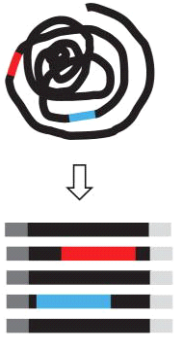
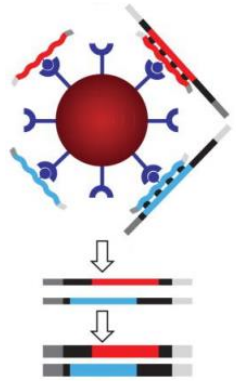


Target enrichment for plant/animal systematics - methodological workshop -



Wet lab work

Roswitha Schmickl, Tomáš Fér, Vojtěch Zeisek, Soňa Píšová

Dept. of Botany, Charles University, Prague

9th-17th June 2025

Hyb-Seq wetlab work

DNA extraction
Invitek Plant Mini Kit

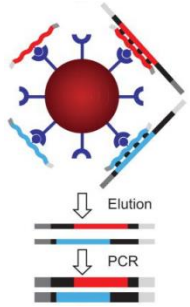
sonication
Covaris M220
1,000 bp

library preparation
NEBNext Ultra Library Kit
gel size selection 400-700

quantification
Qubit, BioAnalyser

libraries pooling
up to 24plex

solution hybridization
MYcroarray MYbaits
biotin-streptavidin capture



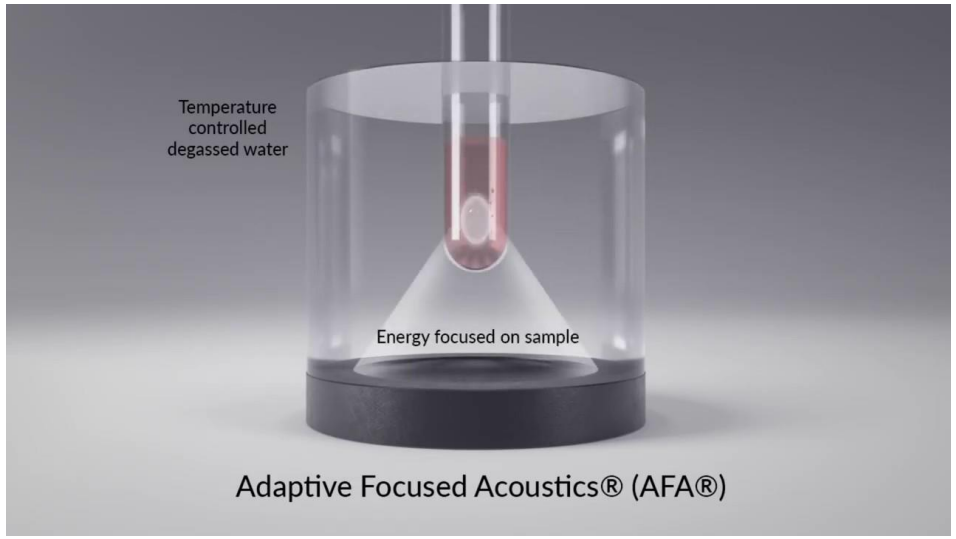
PCR enrichment
9-12 cycles

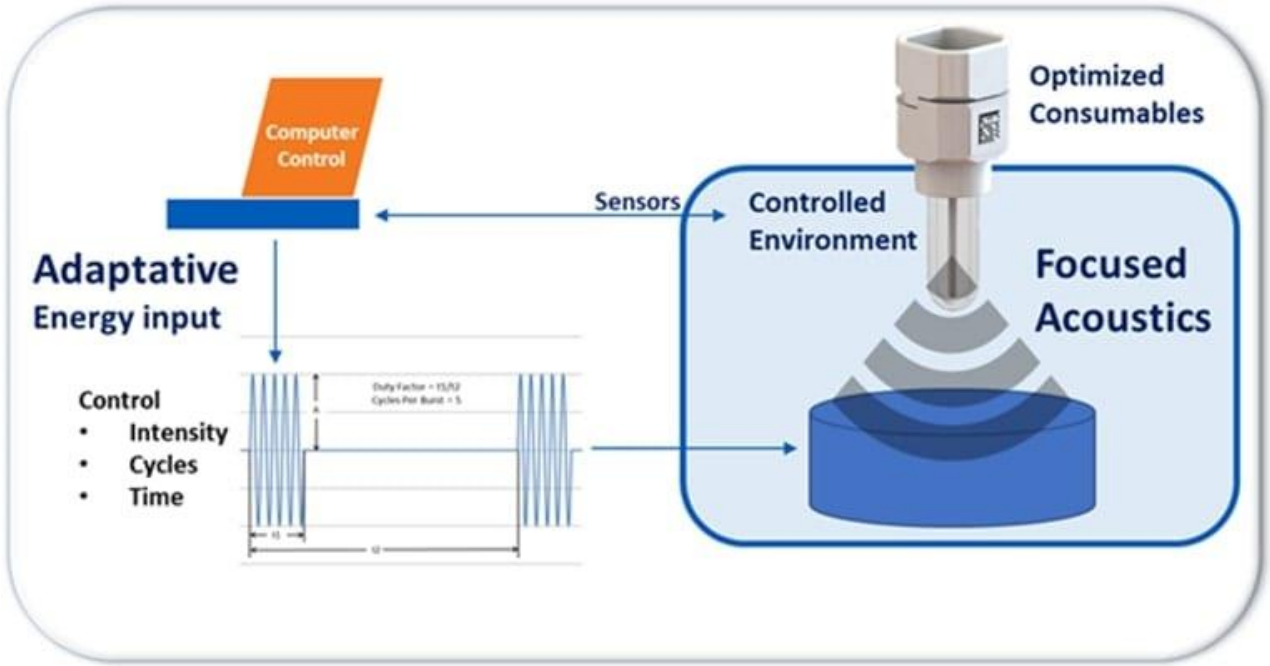
sequencing
Illumina NovaSeq 2x150
BaseSpace demultiplexing

					% reads mapped to		
	Reads	% quality trimmed reads	% duplicates	Reads without duplicates	exons	cpDNA	rDNA
MEAN per sample	1,535,205	5.04	10.20	1,297,644	39.80	1.95	0.13

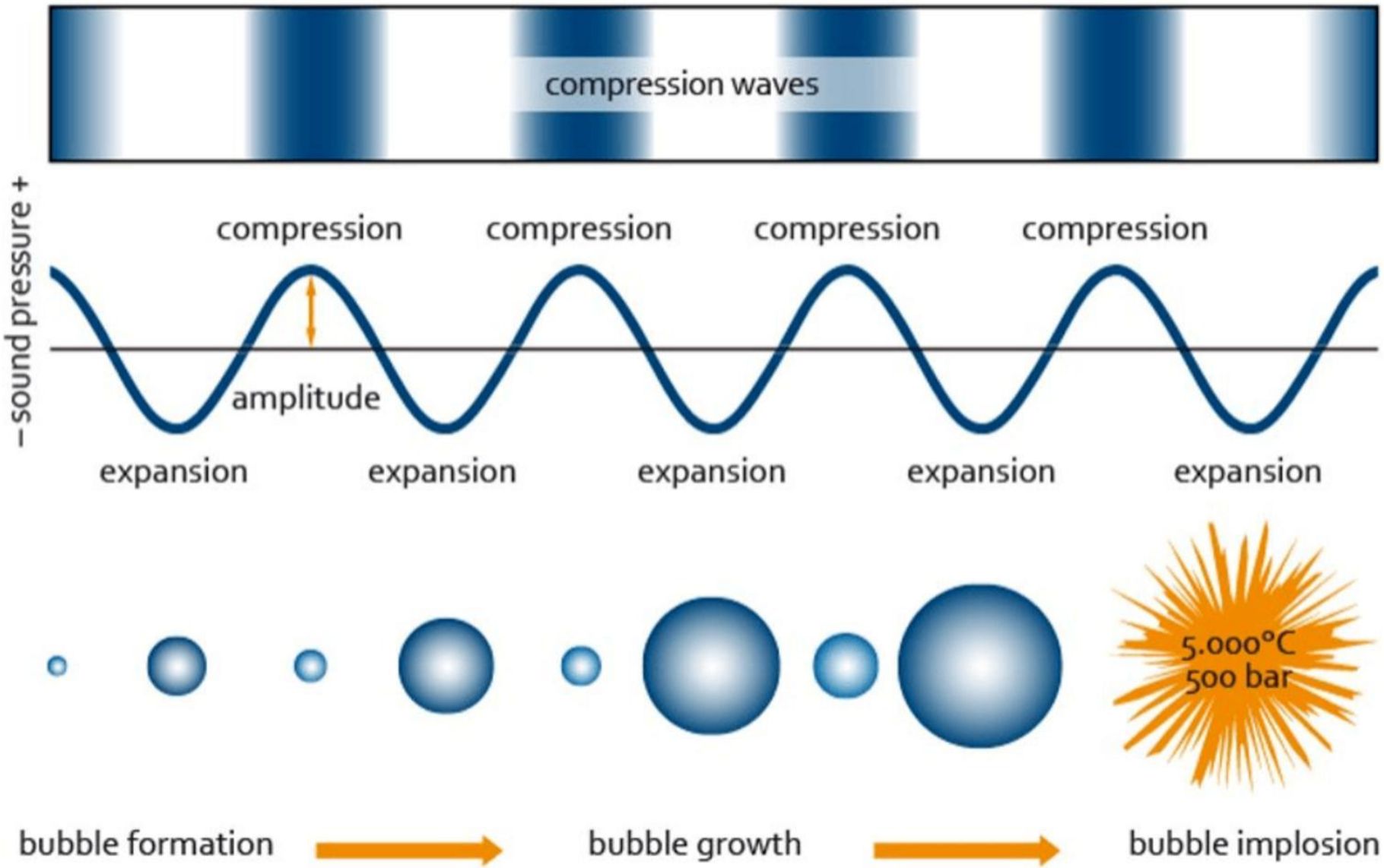
DNA fragmentation using sonication (Covaris M220)

DNA fragmentation using sonication (Covaris M220)

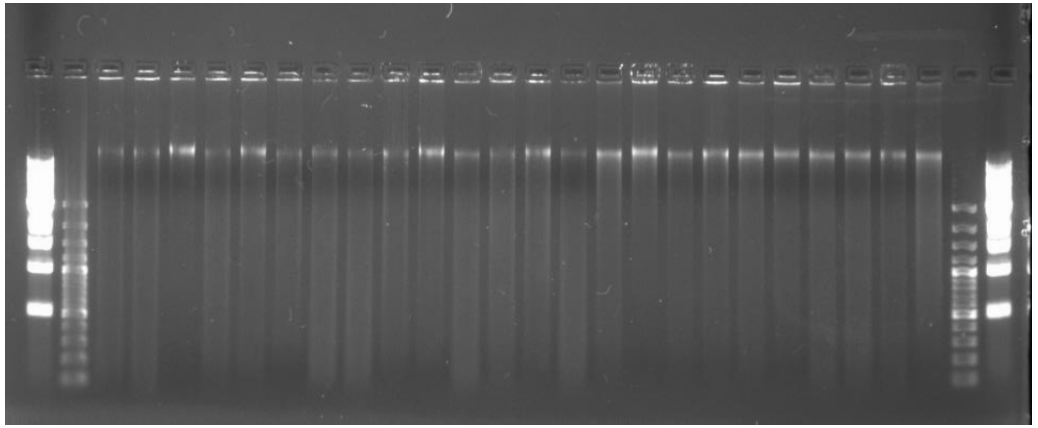




DNA fragmentation using sonication (Covaris M220)



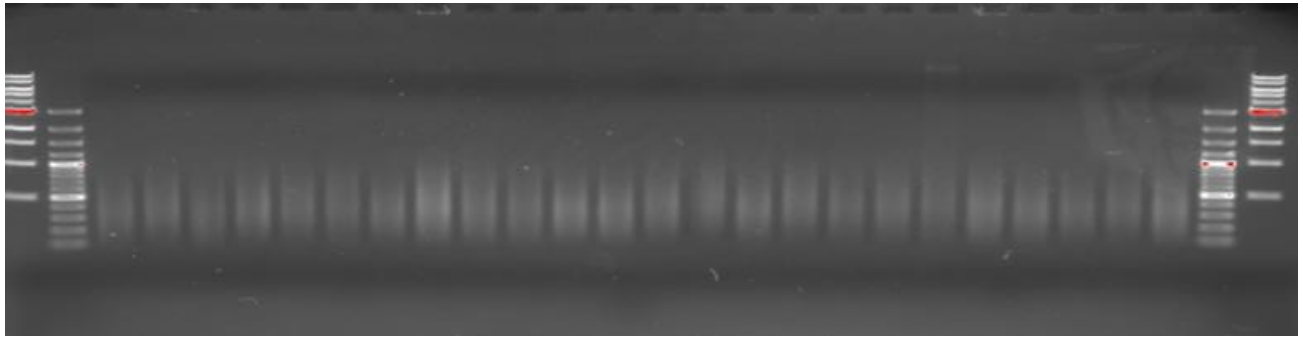
Before sonication



500 bp

After sonication

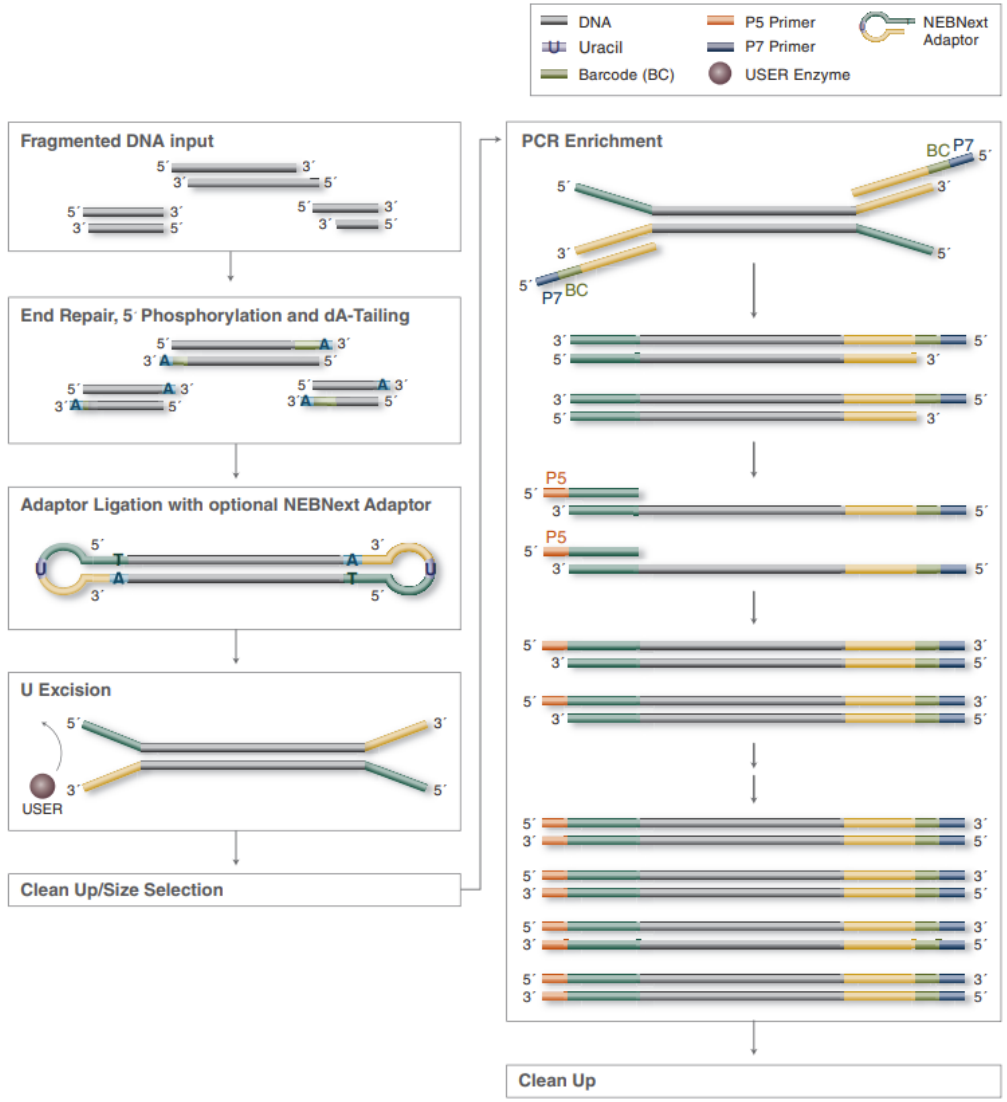
500 bp



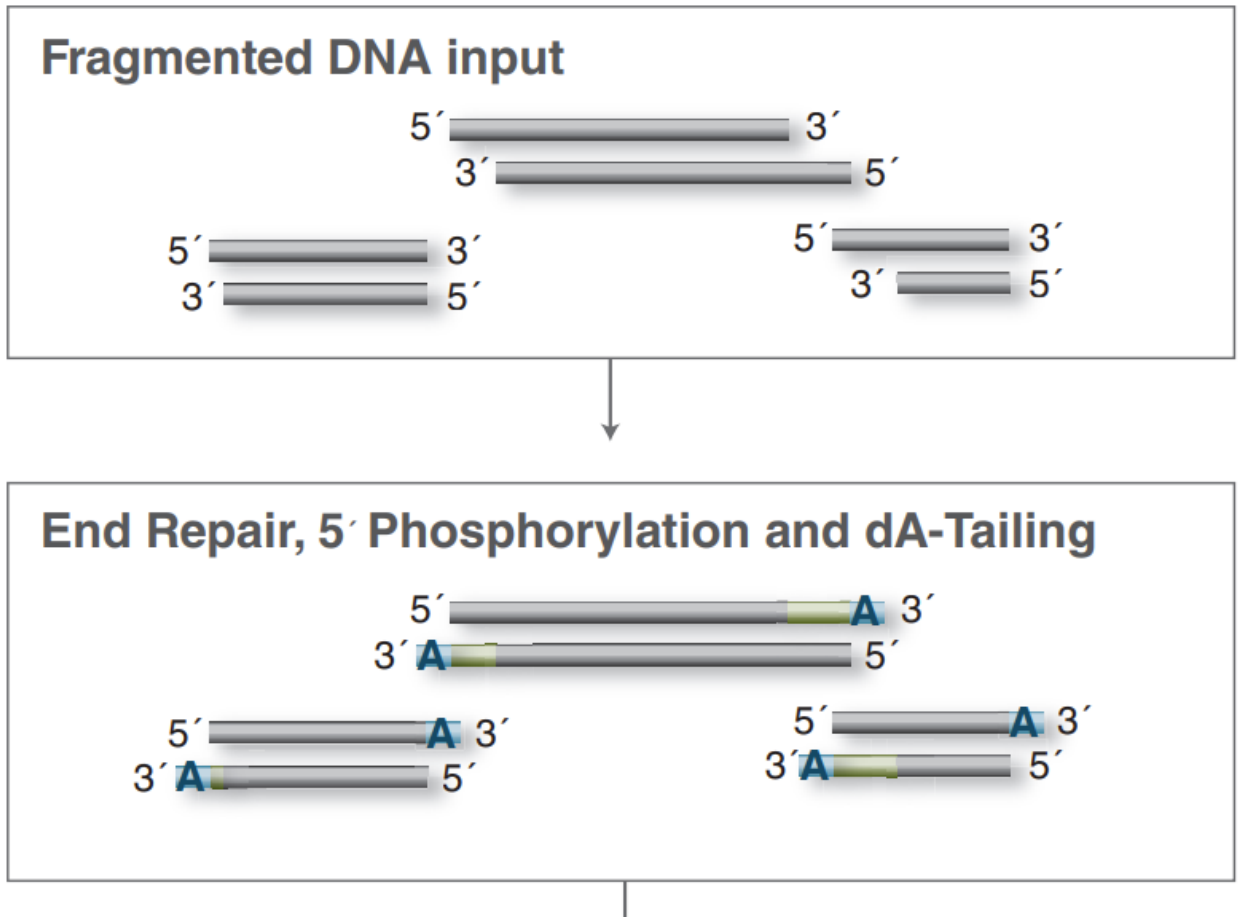
Library preparation – Illumina NEBNext II

- end repair
- adaptor ligation
- U excision
- clean up
- size selection
- PCR enrichment
- clean up

- continue with hybridization…

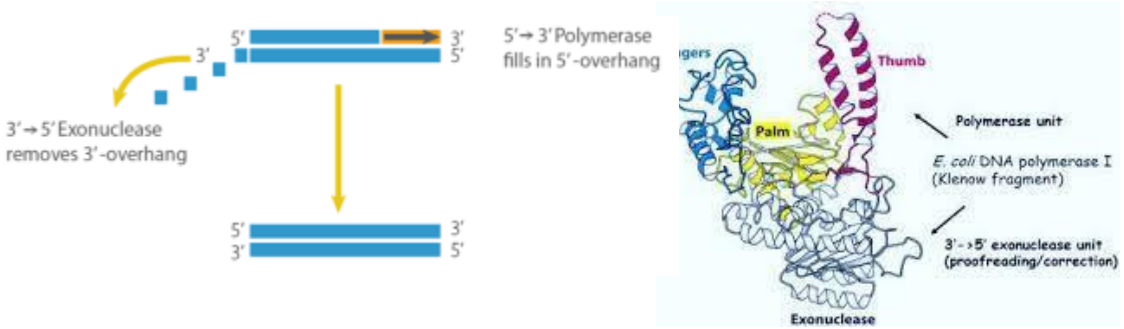


Library end repair

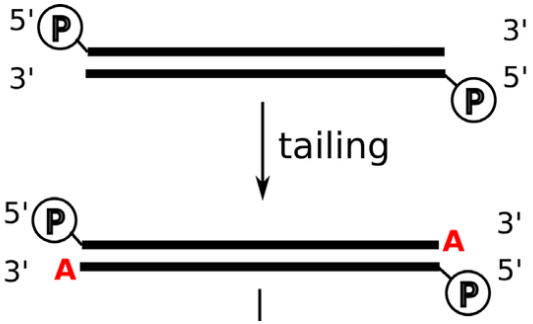


sticky ends after fragmentation

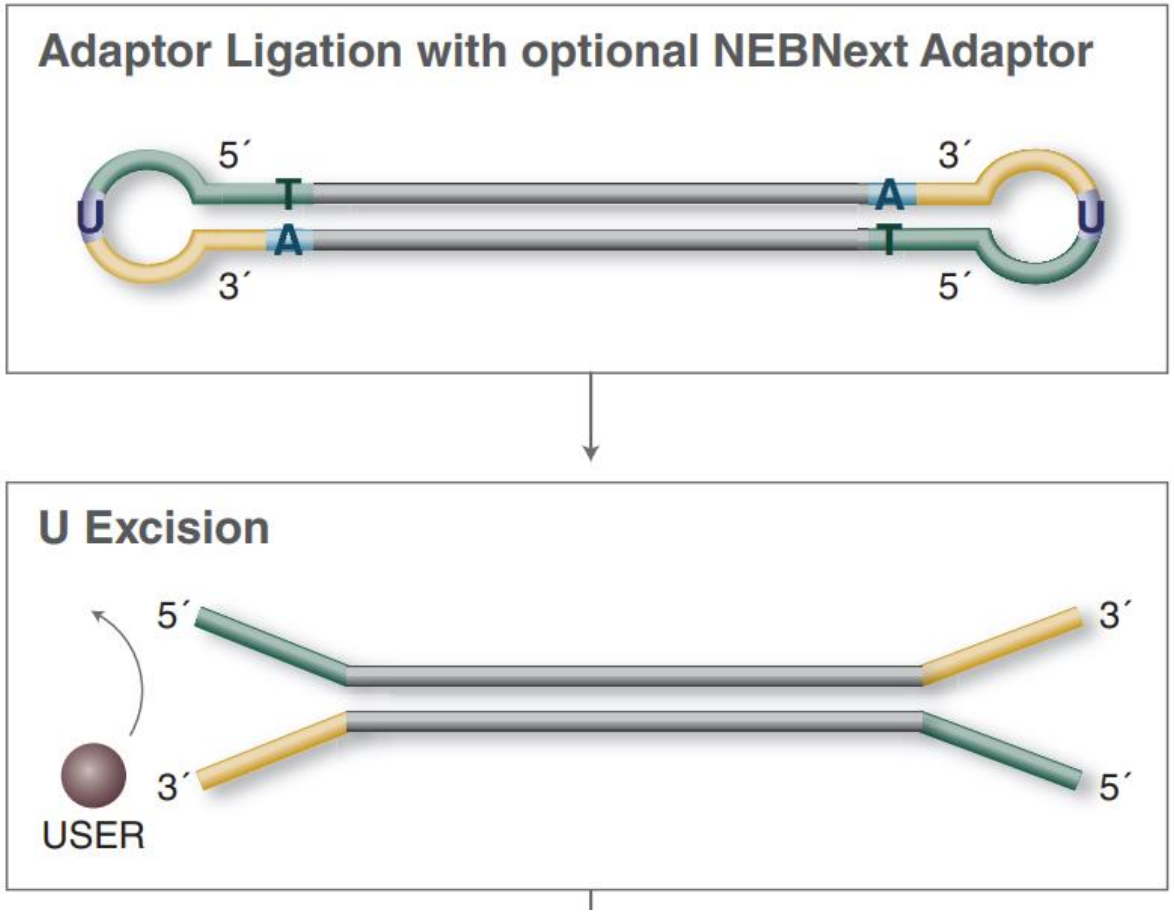
end repair (e.g., Klenow fragment of DNA polymerase I)

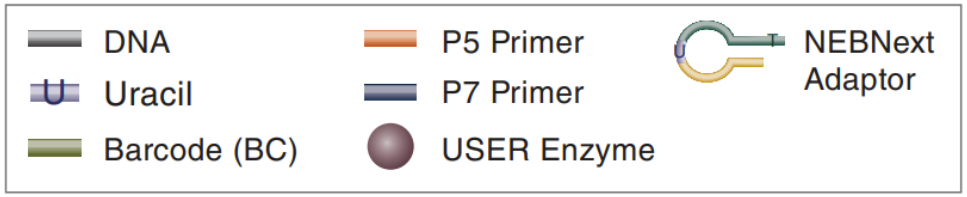


5' phosphorylation and A-tailing



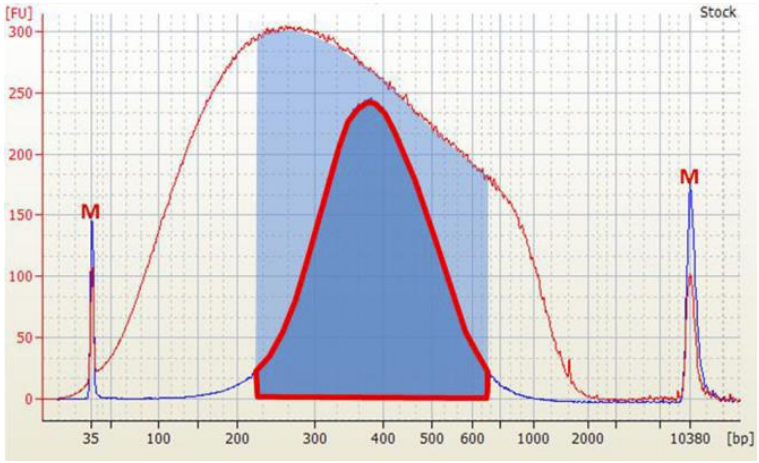
Library adaptor ligation & excision

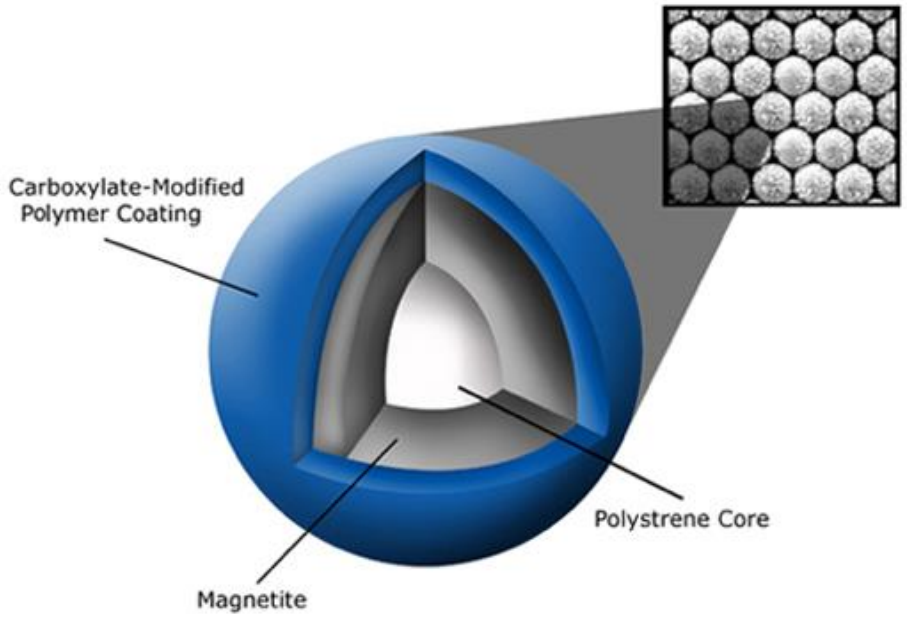


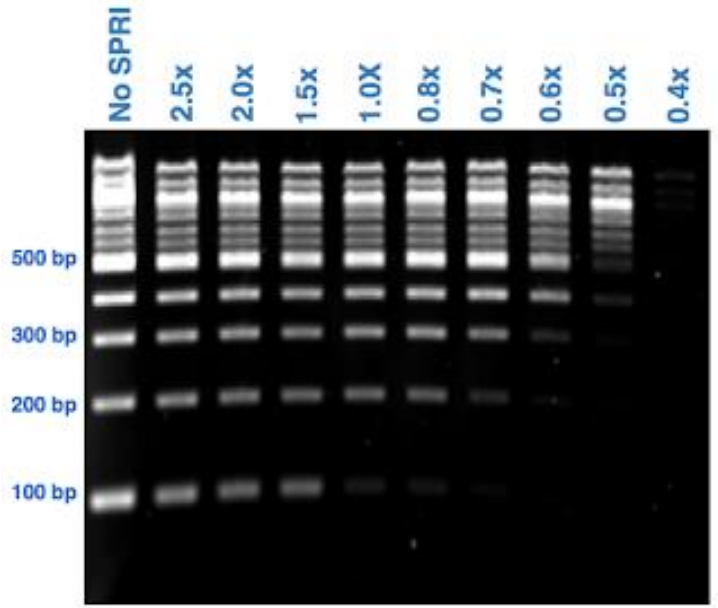


Library fragment size selection

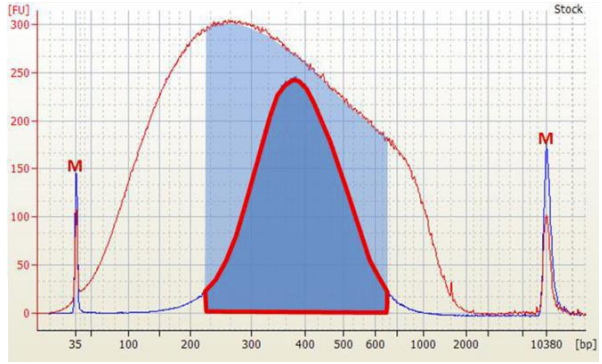
SPRI beads







Library fragment size selection



- By implementing a combination of good shearing with SPRI and "reverse" SPRI, one can select a fairly tight size range *with no gel*:

0.65X SPRI

Fragments >250 bp on beads
Fragments <250 bp in supernatant

KEEP SUPERNATANT
0-250 bp

→

DISCARD BEADS
"Reverse SPRI"

1.0X SPRI

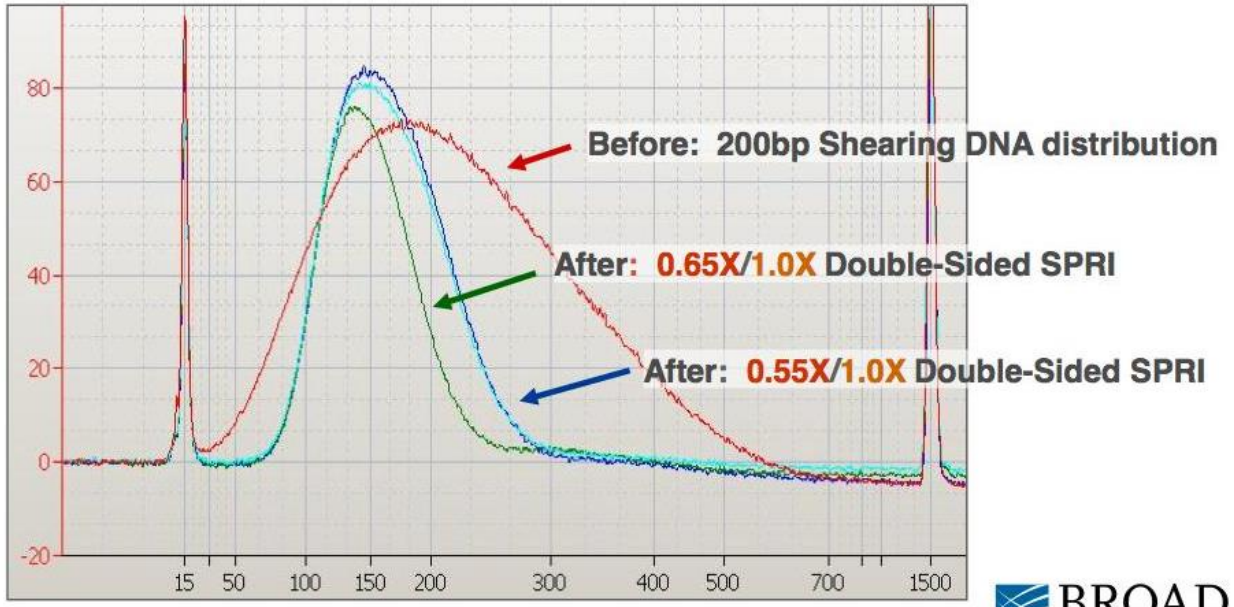
Fragments >100 bp on beads
Fragments <100 bp in supernatant

KEEP BEADS
100-250 bp

→

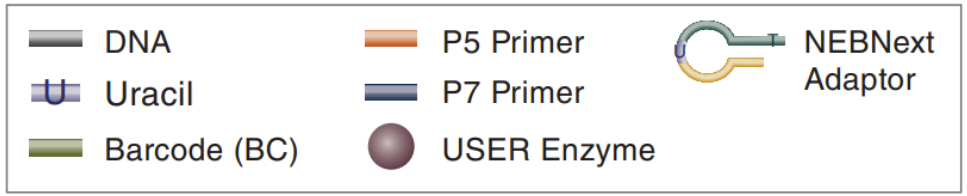
DISCARD SUPERNATANT

Results:

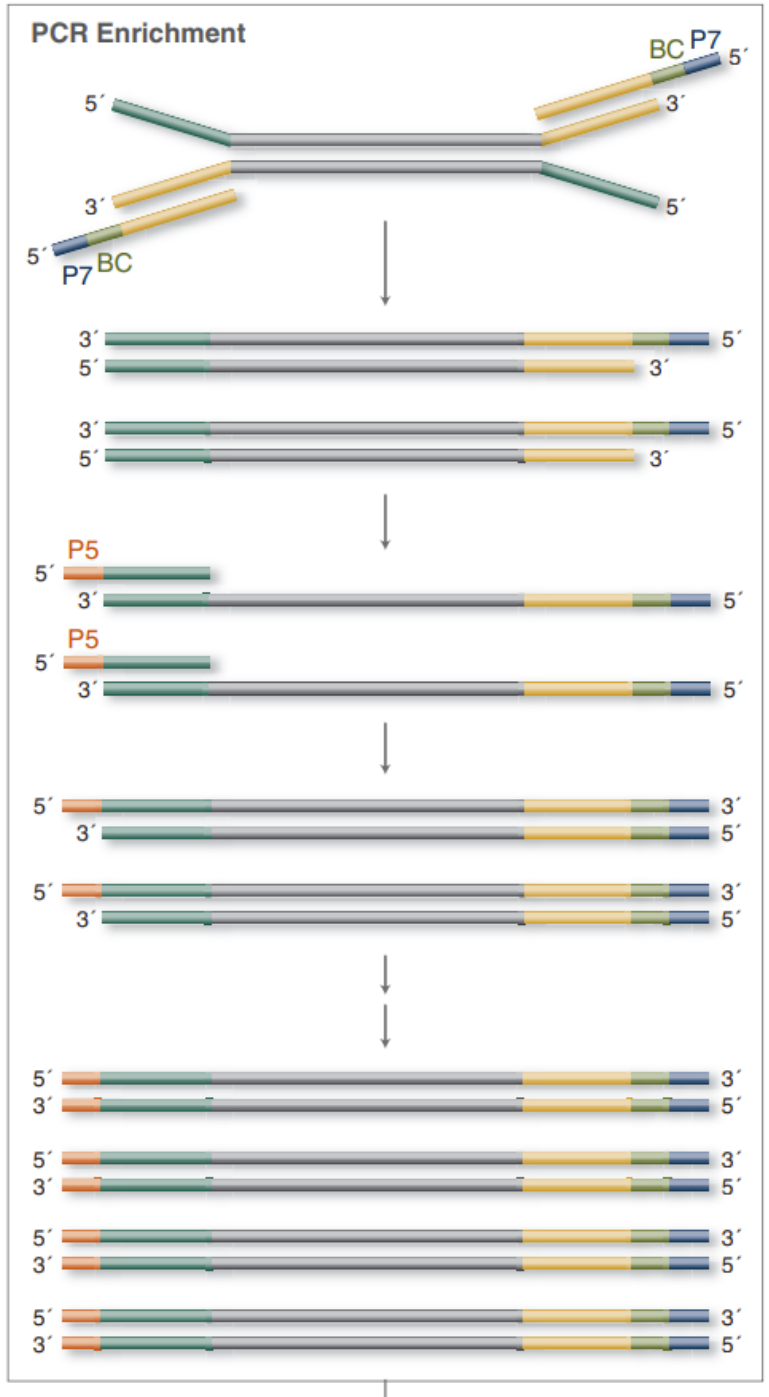


BROAD INSTITUTE illumina

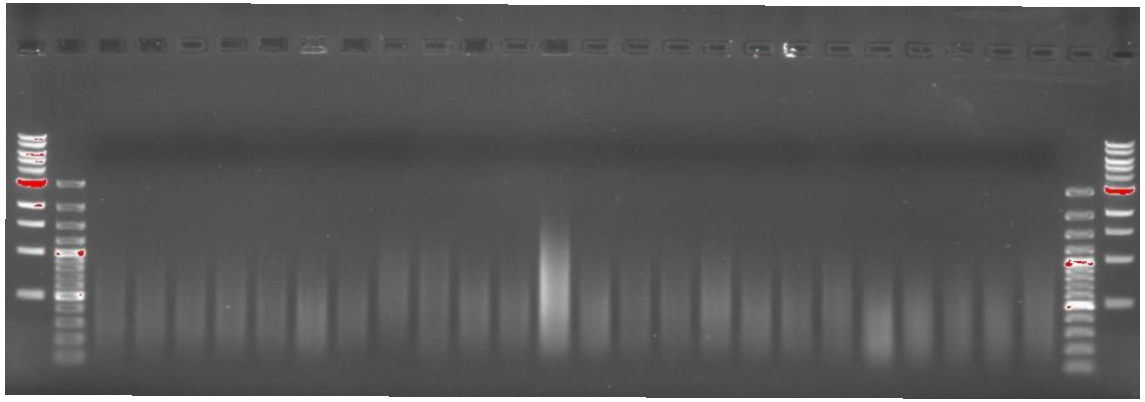
Library PCR enrichment



- to increase DNA amount
- minimize nr. of cycles
  (otherwise too many PCR duplicates)
- might introduce PCR errors
  (PCR-free protocols also exist)



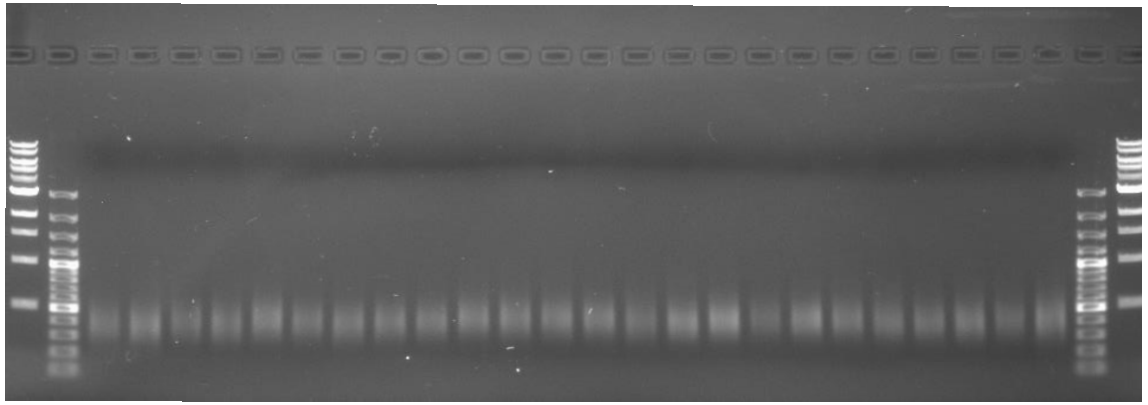
Before size selection



500 bp

After size selection

500 bp



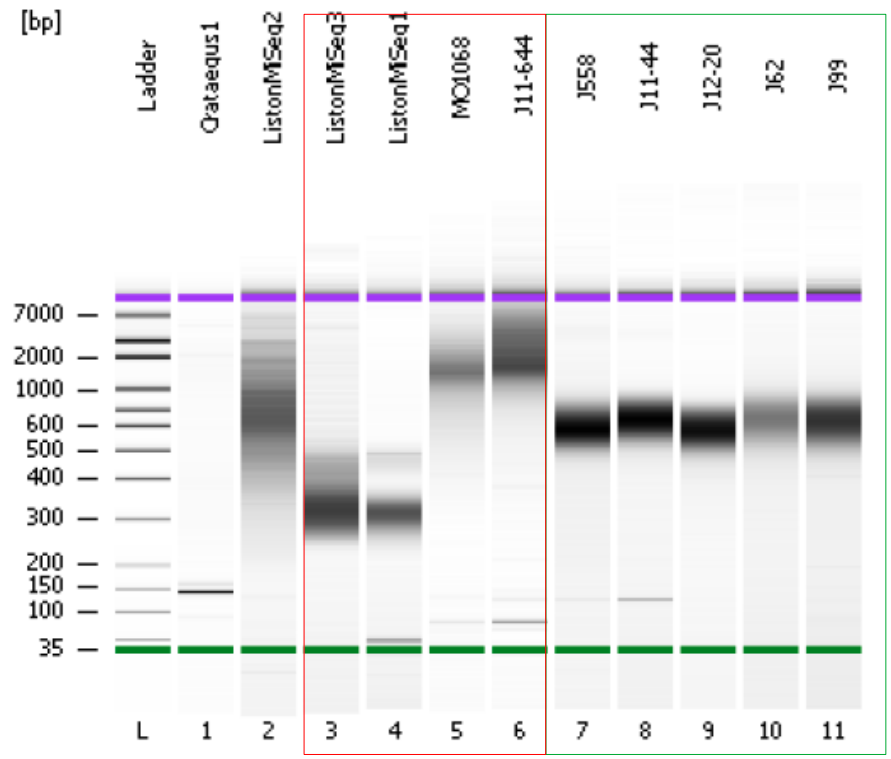
Final libraries (after enrichment PCR)

H2770_Schmickl_2014-05-12_12-06-28.xad Page 1 of 19

Assay Class: High Sensitivity DNA Assay
Data Path: C:\AgilentData\2014-05-12\H2770_Schmickl_2014-05-12_12-06-28.xad

Created: 5/12/2014 12:06:27 PM
Modified: 5/12/2014 12:47:57 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name:	DE24802282	Firmware:	C.01.069
Serial#:	DE24802282	Type:	G2938B

Assay Information:

Assay Origin Path:	C:\Program Files\Agilent\2100 bioanalyzer\2100 expert\assays\dsDNA\High Sensitivity DNA.xsy
Assay Class:	High Sensitivity DNA Assay
Version:	1.03
Assay Comments:	Copyright © 2003-2010 Agilent Technologies

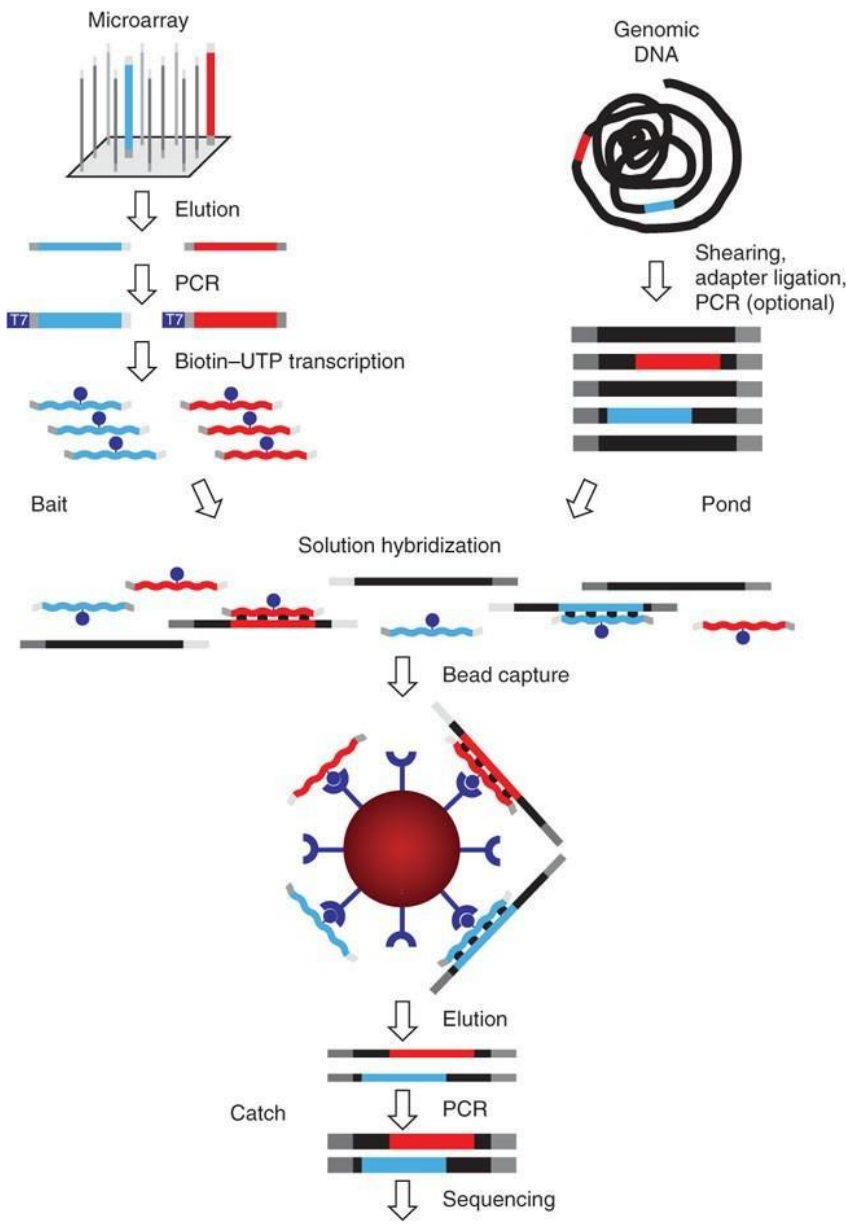
Chip Information:

Chip Lot #:	sa28bk50
Reagent Kit Lot #:	set a
Chip Comments:	HS-DNA | High Sensitivity DNA | Roswitha Schmickl | Jan Suda | 420 271 015 490 | roswitha.schmickl@ibot.cas.cz | Plants | Leaves |

Why are these bad Libraries?

Why are these good Libraries?

Summary of library preparation and bait hybridization

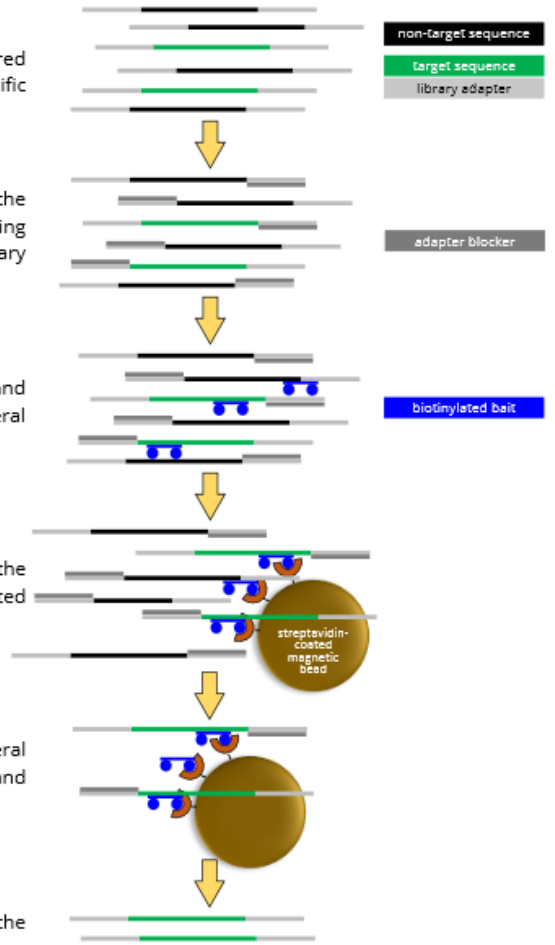


Summary of bait hybridization (Arbor Biosciences)

INTRODUCTION

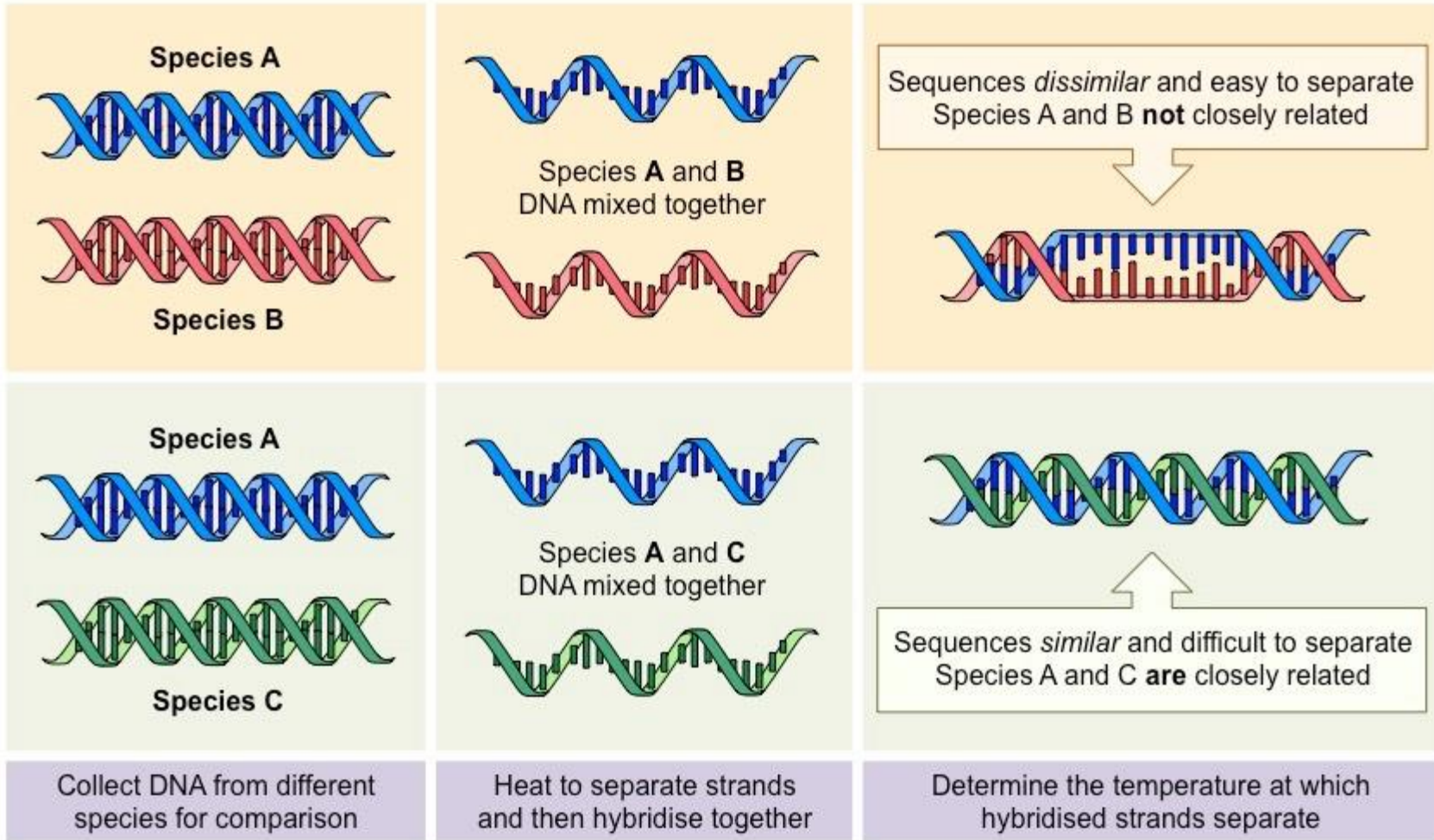
MYbaits® is a fully customizable in-solution DNA capture (targeted enrichment) system. We use our versatile DNA synthesis technology to make oligonucleotides complementary to your specific sequence targets of interest. We then transcribe these oligos into biotinylated RNAs, generating "baits." The MYbaits® kit procedure is similar to Gnirke *et al.* 2009 (doi: 10.1038/nbt.1523) and can be divided into six main steps:

1) DNA sequencing library is heat-denatured in the presence of adapter-specific blocking oligonucleotides
2) Library and blockers are dropped to the hybridization temperature, allowing blockers to hybridize to the library adapters
3) Biotinylated RNA baits are introduced and allowed to hybridize to targets for several hours
4) Bait-target hybrids are pulled out of the solution with streptavidin-coated magnetic beads
5) Beads are stringently washed several times to remove non-hybridized and nonspecifically-hybridized molecules
6) Captured DNA library is released from the beads and amplified

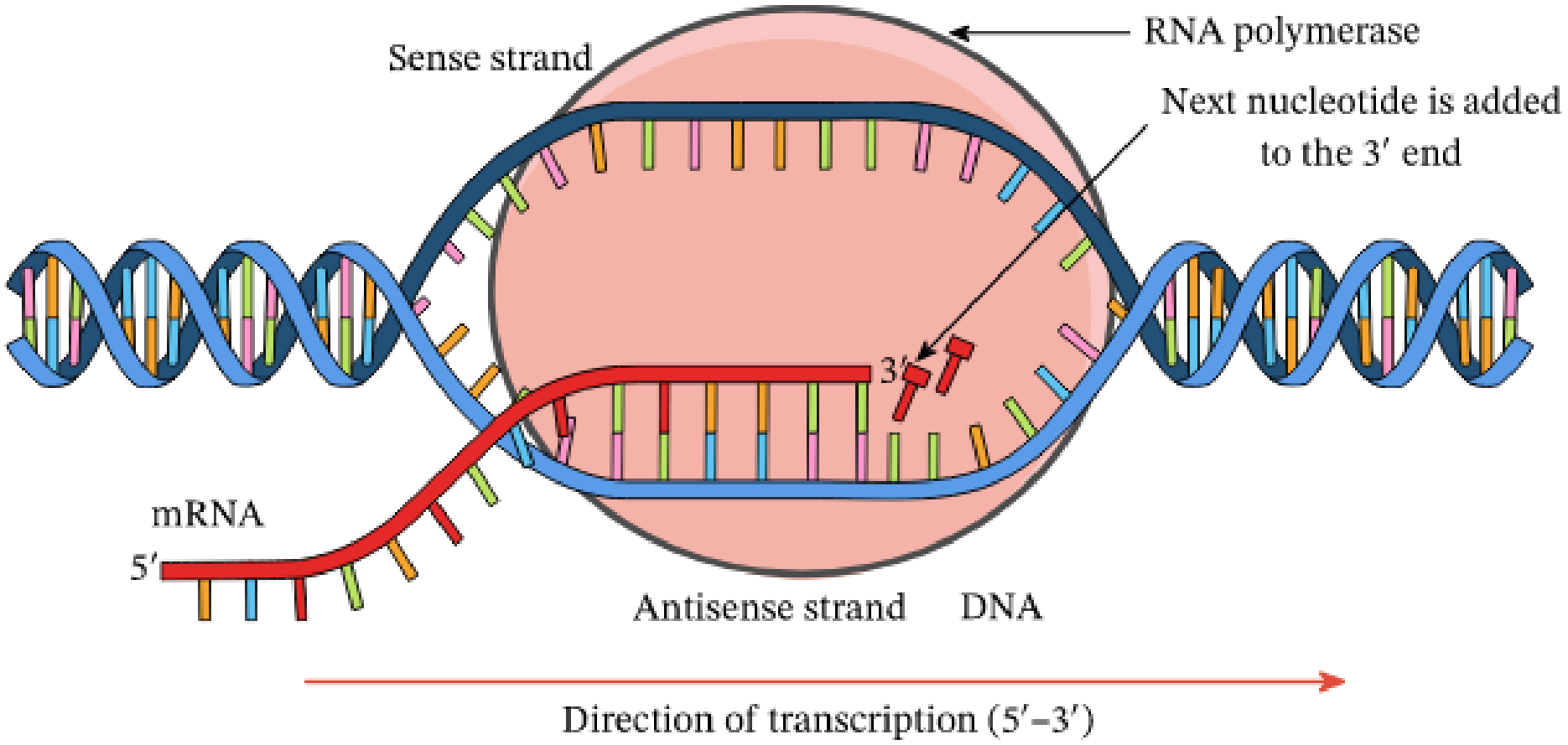


Summary of bait hybridization

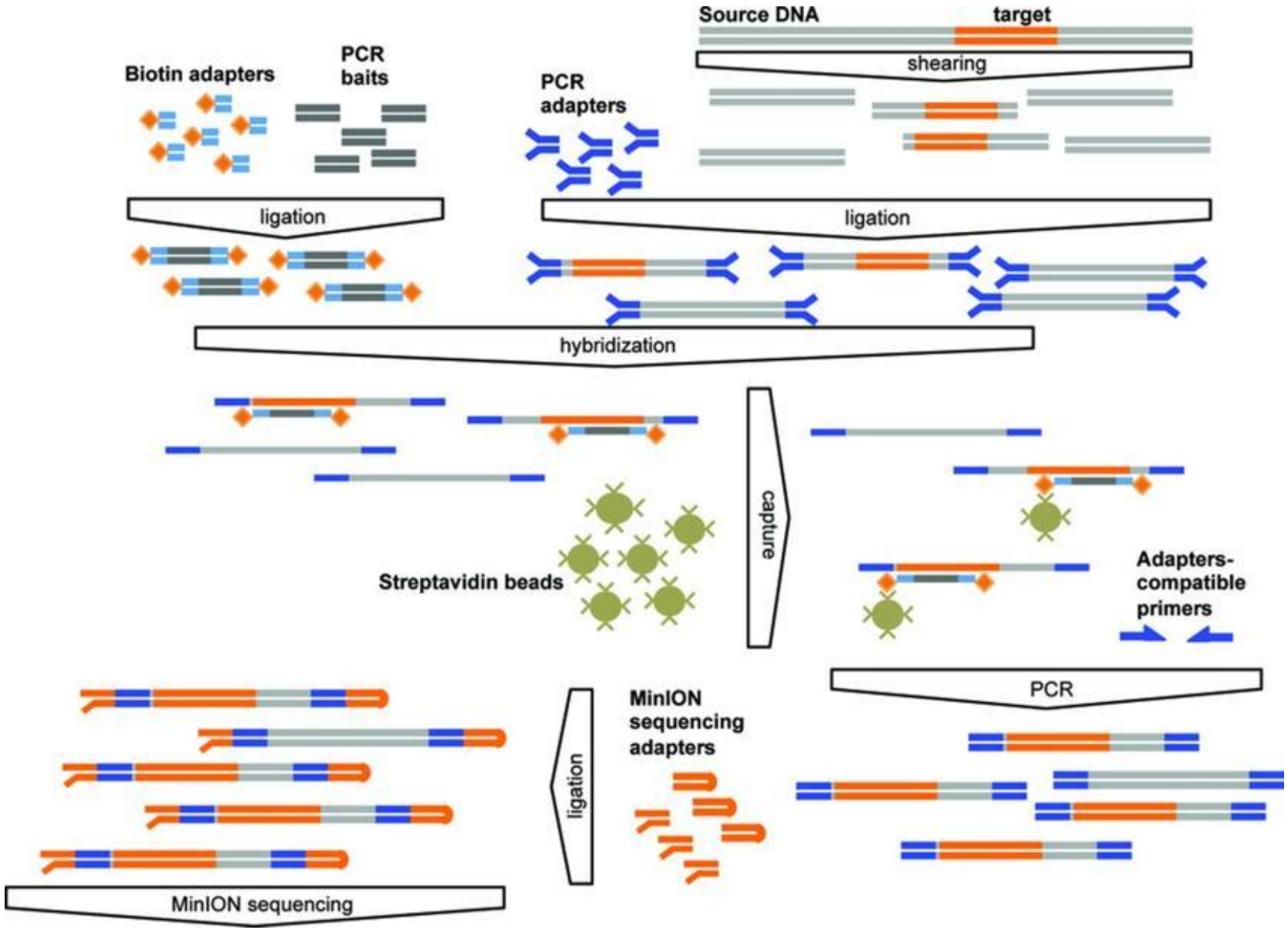
Hybridization between two DNA (or RNA) strands



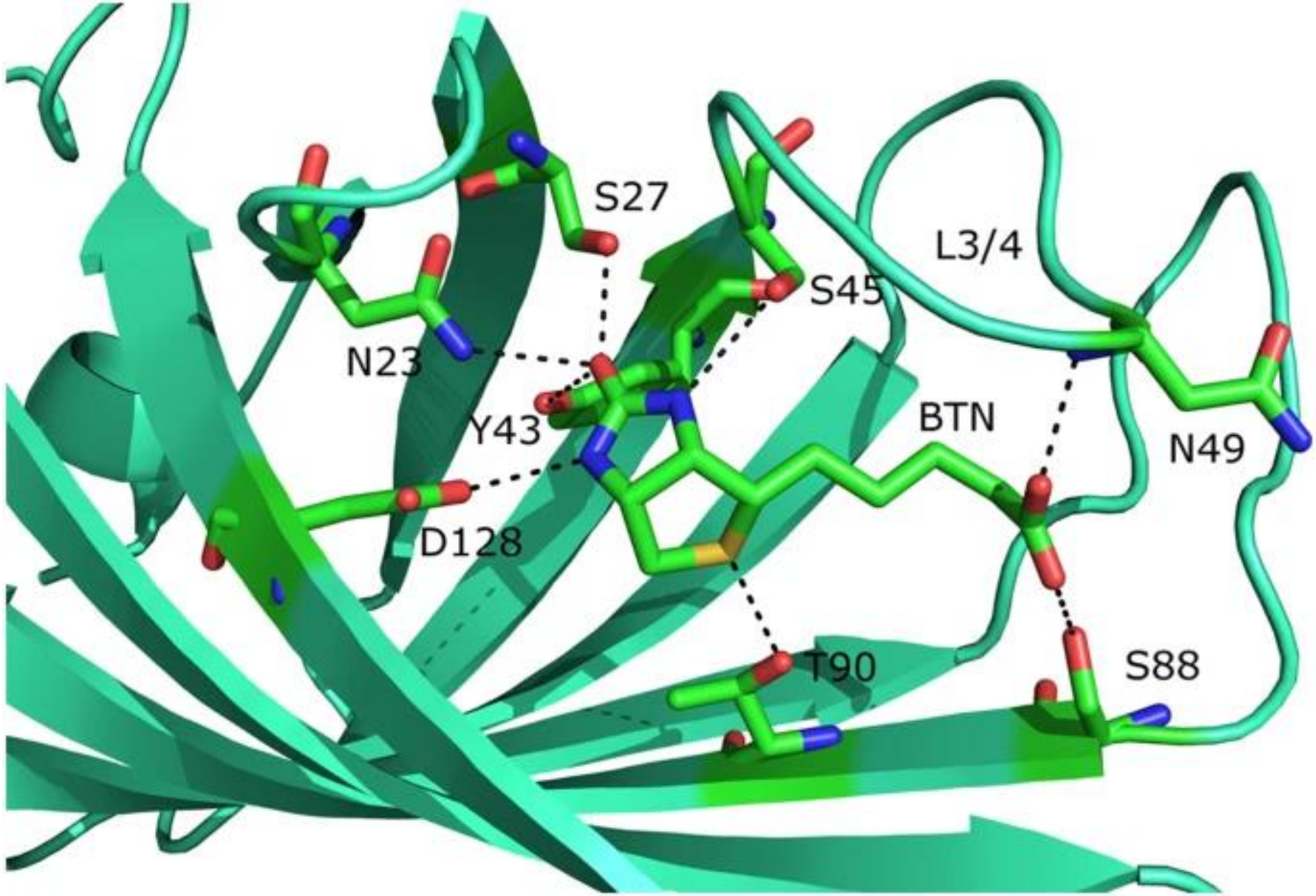
DNA-RNA binding is stronger than DNA-DNA binding



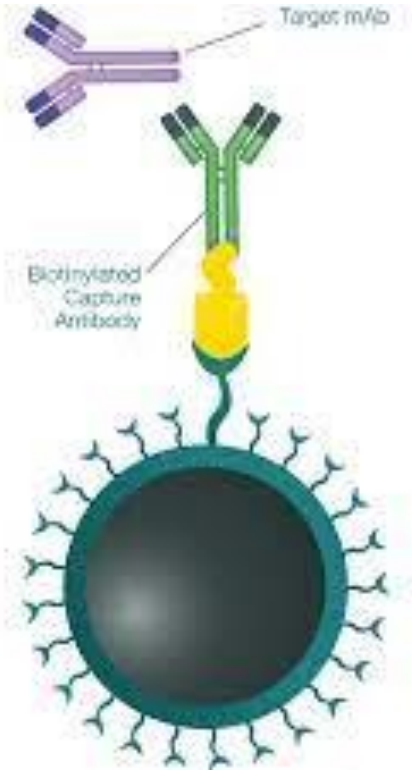
The actual step of „sequence capture“

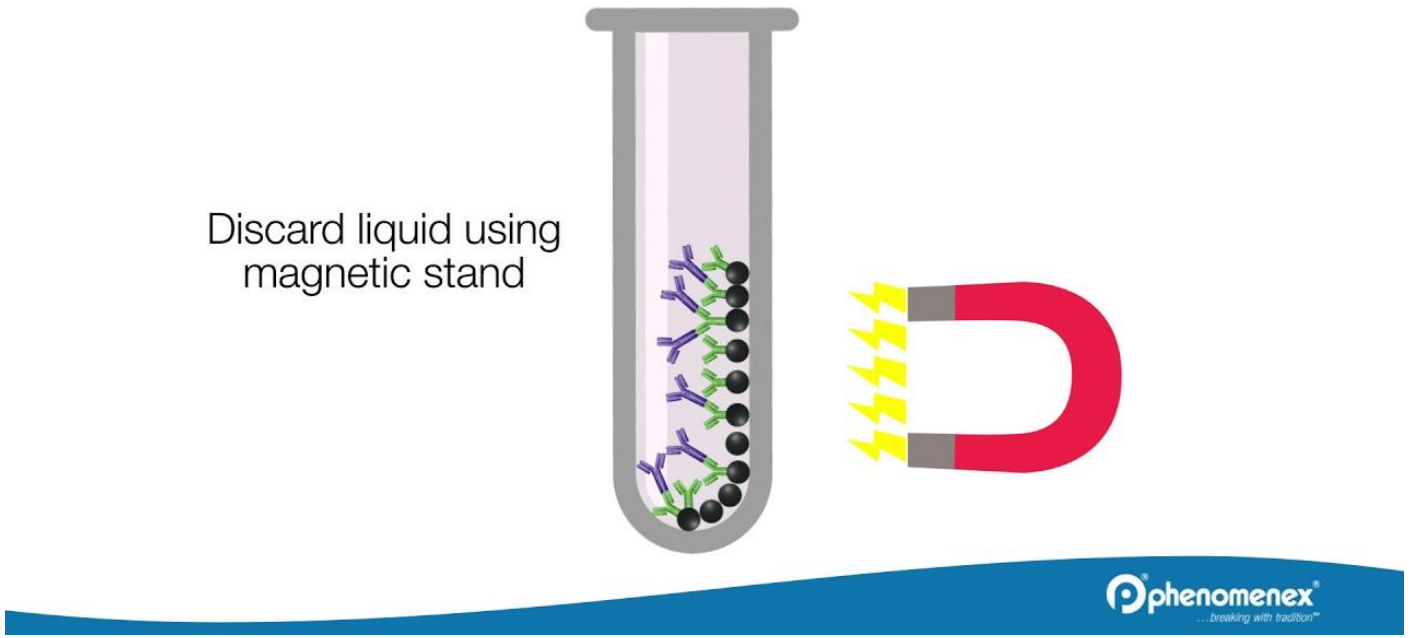


Streptavidin – Biotin interaction

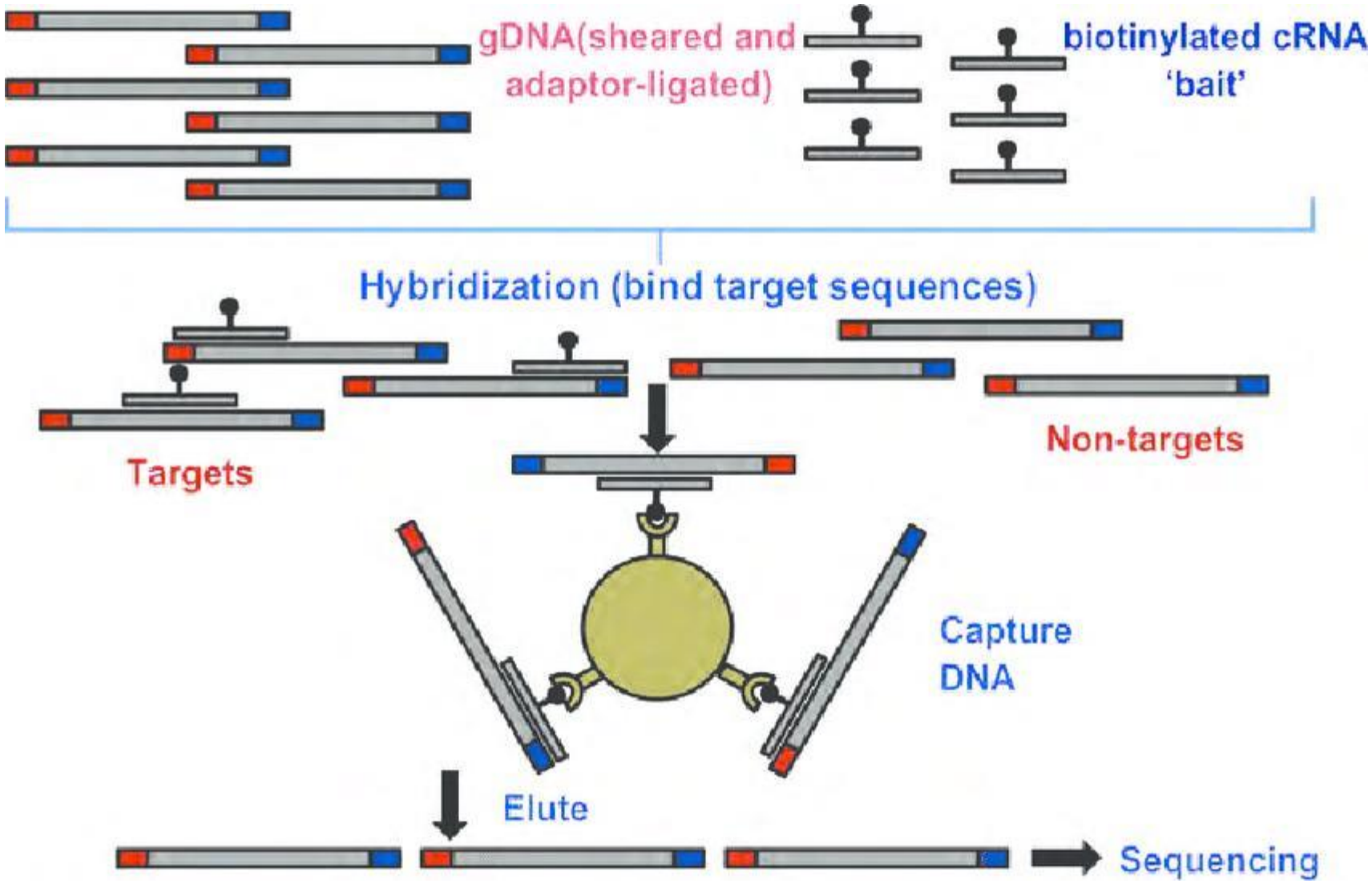


Streptavidin – Biotin interaction and magnetic bead capture





Streptavidin – Biotin interaction is used for sequence capture



Final library PCR enrichment

- to increase DNA amount
- minimize nr. of cycles
  (otherwise too many PCR duplicates)
- might introduce PCR errors
  (PCR-free protocols also exist)