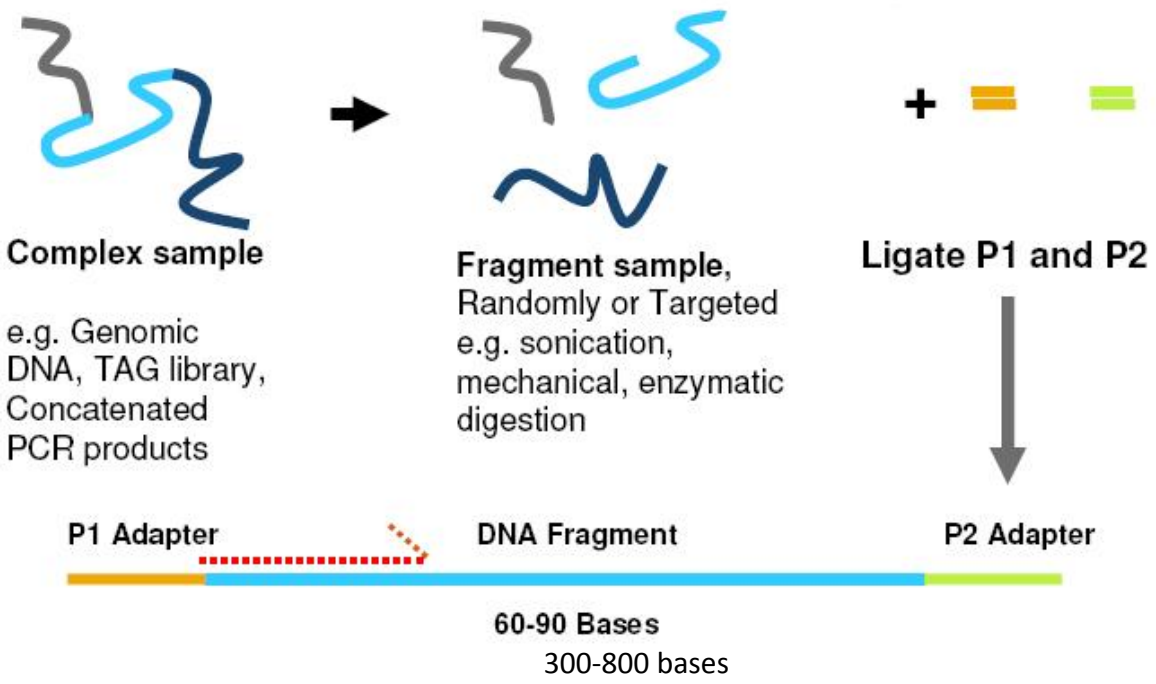
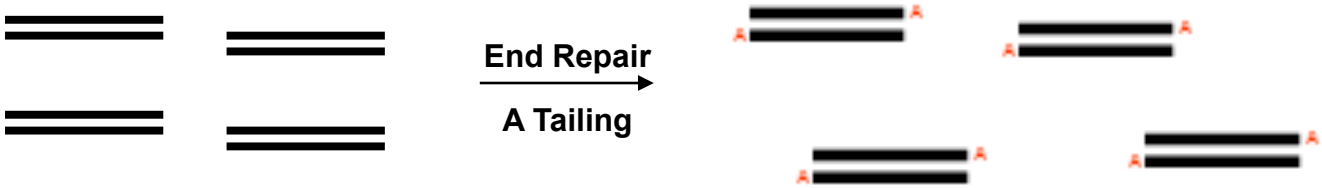
Library Construction



DNA is fragmented and PCR primer adaptors are ligated to the DNA

Rapid Library Preparation (Feb. 2010)

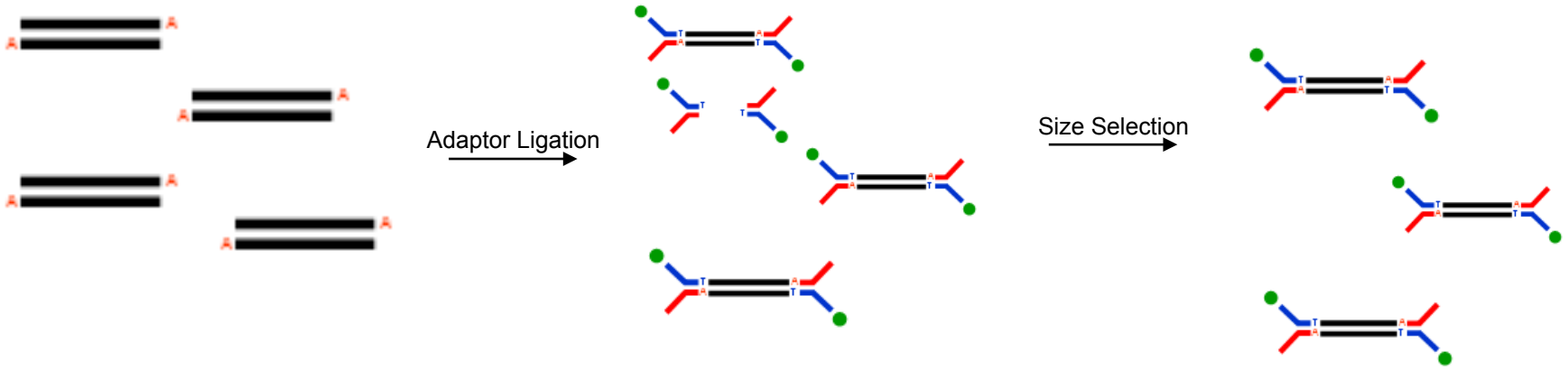
Polishing and A Tailing



- ***Polishing the ends***: fragments are polished to create blunt ends for adaptor ligation
- ***A Tailing***: A tails are added to fragments to allow for TA ligation of the library adaptor

Rapid Library Preparation

Adaptor Ligation and Size Selection



- ***Ligation***: Adaptors are ligated onto polished fragments. Adaptors contain flourscent molecule for direct quantitaiton of the library. Each double stranded adaptor contains both A and B sequences. New key sequence of GATC.
- ***Size Selection***: AMPure bead size exclusion of fragments. No calibration of AmPure Beads required. Wash steps during size selection are critical. Complete removal of buffer during washing steps will ensure removal of residual adaptor molecules

454 adaptors

Adaptor A

Primer A1: 5' – C*C*A*T*CTCATCCCTGCGTG*TCTCCGAC*T*C*A*G*- 3'

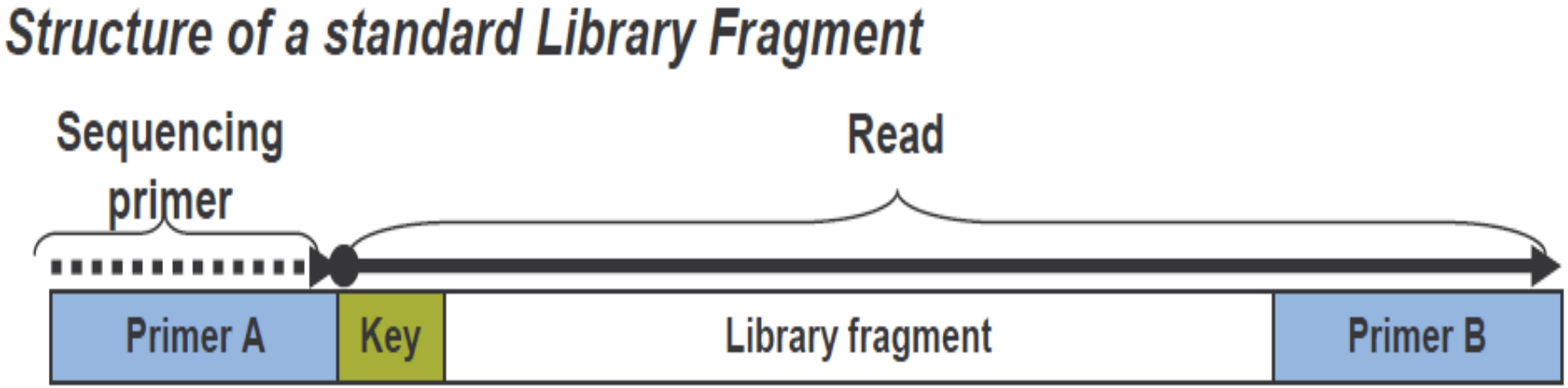
Primer Aprime1: 5'–*C*T*G*A*GTCG*G*A*G*A*-3'

Adaptor B

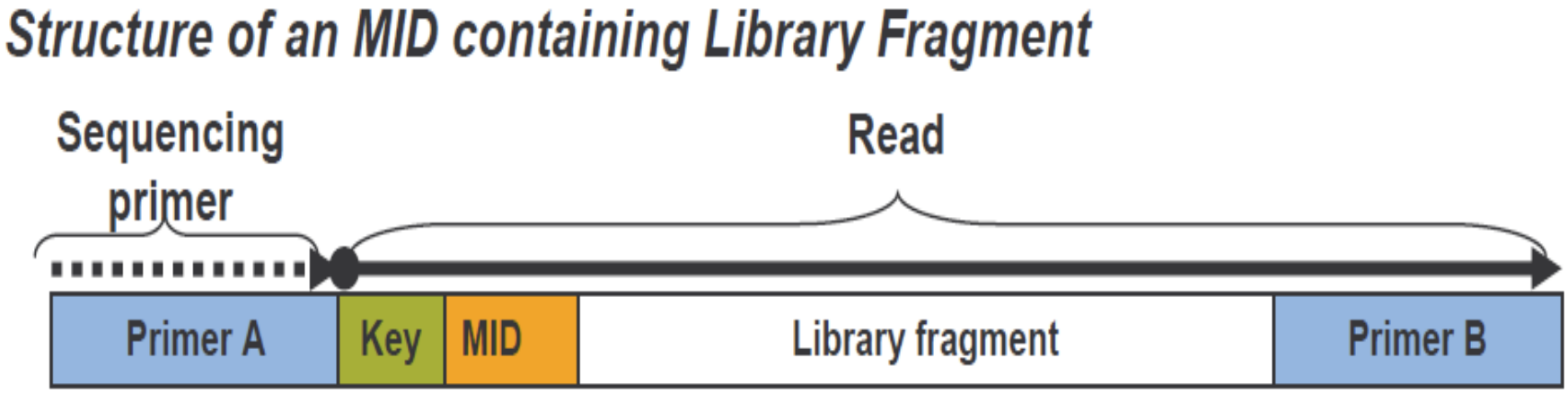
Primer B1: 5'/5BioTEG/C*C*T*A*TCCCCTGTGTGCCTTGGCAGTC*T*C*A*G*- 3'

Primer Bprime1: 5'-*C*T*G*A*GACT*G*C*C*A- 3'*

454 Standard Library Fragment



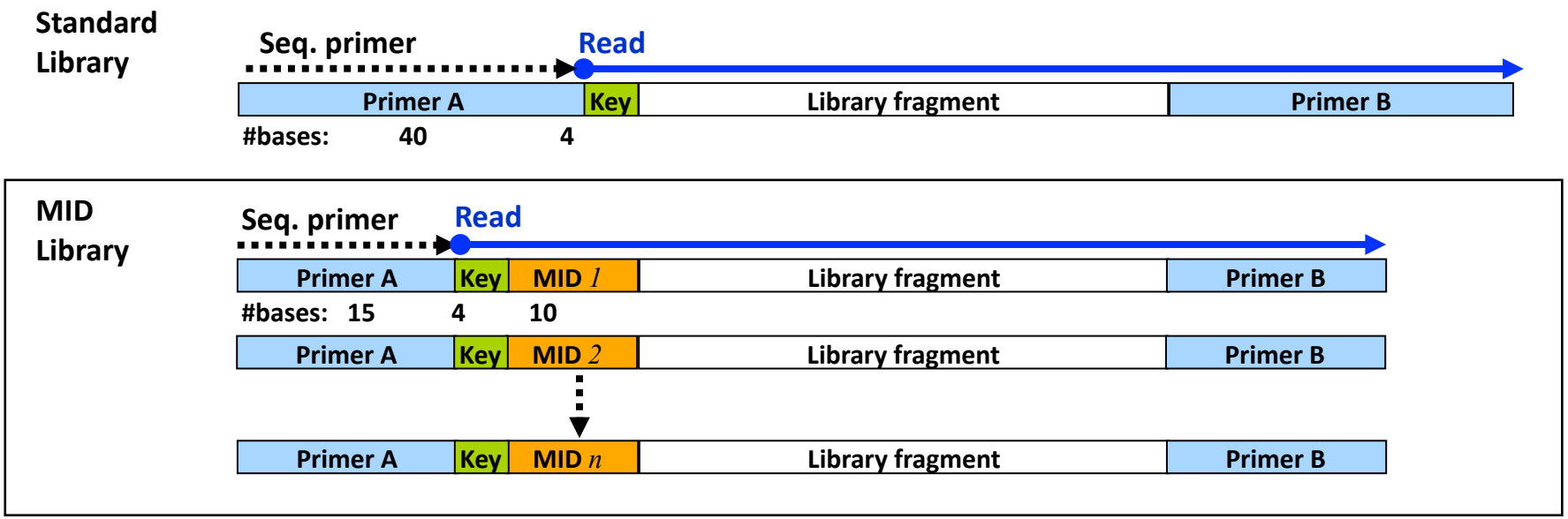
454 Barcode Library Fragment



454 Barcode Sequences

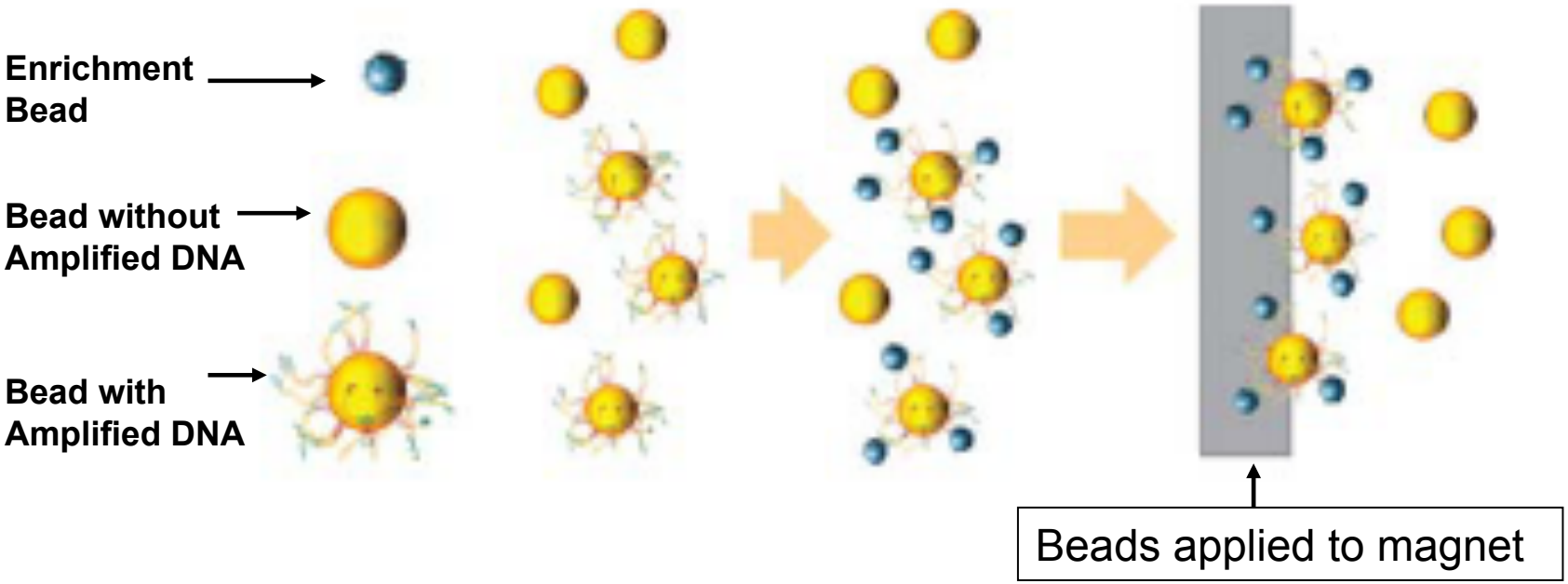
MID1	ACGAGTGCGT
MID2	ACGCTCGACA
MID3	AGACGCACTC
MID4	AGCACTGTAG
MID5	ATCAGACACG
MID6	ATATCGCGAG
MID7	CGTGTCTCTA
MID8	CTCGCGTGTC
MID9	TAGTATCAGC
MID10	TCTCTATGCG
MID11	TGATACGTCT
MID12	TACTGAGCTA

454 Library construct



GS FLX Titanium Chemistry

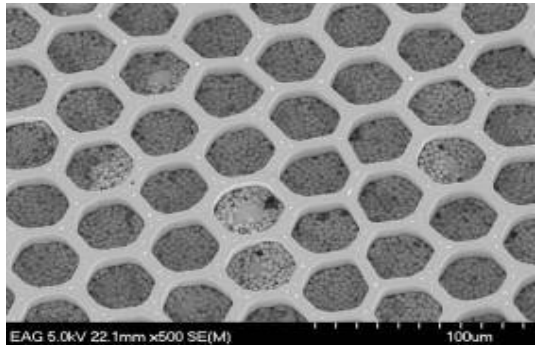
Enrichment



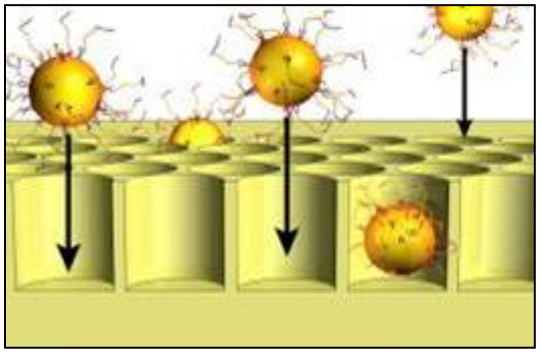
- **Melt Solution added to render PCR products single stranded**
- **Biotinylated Enrichment primer is annealed to fragments on capture beads**
- **Streptavidin coated, magnetic Enrichment beads are added and bind to biotin**
- **Beads with DNA product are extracted using magnet**
- **DNA positive beads recovered by denaturing enrichment primer and collecting non-magnetic DNA beads**
- **Ideally, approximately 10% of beads are recovered**

GS FLX Titanium Chemistry

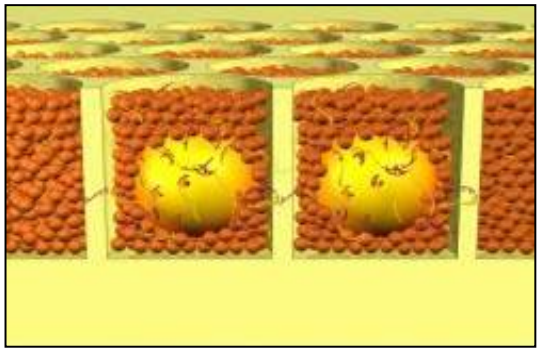
Bead Deposition



PicoTiterPlate ™



DNA beads are loaded into the wells of the PTP.



DNA beads packed into wells with surrounding beads and sequencing enzymes.

*A well diameter of 29 µm allows for a single **20 µm** bead per well*

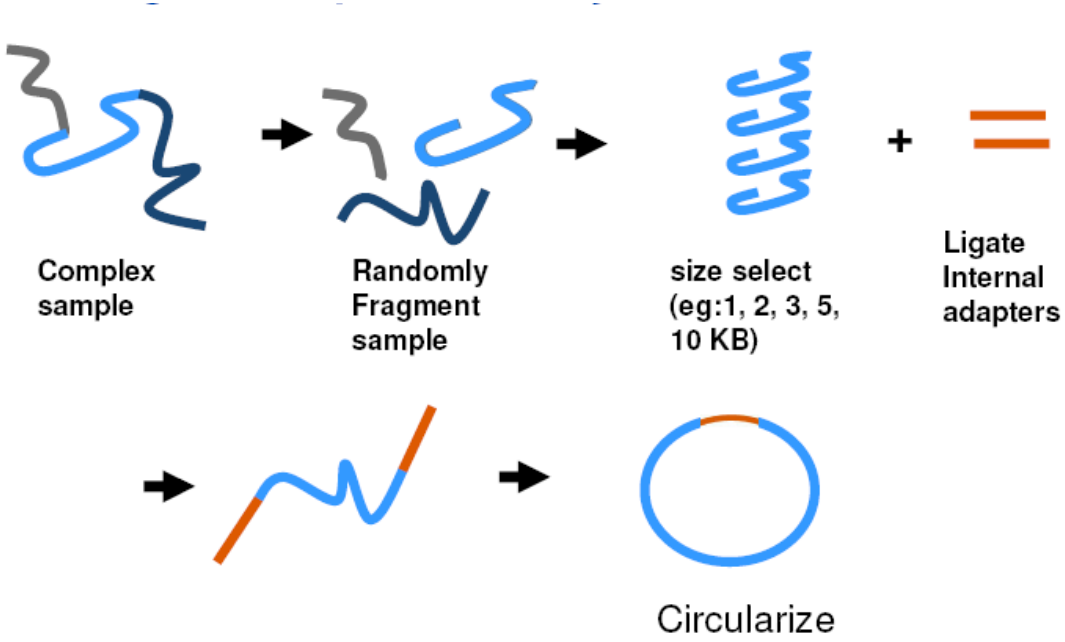
SOLid Standard Library Construction

Fragment Library (directed resequencing)

SOLid Mate-pair Library Construction

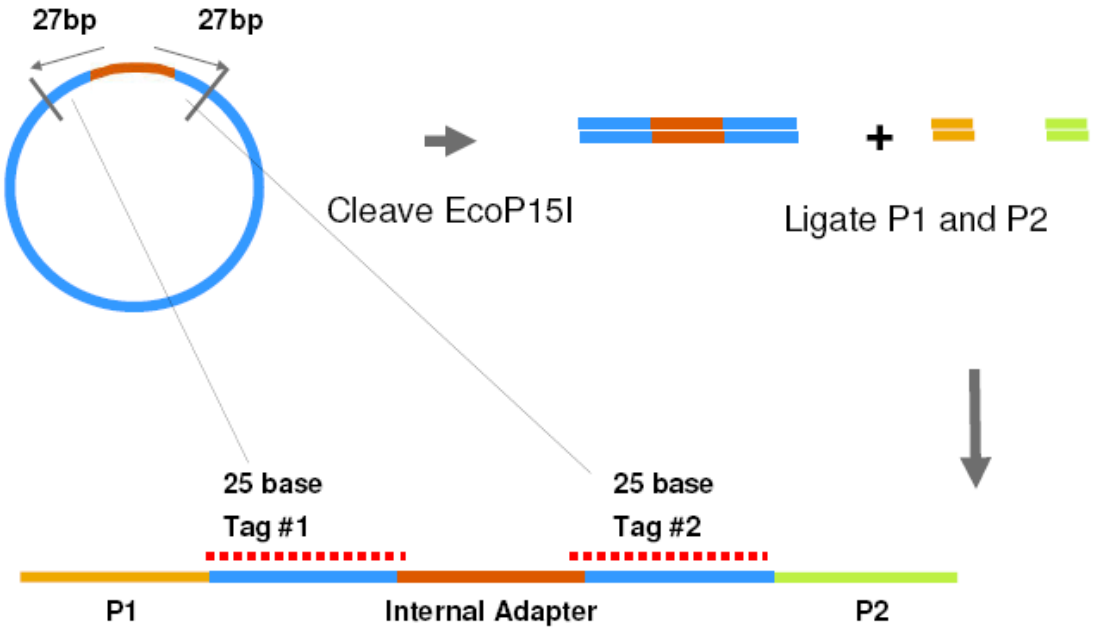
Mate Pair Library (whole genome sequencing)

Mate-Pair Library



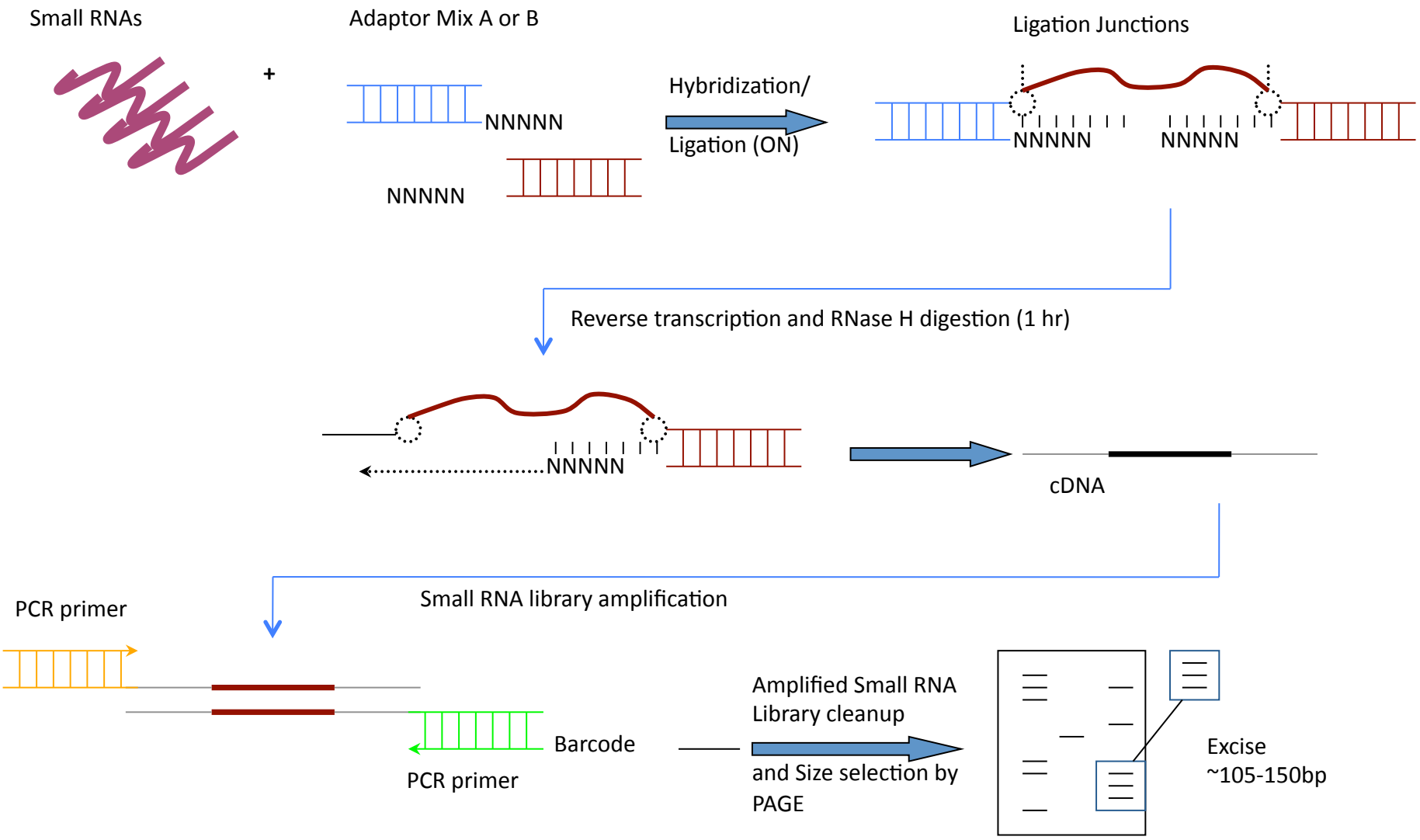
DNA is sheared, selected for a desired input size, and circularized around an internal adaptor.

Mate-Pair Library (cont.)

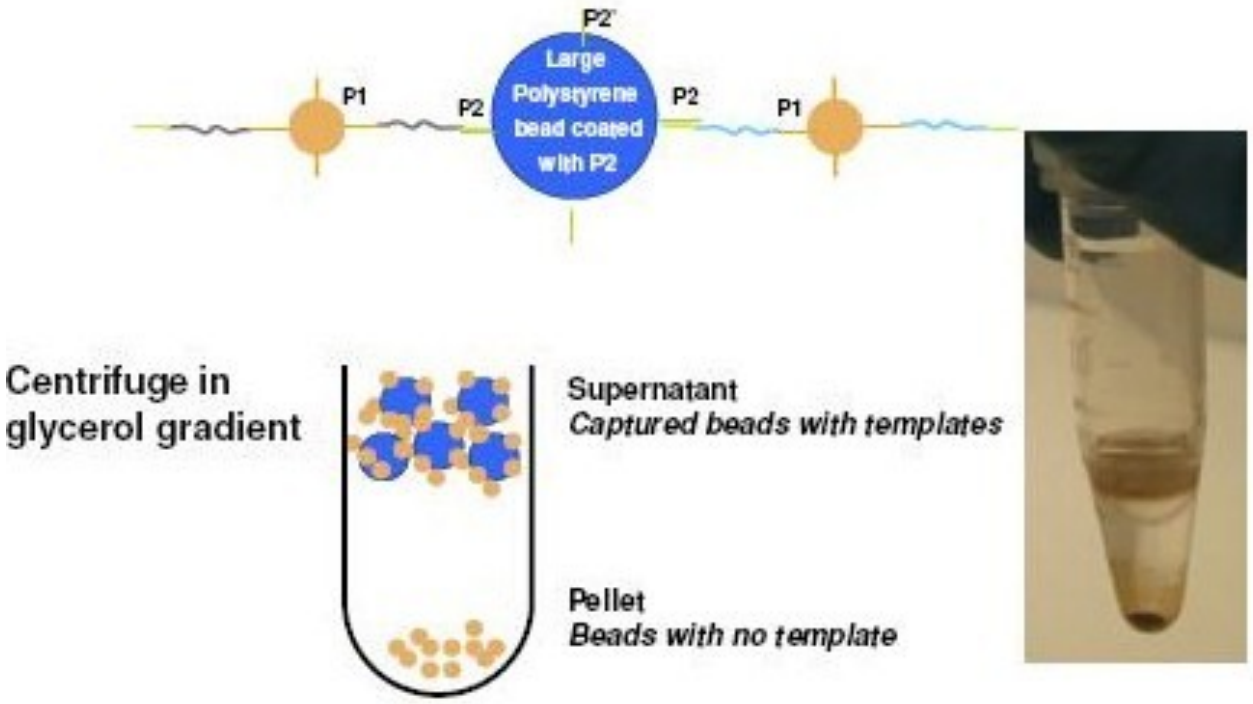


The circularized DNA is enzymatically cleaved to yield 2 DNA fragments separated by an internal adaptor. PCR primer adaptors are ligated on to the end of this piece of DNA.

Library Preparation - Small RNA Library –SREK kit

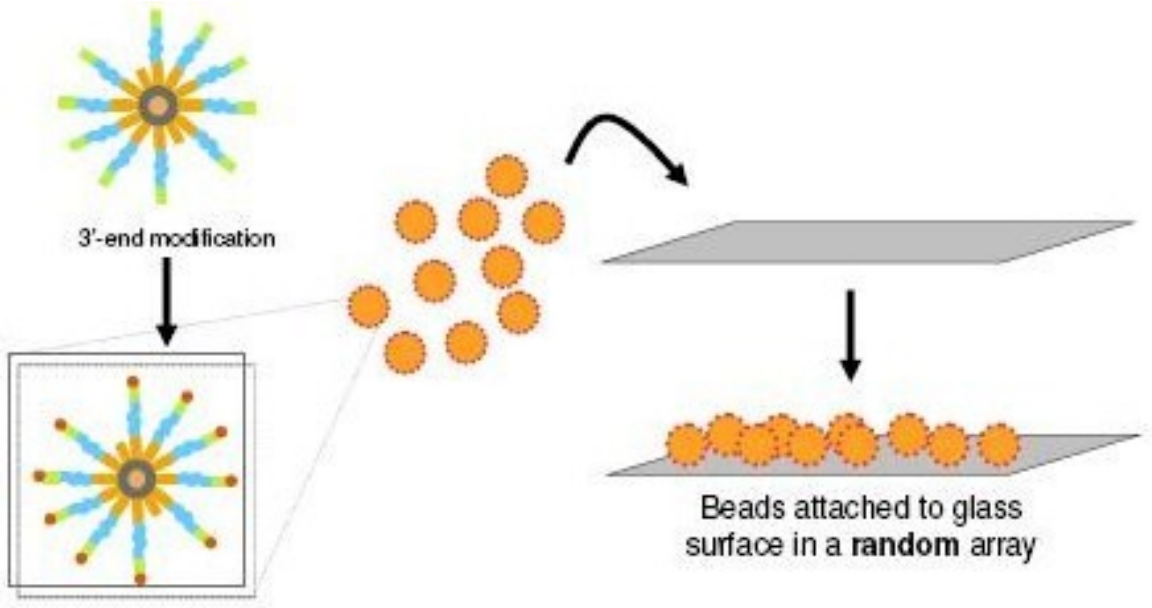


Bead Enrichment



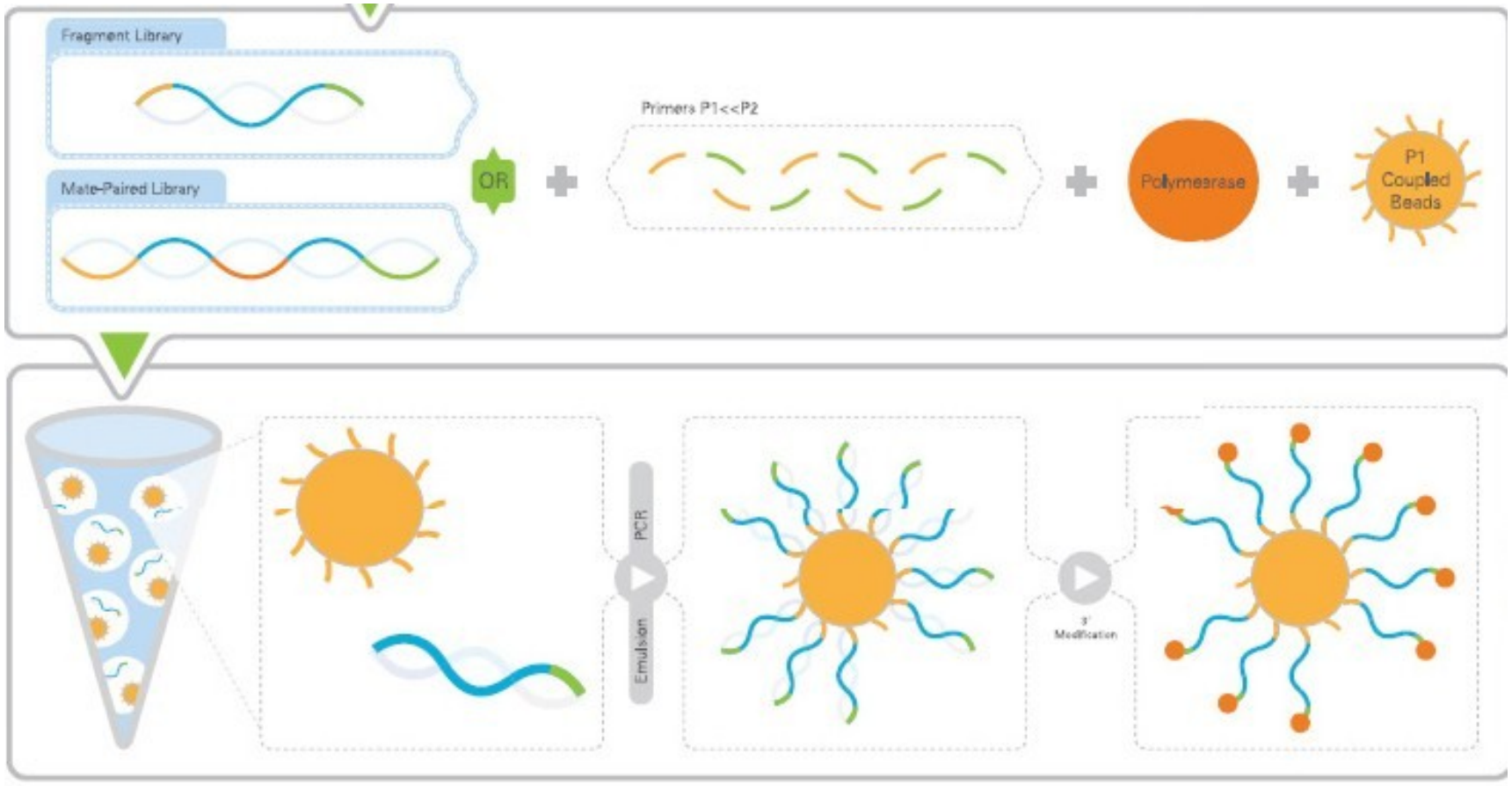
Templated beads are separated from non-templated beads via polystyrene beads

Solid Bead Deposition



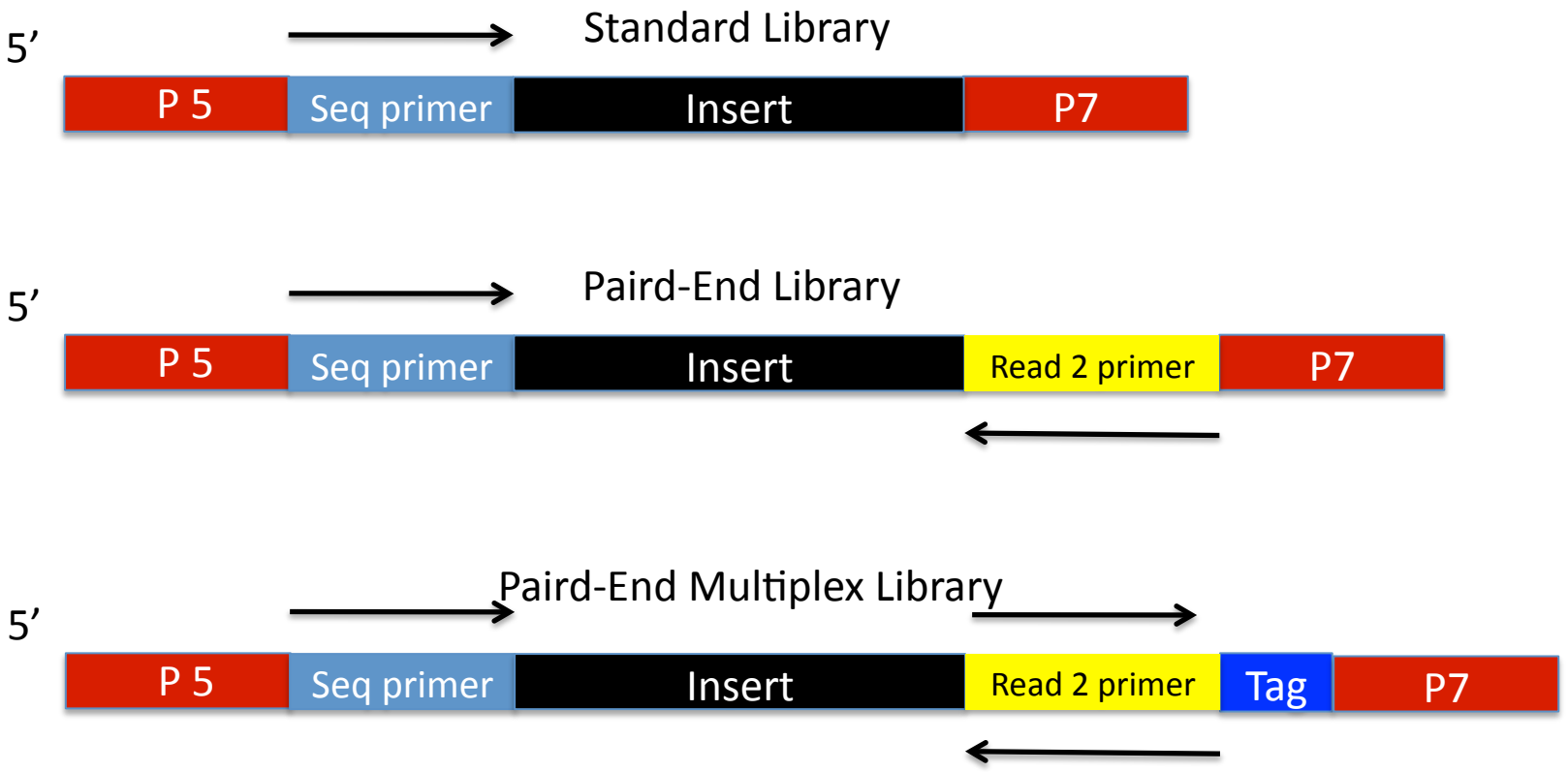
Templated beads are modified at their 3'-end and covalently attached to a glass slide.

Emulsion PCR and Bead Enrichment

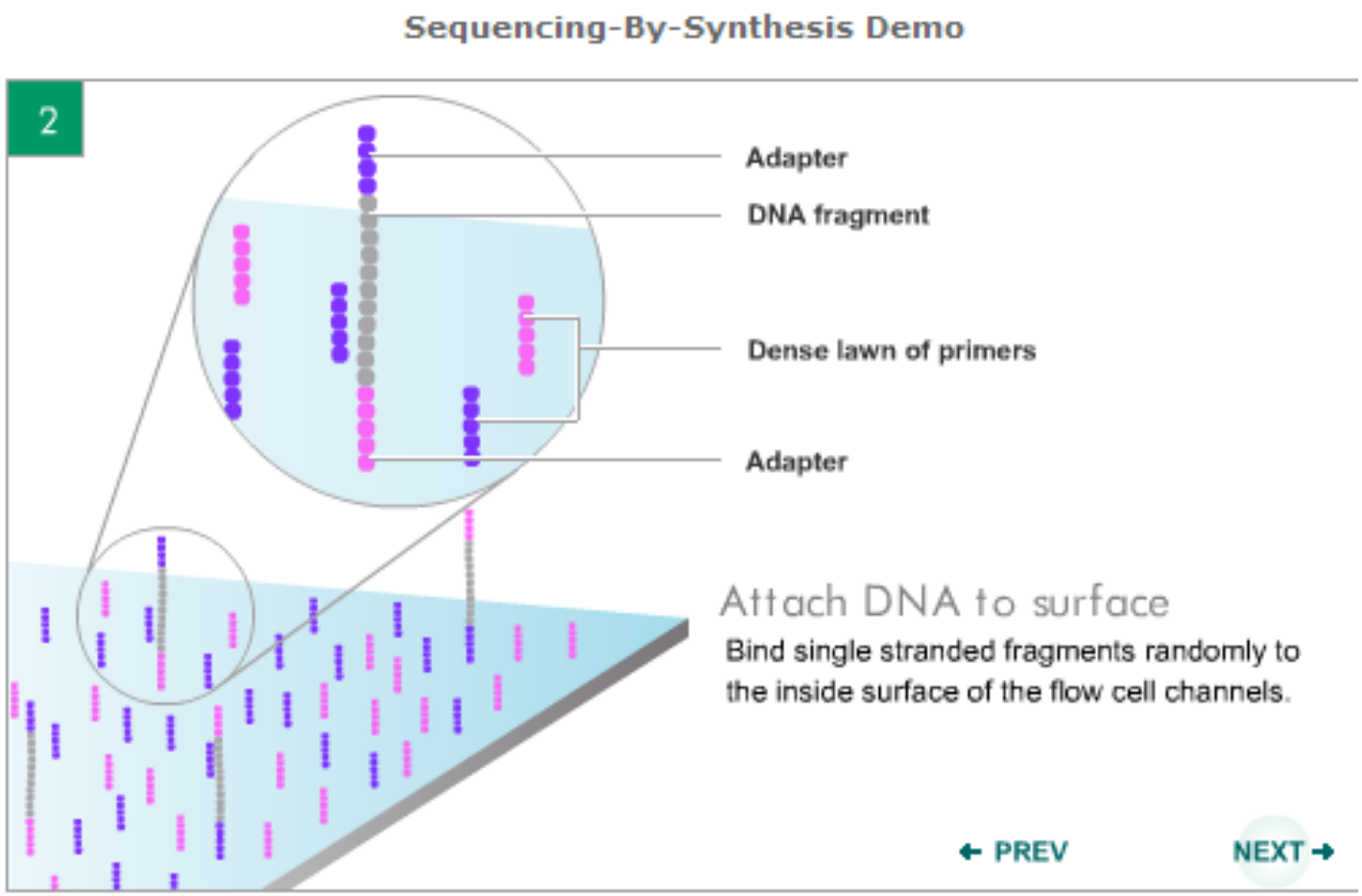


PCR takes place in oil in water microreactors. Post-PCR, templated beads are separated from non-templated beads, and modified at the 3' end to allow covalent linkage to the SOLiD sequencing slide.

Illumina Library construct

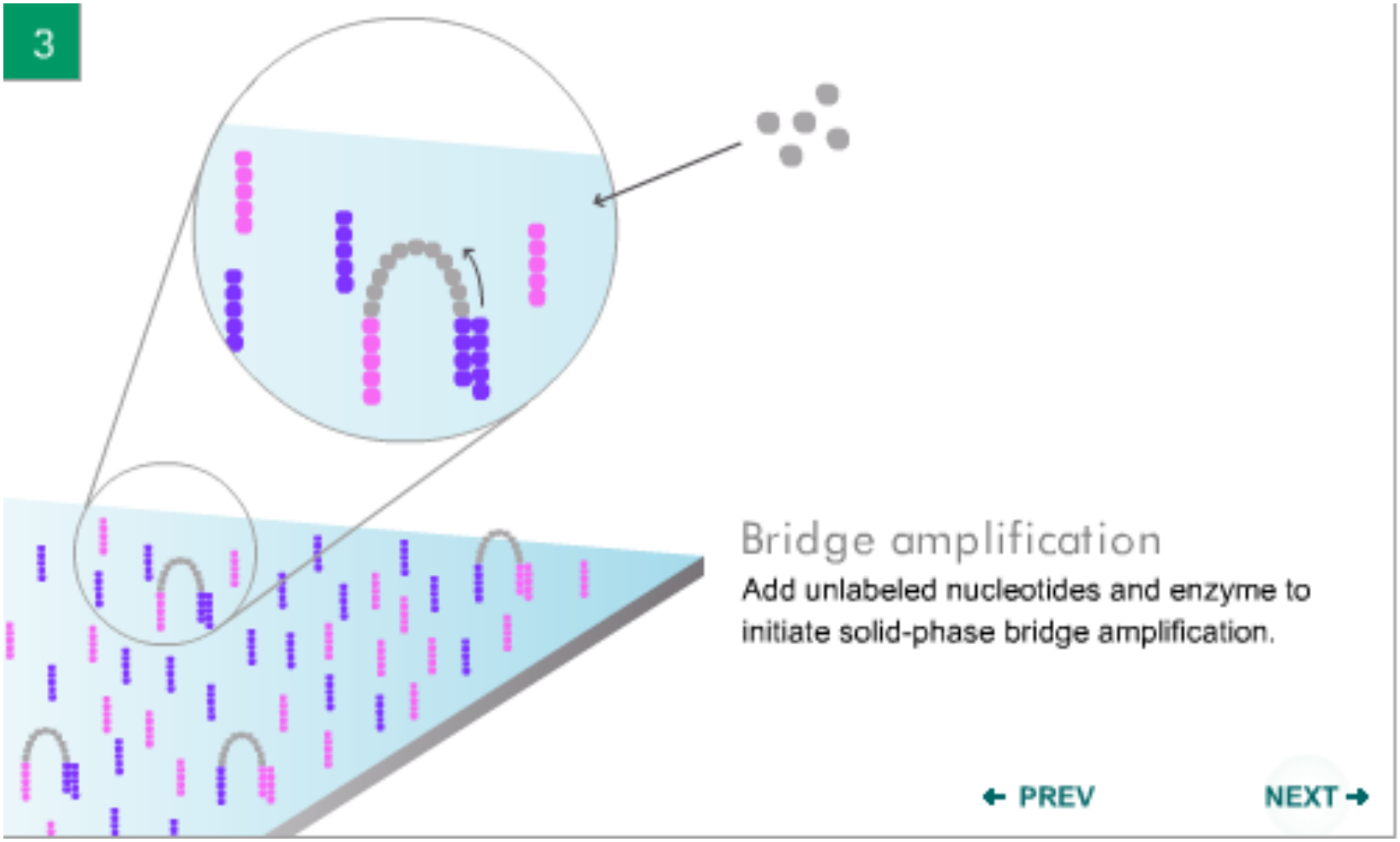


Immobilize DNA to Surface



Source: www.illumina.com

Technology Overview: Solexa Sequencing



Sequence Colonies