



PACIFIC
BIOSCIENCES®

华大基因
BGI

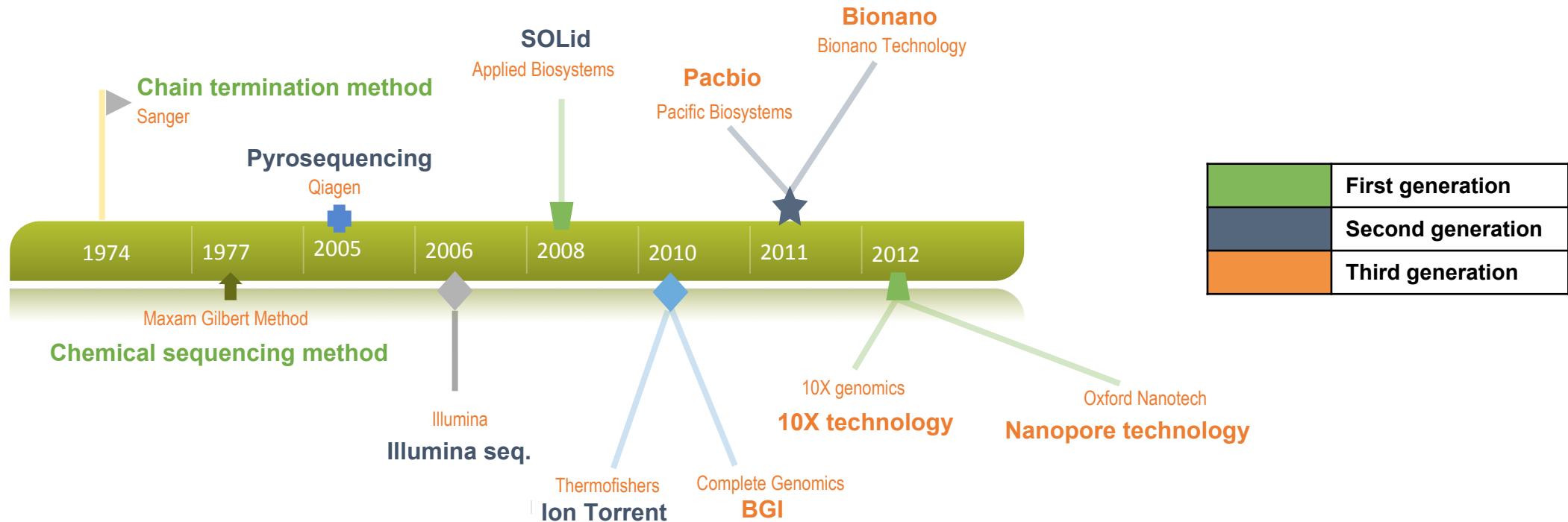
10X GENOMICS

illumina®

Next Generation Sequencing Technologies

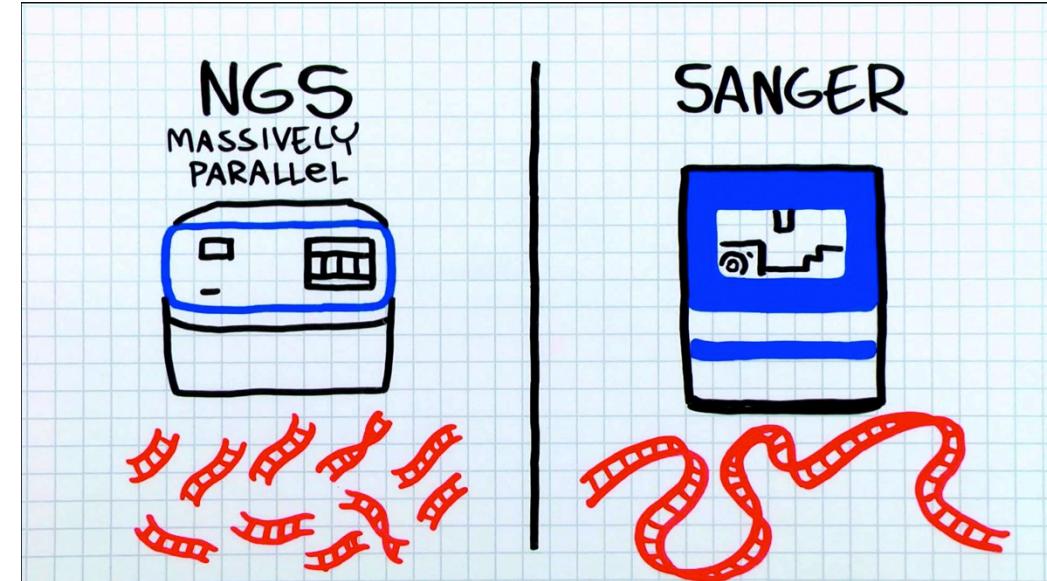
Carla Redila, Chandrima Shyam, Nida Ghori

Evolution of sequencing technologies



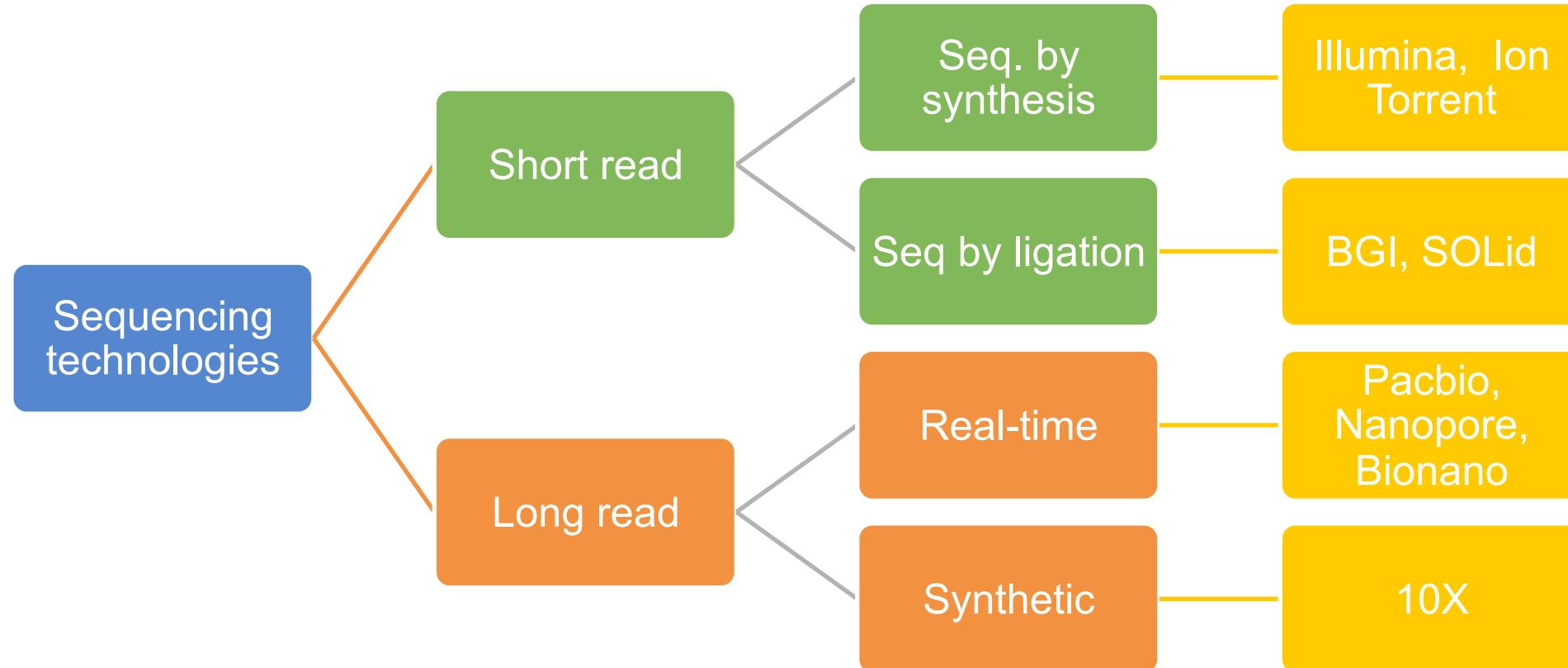
Why NGS when we have Sanger?

- Higher number of genes or gene regions can be identified simultaneously
- Finding novel variants by expanding the number of targets sequenced in a single run.
- Sequencing samples that have low input amounts of starting material example amount of DNA used
- Comprehensive genomic coverage



<https://www.thermofisher.com/blog/behindthebench/when-do-i-use-sanger-sequencing-vs/ngs-seq-it-out-7/>

Different sequencing Approaches



illumina® Workflow

Library Preparation



- Fragment DNA
- End repair
- Purify

Cluster generation

-
- A blue arrow indicating the flow from Cluster generation to Sequencing.
- Hybridized to flow cell and extend the template
 - Bridge amplification

Sequencing



- Sequencing
- Base call generation



Data Analysis

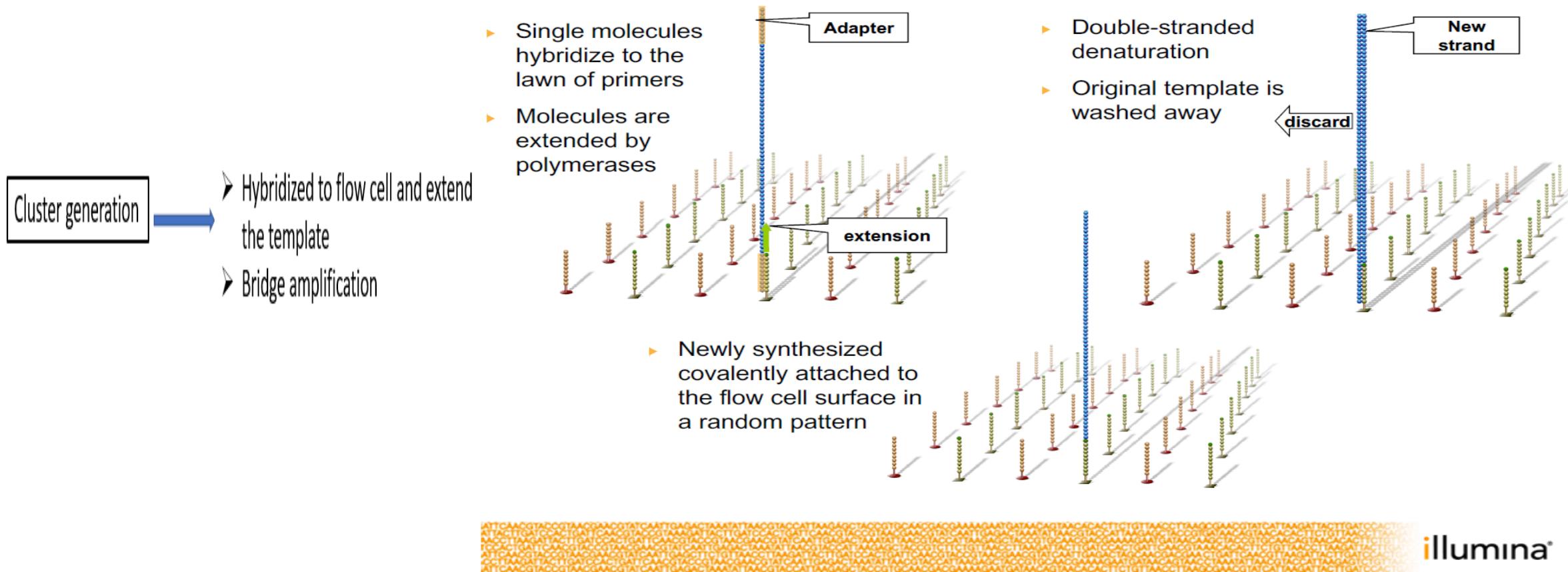


- Images
- Reads
- Alignments

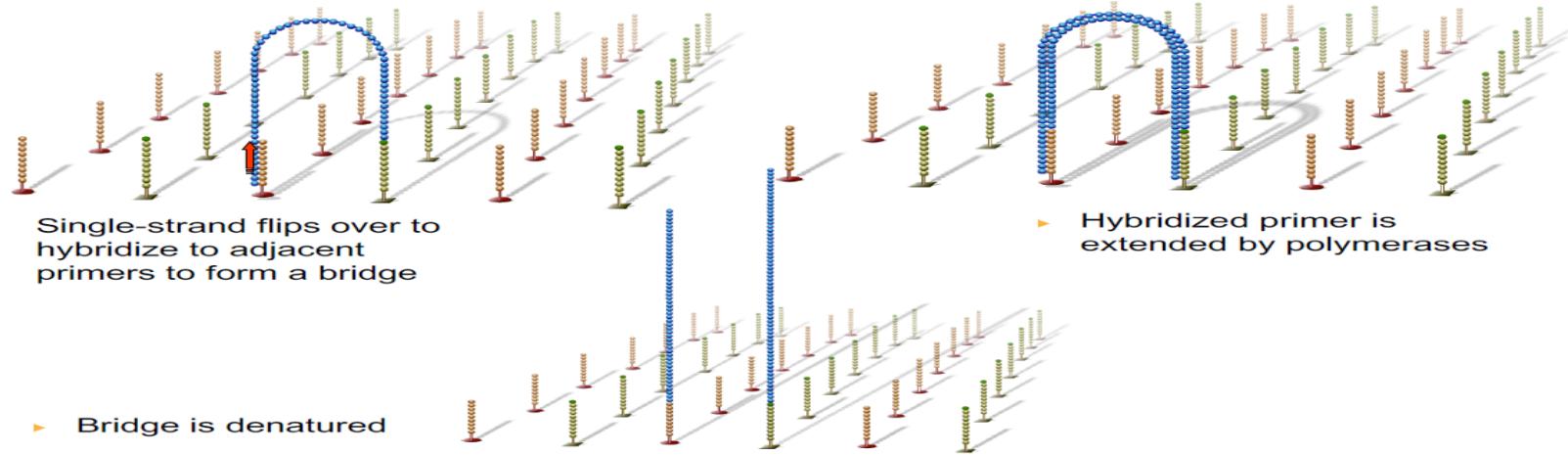


illumina® Workflow

Cluster Generation: *Hybridize Fragment & Extend*



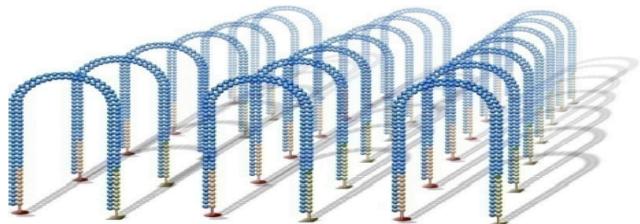
Cluster Generation: *Bridge Amplification*



illumina®

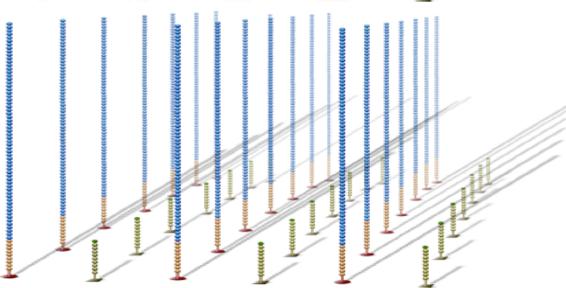
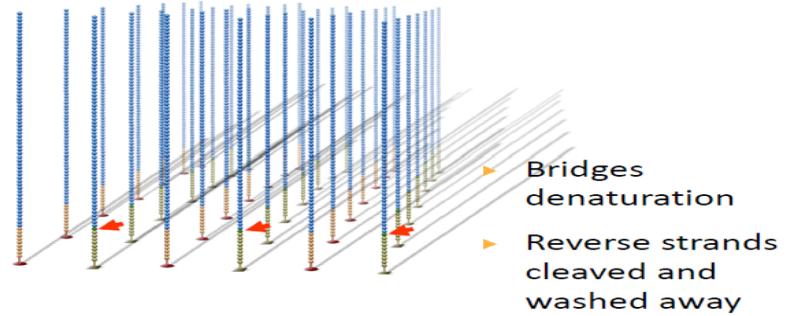
Cluster Generation: *Bridge Amplification*

- Bridge amplification cycle repeated until multiple bridges are formed

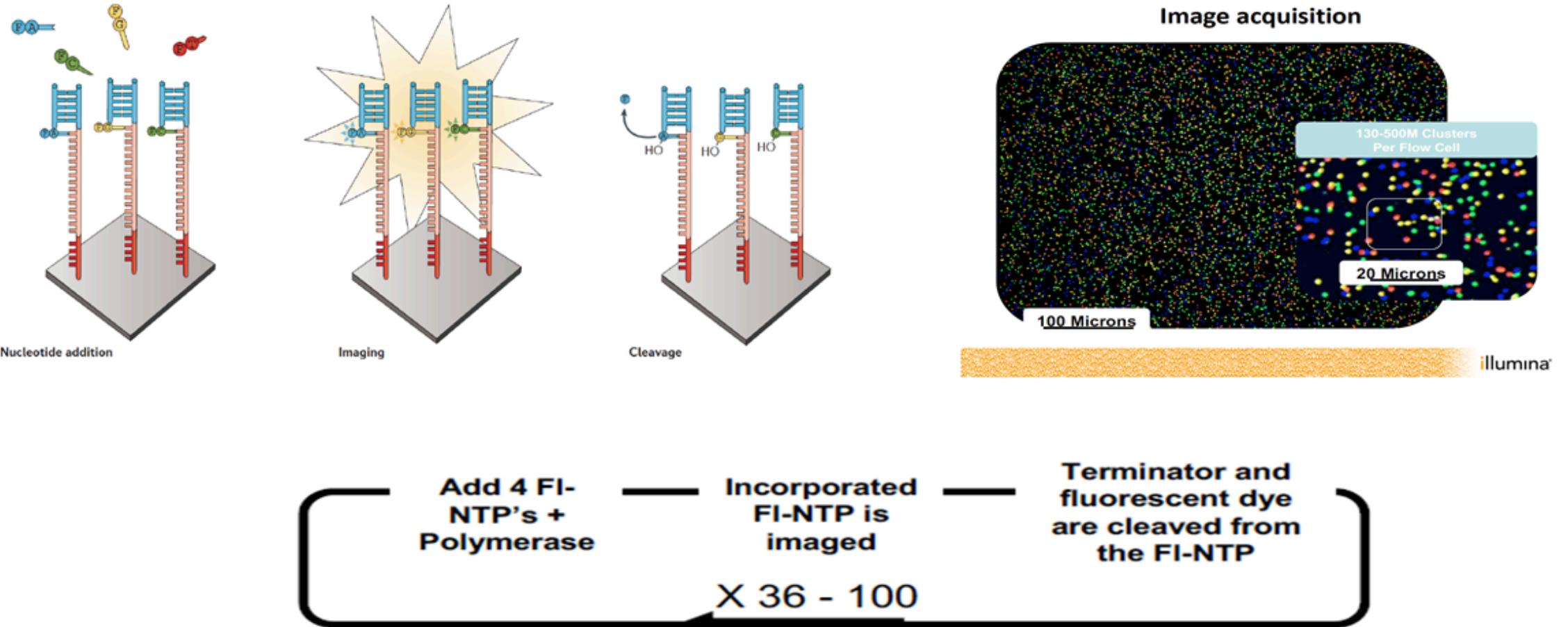


Cluster generation

- Hybridized to flow cell and extend the template
 - Bridge amplification

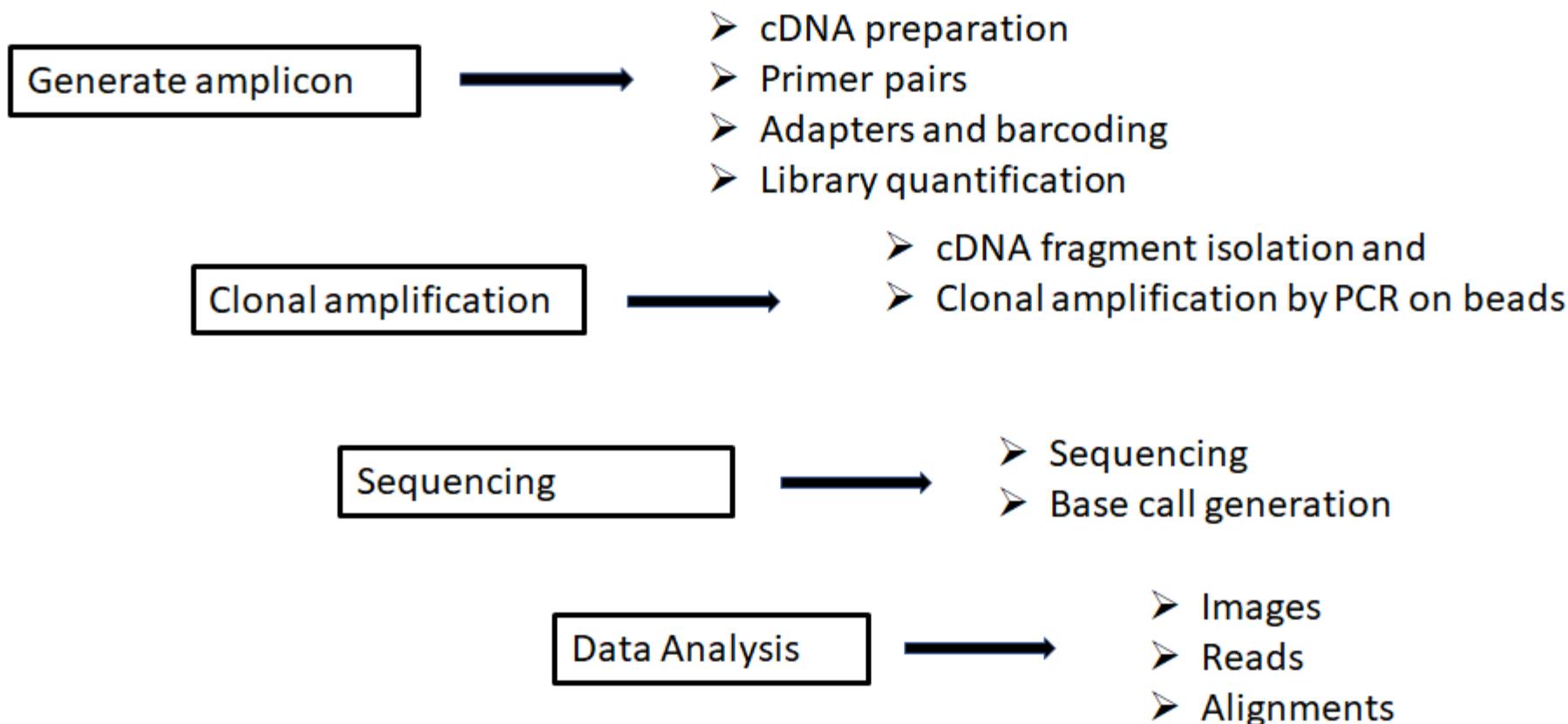


illumina® Workflow

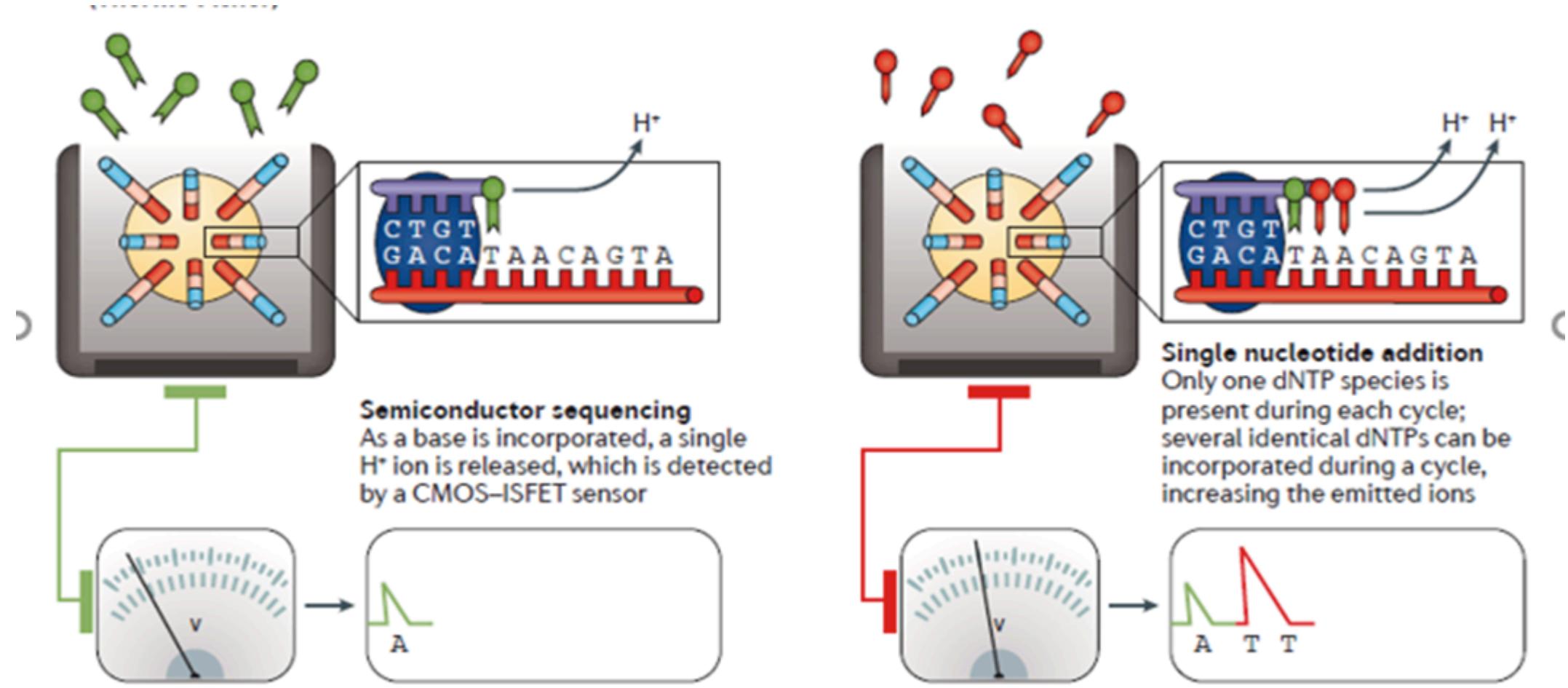


Goodwin et al., (2016), Voelkerding et al., 2009

ion torrent Workflow



ion torrent Workflow



SOLid Workflow

Library Preparation



- Fragmentation of gDNA
- Ligation of adapters

Amplification



- Emulsion based
- Polonies formation

Ligation reactions
and imaging



- Probe hybridization and ligation
- Fluorescence measurement
- Cleaving dye-end nucleotides (3')

Data Analysis

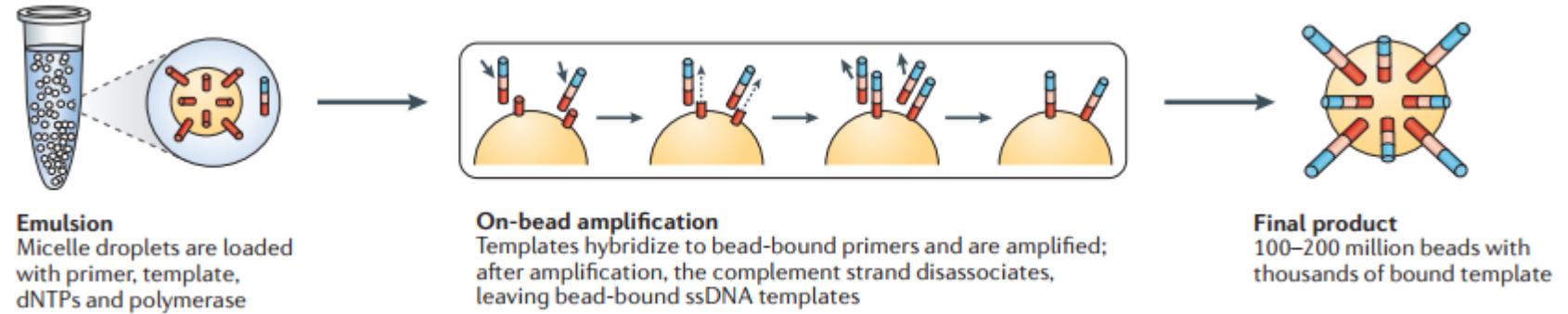


- Images
- Reads
- Alignments



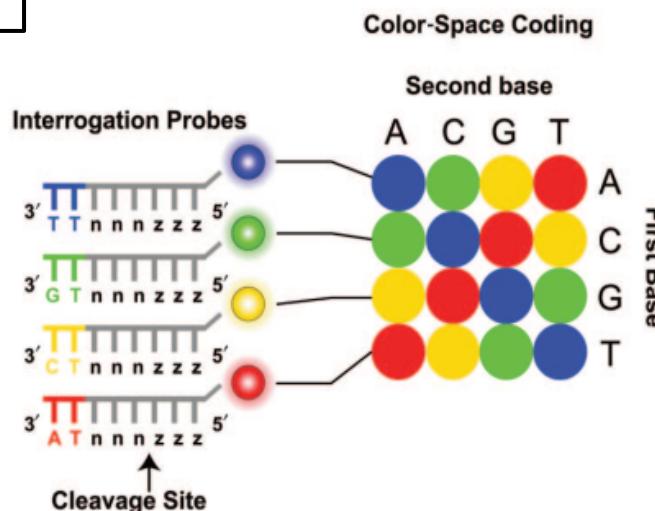
Library preparation

P2

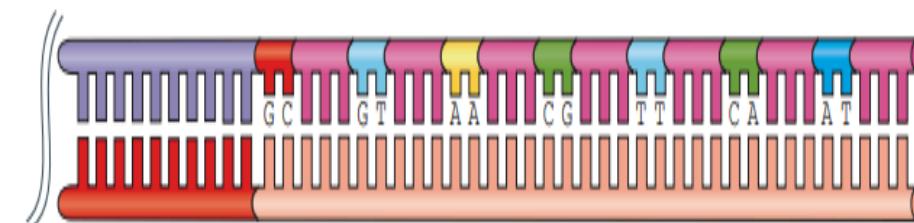
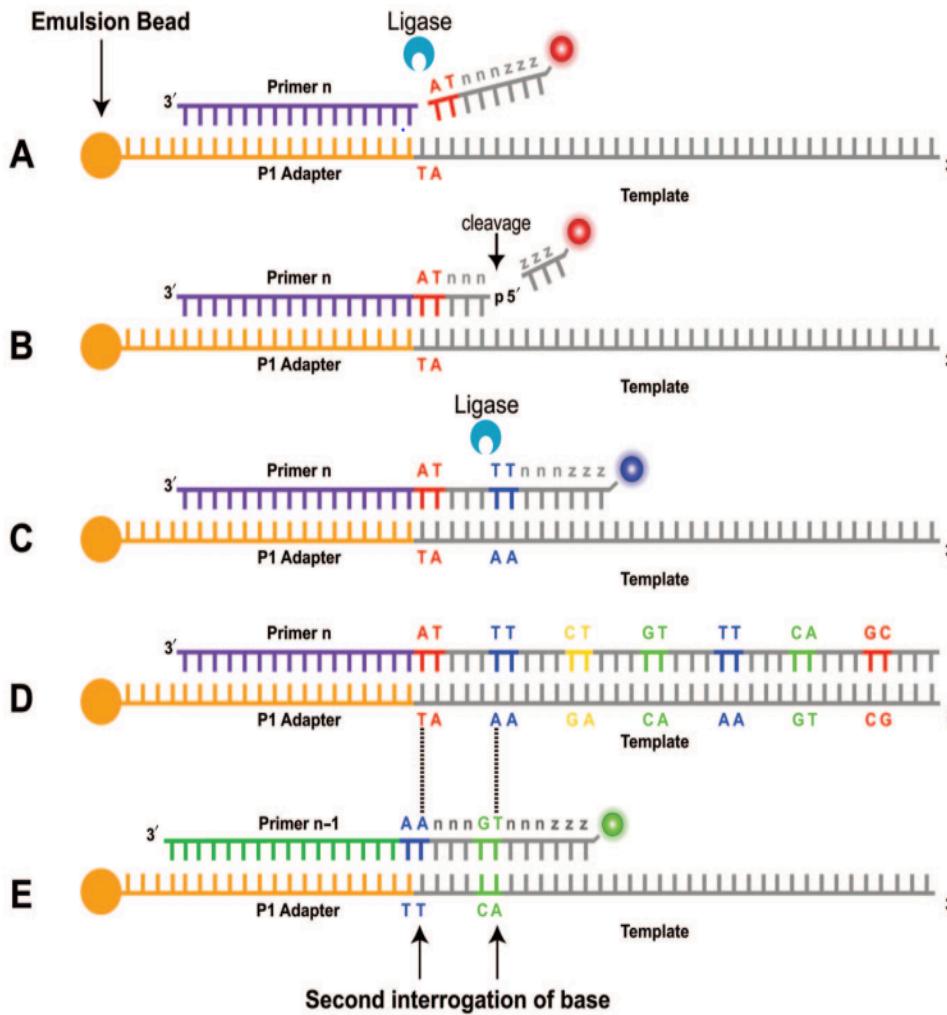


Probe hybridization

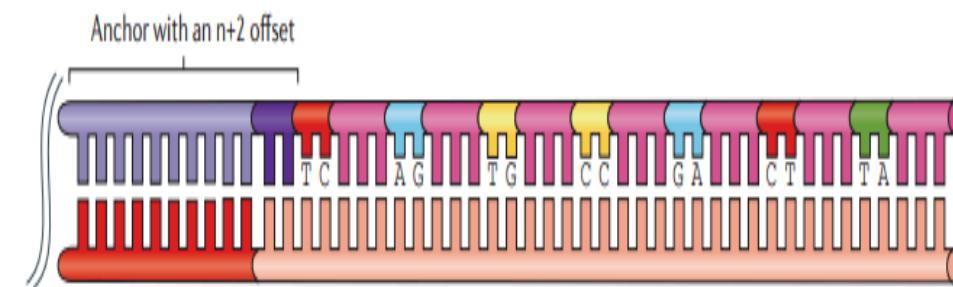
P1



Ligation reactions and imaging



Probe extension
10 rounds of hybridization, ligation, imaging and cleavage identify 2 out of every 5 bases



Reset
After a round of probe extension, all probes and anchors are removed and the cycle begins again with an offset anchor

Library Preparation

- Fragmentation of gDNA
➤ Ligation of adapters

Amplification

- PCR amplification
➤ Circular DNA formation
➤ DNA nanoball formation

Array sequencing

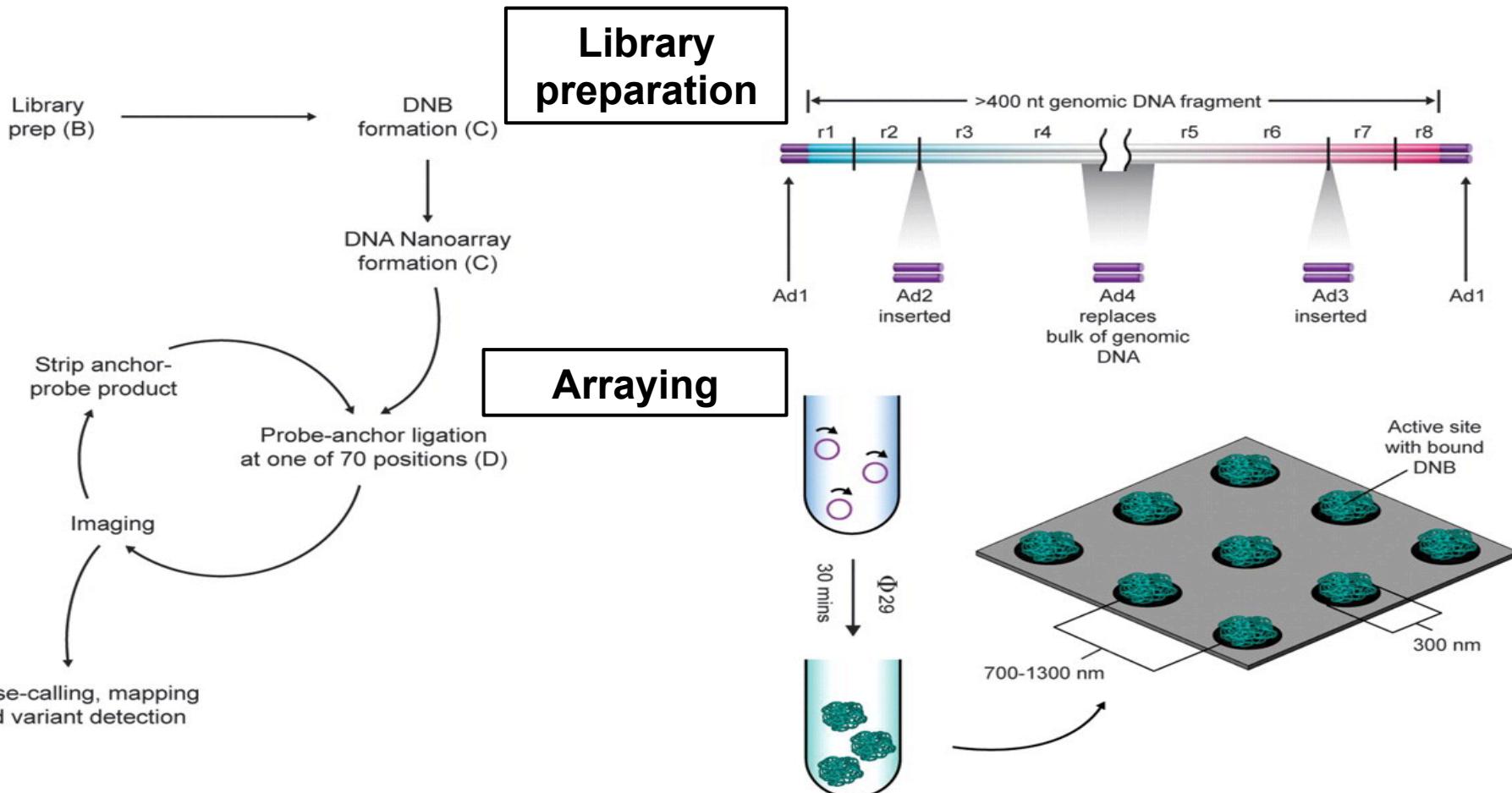
- 1-patterned array flow-cell
➤ Sequence by synthesis

Data Analysis

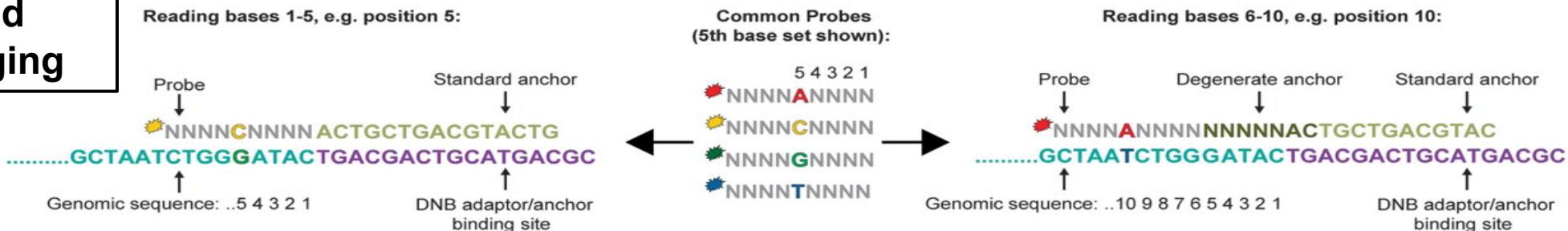
- Images
➤ Reads
➤ Alignments



Summary



Sequencing and imaging





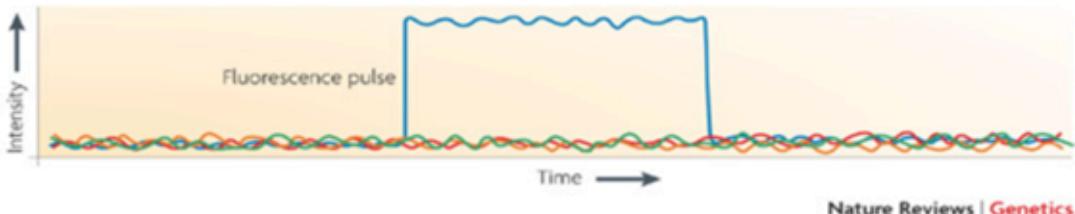
PACBIO® Workflow

Generate amplicon

Adapter ligation

Sequencing

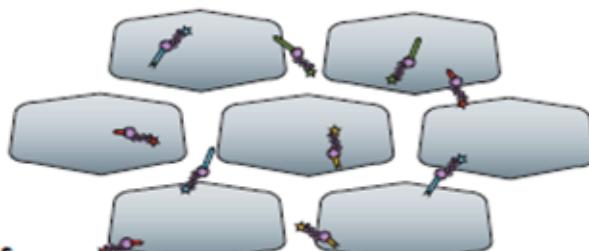
Data Analysis



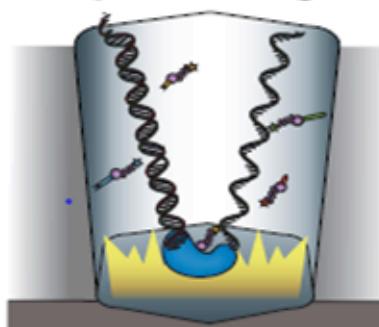
SMRTbell template
Two hairpin adapters allow continuous circular sequencing



ZMW wells
Sites where sequencing takes place



Labelled nucleotides
All four dNTPs are labelled and available for incorporation



Modified polymerase
As a nucleotide is incorporated by the polymerase, a camera records the emitted light



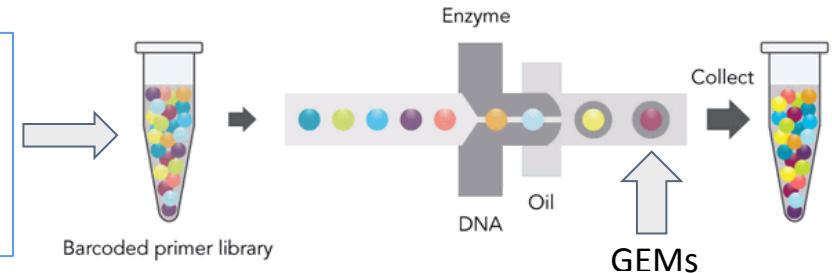
PacBio output
A camera records the changing colours from all ZMWs; each colour change corresponds to one base

Goodwin et al., (2016), Voelkerding et al., 2009

Whole Genome or Exome Sequencing Workflow

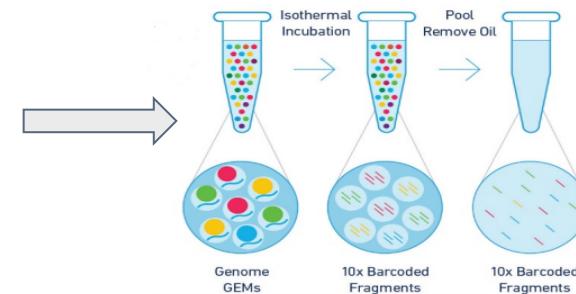
Creation of Genome Gel bead in emulsion (GEMs)

- 1ng DNA loaded into chromium controller
- Cells and enzymes bind to 10x barcoded beads
- Coated in oil



Incubation and Purification

- Isothermal incubation
- Creates 10X barcoded DNA fragments
- Emulsion broken
- Oil removed

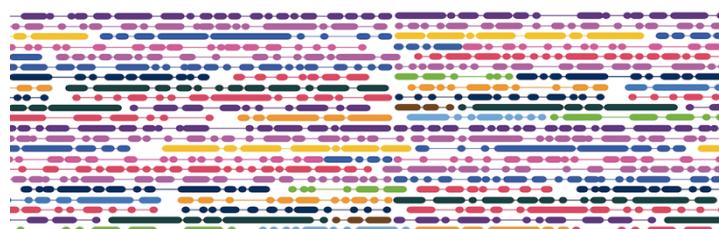


Library Preparation and Sequencing

- Standard Illumina library prep
- Illumina platform for sequencing
- Can do WGS or bait capture for exome sequencing



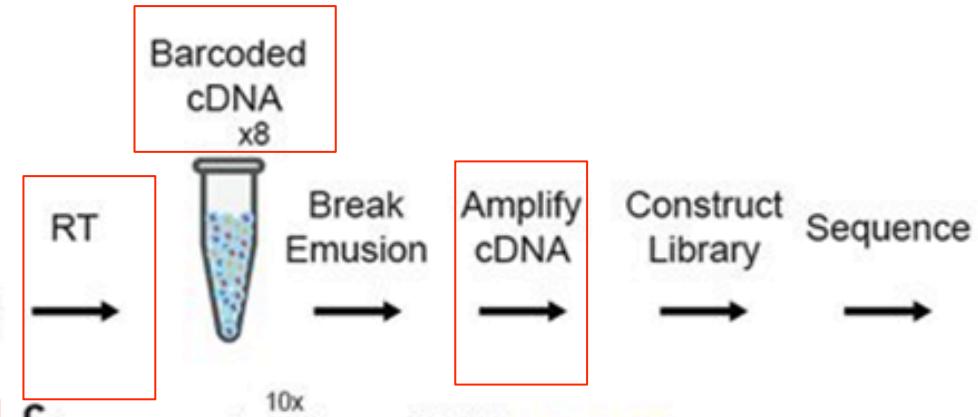
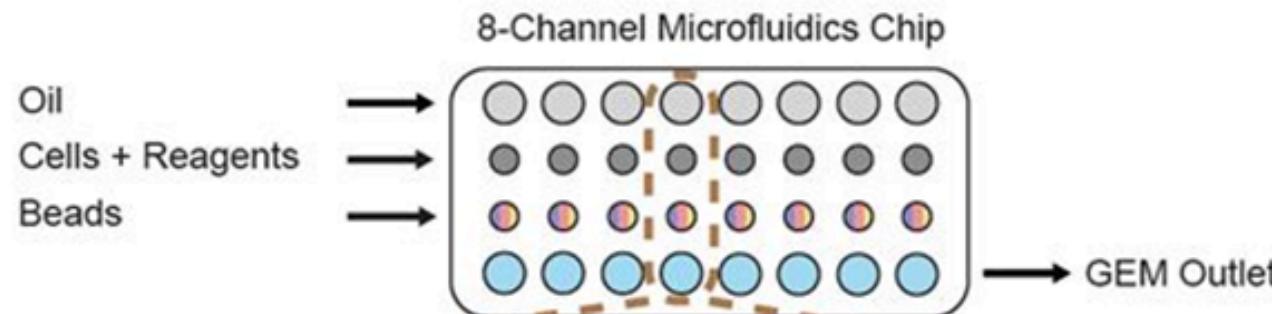
Assemble Linked Reads



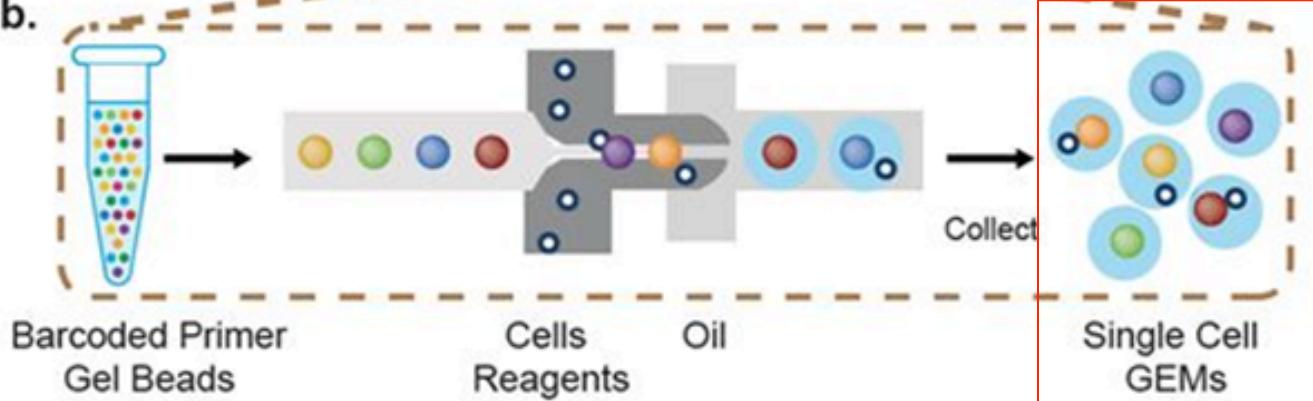


Single Cell Expression Workflow

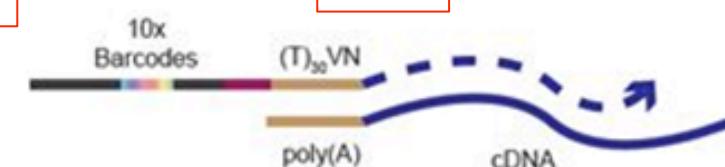
a.



b.



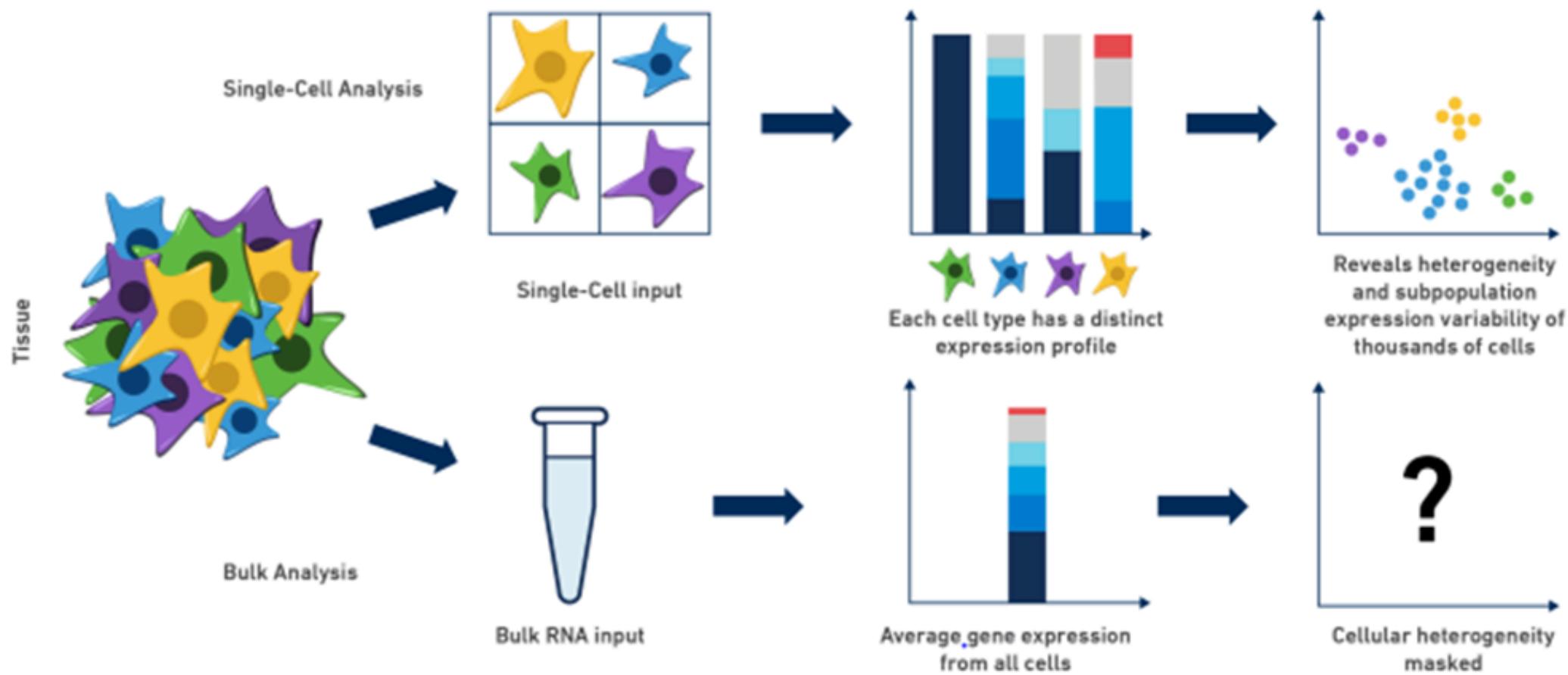
c.



d.



Single Cell Expression





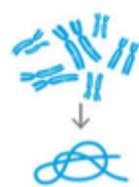
Workflow

Customer Sample

- Blood • Tissue
- Cells • Microbes



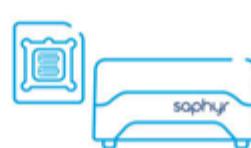
Isolate
High Molecular
Weight DNA



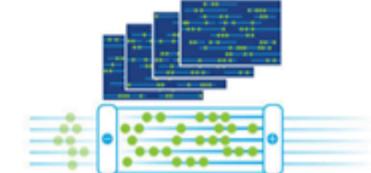
Label Specific
Sequences Across
the Entire Genome



Transfer Labeled DNA
into Cartridge
for Scanning

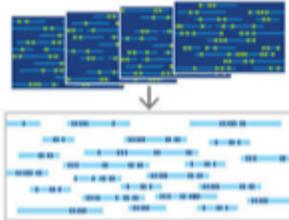


Load, Linearize & Image Labeled
DNA in Repeated Cycling
to Scan Whole Genome

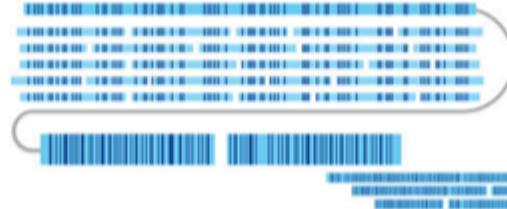


High-throughput, High-resolution Imaging of Megabase Length Molecules

Algorithms
Convert Images
into Molecules



Assembly Algorithms Align
Molecules *de novo* to Construct
Consensus Genome Maps



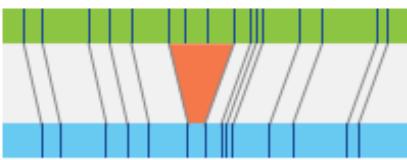
Cross-Mapping Across
Multiple Samples or
to a Reference



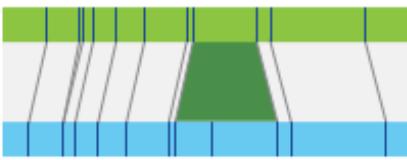
- Automated SV Detection
- Scaffolding

GAIN/LOSS

Deletion

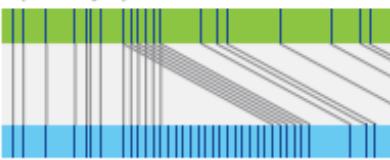


Insertion

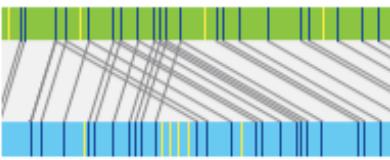


COPY NUMBER CHANGE

Repeat array expansion

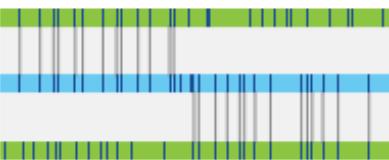


Tandem duplication



BALANCED

Translocation

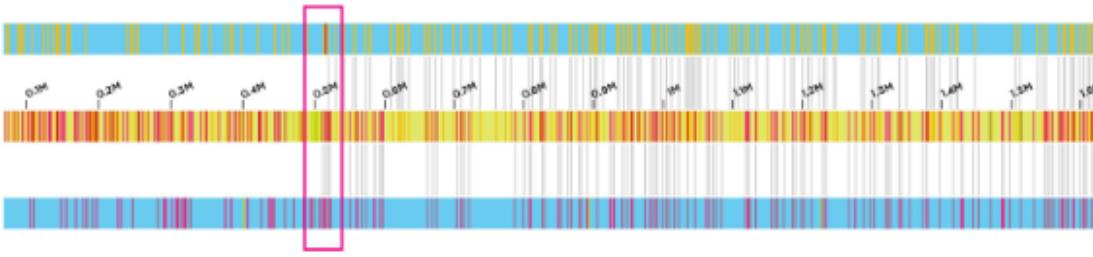


Inversion



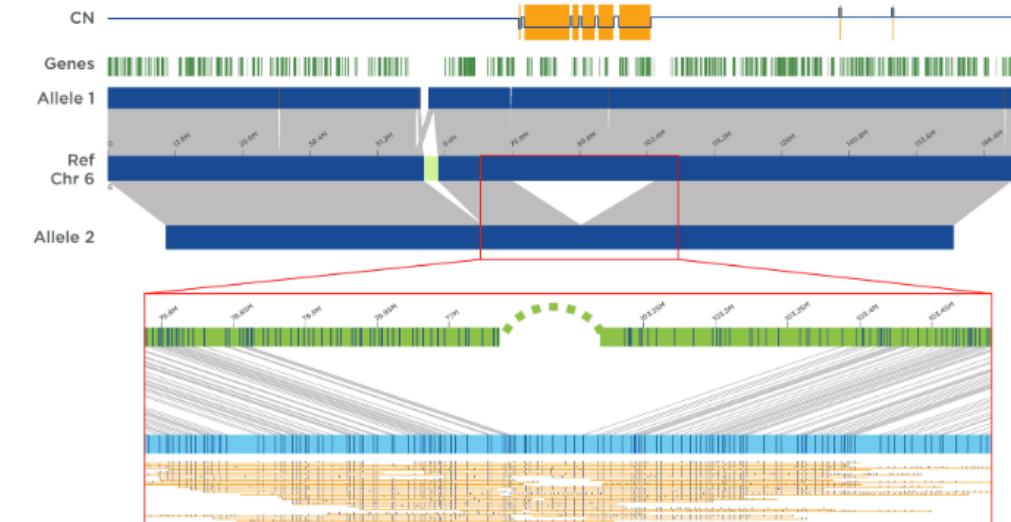
ERROR CORRECTION

Bionano Map BspQI



What type of Structural Variant is shown below?

LEUKEMIA 26.9 MBP





For example, a quote for a 500Mb genome that required additional work to optimize the HMW DNA isolation might look like this:

description	units	price per unit (\$)	total (\$)
project set up	1	570	570
HMWDNA isolation	2	1073	2146
QC	2	393	786
NLR	1	503	503
imaging	1.5	2032	3048
compute maps	7.5hr	114	855
total			7908

Available at K-State's
Bioinformatics Center!

Third Generation Sequencing Summary

Platform:	Advantage:	Disadvantage:
Nanopore	<ul style="list-style-type: none">Up to 2Mb for readsPortable instrumentEasy sample preparation	<ul style="list-style-type: none">Error rate: 10-15%
PacBio	<ul style="list-style-type: none">Up to 70kbNo PCR BiasDirect detection of modified nucleotides	<ul style="list-style-type: none">Input material (100ng for 10kb library)Error rate: 10-13%
10x Genomics	<ul style="list-style-type: none">Compatible with IlluminaLow material input(1-3 ng)	<ul style="list-style-type: none">PCR Bias
BioNano Genomics	<ul style="list-style-type: none">Insertions, deletions and other structural variants found, especially in repetitive genomeNo substitution bias	<ul style="list-style-type: none">Very dependent on the quality of the reference genomeRestriction enzyme and labeling must be efficient

References

- Drmanac et al., (2009) Human Genome Sequencing Using Unchained Base Reads on Self-Assembling DNA Nanoarrays. *Science*, 10.1126/science.1178068.
- Goodwin S et al.,(2016) Coming of Age: ten years of next generation sequencing. *Nature Reviews Genetics*, 17: 333-351.
- Voelkerding KV et al., (2009) Next generation sequencing: from basic research to diagnostics. *Clinical Chemistry*, 55: 641-658.