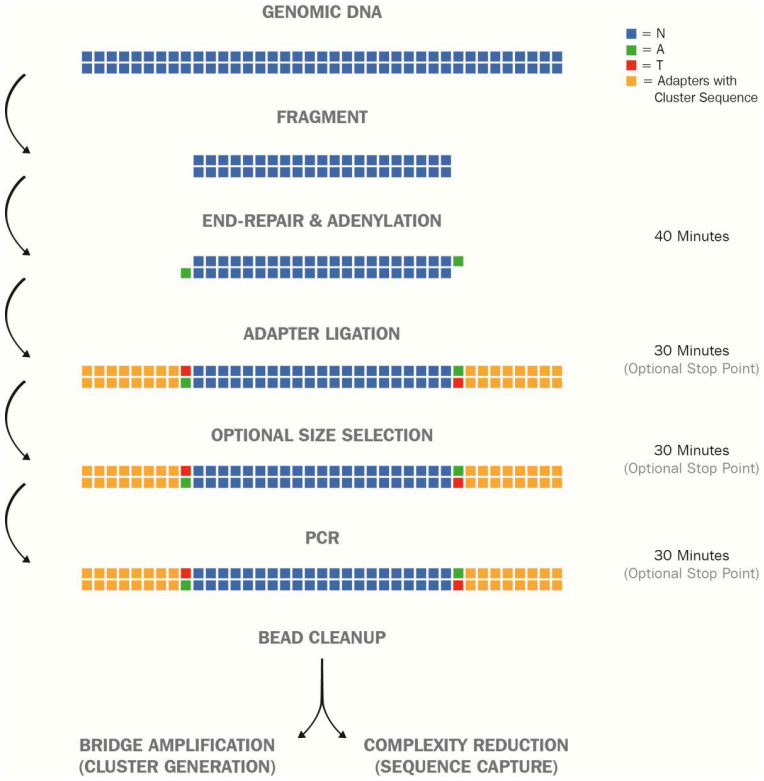
Wednesday 18 September

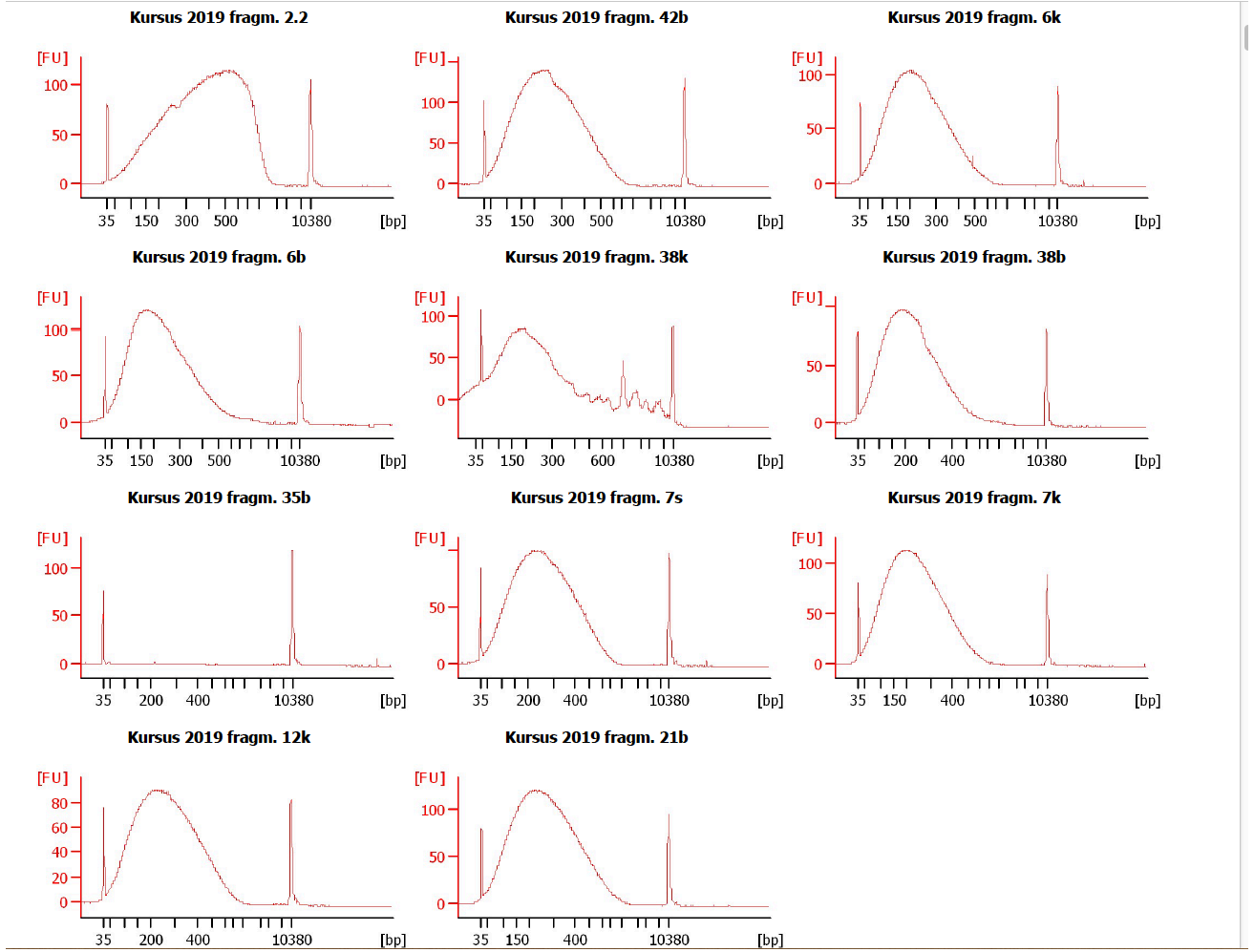
Library preparation

Workflow for purification

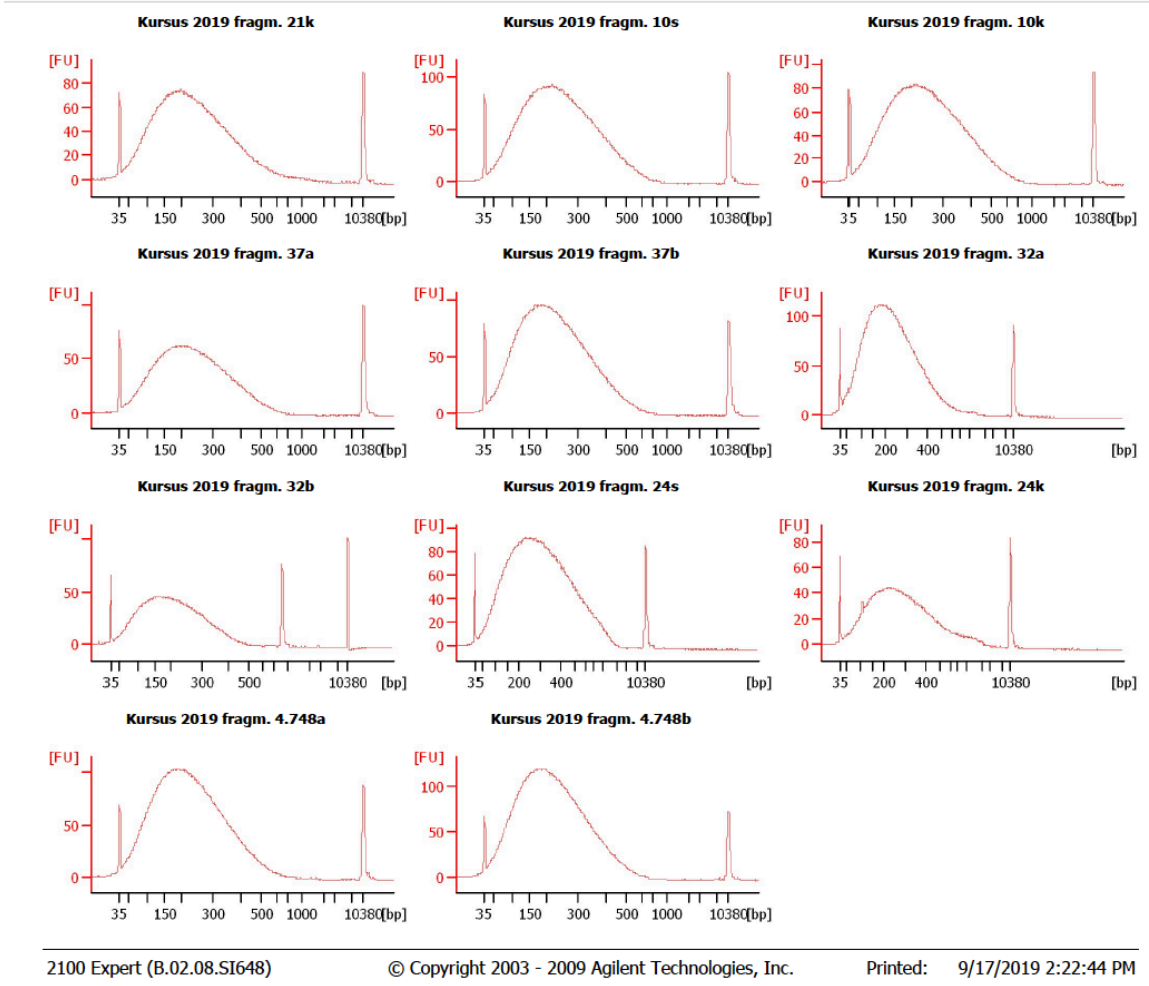


DNA shearing

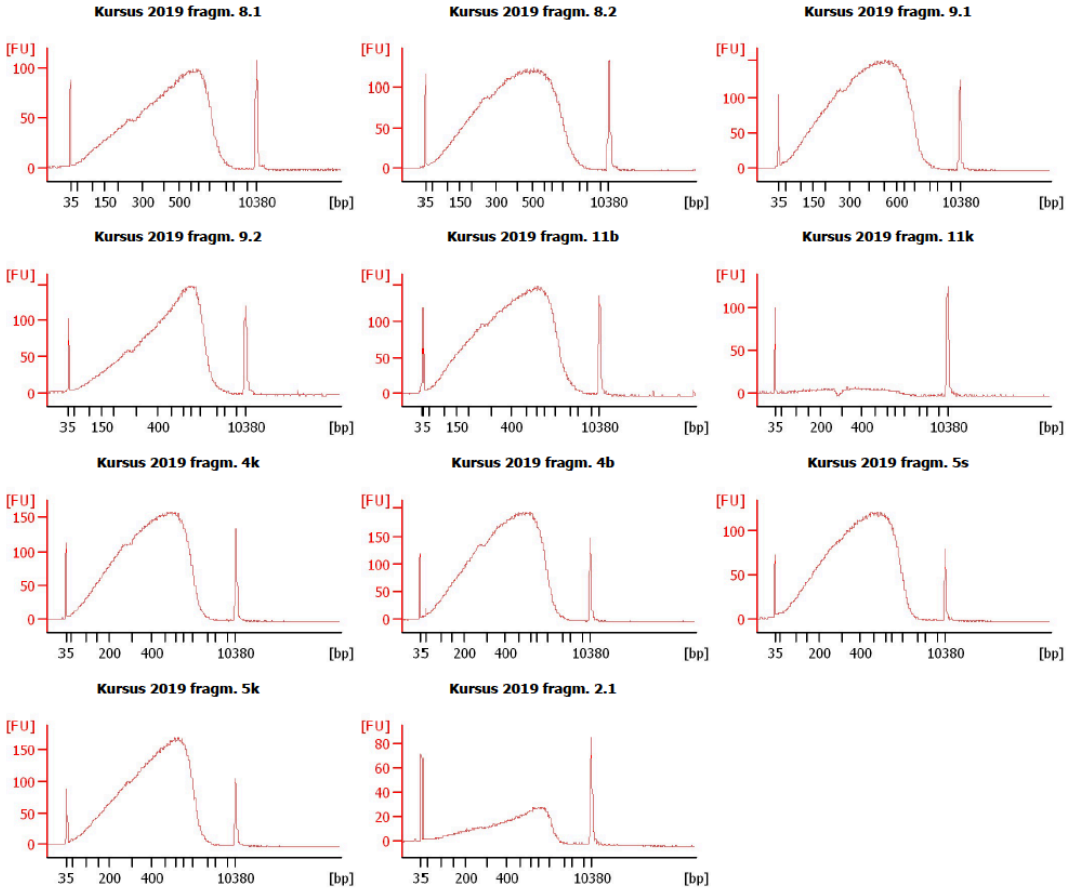
Bioanalyser results



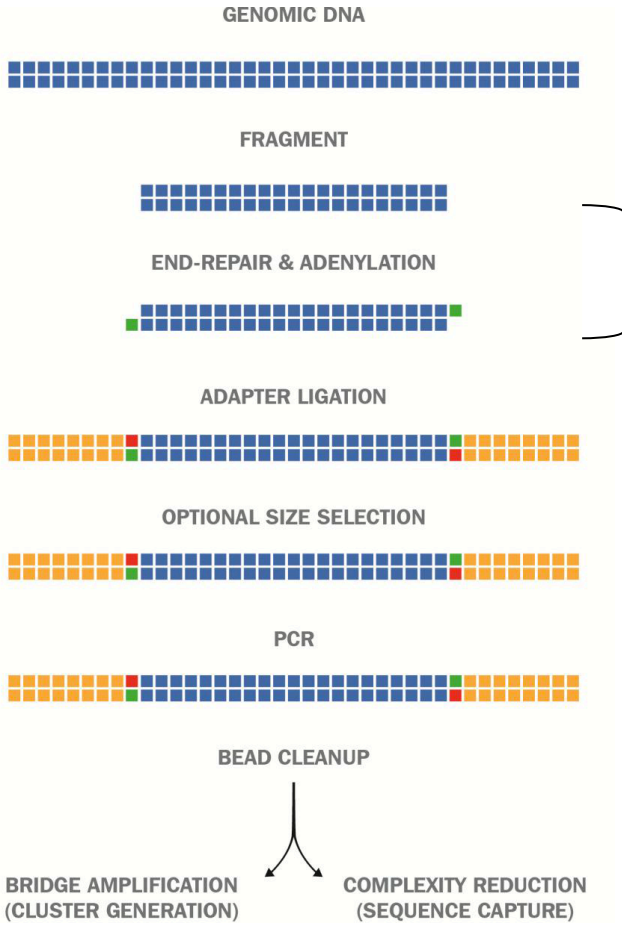
Bioanalyser results



Bioanalyser results

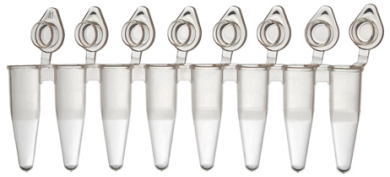


Step 1: End repair and Adenylation

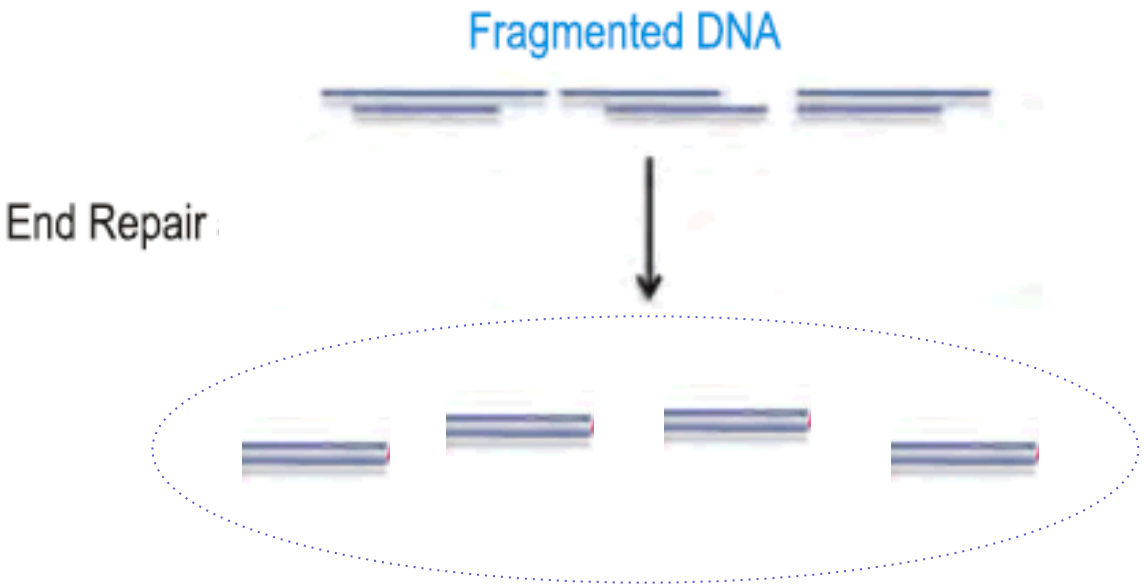


- In PCR tubes, strip tubes, or plates, prepare the reaction mix
- Add 32µL fragmentised DNA
- Add 18µL Nextflex End repair & adenylation buffer/Enzymemix
- Gently pipette the entire volume up and down 10 times.

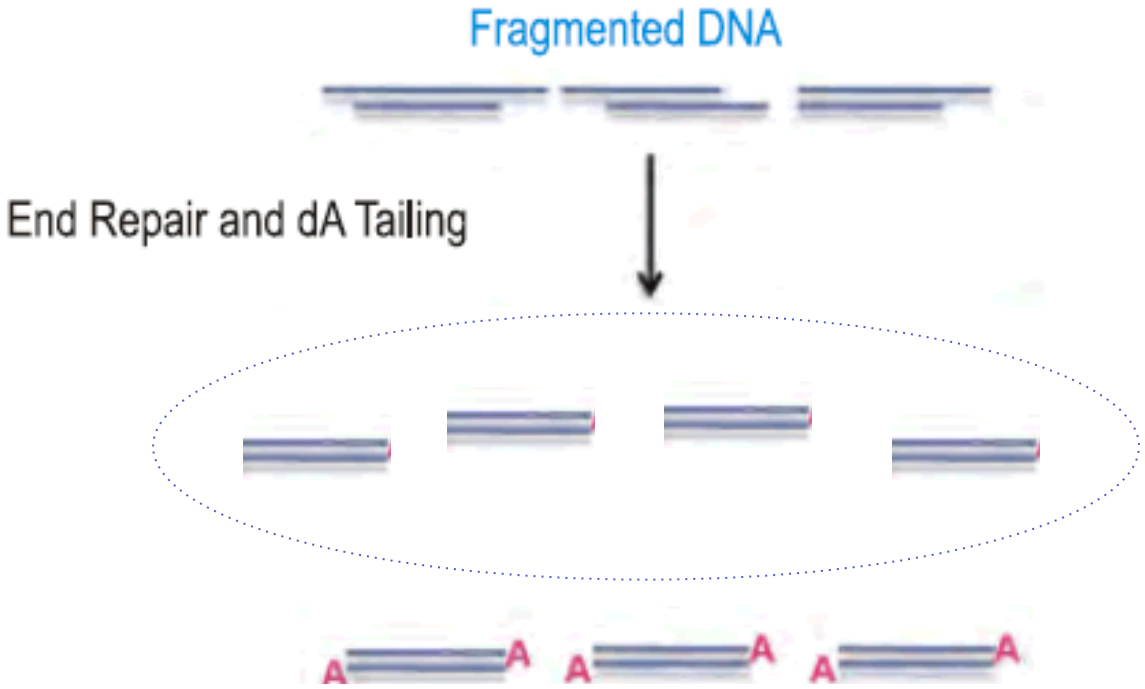
- Place the samples in a thermo cycler and run the following program.
- Without lid on for the first 20 minutes (22°C) and 20 minutes (72°C) with the lid back on



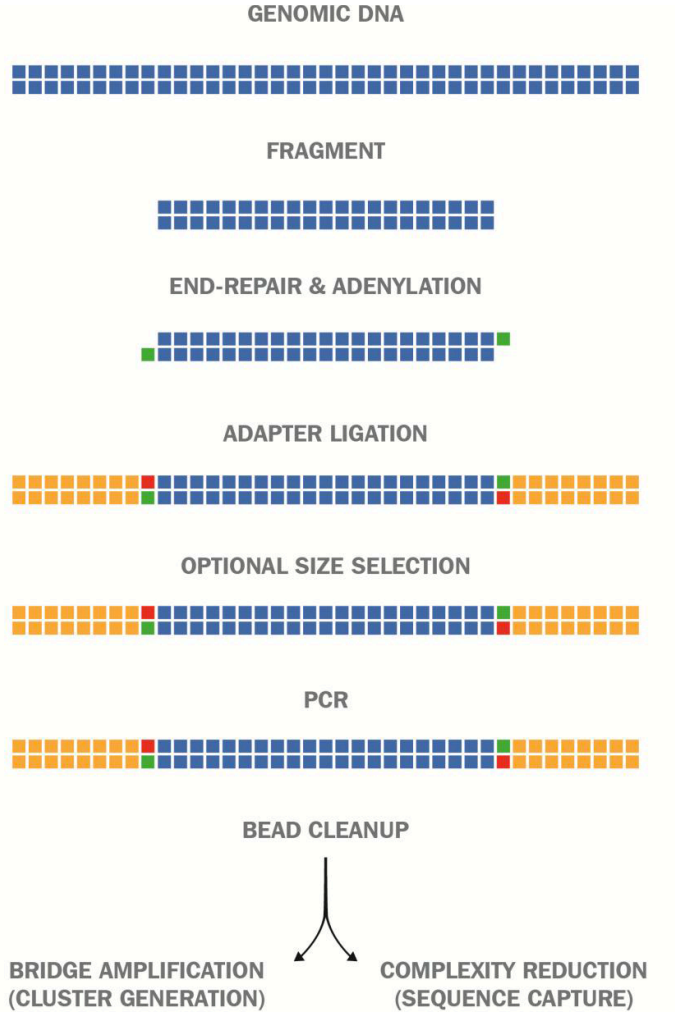
Step 1: DNA Fragmentation/ End repair



Step 1: DNA Fragmentation/ End repair



Step 2: Adaptor ligation



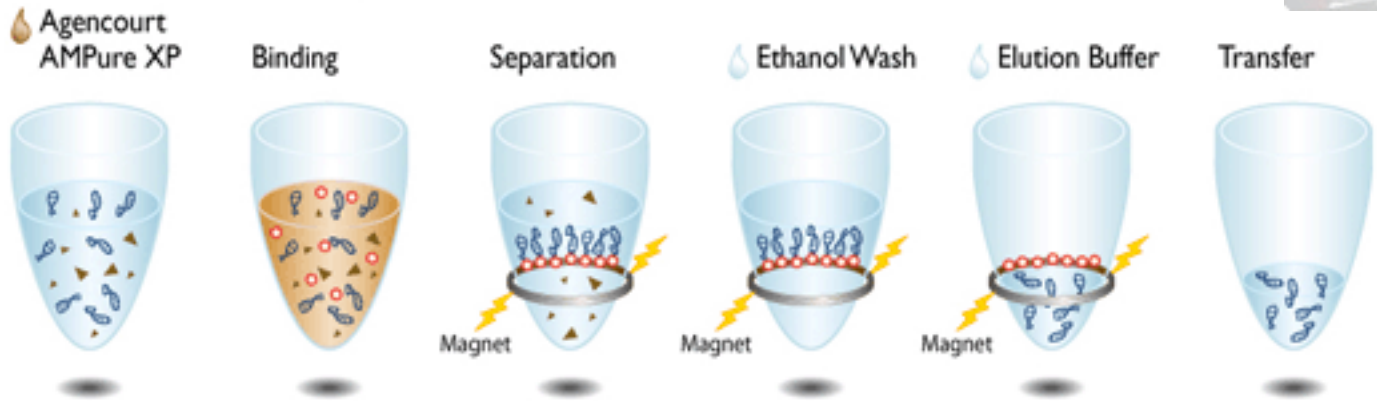
- Bring the NEXTflex Ligase mix to room temperature, vortex NB!! Do not spin down
- Add 47.5 µL Nextflex Ligation Mix
- Add 2.5µL Nextflex DNA Barcodes Adaptor (according to the Adaptor table)
- Gently pipette the entire volume up and down 10 times.

Place the samples in a thermo cycler and run the following program. With out lid on.

Clean up

Size selection and clean-up using SPRI Beads

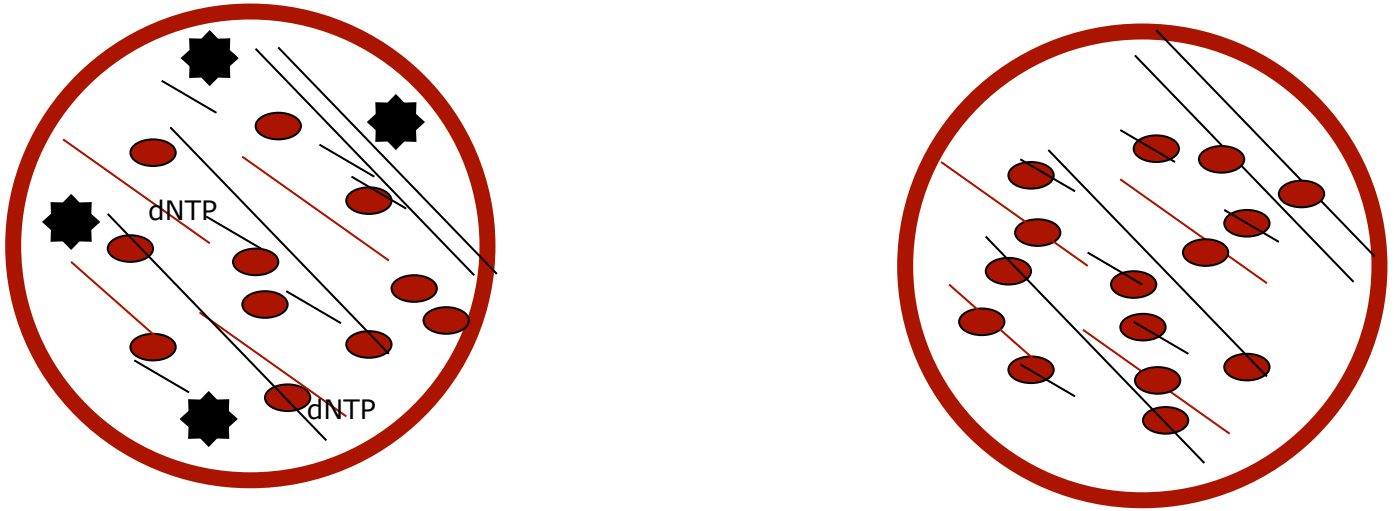
SPRI = Solid Phase Reversible Immobilization



Ratio of SPRI beads to sample determines size cut off

Purification

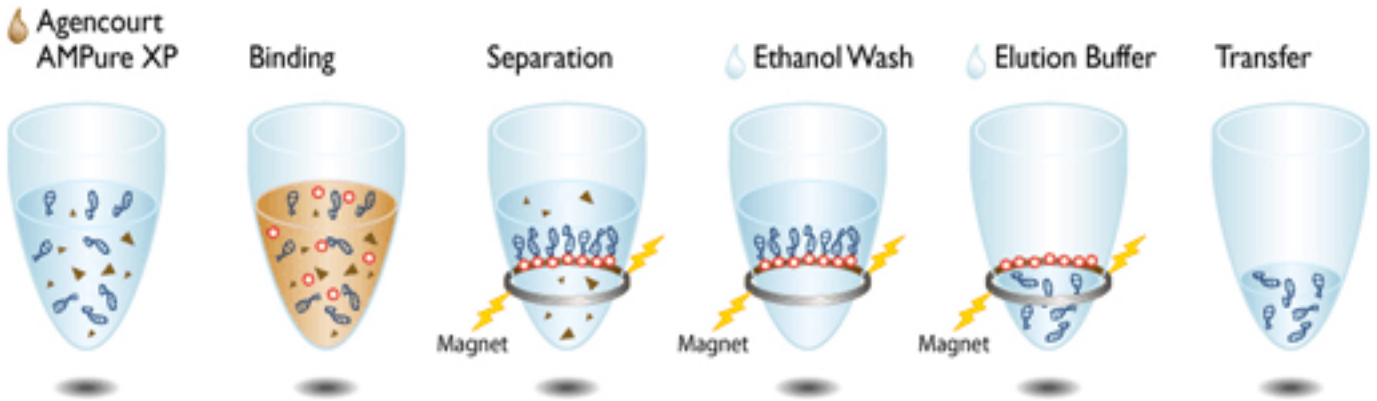
50µL



Step 3: Adaptor ligation clean up

Size selection and clean-up using SPRI Beads

SPRI = Solid Phase Reversible Immobilization



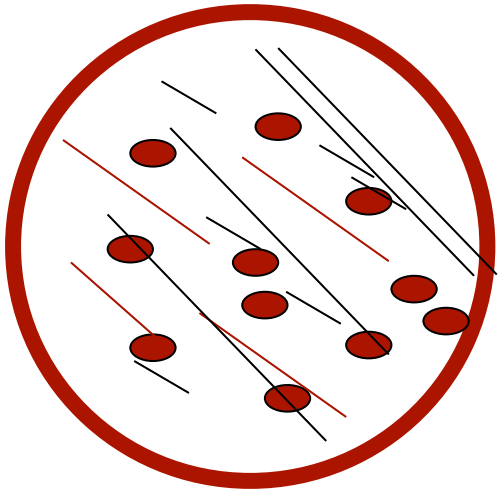
Ratio of SPRI beads to sample determines size cut off

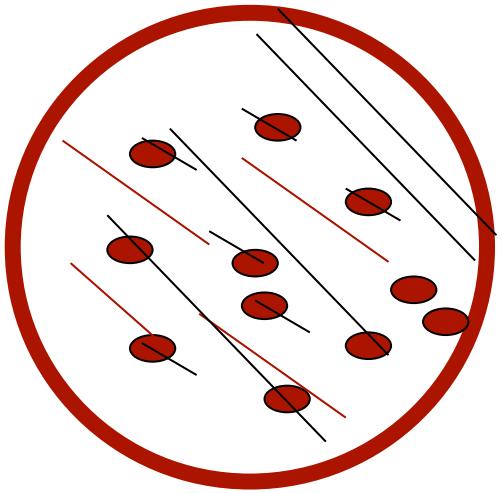
Low cutoff - 35µL AMPure Beads

High cut off -12µL AMPure Beads

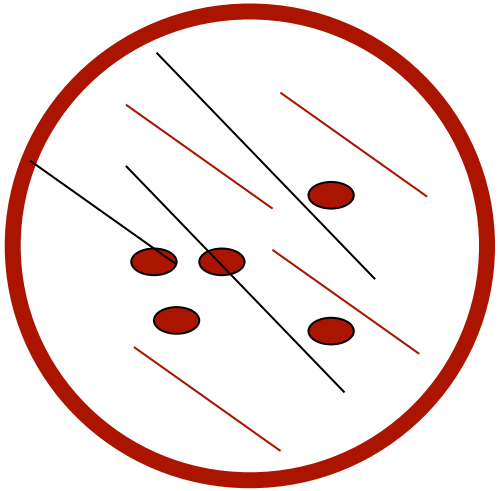
Size selection

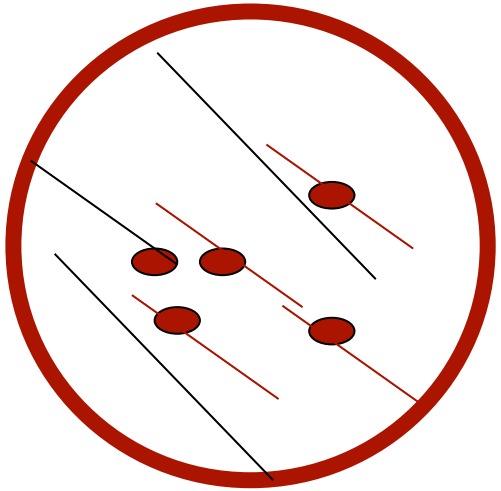
32µL



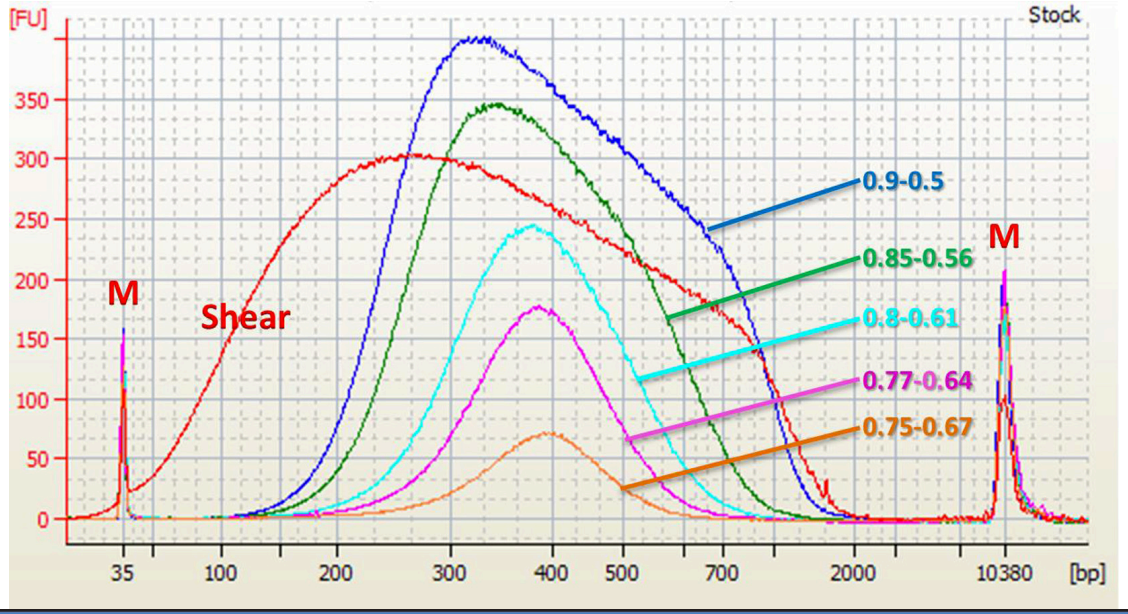


9µL



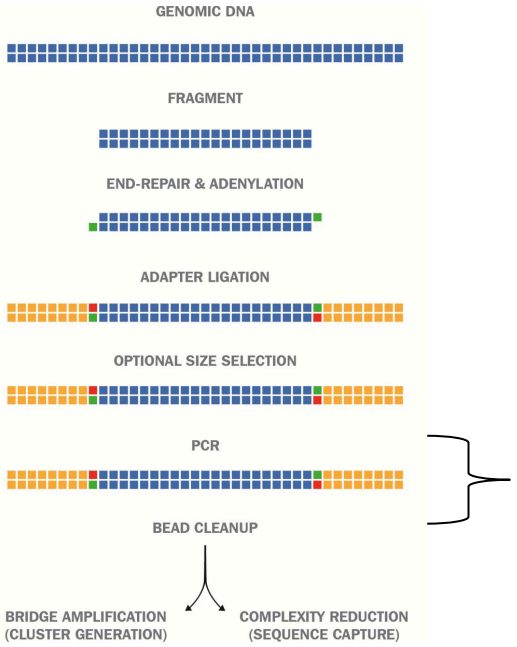


AMPure Beads



Ratios (Left-Right)	bp Region	Selection Delta (bp)	bp Region's % of Shear	Recovered % of bp Region	Recovered Region's % of Shear
Shear	40-3000	2960	100.0%	100.0%	100.0%
0.9-0.5	175-1300	1125	72.7%	60.4%	43.9%
0.85-0.56	200-700	500	61.8%	49.6%	30.6%
0.8-0.61	230-660	430	52.1%	33.4%	17.4%
0.77-0.64	260-575	315	40.8%	21.1%	8.6%
0.75-0.67	280-540	260	33.7%	10.1%	3.4%

Step 4: PCR amplification



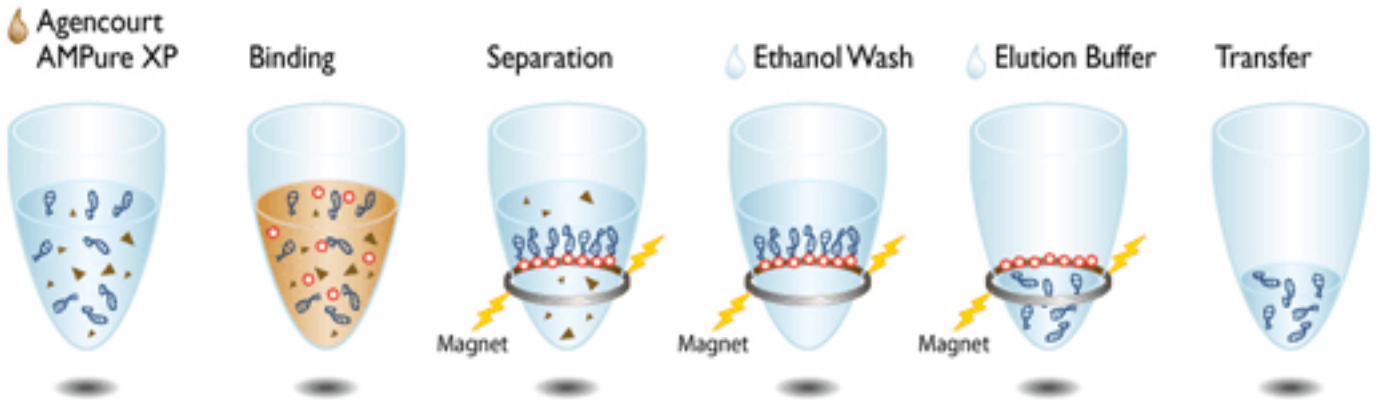
- Prepare the PCR reaction mix ligated DNA to a new PCR tube
- Transfer 20µL Adaptor ligated DNA to a new tube
- Add 30µL PCR master mix to each sample
- Gently pipette up and down 10 times

Amplify using PCR

Step 5: Adaptor ligation clean up

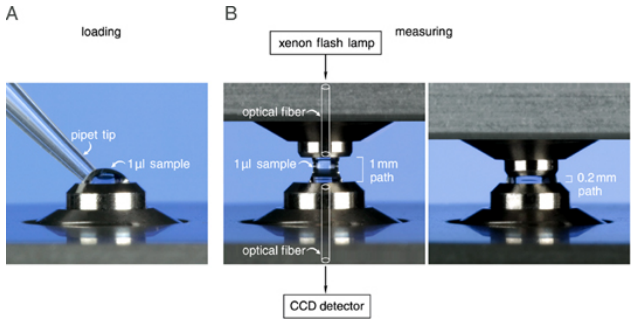
Size selection and clean-up using SPRI Beads

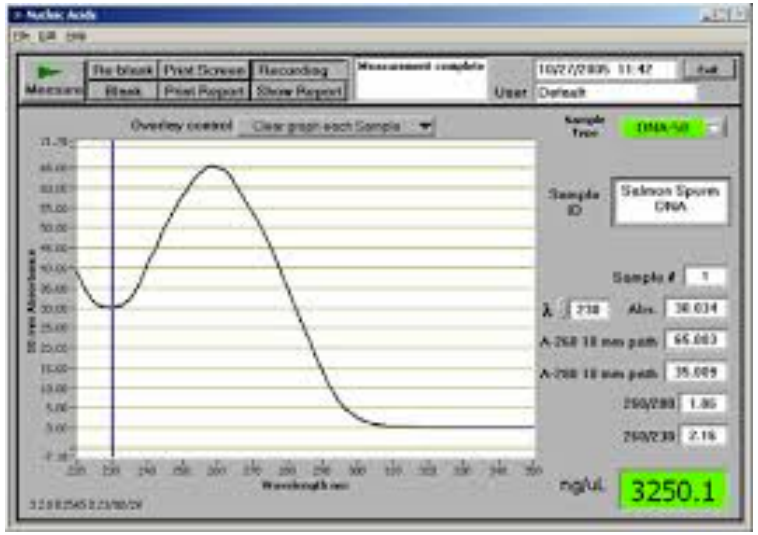
SPRI = Solid Phase Reversible Immobilization



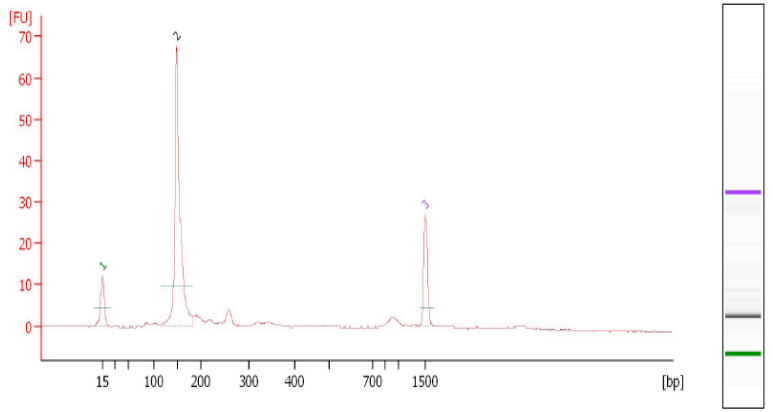
Ratio of SPRI beads to sample determines size cut off

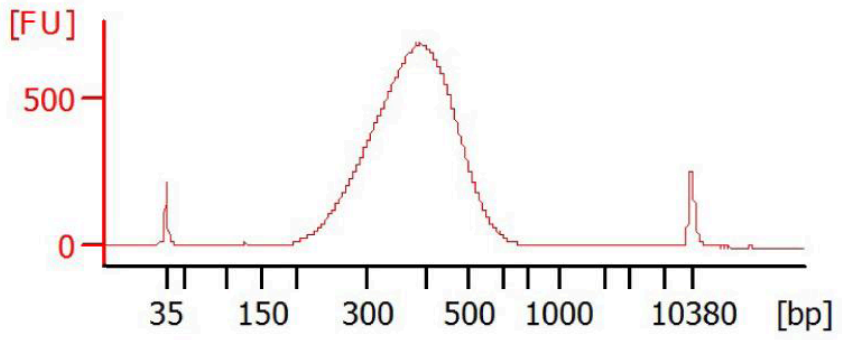
Measure the concentration of DNA



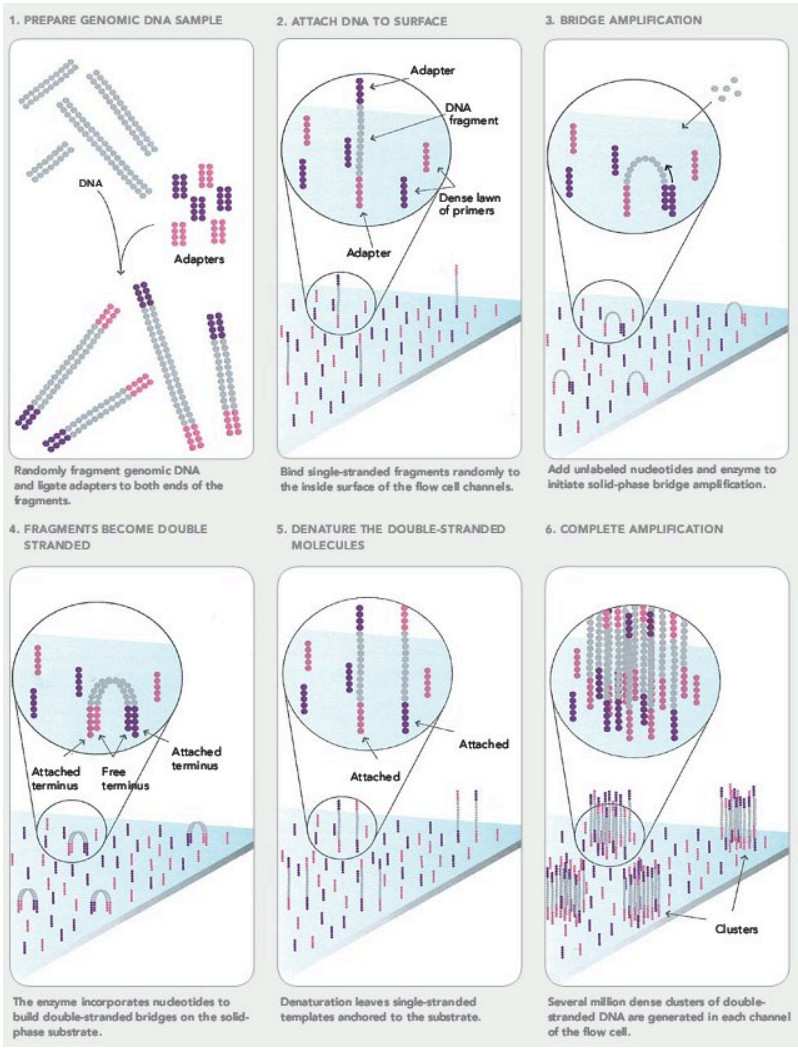


Bioanalyser profile



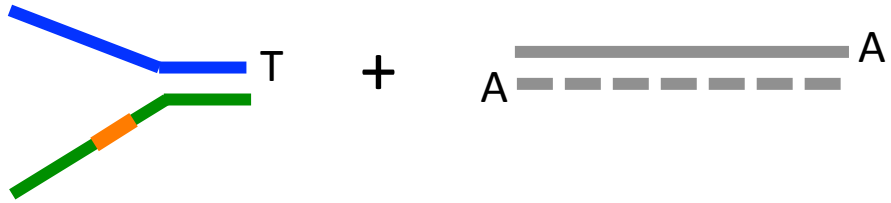


Illumina


1. PREPARE GENOMIC DNA SAMPLE
DNA
Adapters
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.
2. ATTACH DNA TO SURFACE
Adapter
DNA fragment
Dense lawn of primers
Adapter
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.
3. BRIDGE AMPLIFICATION
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.
4. FRAGMENTS BECOME DOUBLE STRANDED
Attached terminus
Free terminus
Attached terminus
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.
5. DENATURE THE DOUBLE-STRANDED MOLECULES
Attached
Attached
Denaturation leaves single-stranded templates anchored to the substrate.
6. COMPLETE AMPLIFICATION
Clusters
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

Adaptor: “ TruSeq –style” indexed adaptors

NEXTflex™	Sequence
DNA Adapter	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG
Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
Primer 2	5'CAAGCAGAAGACGGCATACGAGAT



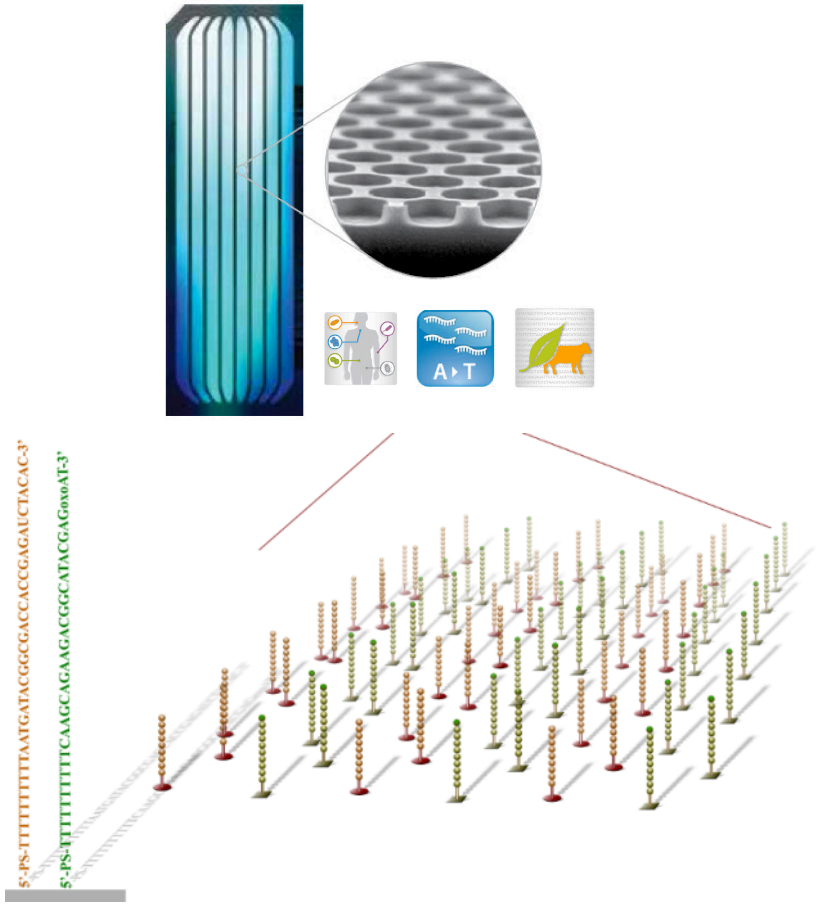
5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC

GCTCTTCCGATCT

CGAGAAGGCTAG

3’GTTCGTCTTCTGCCGTATGCTCTATGTAGCCACTGACCTCAAGTCTGCACA

Cluster



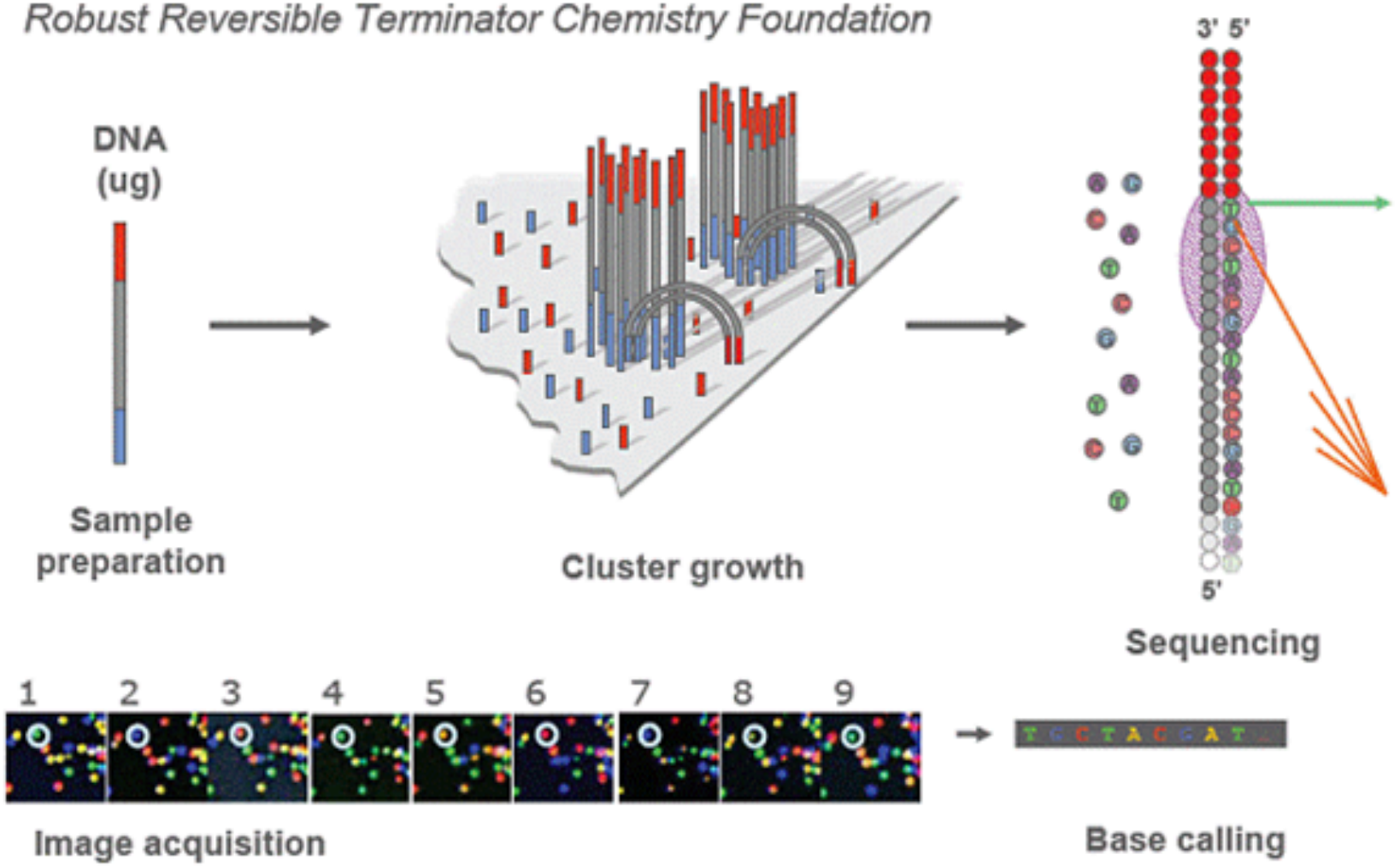
Clusters in a contained

Environment (no need for clean rooms)

Sequencing performed in the flow cell on the clusters

Illumina Sequencing Technology

Robust Reversible Terminator Chemistry Foundation

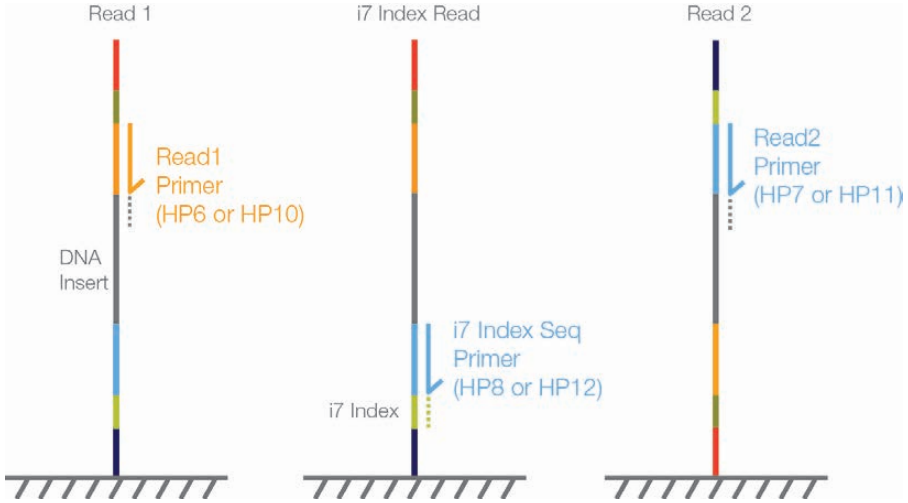


Single index

Single-Indexed Sequencing Overview

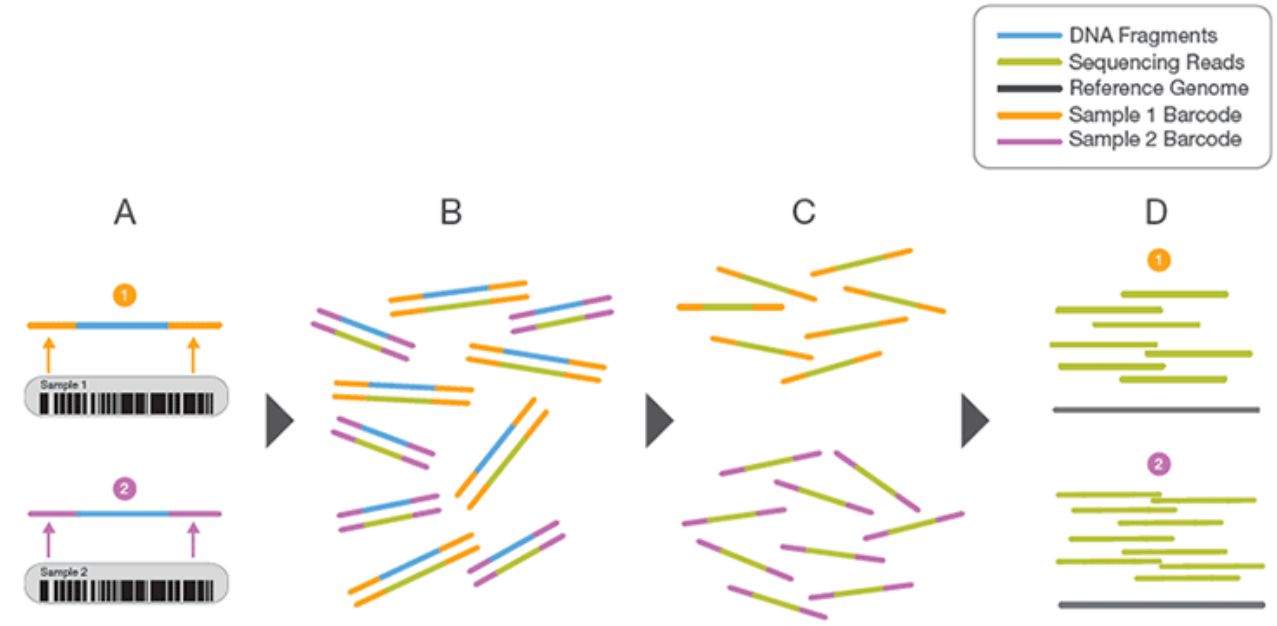
Single-indexed sequencing includes one Index Read following Read 1.

Figure 1 Single-Indexed Sequencing

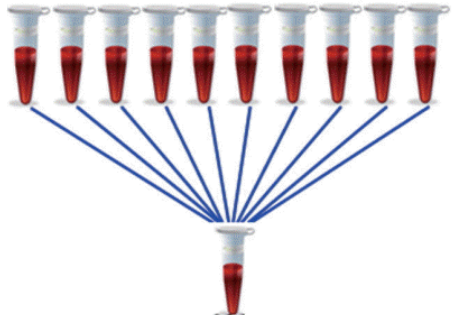


Barcoding and pooling

Figure 2: Conceptual Overview of Sample Multiplexing



A. Two representative DNA fragments from two unique samples, each attached to a specific barcode sequence that identifies the sample from which it originated.
B. Libraries for each sample are pooled and sequenced in parallel. Each new read contains both the fragment sequence and its sample-identifying barcode.
C. Barcode sequences are used to de-multiplex, or differentiate reads from each sample.
D. Each set of reads is aligned to the reference sequence.



De-Multiplexing

```
@FCC5TVWACXX:8:1101:2978:19[illegible]7#ACCTCCAA/2
GCCAAGGATGAAGCAGAGTTCCAAACCT[illegible]GCAGATTGTCAAC[illegible]AAATGATGGCAAGAAAAATTGAATTTCTTCCTGTTGATATCTACAAATCCGAGG
CTA
+
___eeeeeggggiiiiihihiihiiiiihhfifgihiiiiiiddfhihhagghdfhffhihidfhihihgggggddeeeeeeddbbdbdcbccacc
ccc
```