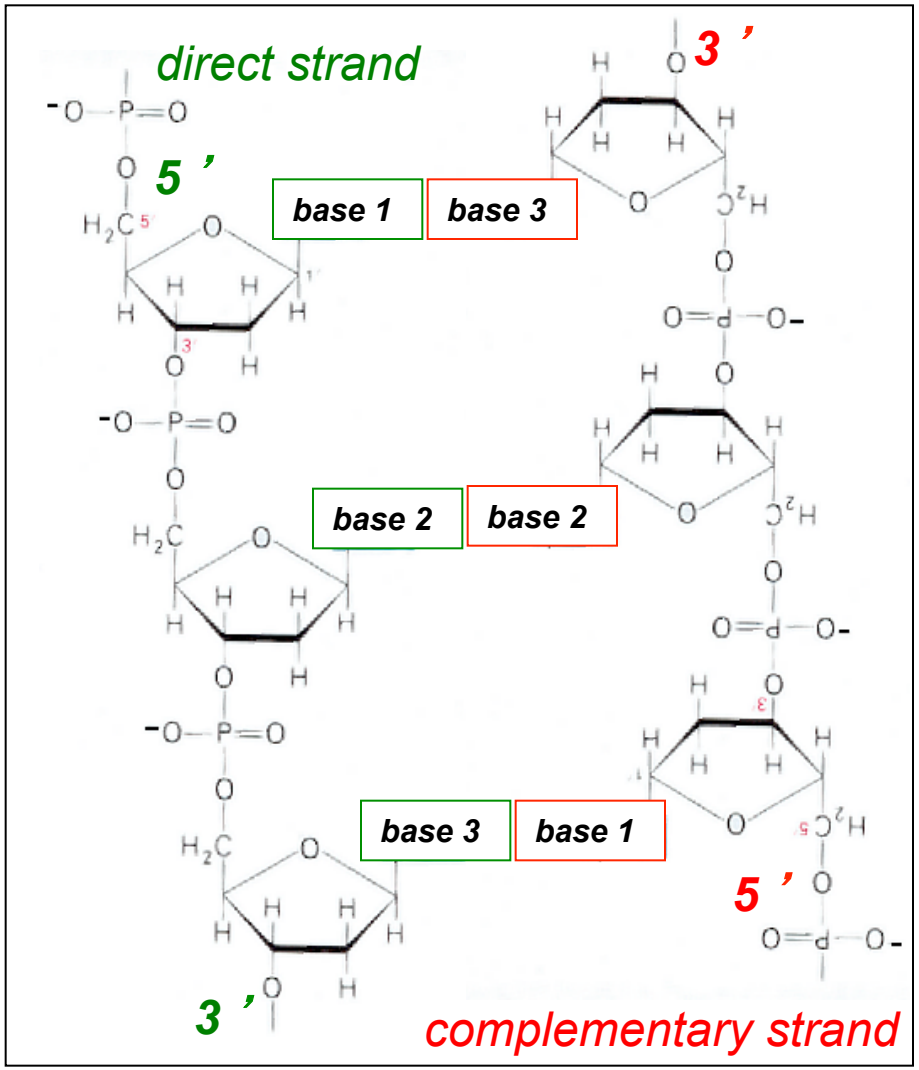
How naming a double stranded DNA?



Naming of a ‘direct’ strand:

5 ’ AACTGGGTCAATTCCG 3 ’

Naming of the ‘complementary’ st:

3 ’ TTGACCCAGTTAAGGC 5 ’ ou
5 ’ CGGAATTGACCCAGTT 3 ’

which is different to:

5 ’ TTGACCCAGTTAAGGC 3 ’

DNA solid phase synthesis

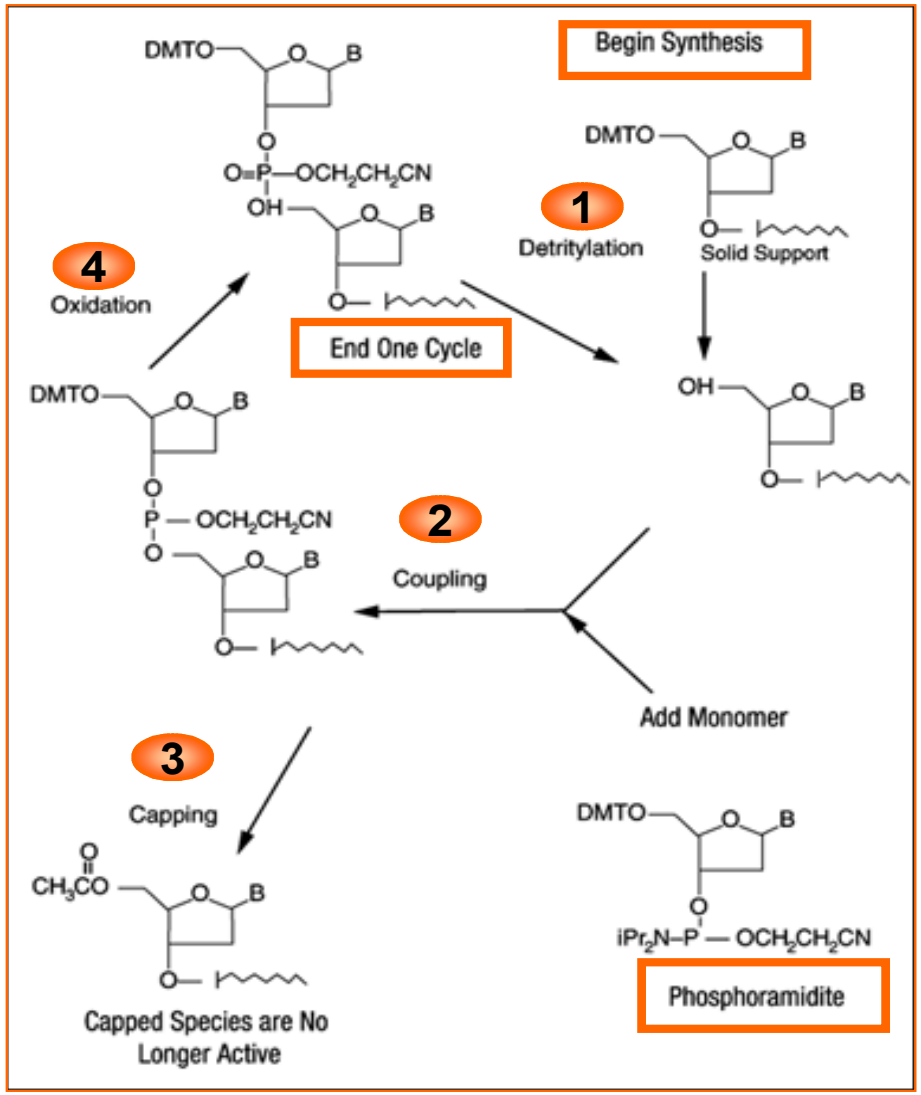
-short fragments (<100 bases)

-RNA and DNA, and NA analogs as well ! All single stranded

-modifications can be incorporated everywhere (fluorescent labeling, biotin, amines, carboxylic acids, thiols, etc)

-many suppliers, cheaper and cheaper

-quality control required

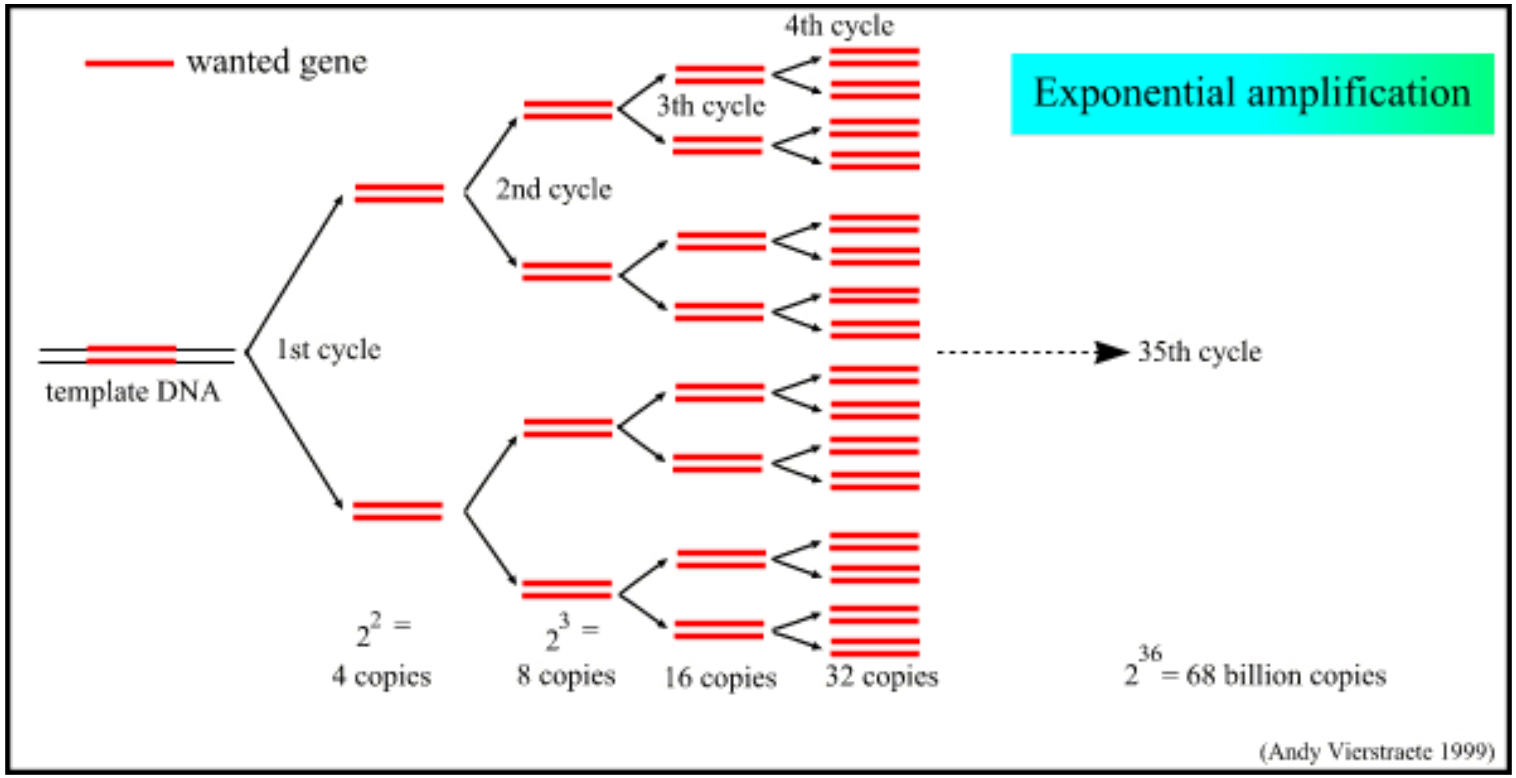


DNA PCR-based synthesis

- PCR amplifies DNA
  - Makes lots and lots of copies of a few copies of DNA
  - Can copy different lengths of DNA, doesn't have to copy the whole length of a DNA molecule
    - One gene
    - Several genes
- Artificial process which imitates natural DNA replication
- Requires a DNA template and specific DNA primers

Routine techniques in molecular biology: NUCLEIC ACIDS

Polymerase Chain Reaction



Routine techniques in molecular biology: NUCLEIC ACIDS

Biological synthesis: PCR - Polymerase Chain Reaction

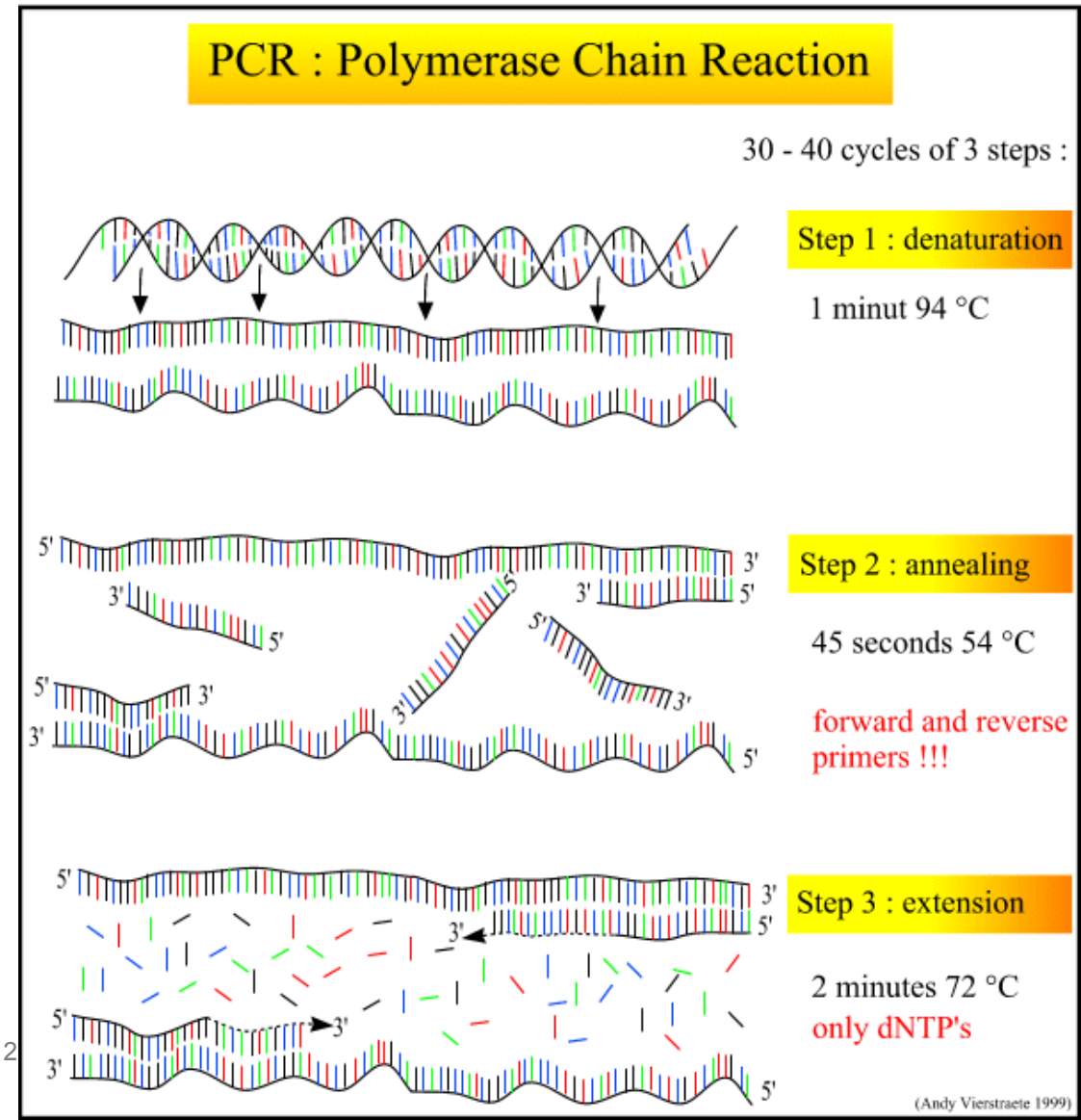
-requires starting material (DNA or RNA sequence)

-primer design needed. They are obtained by solid phase synthesis

-fully automated, pretty affordable

-yields double stranded DNA

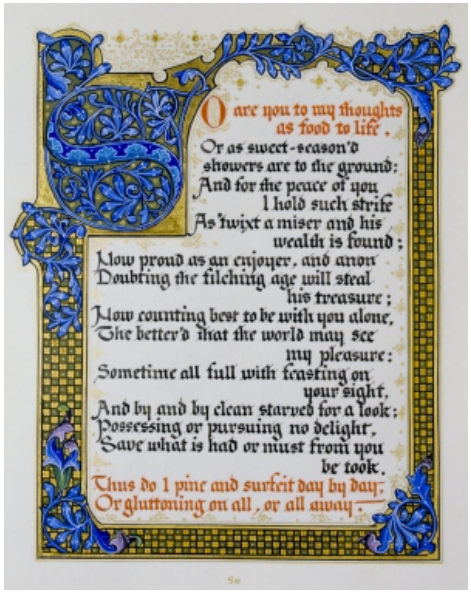
-no incorporation of modifications (except in the primers)

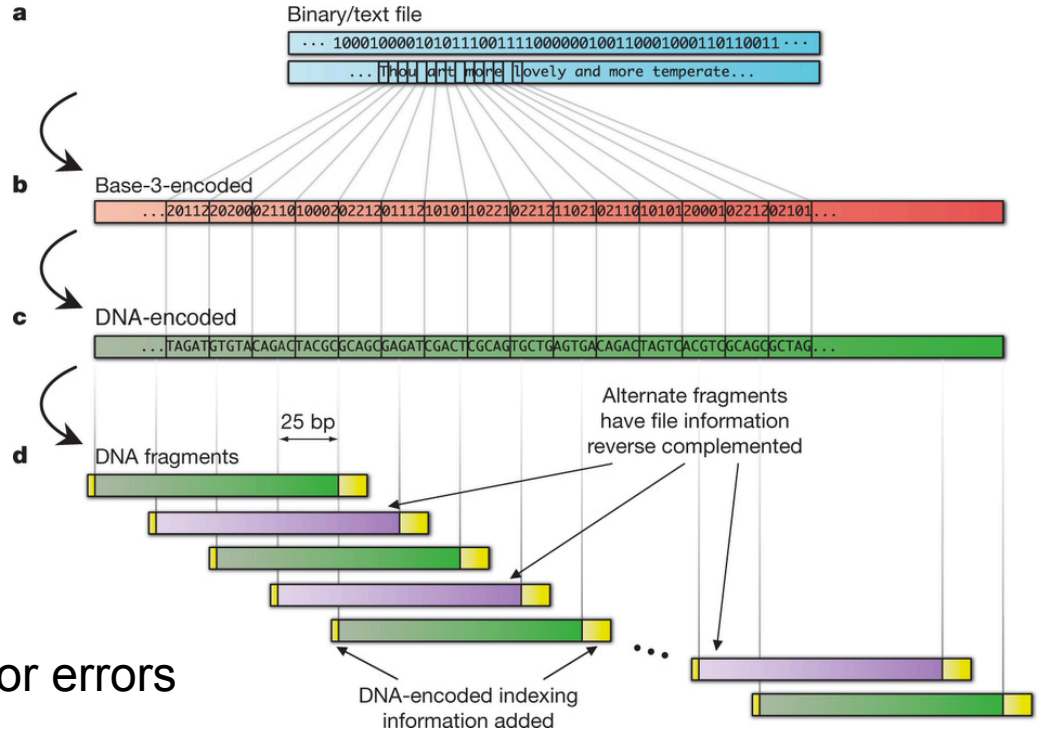


Data storage using DNA

Encoding of 5 files :

1. *All 154 of Shakespeare's sonnets, a classic scientific paper,*
2. *A classic scientific paper*
3. *A medium-resolution colour photograph (JPEG 2000 format),*
4. *26-s extrcact from Martin Luther King's 1963 'I have a dream' speech (MP3 format)*
5. *The Huffman code used in this DNA storage process*

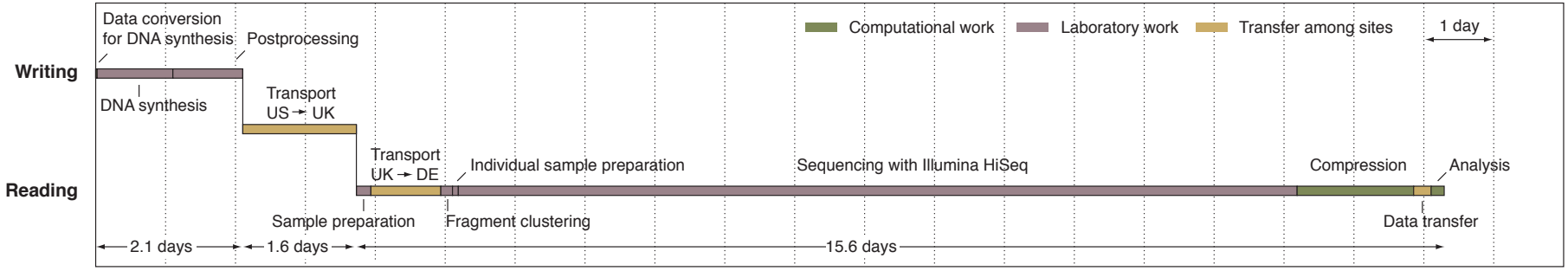




- New DNA coding (self-checking for errors
- No runs >2 identical bases
- Fourfold redundancy
- *=> 739 kilobytes cded in 79.6 x 10^6 DNA fragments of 104 bases in length*

Goldman, N. *et al*., *Nature*, **494**, 77–80 (2013).

Data storage using DNA

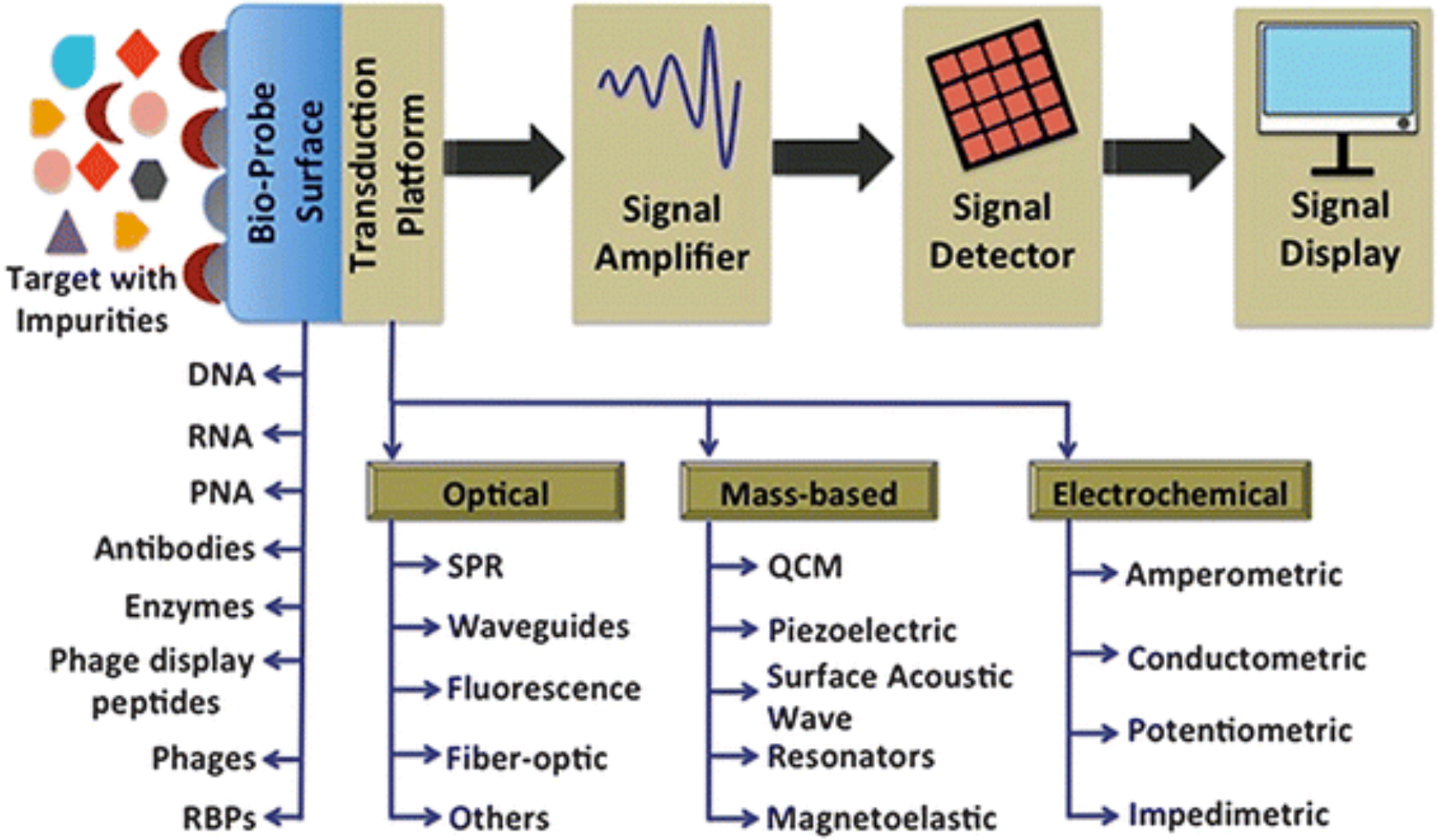


Supplementary Figure 9 | Timeline of DNA-storage experiment. We report only periods of active work on the experiment. We have omitted time taken to devise repairs for the file with two information gaps (above).

Information storage density. We recovered 757,051 bytes of information from 337 pg of DNA (above), giving an information storage density of ~2.2 PB/g (= $757{,}051/337 \times 10^{-12}$). We note that this information density is enough to store the US National Archives and Records Administration's Electronic Records Archives' 2011 total of ~100 TB (ref. 55) in < 0.05 g of DNA, the Internet Archive Wayback Machines's 2 PB archive of web sites[56] in ~1 g of DNA, and CERN's 80 PB CASTOR system for LHC data[25] in ~35 g of DNA.

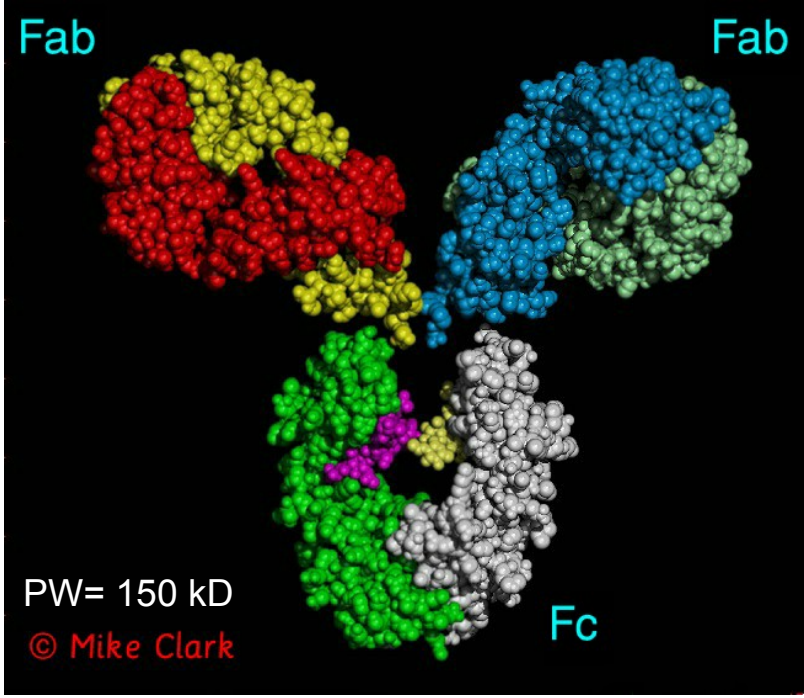
Goldman, N. et al. Nature 494, 77–80 (2013).

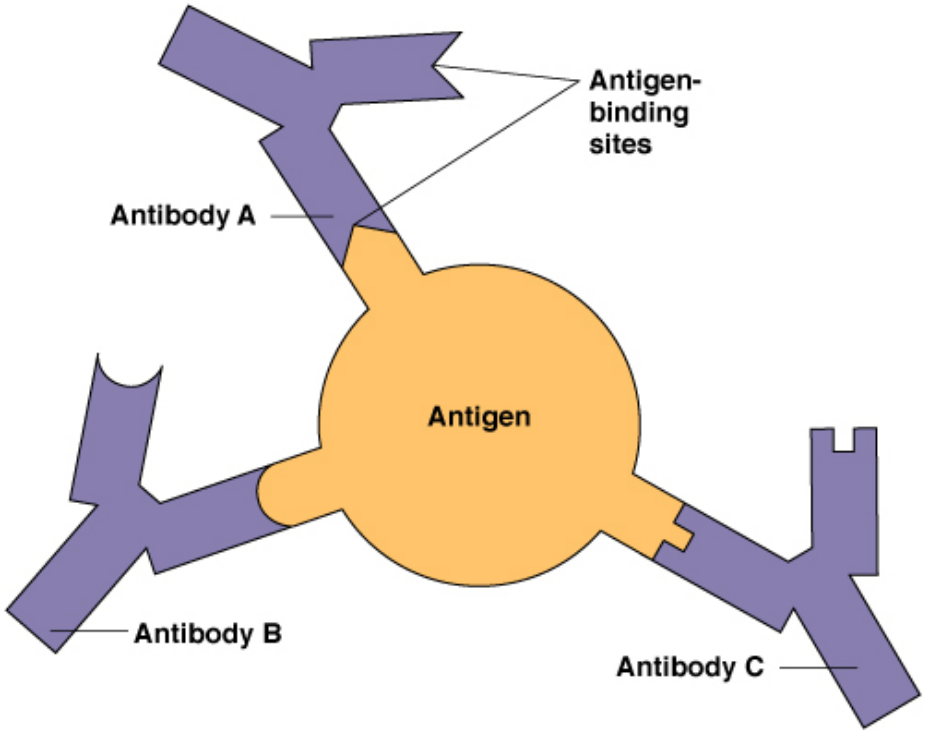
Biosensors & Microarrays



Antibodies used as bioprobes

Capable of specific binding to a dedicated molecular structure (epitope) to neutralize/eliminate a pathogen

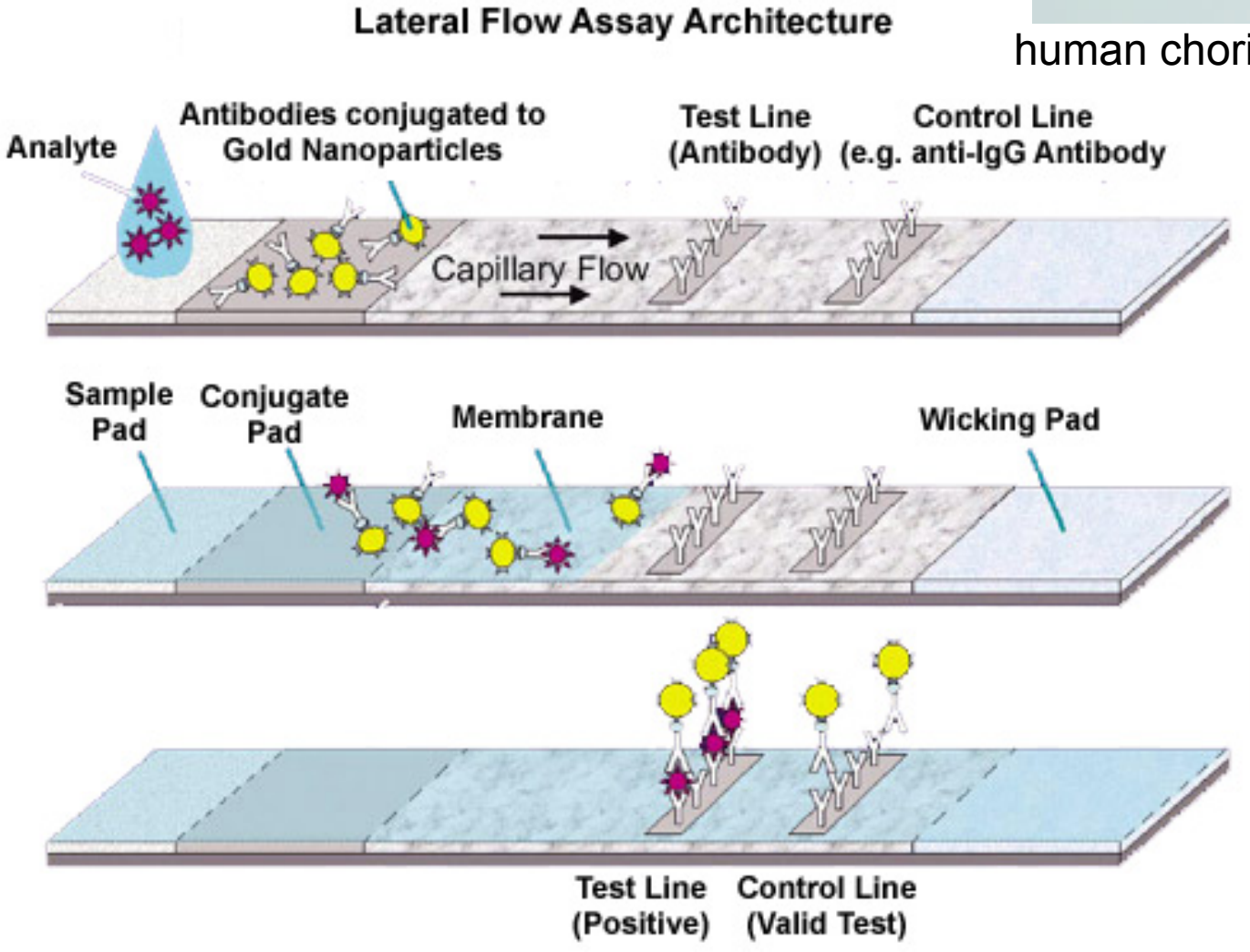




- Monoclonal vs Polyclonal
- Specific to a large variety of targets
- But some limitations:
  - *-toxins*
  - *-small molecules*
  - *-antibody stability*
  - *-cost*

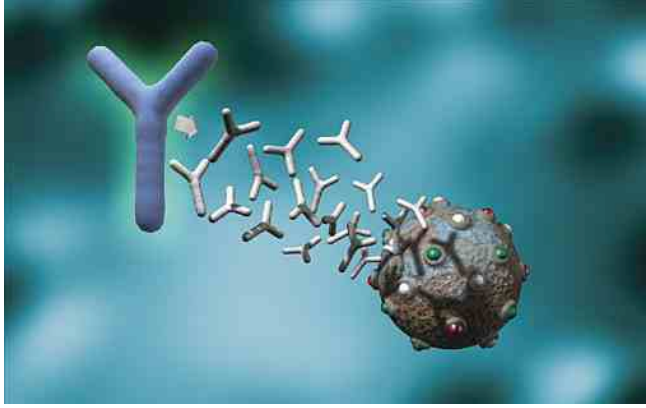
Biosensor with antibodies: immunoassays

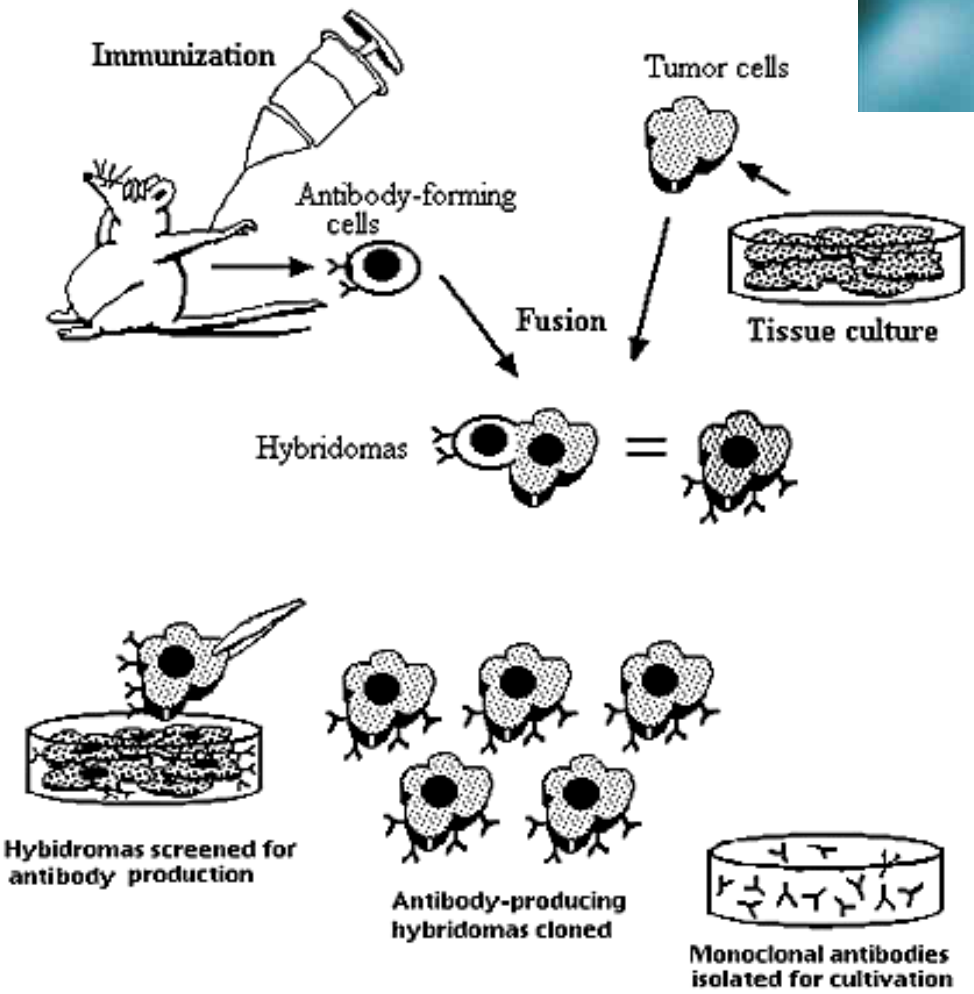
human chorionic gonadotropin



TARGET
PROBE
TRANSDUCER

Antibody production:
a long and expensive process!





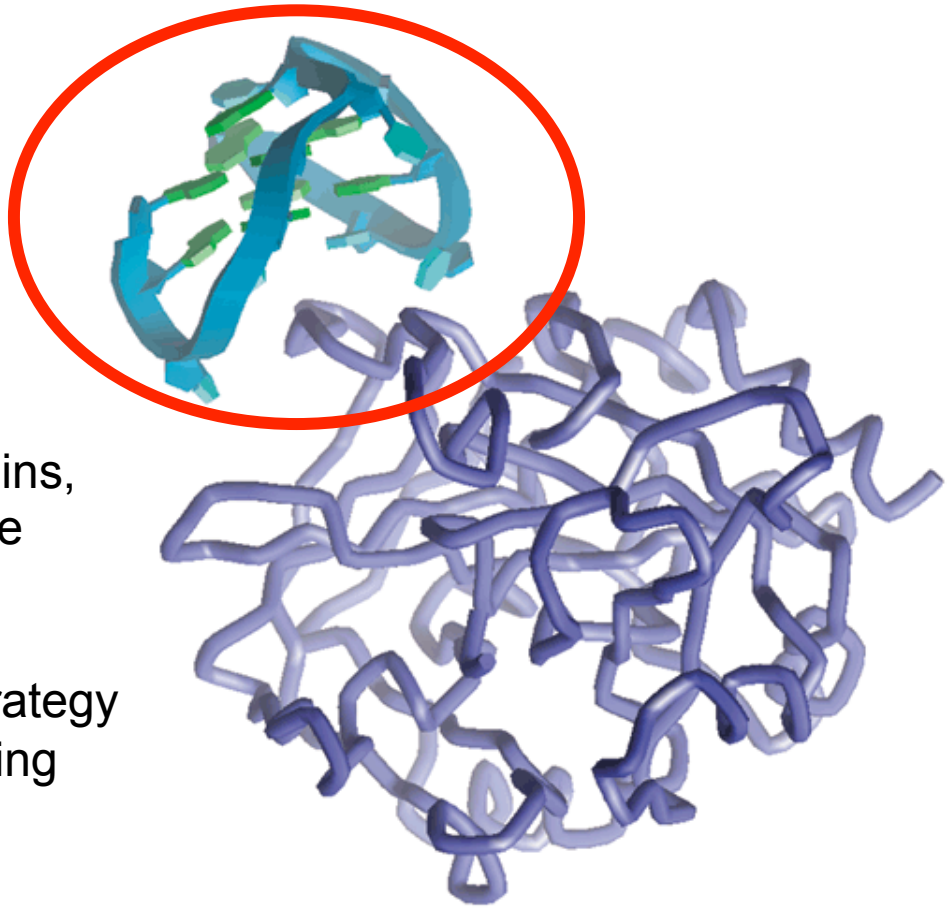
Monoclonal Antibody Production

Toward new ARN/DNA functionalities: aptamers

Objective: Aims at mimicking antibodies

Principle: NA are more stable than proteins, they are cheaper and easier of synthesize
Many target

Method: Production using the SELEX strategy although this approach remains challenging (artefact)

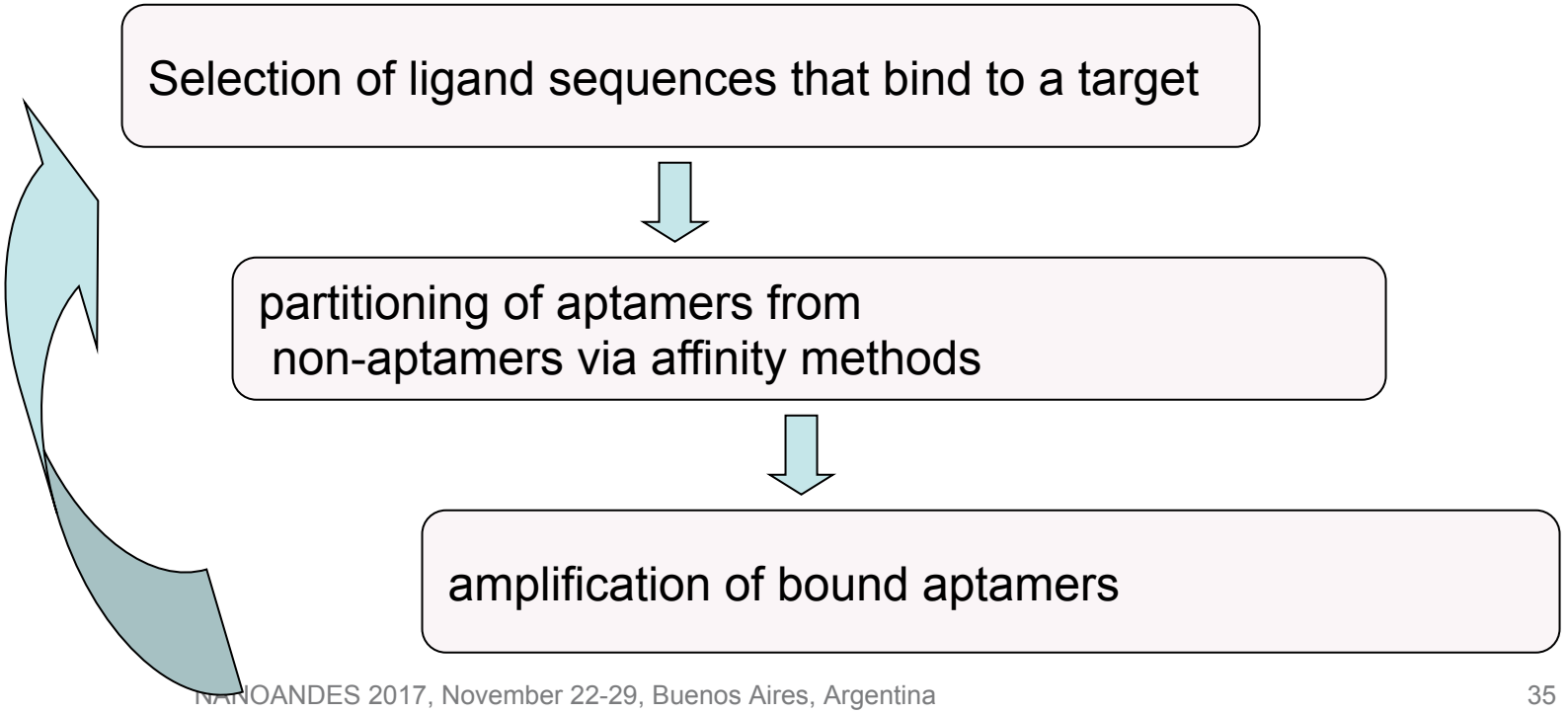


The **thrombin aptamer** forms a specific binding surface with the thrombin protein (blue).
Thiel, Nature Biotechnology 22, 649 - 651 (2004)

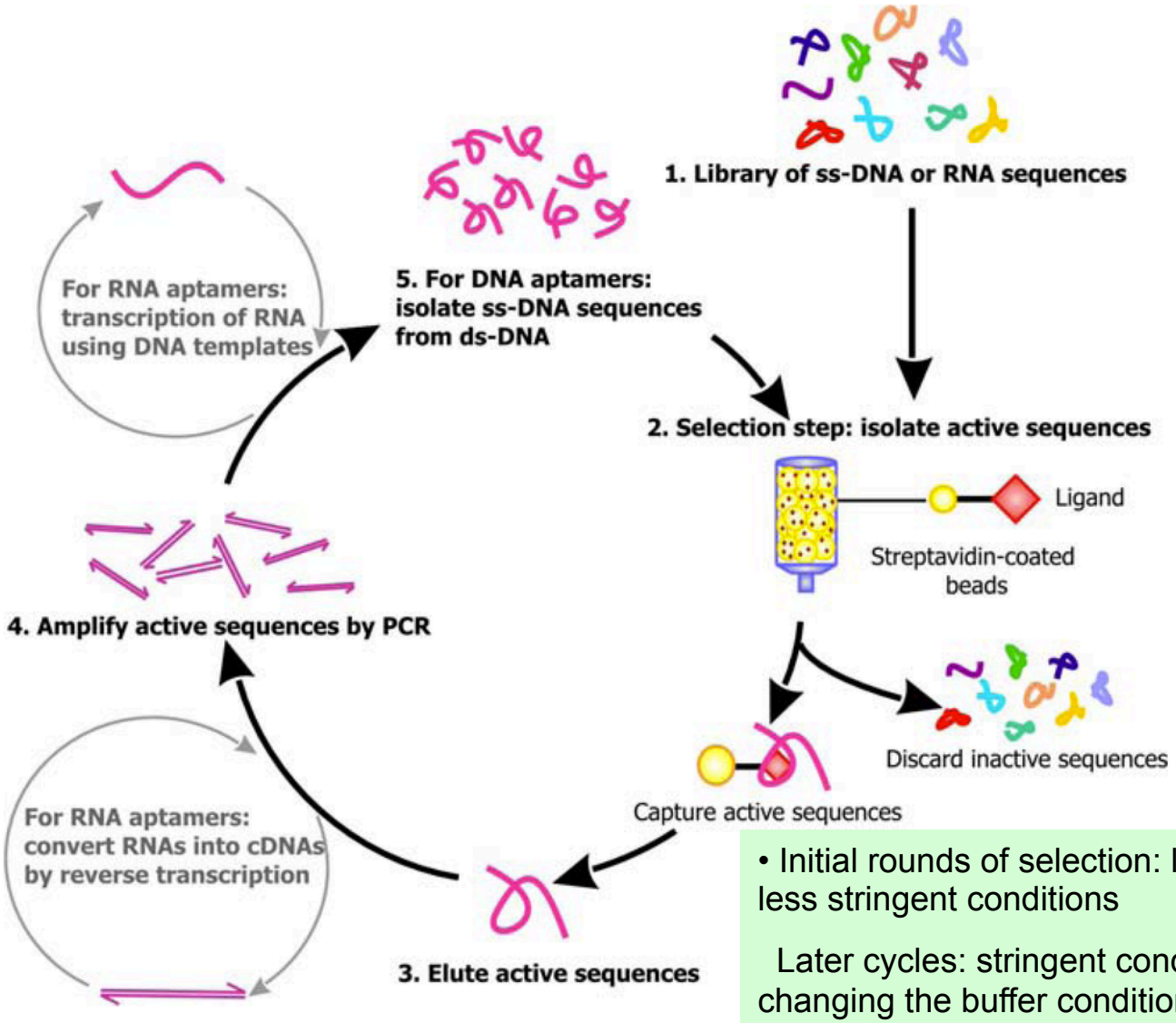
Toward new ARN/DNA functionalities: aptamers

SELEX (systematic evolution of ligands by exponential enrichment) is a process that involves the progressive purification from a combinatorial library of nucleic acid ligands with a high affinity for a particular target by repeated rounds of partitioning and amplification.

Three Processes



Toward new ARN/DNA functionalities: the SELEX



- Initial rounds of selection: long incubation times & less stringent conditions

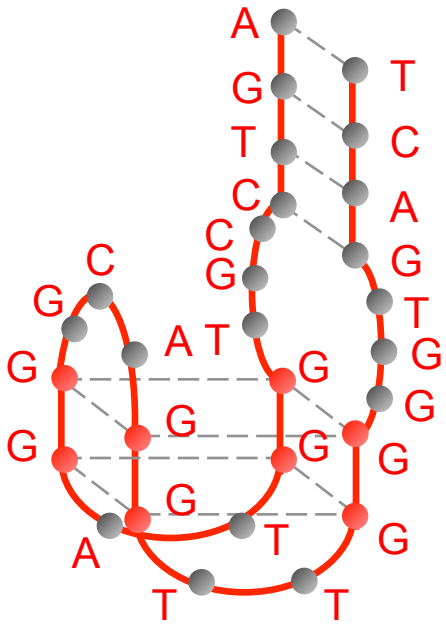
  Later cycles: stringent conditions, such as changing the buffer conditions, reaction volume and time of incubation.

- Monovalent & divalent cations

- Pre-negative selection

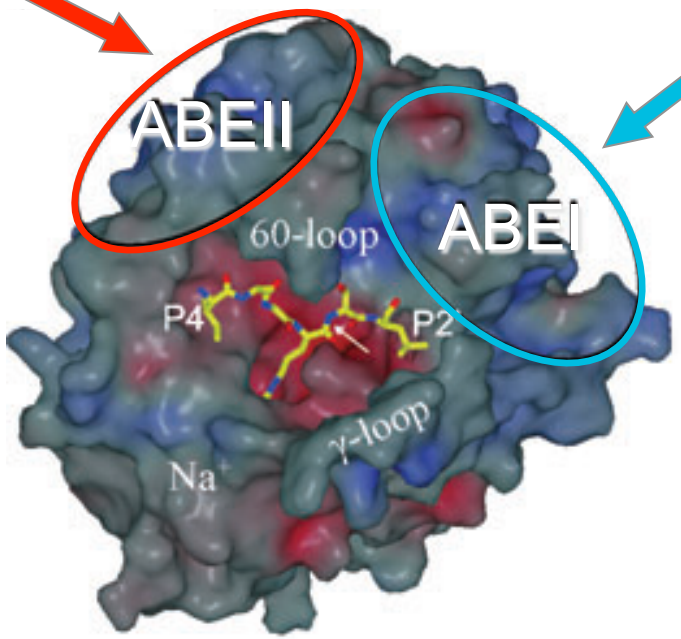
Aptamers raised againt a targeted protein

Thrombin = first protein targeted for DNA aptamer recognition

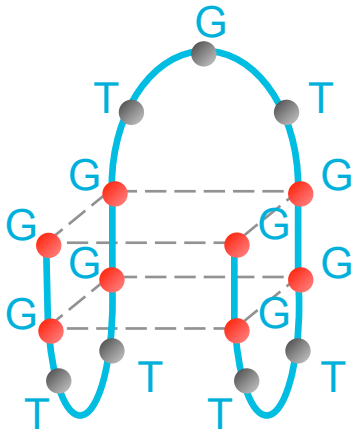


APT 2
(Tasset, 1997)

29 bases
K_D : 0.5 – 250 nM



Source : Huntington, Nature, 2000



APT 1
(Bock, 1992)

15 bases
K_D : 2 – 200 nM

Huang *et al.,* Talanta, 2010
Zhao *et al.,* Biosens. Bioelectron., 2011
Edwards *et al.,* Anal. Bioanal. Chem., 2010…

Small molecule detection

- *Antibodies can hardly be raised against small molecules*
- *Aptamers may address this issue*

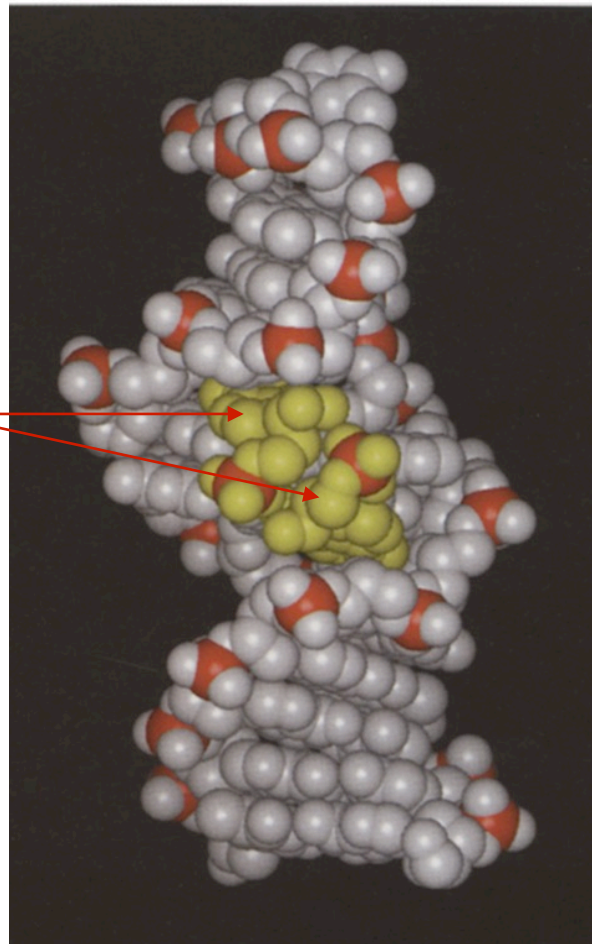
Target	Binding affinity (K_d)	Year
Adenosine TriPhosphate	6 μM	1995
Dopamine	700 nM	2009
Bisphenol A	8.3 nM	2011
Kanamycin	78.8 nM	2011
Ampicillin	9.4–13.4 nM	2012
Cellobiose	600 nM	1998
Cholic acid	5–67.5 μM	2000

Examples of small molecules reported in the literature that have been confirmed to bind specific aptamers.

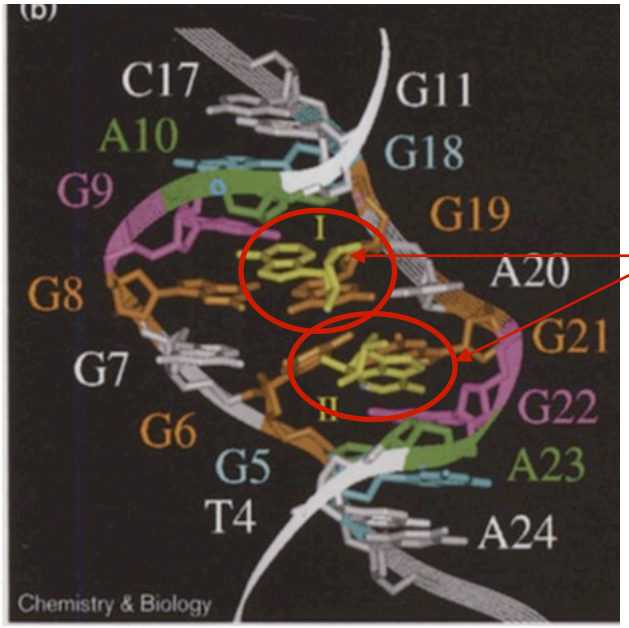
M. McKeague, M. DeRosa, *Journal of Nucleic Acids*, vol. 2012.

The most famous aptamer against small targets

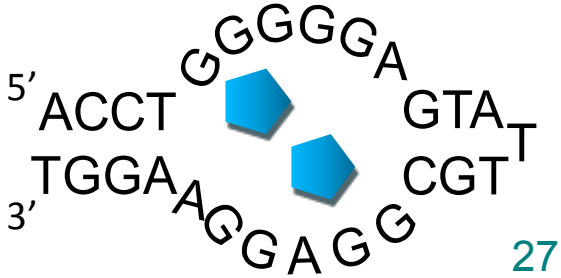
2 Adenosines molecules



Lin et al., *Chemistry & Biology*, 1997.



Adenosine



27 bases

K_D : 6 µM

Huizenga et al., *Biochemistry*, 1995.