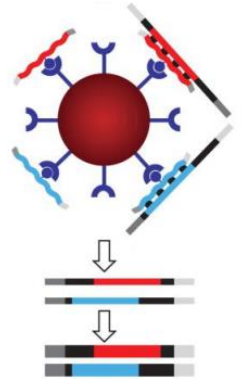


Target enrichment principle

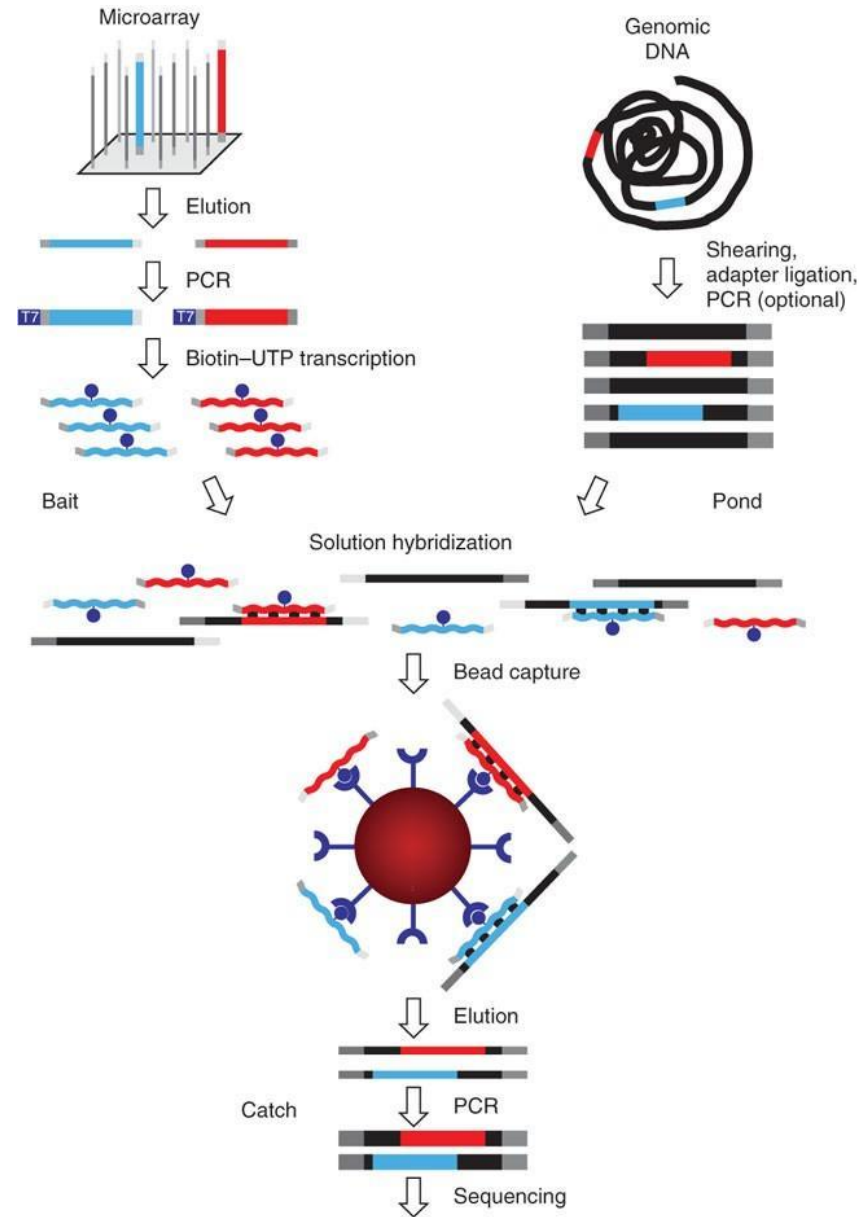


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Summary of library preparation and bait hybridization



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

Prepped Library Fragments



Blocked Library Fragments



Hybridized Library Fragments



Bead-Bound Target Sequences



Universal Blockers and COT-1







Twist DNA Probes

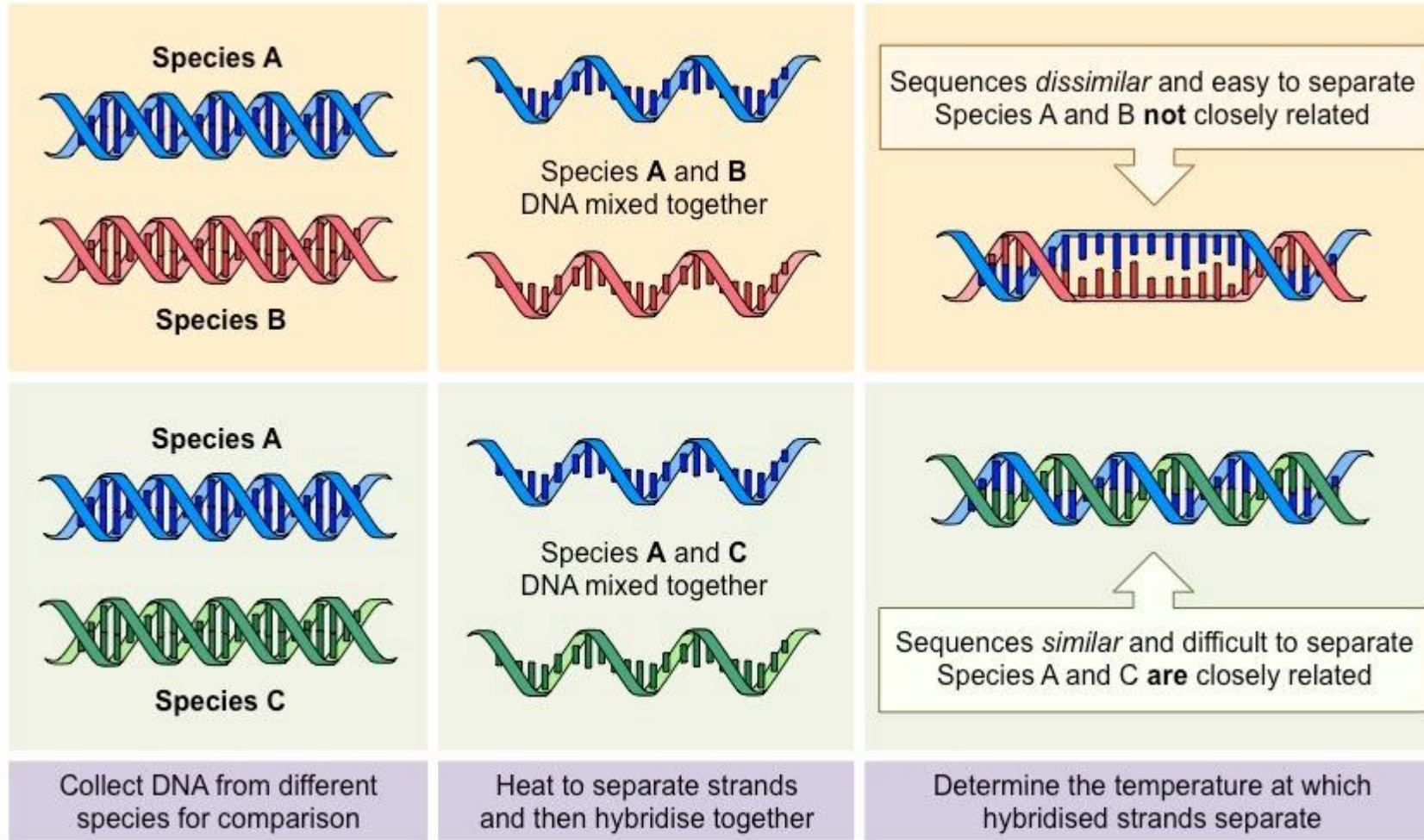


Streptavidin Beads

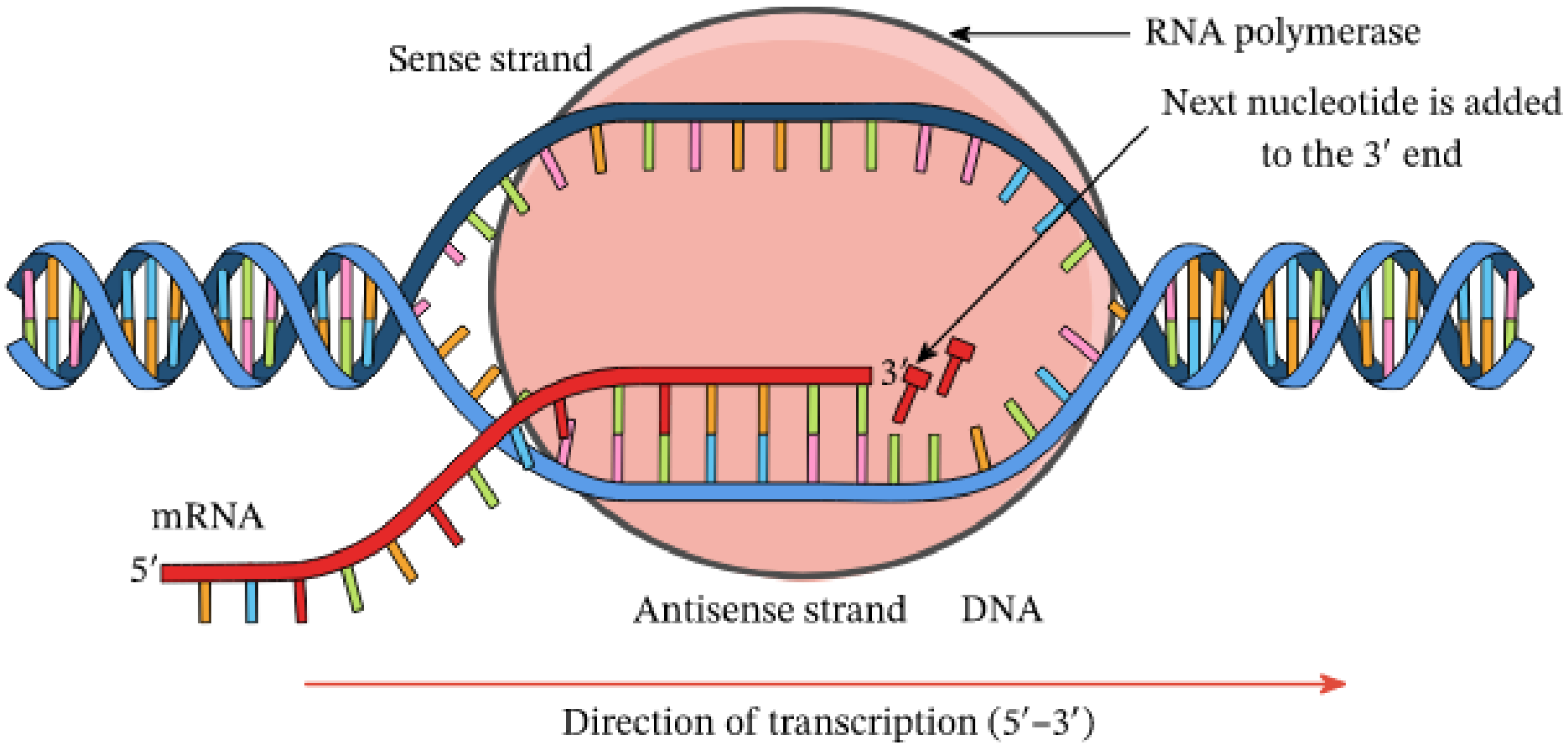


-  Sense Strand
-  Anti-Sense Strand
-  Dual Adapters
-  Biotin

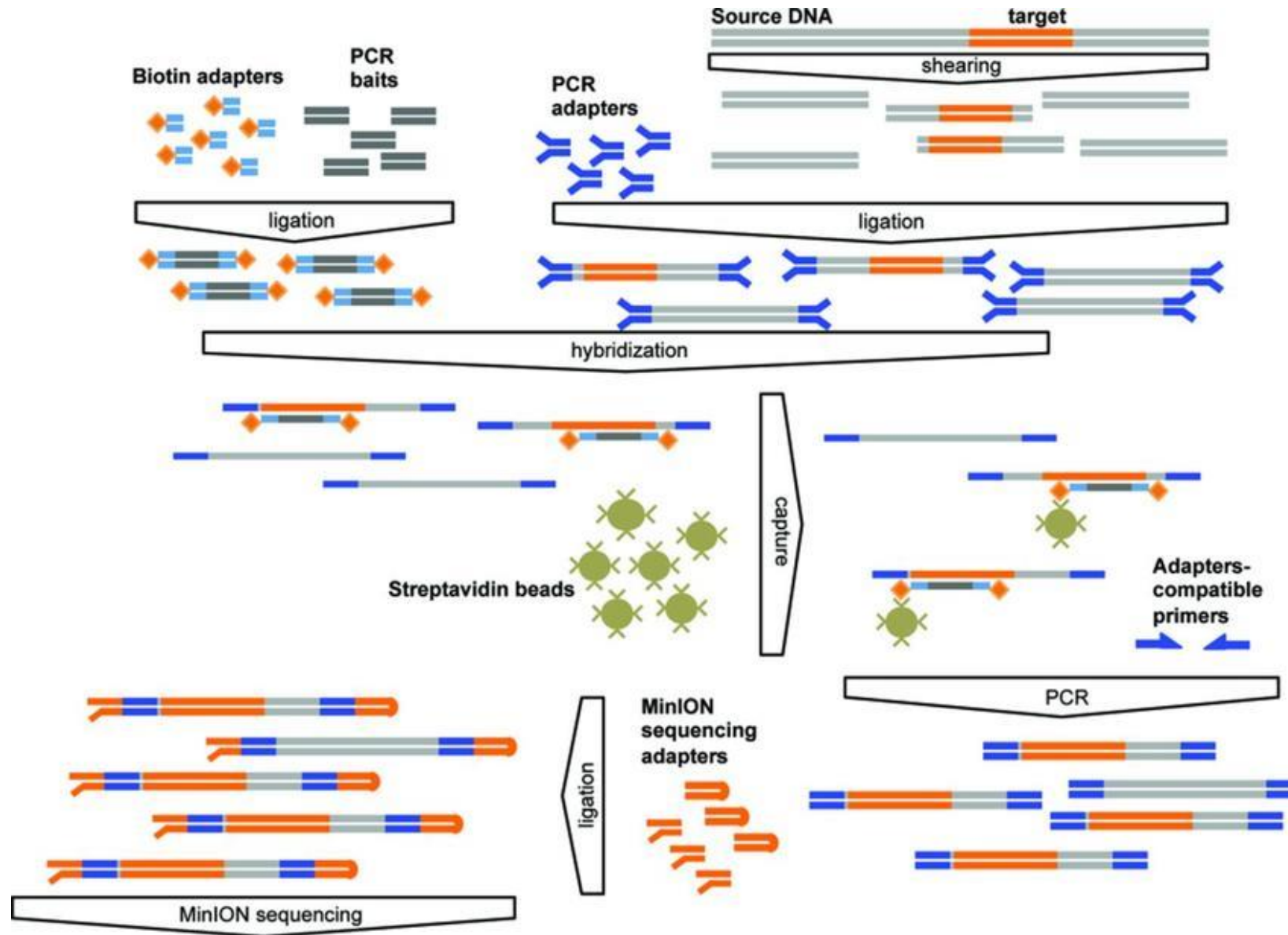
Hybridization between two DNA (or RNA) strands



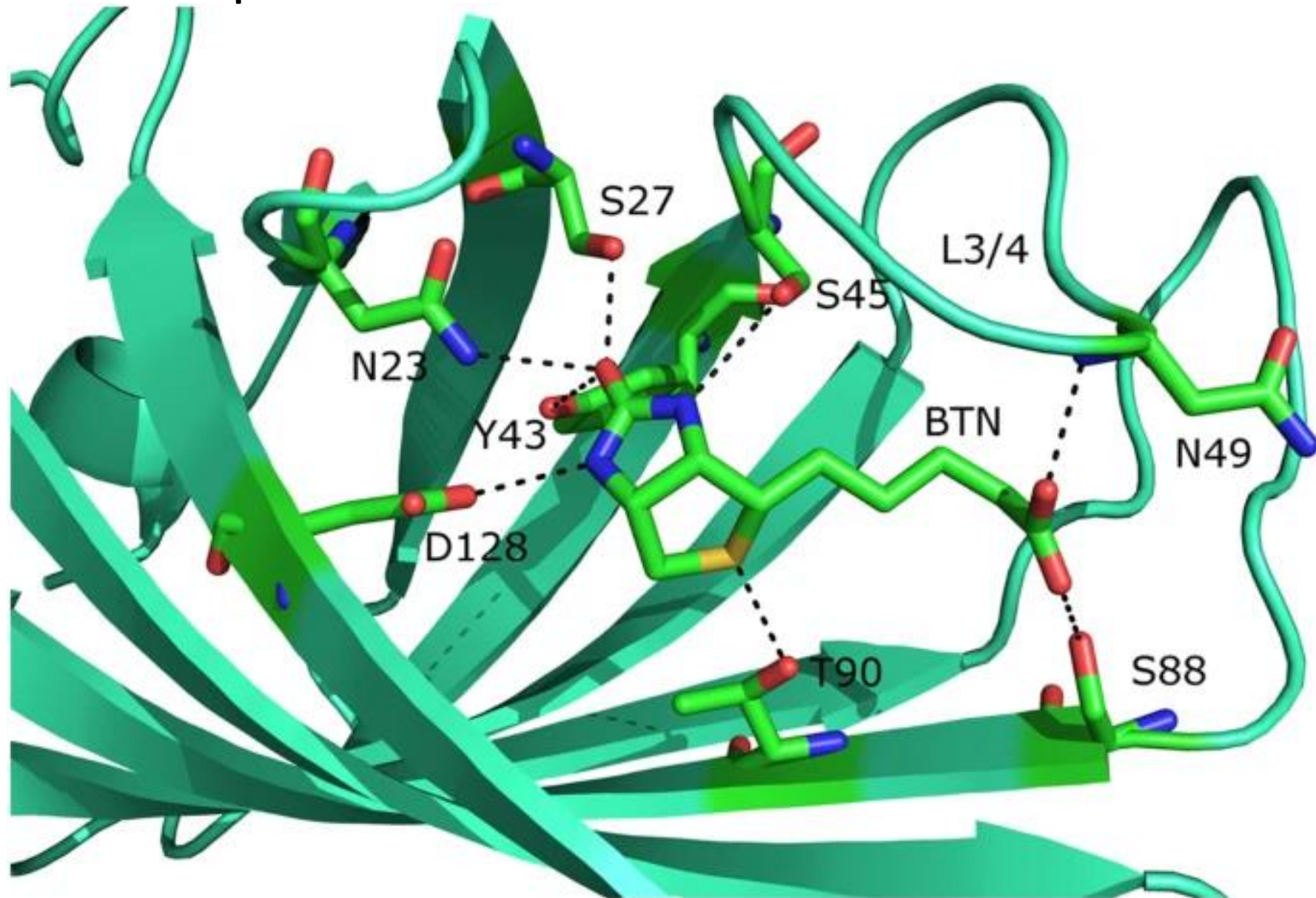
DNA-RNA binding is stronger than DNA-DNA binding



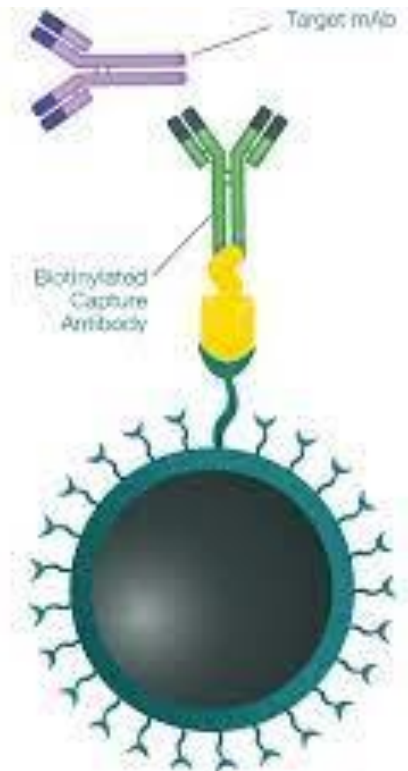
The actual step of „sequence capture“



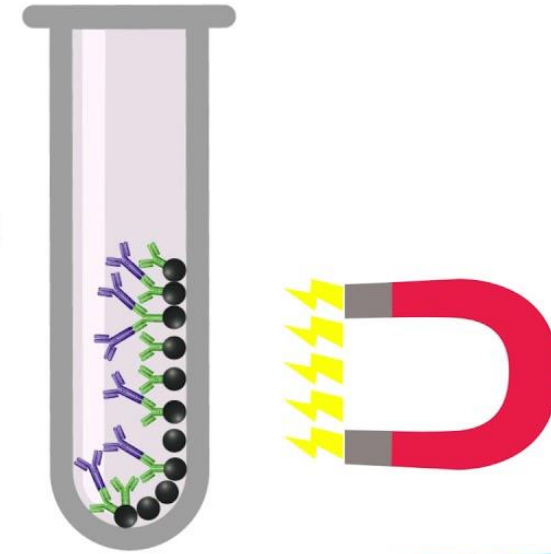
Streptavidin – Biotin interaction



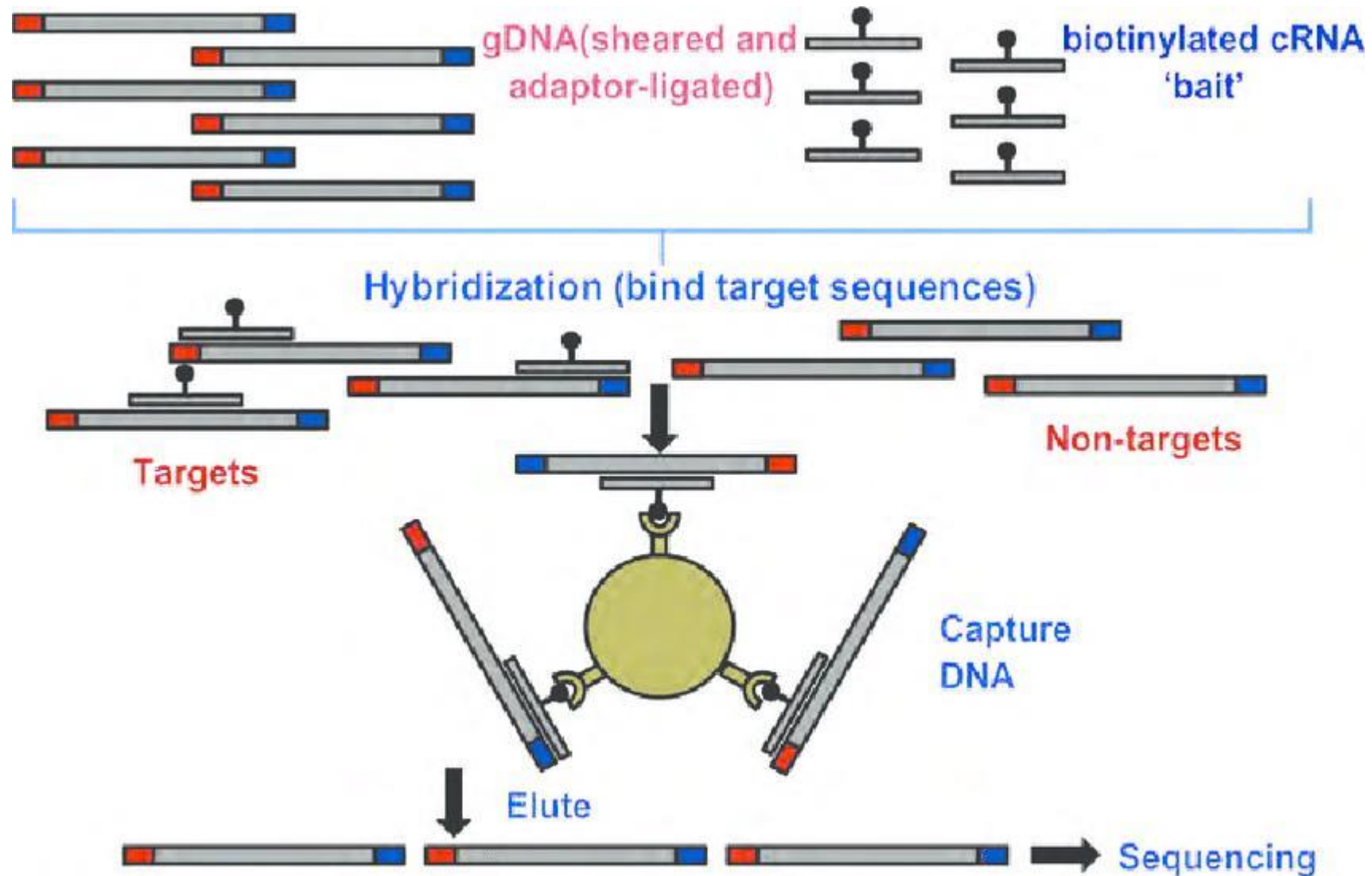
Streptavidin-Biotin interaction and magnetic bead capture



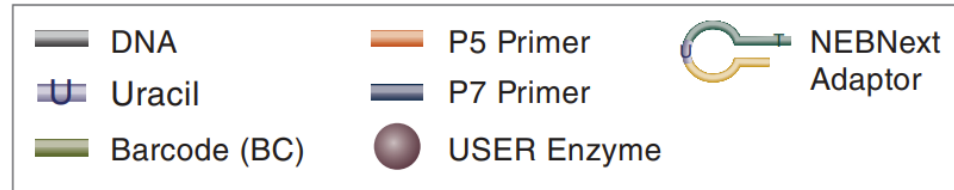
Discard liquid using magnetic stand



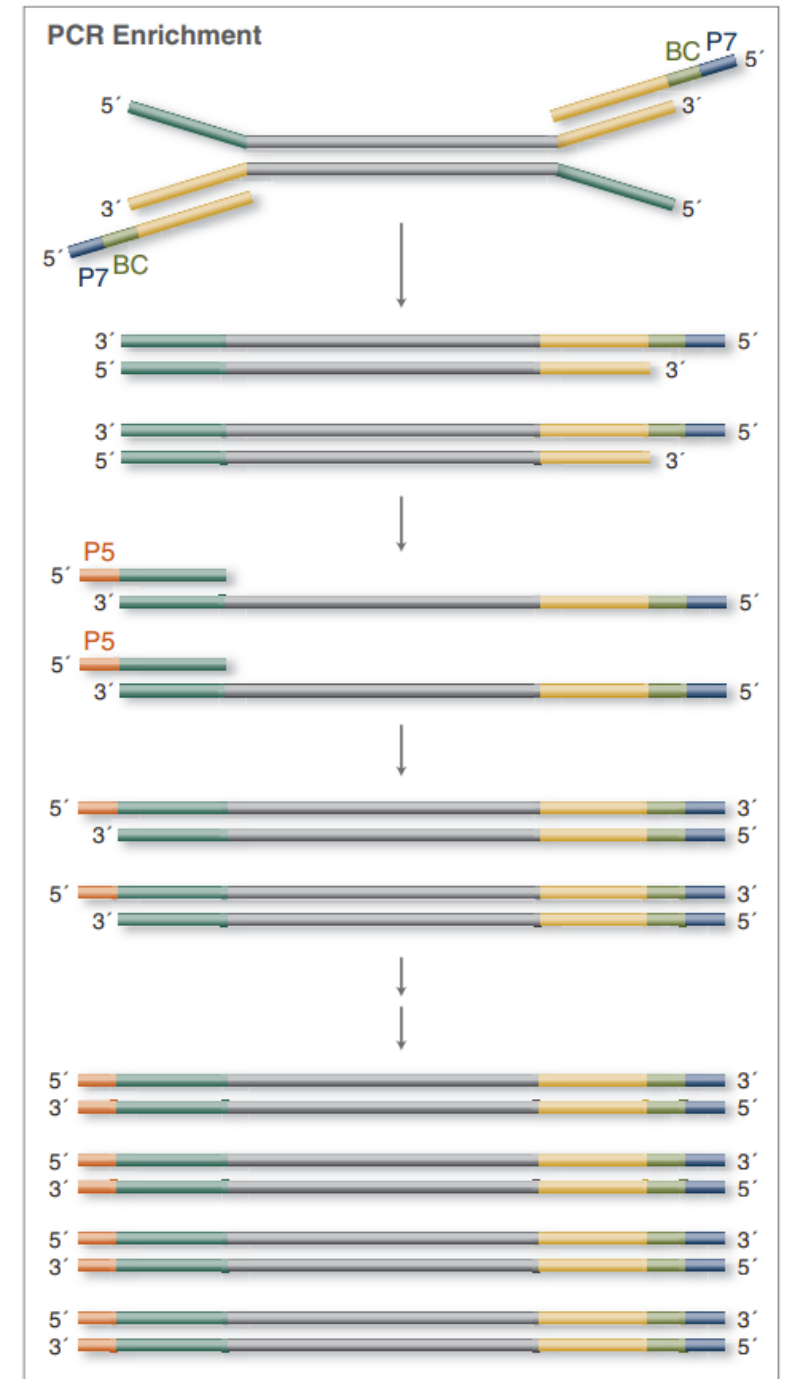
Streptavidin – Biotin interaction is used for sequence capture



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)



Illumina sequencing - solid-phase amplification (= bridge PCR)

