DNA Aptamers

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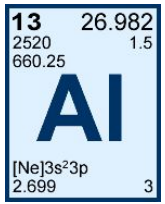
What Are Aptamers?

- Diagnostic and therapeutic tool
- Sequences of nucleic acids that are capable of recognizing and binding to a specific target



Three types: DNA, RNA and peptide aptamers

Xing et. al., *Current opinion in chemical engineering,* 2014, **4**, 79**.**
(Inorganic ventures, 2013)
Created with BioRender

Function: DNA Aptamers

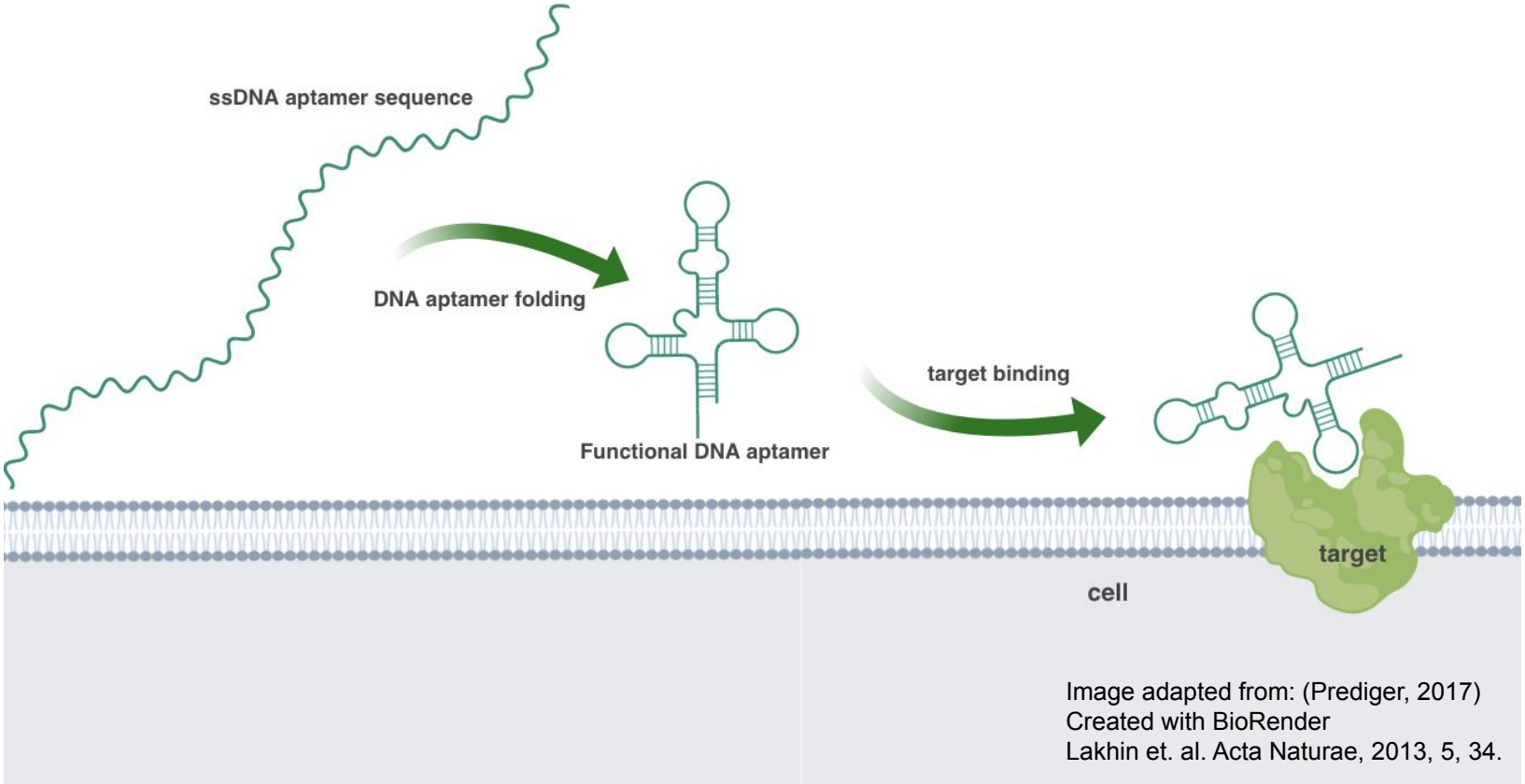


Image adapted from: (Prediger, 2017)
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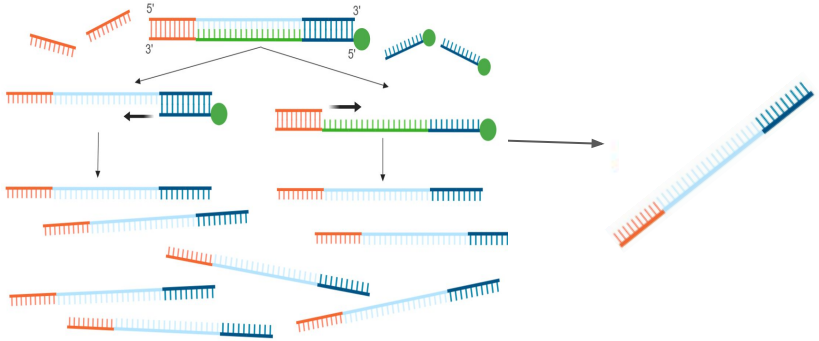
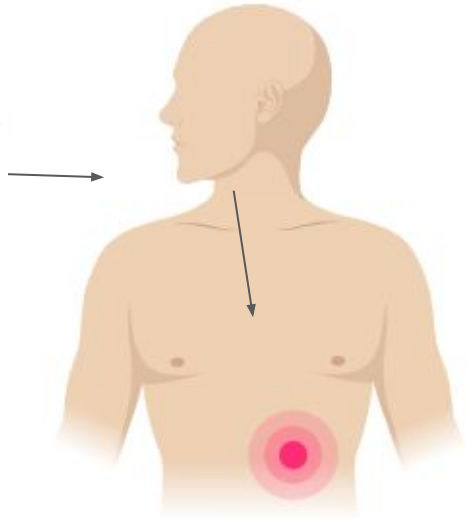
Lakhin et. al. Acta Naturae, 2013, 5, 34.

Pros & Cons: DNA Aptamers



Accurate & Reproducible Synthesization



Thermal stability

High Specificity

Non-immunogenic

Cons: Difficulty delivering, unable to cross-react

Xing et. al., *Current opinion in chemical engineering,* 2014, **4**, 79.
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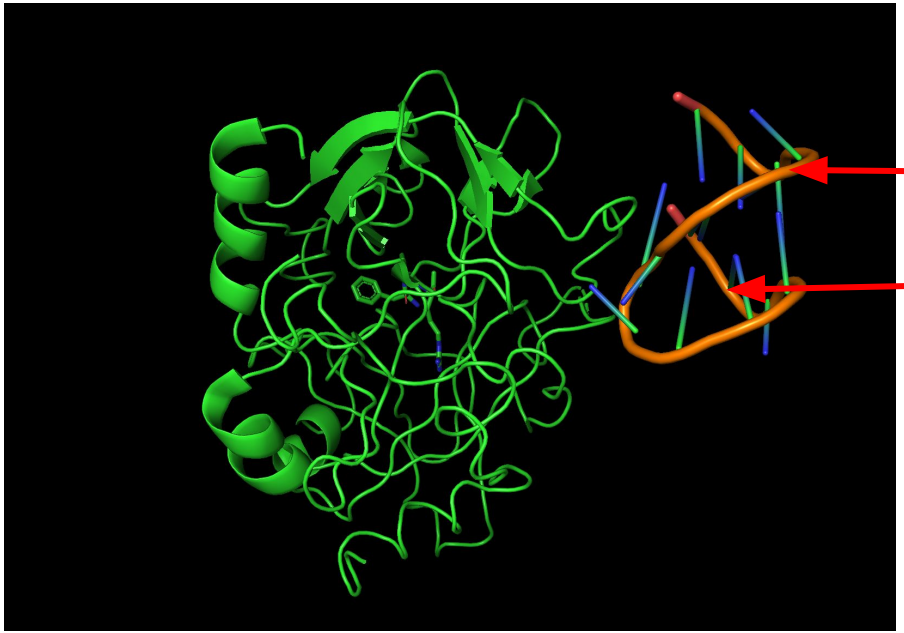
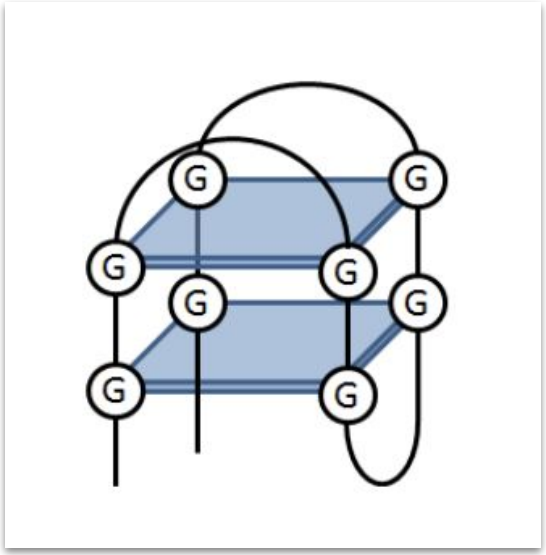
Anti-thrombin ssDNA Aptamer





G-quartet structure

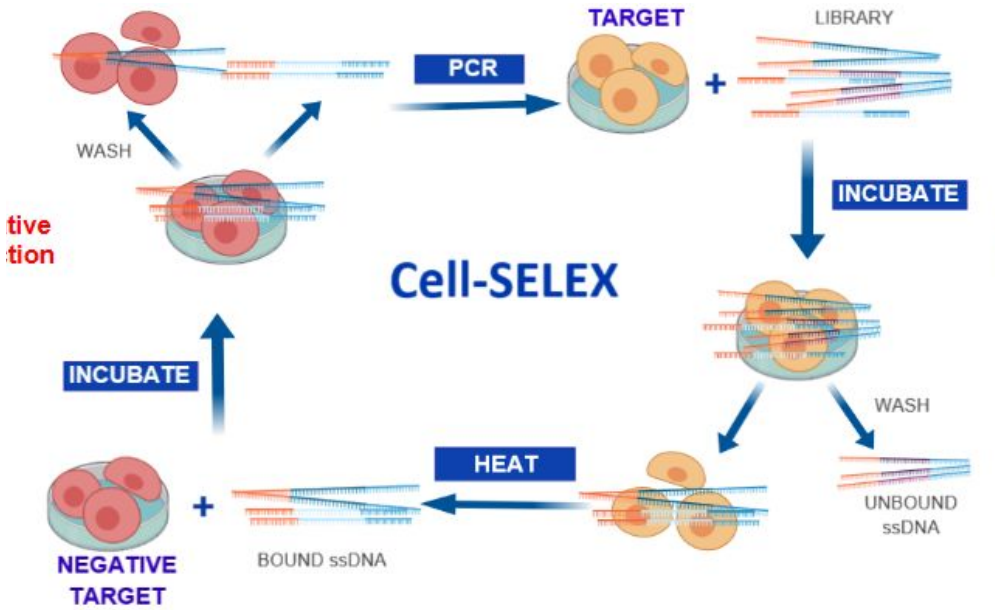
Image: Adapted from: (Aptagen, 2008)
Thrombin-Aptamer complex created with PyMOL
White et al. *Journal of Clinical Investigation*, 2000, 106, 8.

SELEX

- Scientific process to create DNA aptamers
- Several different methods

Our focus: **Cell-based SELEX**

1. Selection
2. Partitioning
3. Amplification

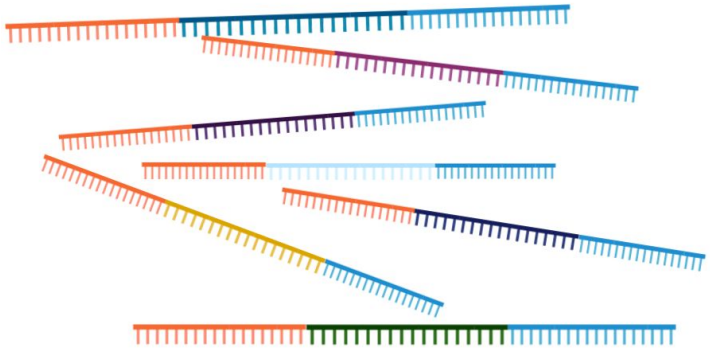


Adapted from: (Npj Precision Oncology (2017)1:37)

Zhuo et. al., *Int J Mol Sci*, 2017, **18**, 10.

STEP ONE: Selection

- Library of ssDNA oligonucleotides
  - Flanked by conserved primer binding sites



LIBRARY

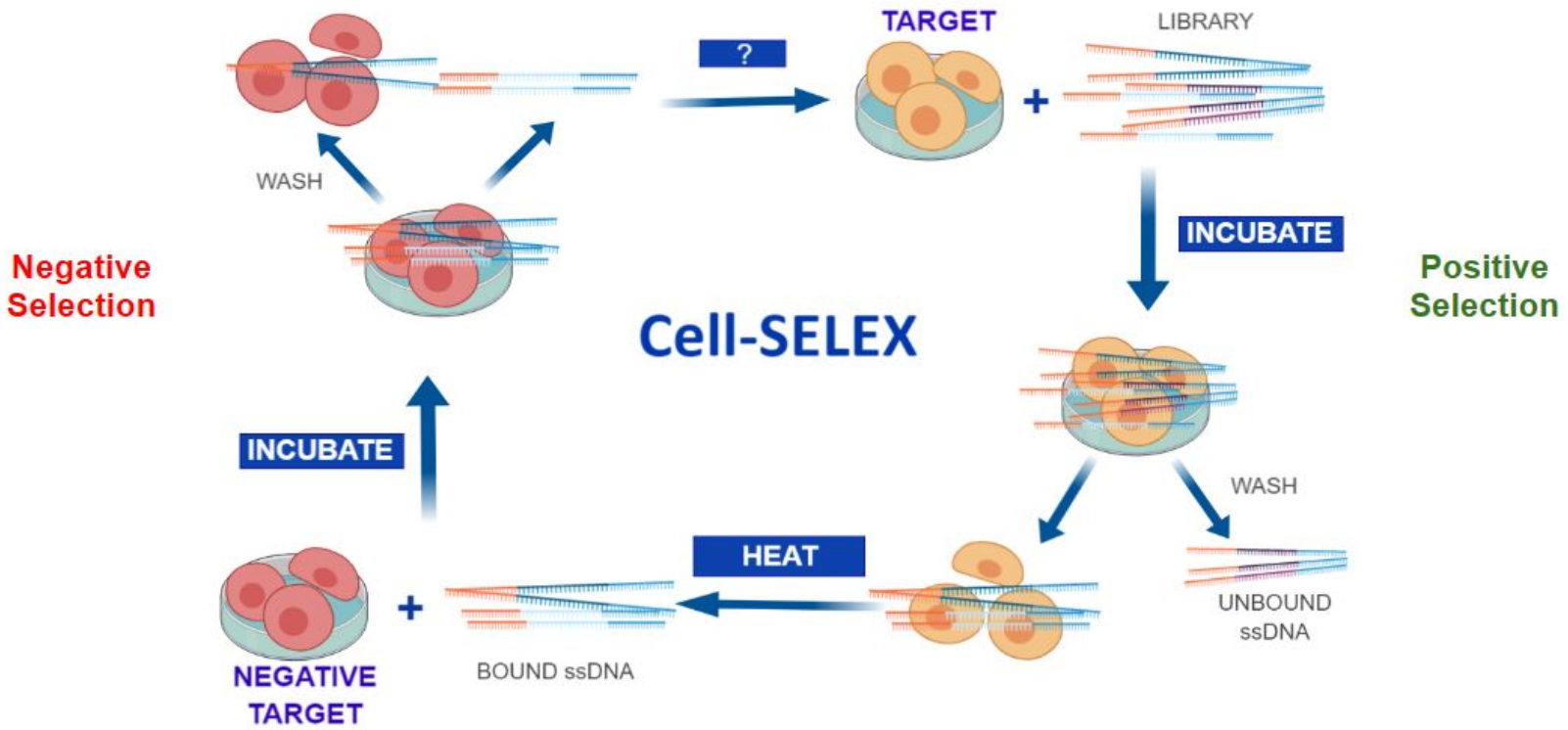
- Incubated with target molecules
  - Eg. cells
  - Conditions depend on application of the aptamers

TARGET

Created with BioRender

Zhuo et. al., *Int J Mol Sci*, 2017, **18**, 10.

STEP TWO: Partitioning



Adapted from: (Npj Precision Oncology (2017)1:37)
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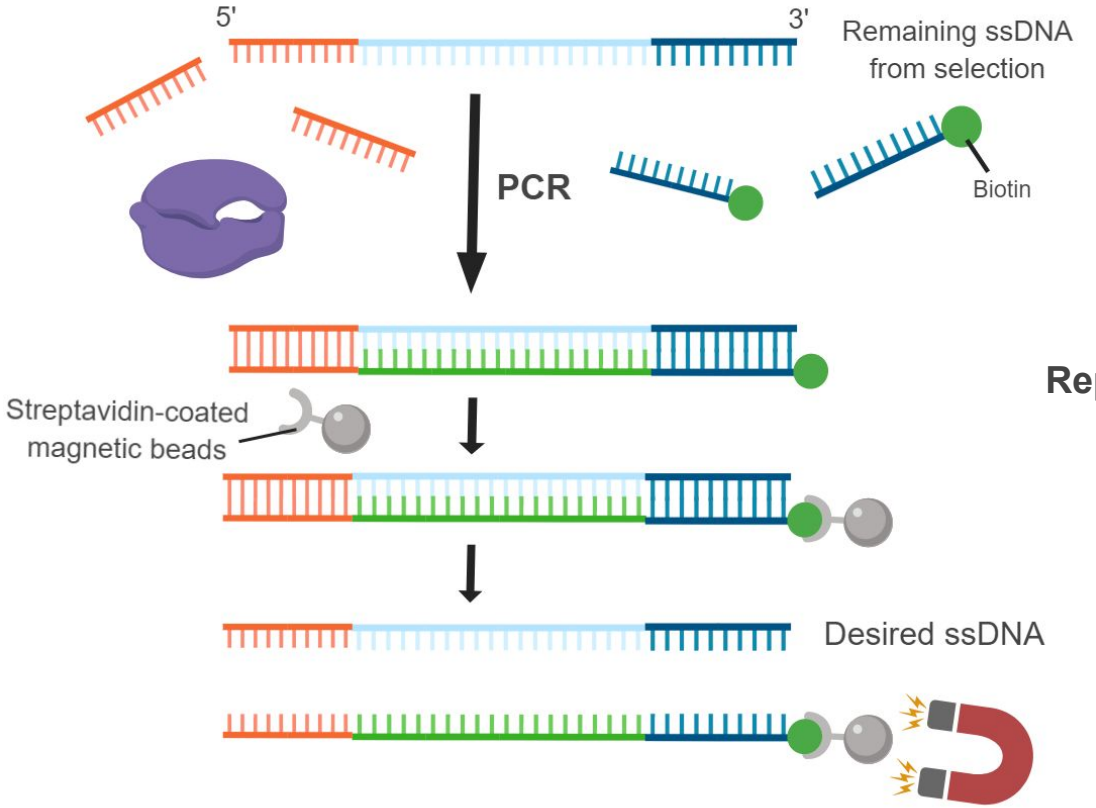
Marimithu et al. *The Analyst*, 2012, **137**, 6.

STEP THREE: Amplification



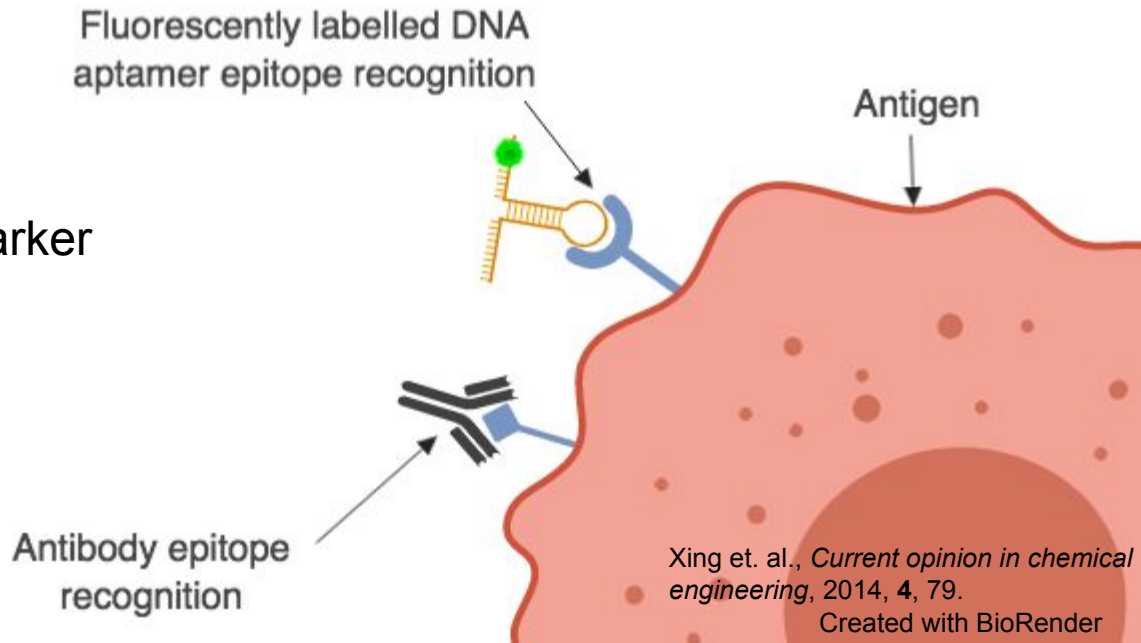
Repeated 10-15 times!

Adapted from: (Marimithu et al., 2012)
Created with BioRender

DNA Aptamers as Monoclonal Antibody Alternative

- Monoclonal Antibodies target and bind to antigen epitopes

- Diagnostic Use through Biomarker Identification

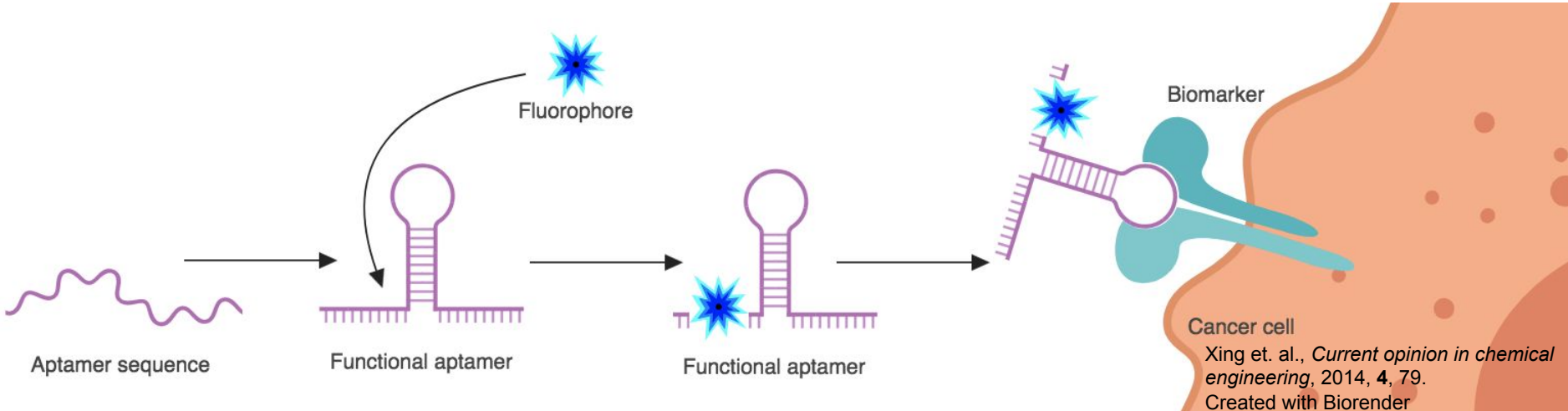


Xing et. al., *Current opinion in chemical engineering*, 2014, **4**, 79.
Created with BioRender

DNA Aptamer-based Techniques for Cancer Diagnosis

- Benefits
  - SELEX technique
  - Cancer cell identification **as early as possible**
  - DNA aptamer as a **diagnostic tool**

- As cancer probes:
  - Modified with **fluorophores** for imaging of tumours
  - Targets cancer cells and tumour-initiating cells

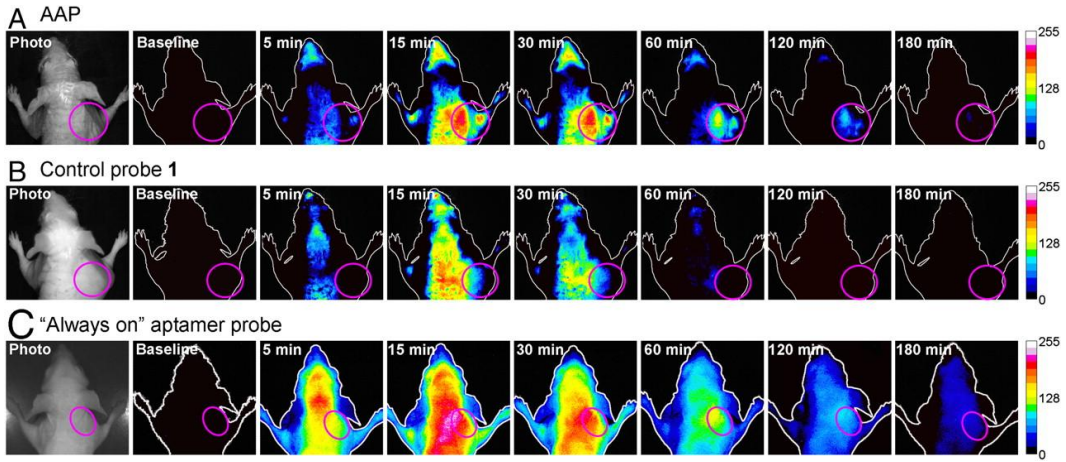


Xing et. al., *Current opinion in chemical engineering*, 2014, **4**, 79.
Created with Biorender

Aptamer-containing DNA as Cancer Probes

Activatable aptamer probe (AAP)

- Quenched fluorescence in its free-state
- Binding activates fluorescence
- Enhancement in medical imaging

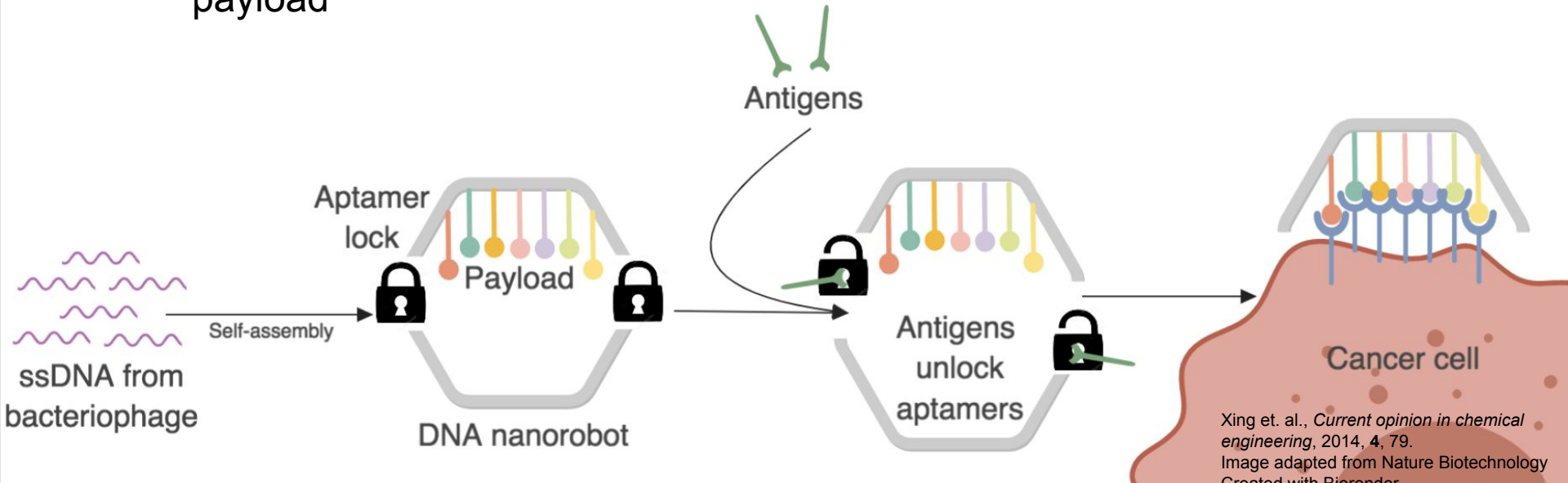


Xing et. al., *Current opinion in chemical engineering*, 2014, **4**, 79.
Image: Shi et al., *National Academy of Sciences*, 2011, **10**, 3900.

Aptamer-containing DNA Nanostructures as Cancer Probes

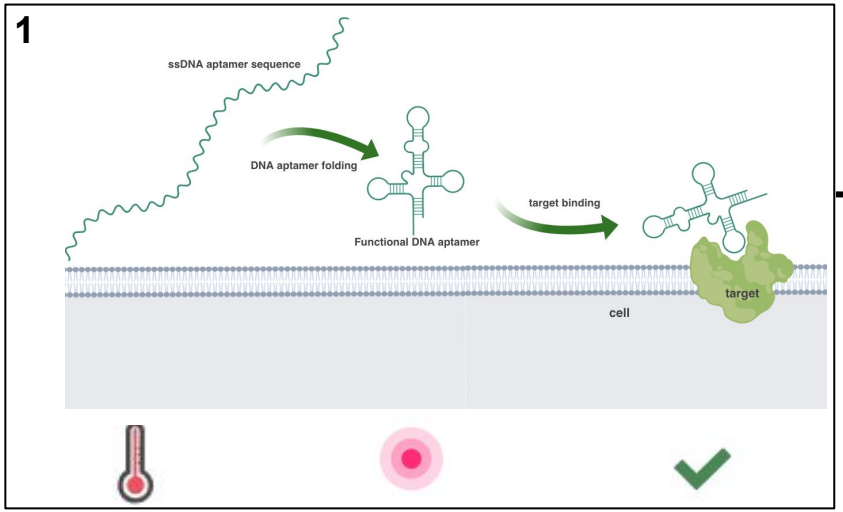
DNA-based nanorobot

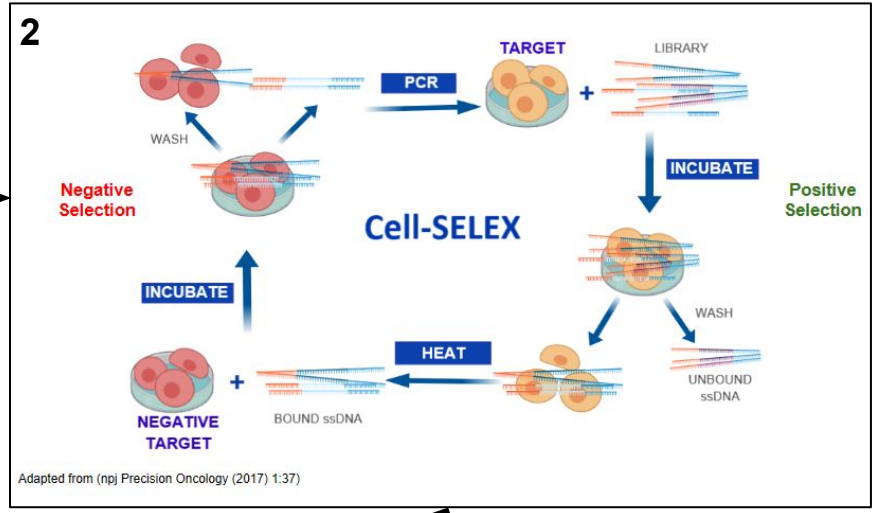
- Engineering nanostructure used to carry molecular cargo for cell-targeting
- Aptamer-encoded logic gate allows controlled release of biological payload

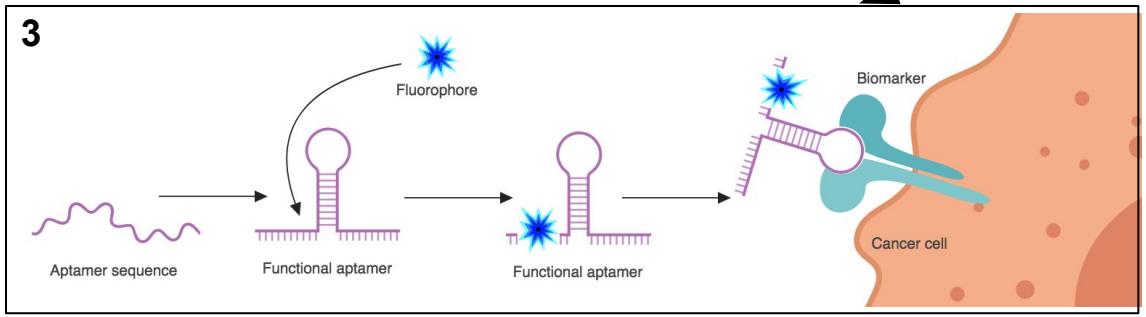


Xing et. al., *Current opinion in chemical engineering*, 2014, **4**, 79.
Image adapted from Nature Biotechnology

Summary


1
ssDNA aptamer sequence
DNA aptamer folding
Functional DNA aptamer
target binding
target
cell


2
TARGET
LIBRARY
PCR
WASH
INCUBATE
Negative Selection
Positive Selection
Cell-SELEX
INCUBATE
WASH
HEAT
UNBOUND ssDNA
NEGATIVE TARGET
BOUND ssDNA
Adapted from (npj Precision Oncology (2017) 1:37)


3
Fluorophore
Biomarker
Aptamer sequence
Functional aptamer
Functional aptamer
Cancer cell

Acknowledgements

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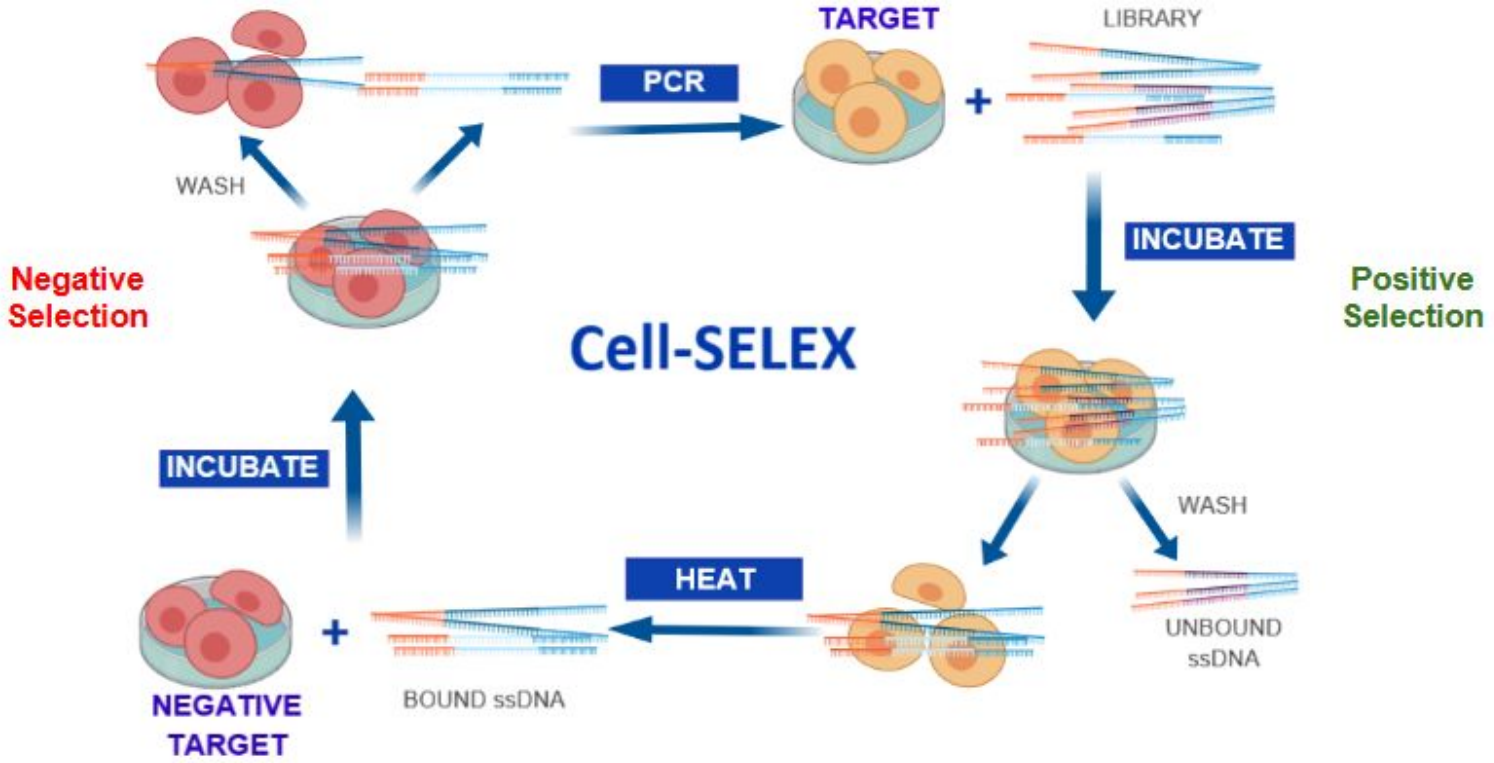
Systematic Evolution of Ligands by EXponential enrichment



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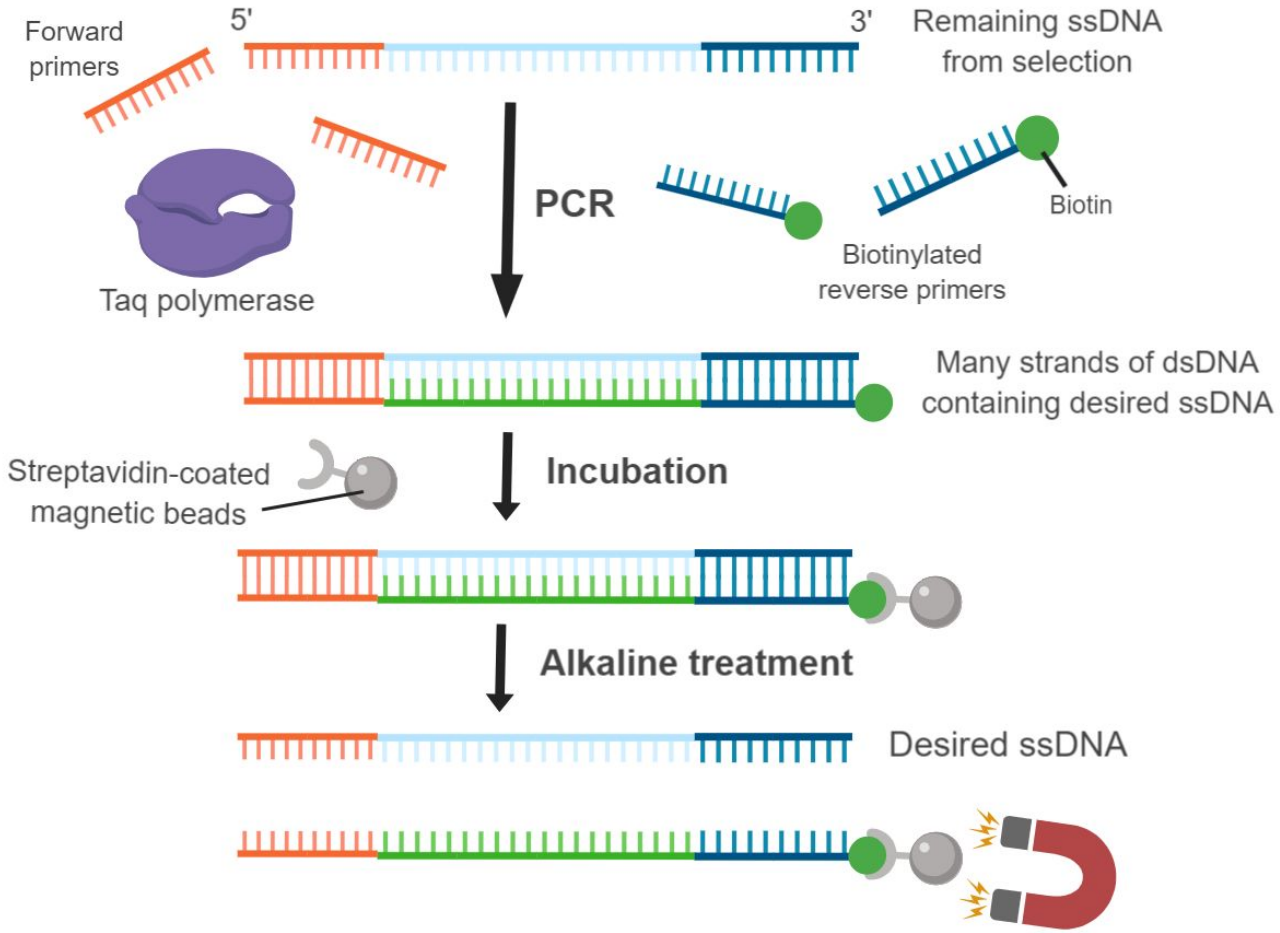
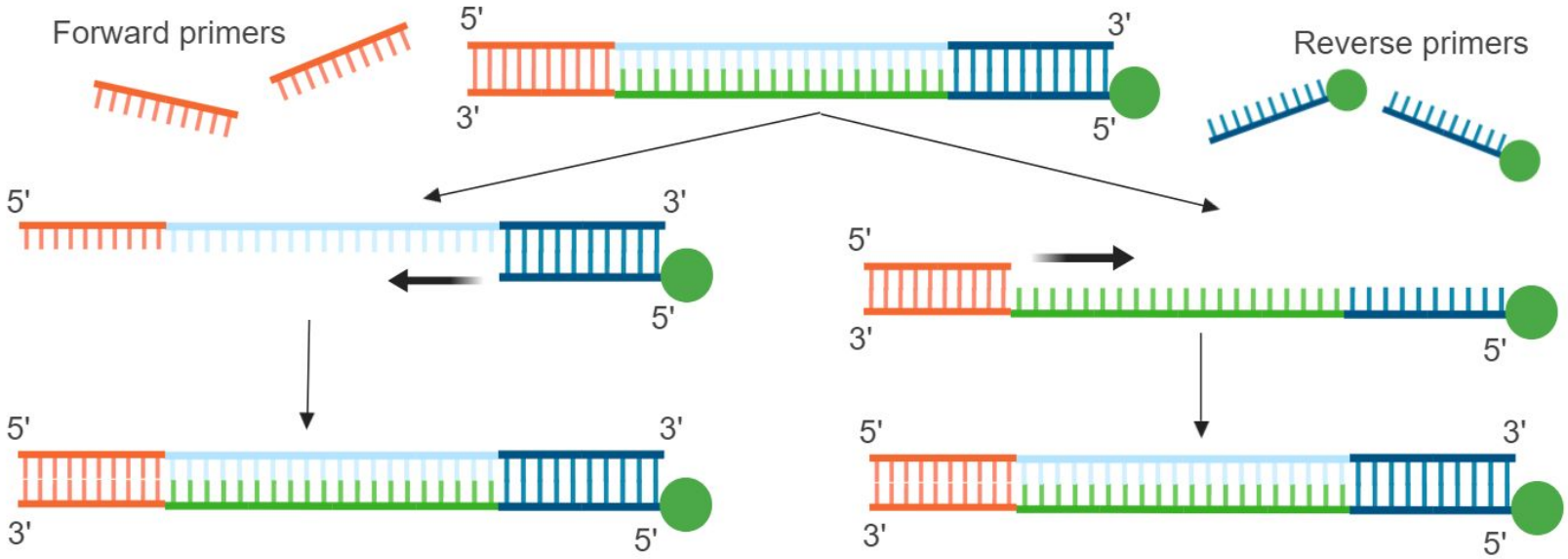

Forward primers
5'
3'
Remaining ssDNA from selection
Taq polymerase
PCR
Biotin
Biotinylated reverse primers
Many strands of dsDNA containing desired ssDNA
Streptavidin-coated magnetic beads
Incubation
Alkaline treatment
Desired ssDNA



Having only one biotinylated primer allows only that strand to be isolated by the magnet

Next steps

- Perform SELEX with oligonucleotide
- Modify 3’- and 5’- of aptamers
- Develop mirror aptamers (Spielgemer:) transform D-aptamer to L-aptamer (backbone with L-deoxyribose)
- Aptamer displacement screening: screen aptamers by comparing their binding affinity with low molecular weight compounds