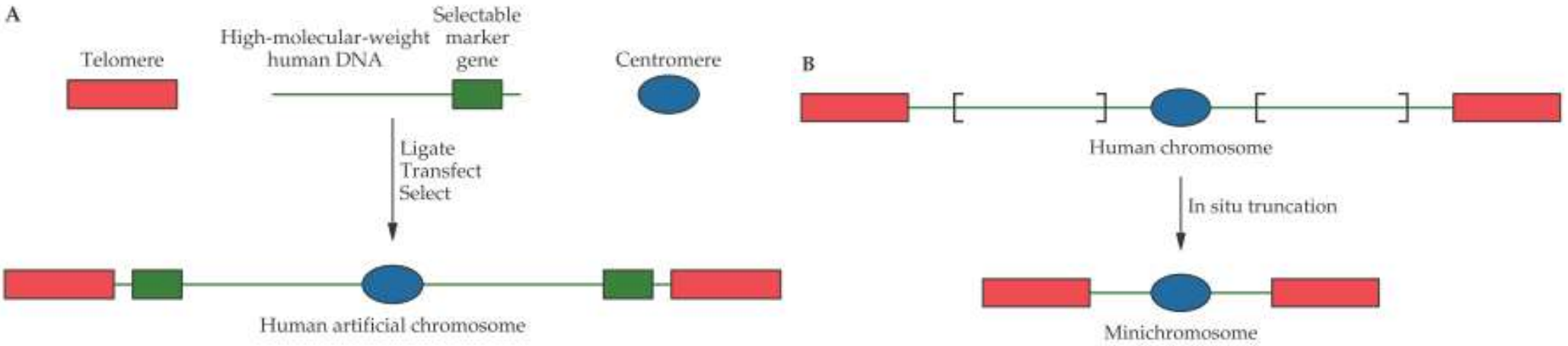
Peptide Nucleic Acids

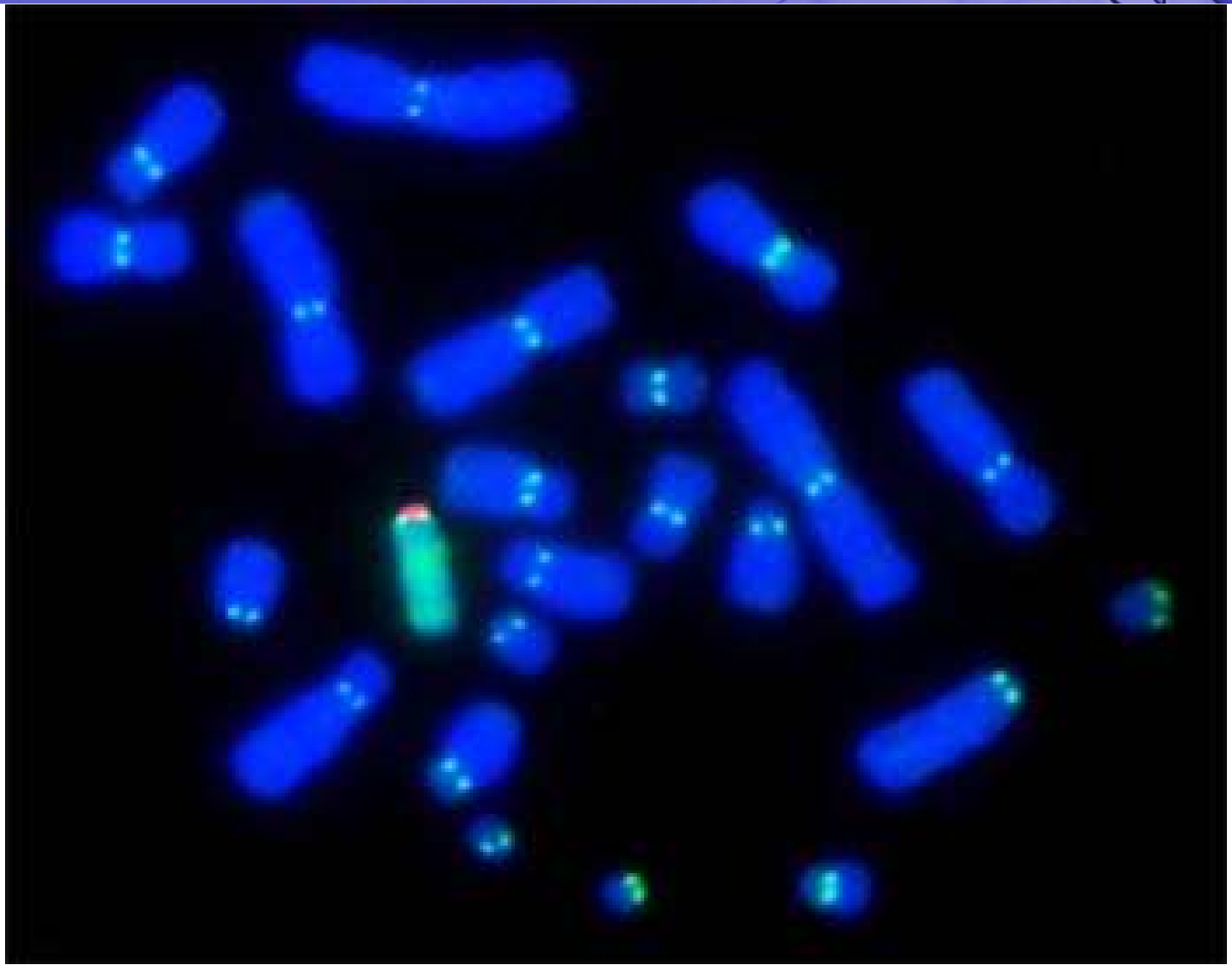
- **Invented in 1991**
- **Chemically stable and resistant to hydrolytic cleavage**



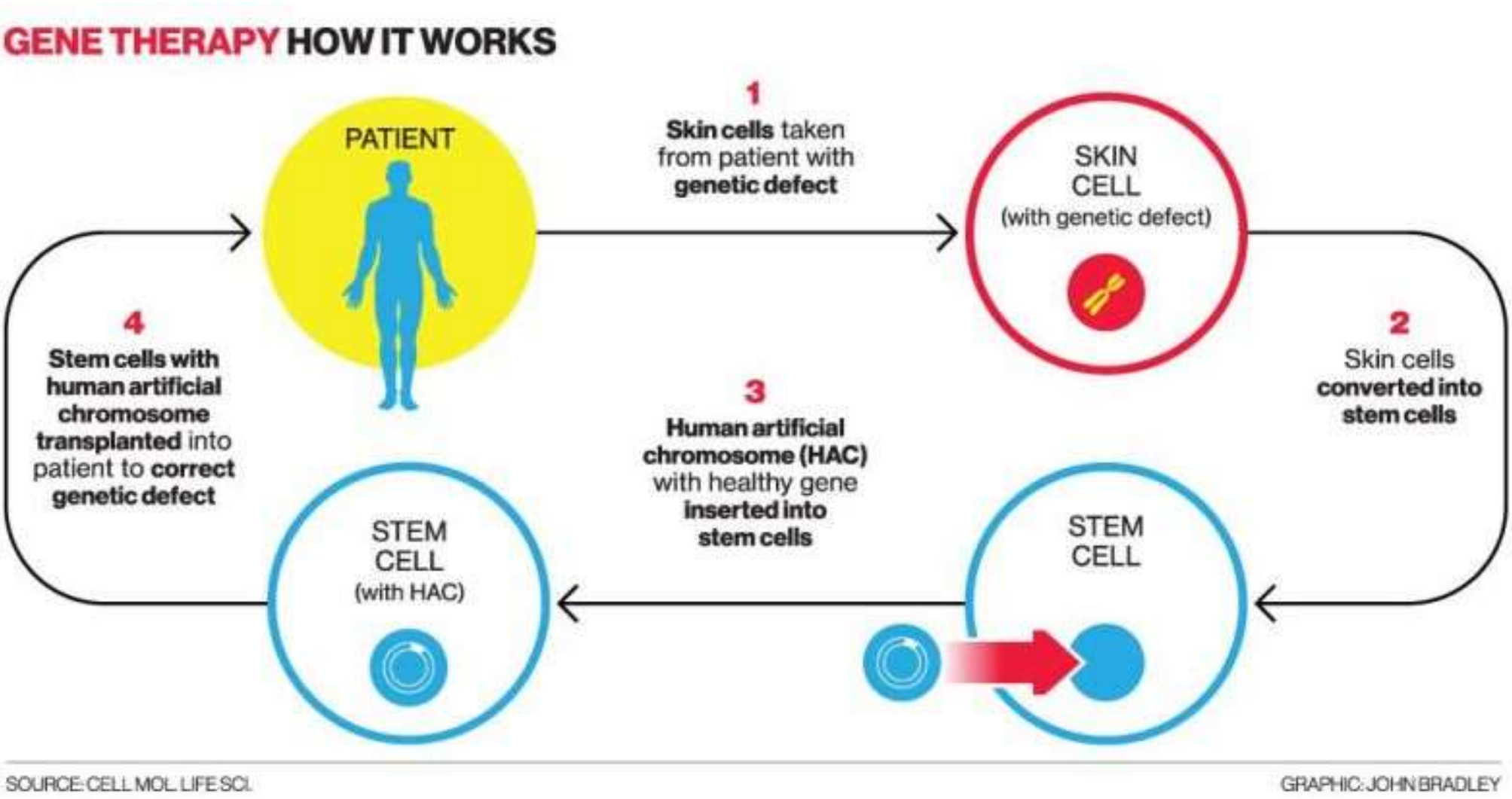
Human Artificial Chromosomes (HAC)

- **Two ways for creation**
  - **Ligation of individual chromosome components (telomers, centromere, origins of replicon)**
  - **Paring down an existing human chromosome by deleting material to form "minichromosome"**

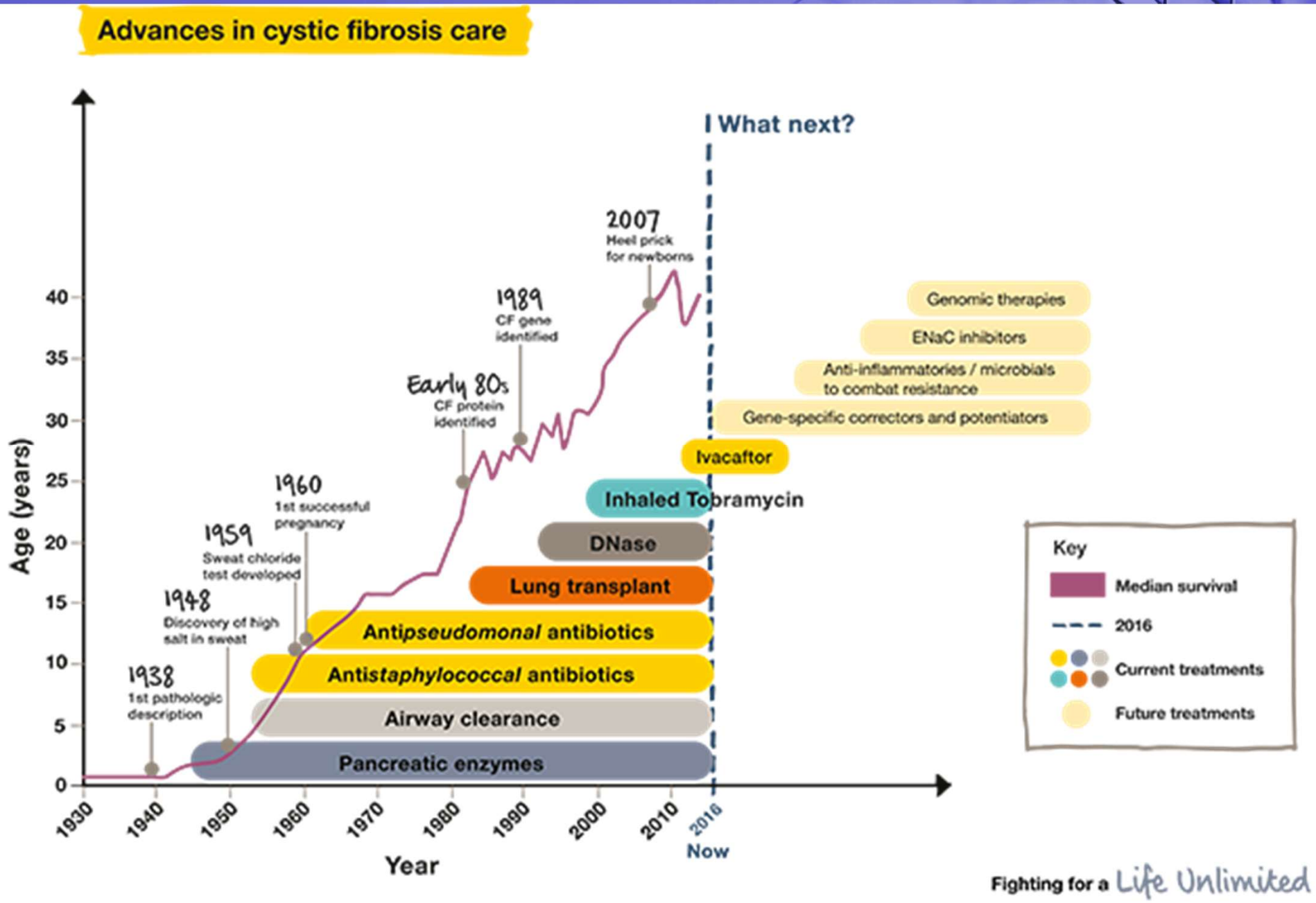




Gene Therapy using HAC



Cystic fibrosis treatment/ hopefully near future



Clinical Trials

- Phase I
  - first-in-man trials
  - Usually, small group 20-80
  - Screening for safety and dosage
- Phase II
  - Larger group (200-300)
  - Determine efficacy, usually against placebo
- Phase III
  - Large group (1000-3000)
  - Confirmation of safety and efficacy (compare to commonly used treatments)

Reading

- **Vogenberg FR, Isaacson Barash C, Pursel M. Personalized Medicine: Part 1: Evolution and Development into Theranostics. Pharmacy and Therapeutics. 2010;35(10):560-576.**

Personalized Medicine

Part 1: Evolution and Development into Theranostics

F. Randy Vogenberg, PhD, RPh; Carol Isaacson Barash, PhD; and Michael Pursel, RPh, MBA

This article is the first in a three-part series on the topic of medicine that is geared toward the individual patient. Part 2 will explore key ethical, legal, and regulatory issues facing the future of personalized medicine, and Part 3 will cover the anticipated challenges in implementing pharmacogenomics and genetic testing into routine clinical practice.

Key words: personalized medicine, pharmacogenomics, pharmacogenetics, pharmacodiagnostics, theranostics, personal genomics, human genome, gene testing

INTRODUCTION

Personalized medicine (PM) has the potential to tailor therapy with the best response and highest safety margin to ensure better patient care. By enabling each patient to receive earlier diagnoses, risk assessments, and optimal treatments, PM holds promise for improving health care while also lowering costs.

HISTORY AND LANDSCAPE

Over the past six decades, much evidence has emerged indicating that a substantial portion of variability in drug response is genetically determined, with age, nutrition, health status, environmental exposure, epigenetic factors, and concurrent therapy playing important contributory roles. To achieve individual drug therapy with a reasonably predictive outcome, one must further account for different patterns of drug response among geographically and ethnically distinct populations.

These observations of highly variable drug response, which began in the early 1950s, led to the birth of a new scientific discipline arising from the confluence of genetics, biochemistry, and pharmacology known as pharmacogenetics. Advances in molecular medicine have spawned the newer field of pharmacogenomics, which seeks to understand all of the molecular underpinnings of drug response. Commercialization of this research application is now known as personalized medicine (PM). Demonstrated success is emerging for several conditions and treatments, but whether PM will achieve