Weill Cornell Medicine

Core Laboratories Center

Epigenomics Core Facility:
"I have samples for sequencing", what does it mean?

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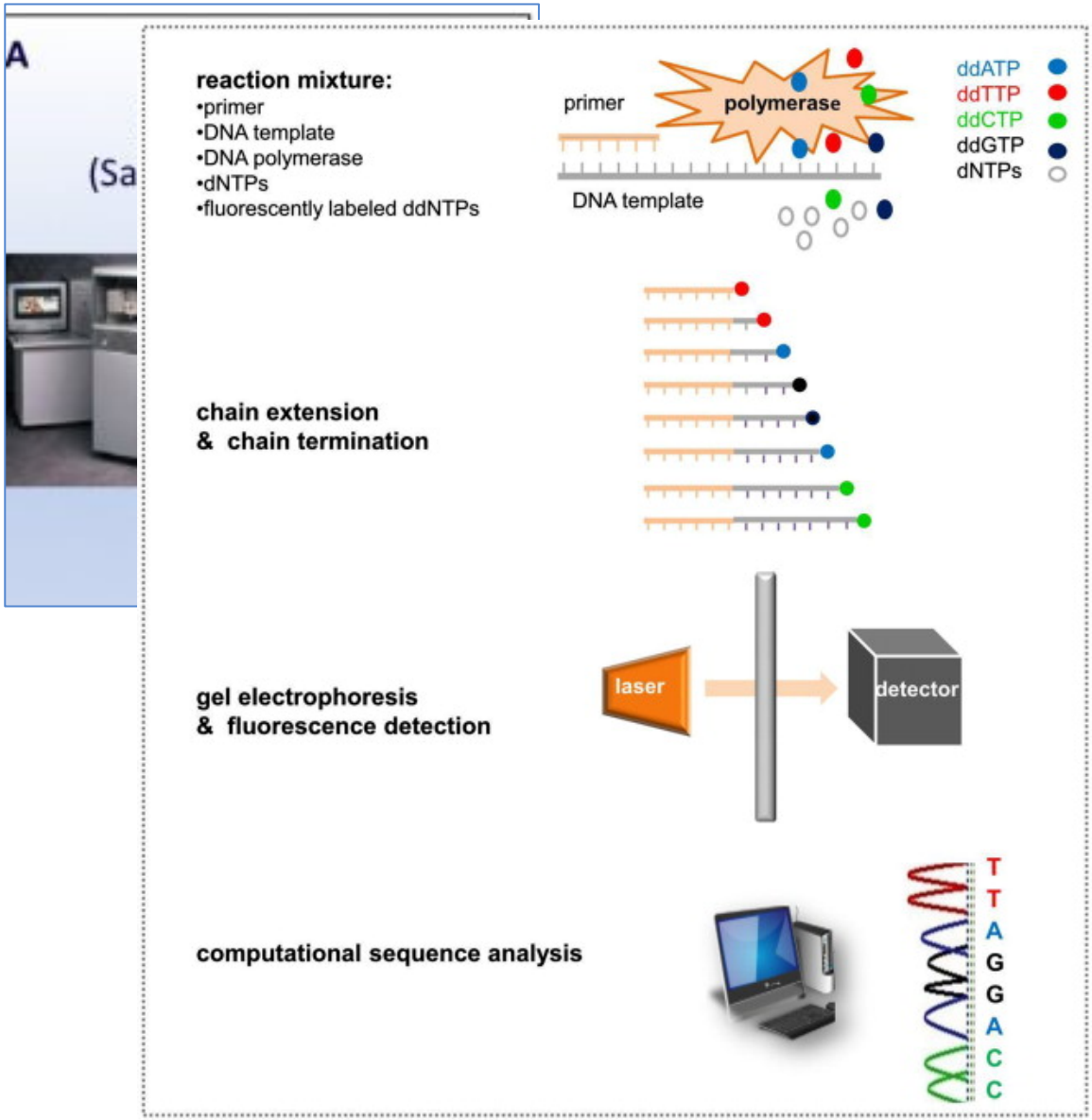
Genomics and Epigenomics Core Facility
Assistant Research Professor, Department of Hematology and Oncology

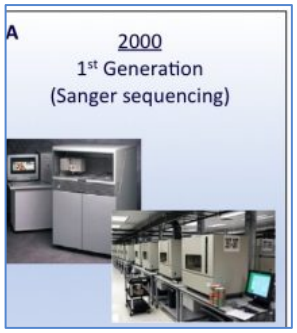
04022019-ICB students

Evolution of Sequencing Technologies

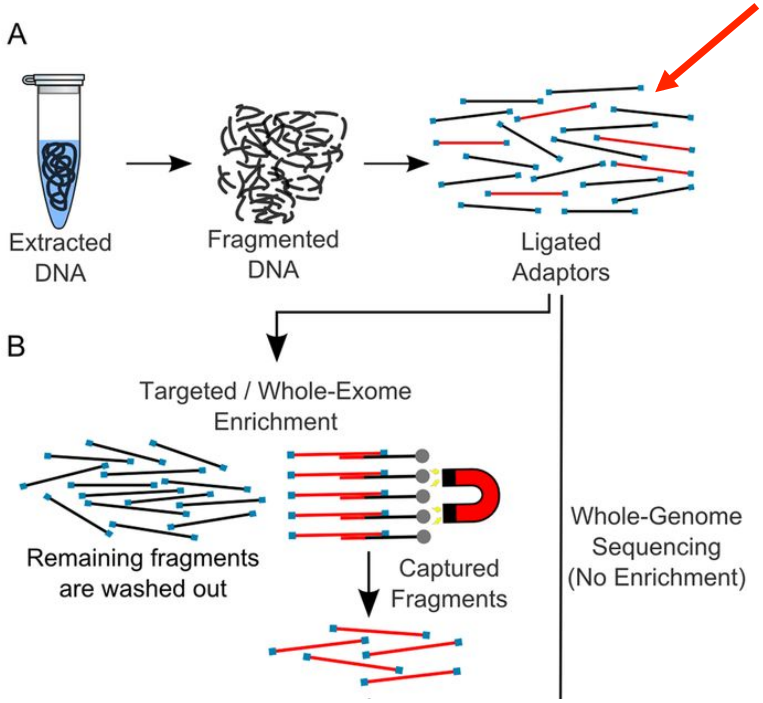


- Custom sequencing (targeted)
- Sequencing length: 300-1000bp
- Output: 384 independent samples at a time
- ABI Prism 375 (Applied Biosystems)

Evolution of Sequencing Technologies



Making a "library"

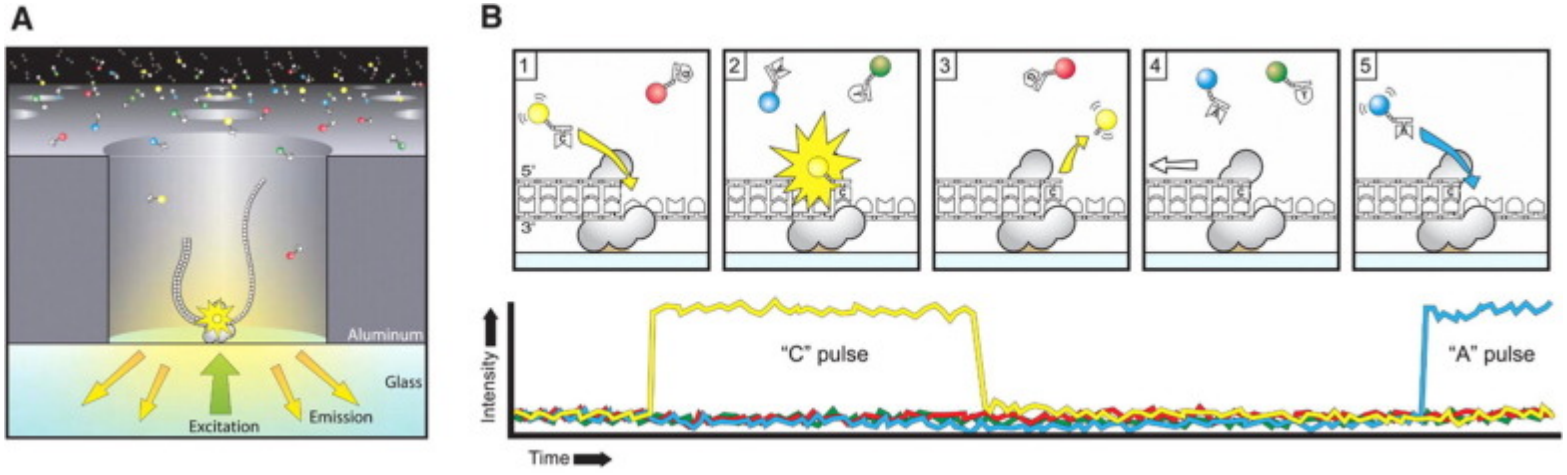


- Generation of many millions of short reads **to be sequenced** in parallel (150-600bp)
- Speed of sequencing (compared to 1st generation)
- Cost of sequencing (lower per base)
- Output is detected directly (ie no electrophoresis)
- Output varies from 15M – 400B "reads"
- Short reads are aligned to a **previously sequenced genome**
- Examples of 2nd generation competing technologies
  - Roche/454 (pyrosequencing – extinct- up 1000bp, 1Gb
  - Ion torrent (detects the Hydrogen ion released, change of pH-Life Technologies, 2010) 200-600bp, 10Gb (2-8 hours)
  - ABI/SOLiD 35-75 bp 30Gb/run (each base is read 2x, high accuracy)
  - ***Illumina/Solexa sequencing: Sequencing by Synthesis SBS (50-250bp)***

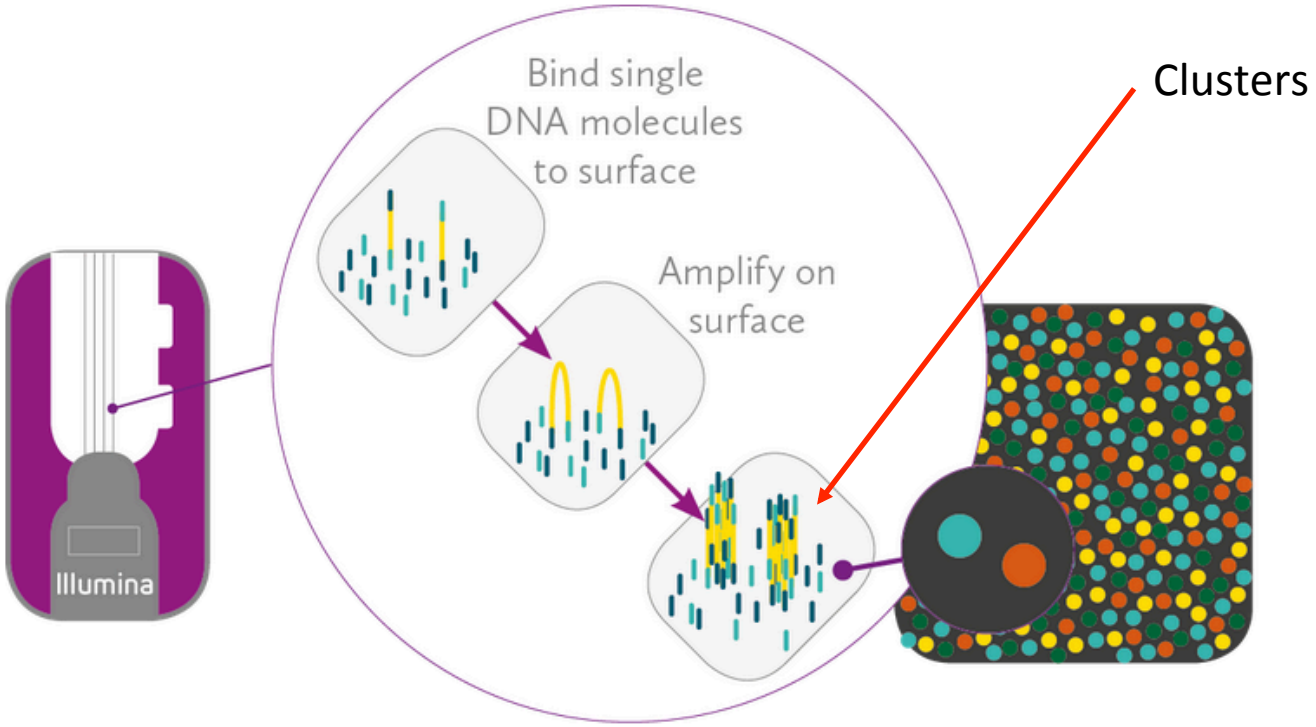
Evolution of Sequencing Technologies

SMRT : single molecule real time sequencing

- Pacific Biosciences: Sequel
- Oxford Nanopore: MinION, PromethION
- Sequencing length: up to 100kb
- Allows "de novo" mapping
- Sequencing of modifications of the bases (methylation for example)



Illumina Technology for 2nd Generation Sequencing Rules the Field!



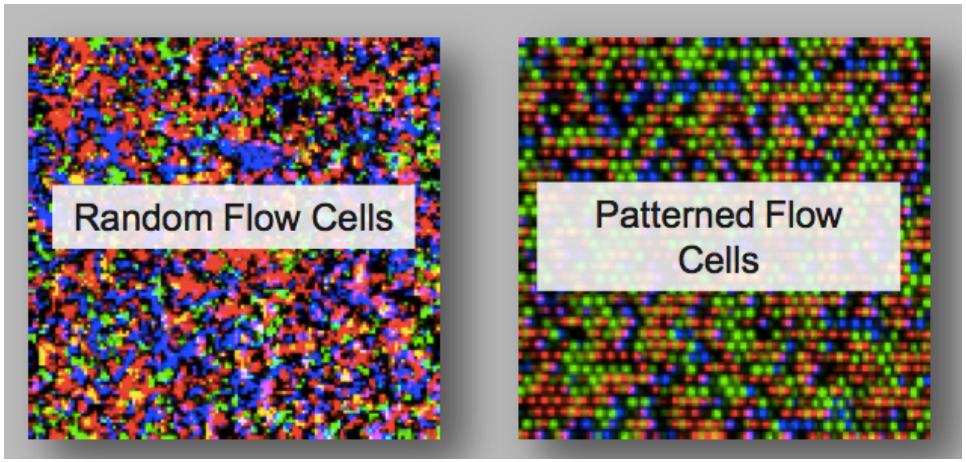
Processing: base calls are made directly from the signal intensity using Illumina's RTA software, using Casava 2.1.7 raw reads and quality scores are generated.

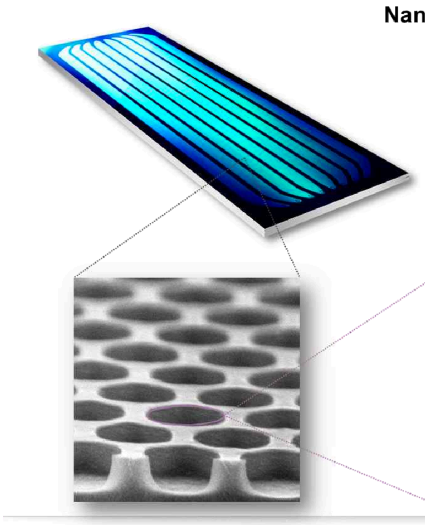
 # Clusters: varies with the sequencer used and the chemistry used
A HiSeq 2500 (our most common sequencing run) gives ~ 800k/mm2

 # reads: total number of clusters X read length
for SR50, our most common length ~280M reads, 7GB per lane, 8 lanes

Illumina Technology continues improving!

Efficient clustering





Illumina Technology continues improving!

Faster “sequencing”

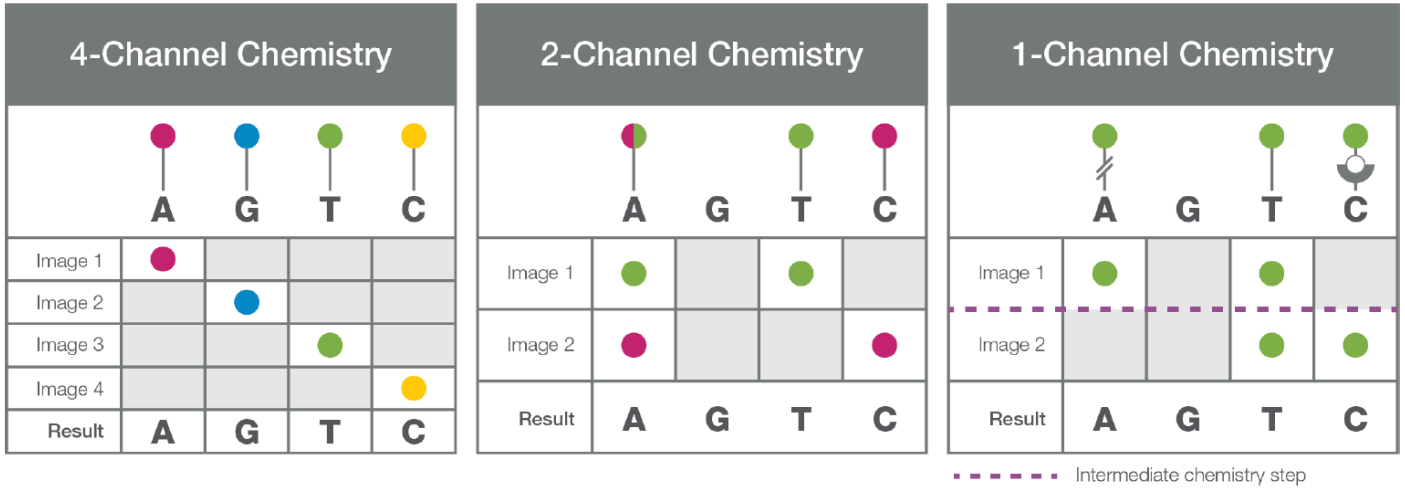
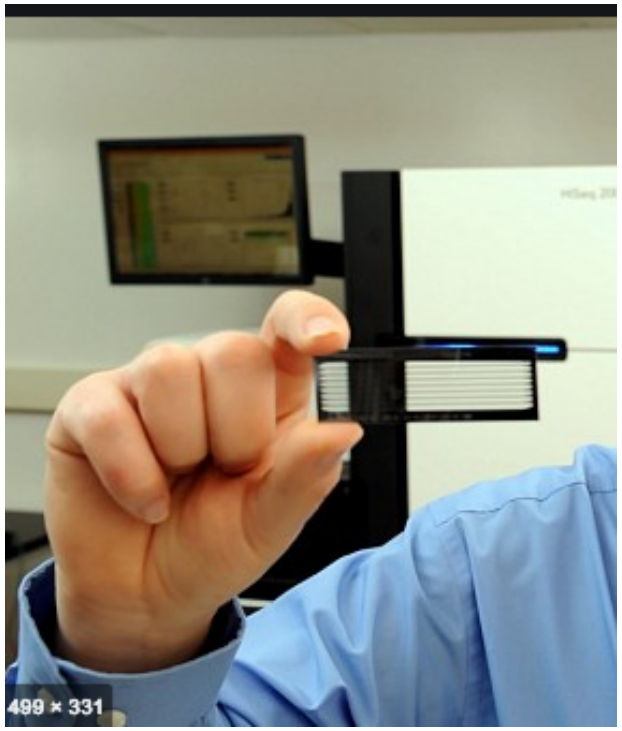


Figure 2: Four-, Two-, and One-Channel Chemistry—Four-channel chemistry uses a mixture of nucleotides labeled with four different fluorescent dyes. Two-channel chemistry uses two different fluorescent dyes, and one-channel chemistry uses only one dye. The images are processed by image analysis software to determine nucleotide identity.

Increasing throughput

Sequencing System	iSeq™	MiniSeq™	MiSeq®	NextSeq®	HiSeq® 4000	HiSeq® X Five/Ten	NovaSeq® 6000
Output per run	1.2 Gb	7.5 Gb	15 Gb	120 Gb	1.5 Tb	1.8 Tb	1 Tb - 6 Tb[1]
Instrument price	$19.9K	$49.5K	$99K	$275K	$900K	$6M[2]/$10M[2]	$985K
Clustering	**patterned**	random	random	random	random & patterned	random	**patterned**
Chemistry	1-color	4-color	4-color	2-color	4-color	4-color	2-color

Illumina Technology continues improving!



Illumina Technology continues improving, the need to MULTIPLEX!

- Coverage (or depth):
  number of reads that are likely to be aligned at a given reference position

$$\textbf{Coverage} = \frac{\text{read length} \times \text{number of reads}}{\text{genome length (haploid)}}$$

- How many reads would one need to sequence ONE human genome at 30x coverage in a PE100 run?

$$\text{number of reads} = \frac{30 \times 3\times10^{9}}{200}$$

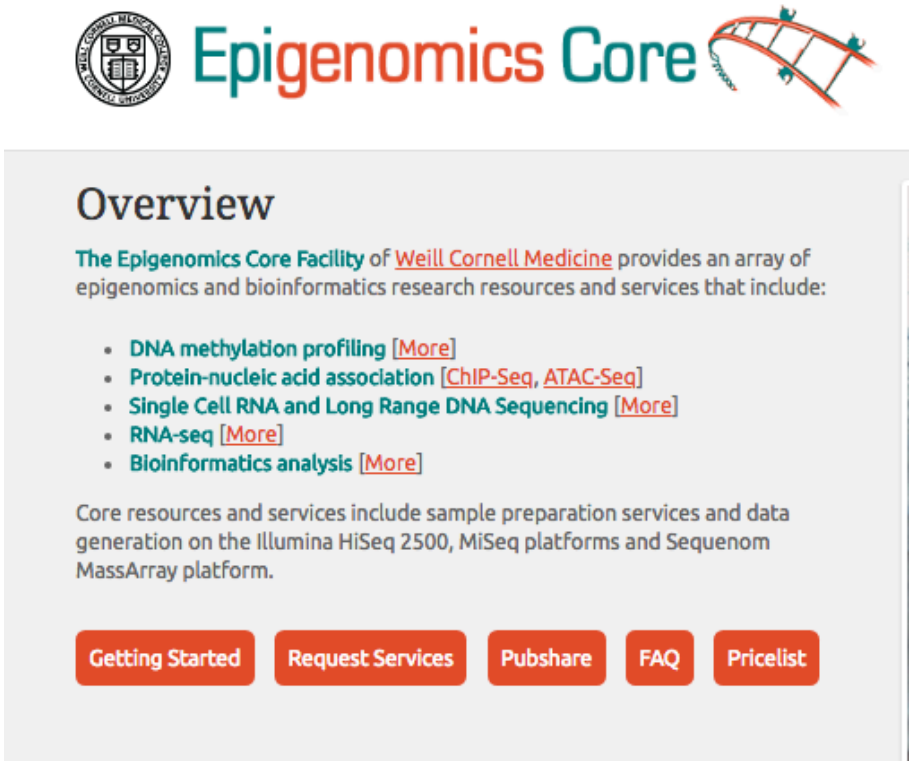
450M reads

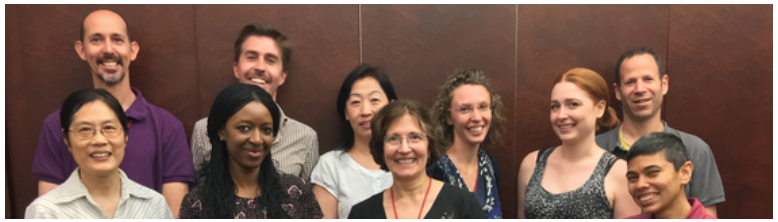
On a HiSeq 2500: two sequencing lanes	~$5,000
On a HiSeq 4000: one sequencing lane	~$2,500
On a Novaseq S4: 1/6 of a sequencing lane	~$1,000

- Multiplexing: being able to physically pool libraries from different origins, sequence and then bioinformatically re-assign each library to its origin

For ChIPseq, 40M reads

http://epicore.med.cornell.edu/





Wet Lab

Scientific Director	Alicia Alonso, PhD
Lab Manager	Yushan Li
Research Specialists	Marisa Mariani, PhD
	Caroline Sheridan
Research Technician	Yuan Xin

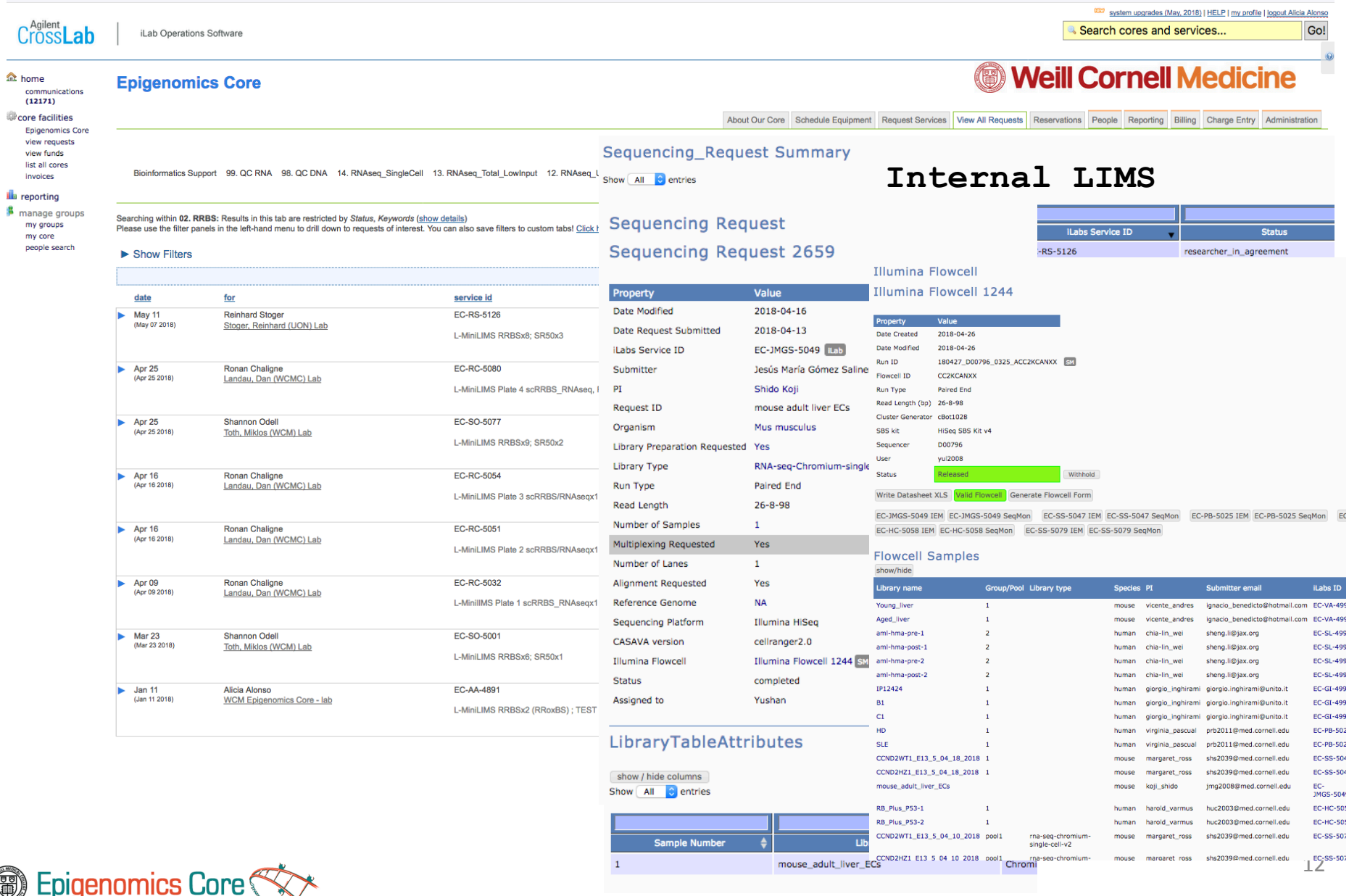
Dry Lab

Bioinformatics Director:	Doron Betel, PhD
Computational Biologist:	Piali Mukherjee
Software Developers:	Thadeous Kacmarczyk, PhD
	Simon Johnson

A project with the Epigenomics core includes

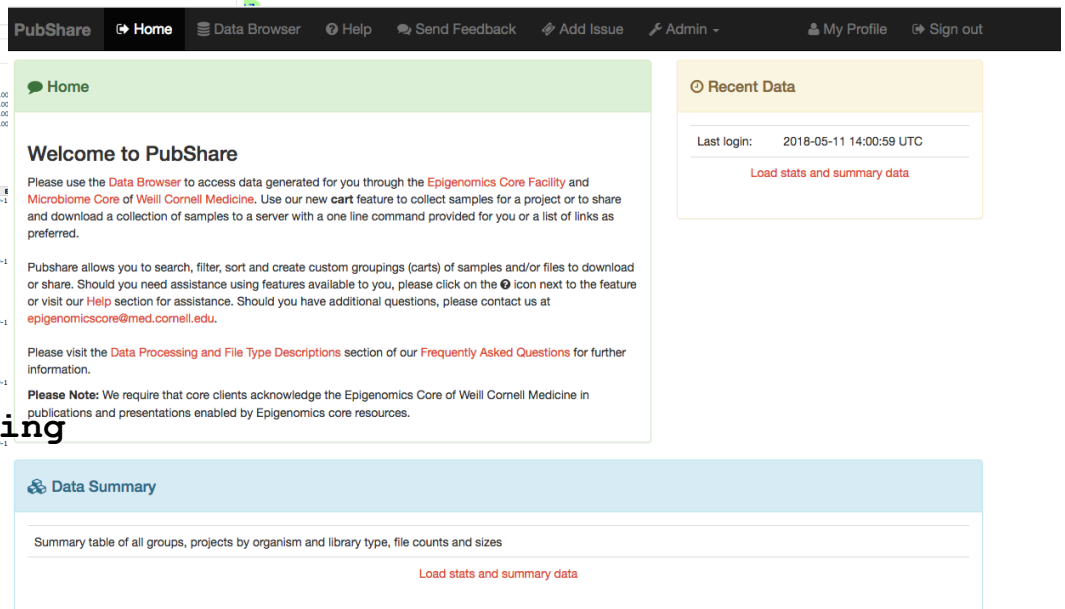
- Consultation
- Sample submission
- Library prep
- Sequencing
- Data release
- Bioinformatics support

Sample Submission Tracking



Monitoring of Sequencing and Analysis

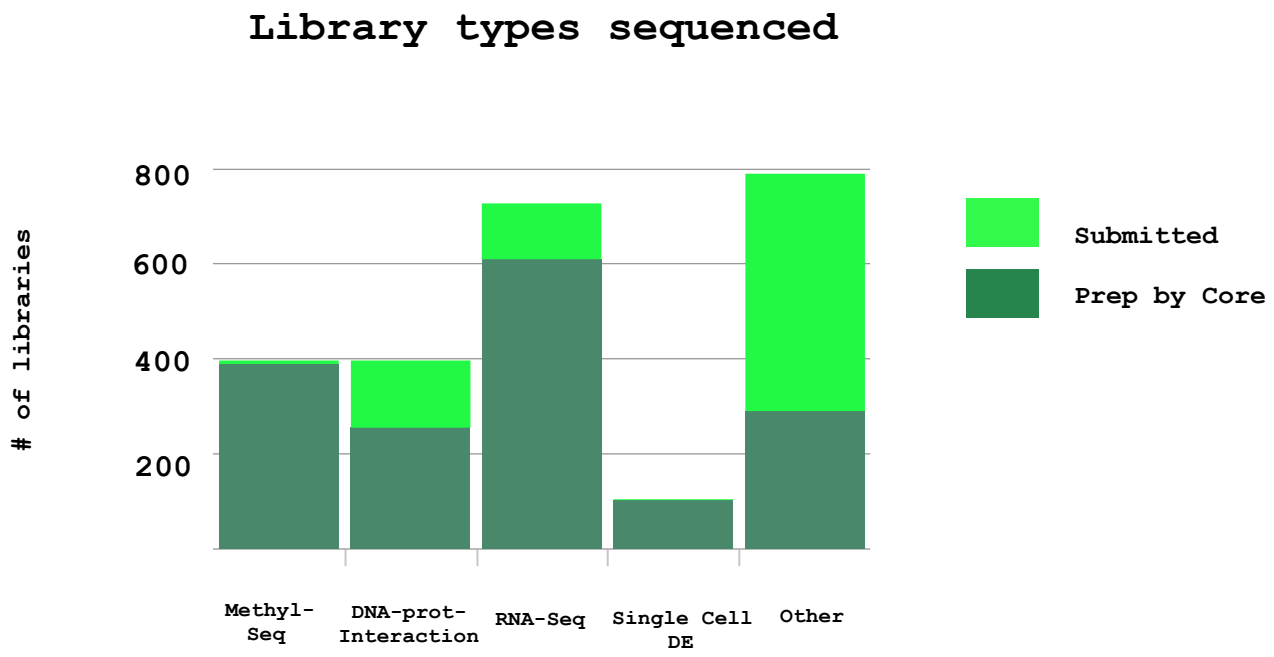
Doron Betel



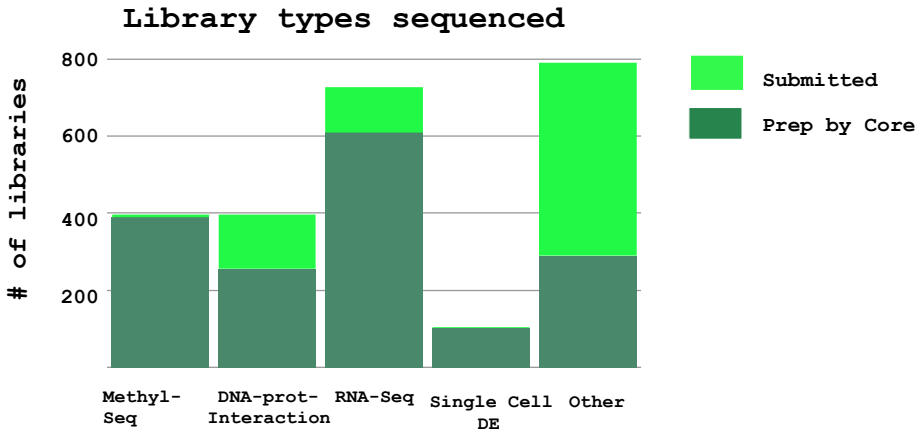
- **Methylation sequencing**
- **DNAseq**
- **RNAseq**

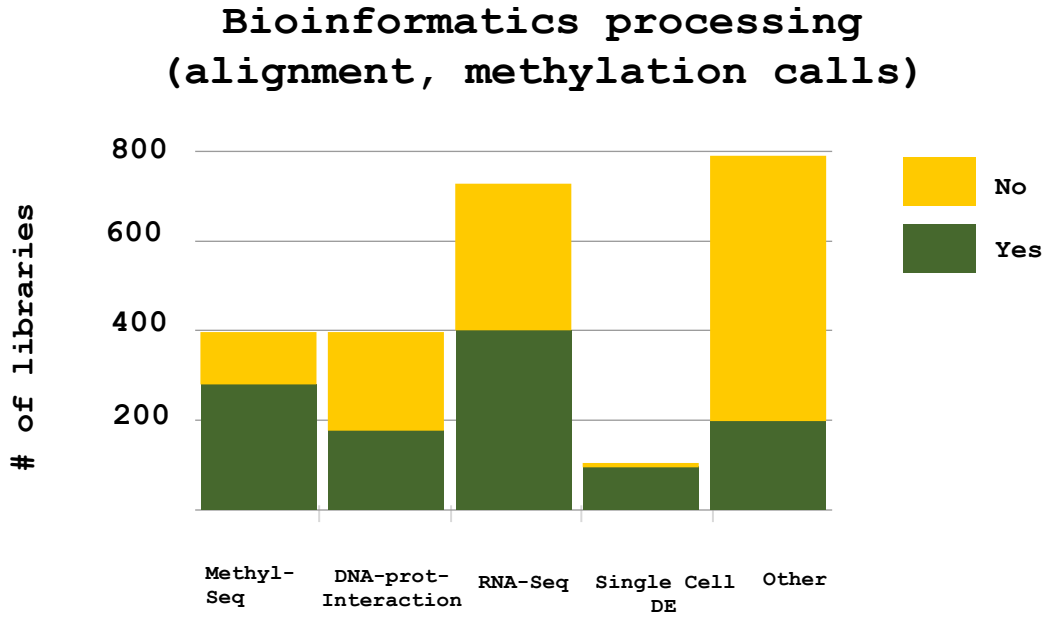
Epigenomics Core

Epicore metrics FY18

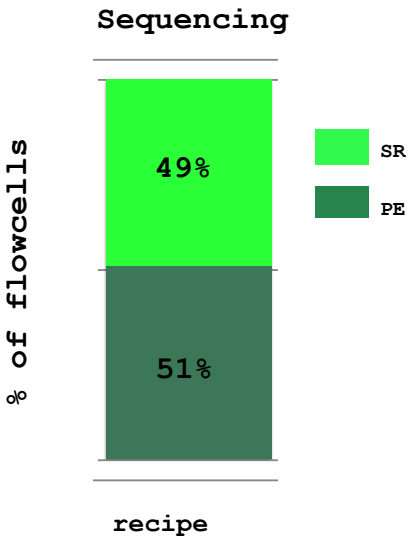


Epicore metrics FY18

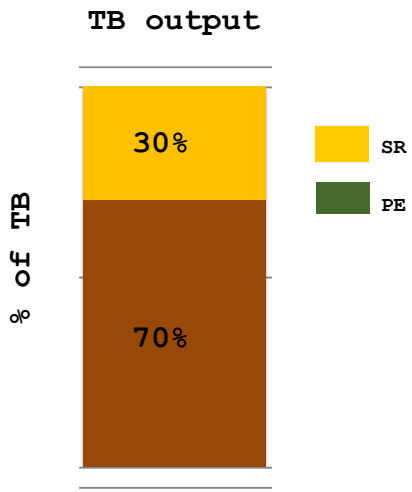




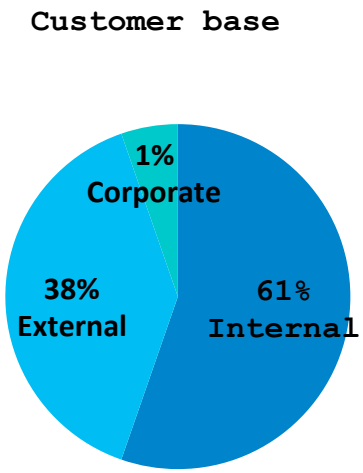
Epicore metrics FY18



90% HiSeq2500
9% HiSeq4000
1% MiSeq

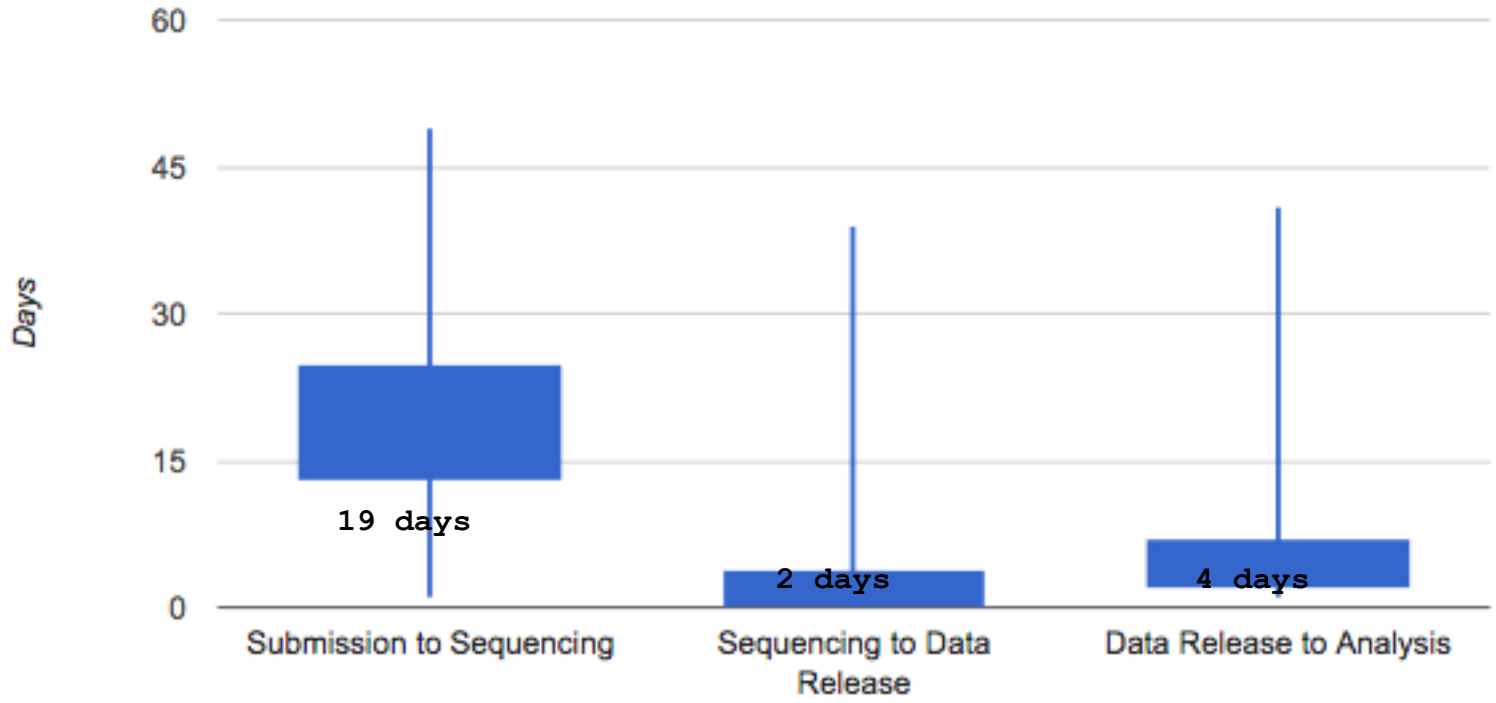


~32TB



~100 labs

Processing time FY18



Illumina NGS workflow

- **Good quality starting material**: DNA and RNA, in the past 2 years .. Add single cell!
  - DNA eukaryotic or prokaryotic, DNA methylation, from immunoprecipitations, from ATACseq, from Hi-C
  - Total RNA, immunoprecipitated RNA, RNA from FFPE (degraded)
  - Single cell suspensions

- **Library preparation**: (from DNA)
  - random fragmentation of DNA, addition of 5' and 3' adapters

  (which will allow binding to the Illumina flowcell) and then undergo PCR amplification if needed.

Sequencing indexing strategy must be thought out before the libraries are made

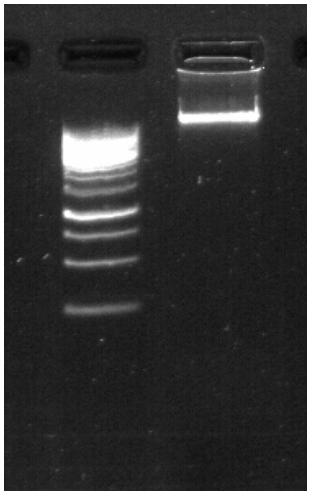
- **Cluster Generation**:
  - library is loaded into an Illumina flowcell, DNA fragments are captured on a lawn of surface bound oligos complementary to the library adapters. Fragments are amplified into clonal clusters through bridge amplification.

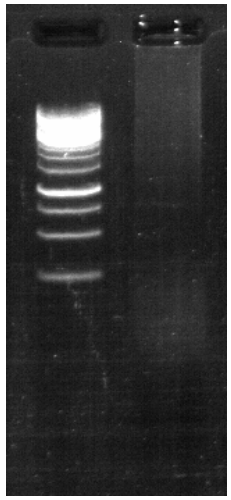
- **Sequencing**:
  - proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands.

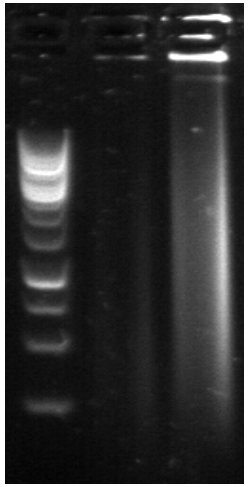
Quality Control of DNA

1. Genomic DNA sample QC: ~40kb, no RNA contamination

(whole genome sequencing, targeted sequencing, methylation sequencing)

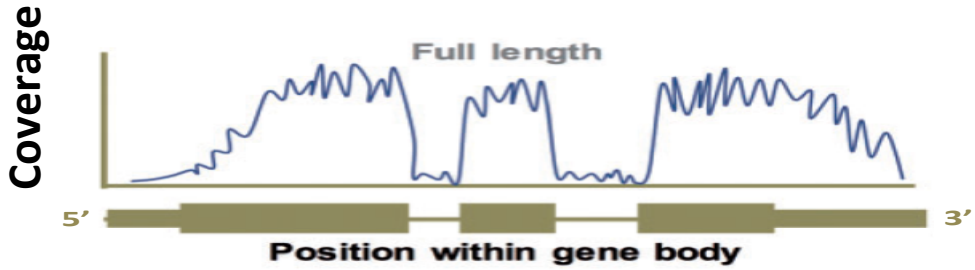






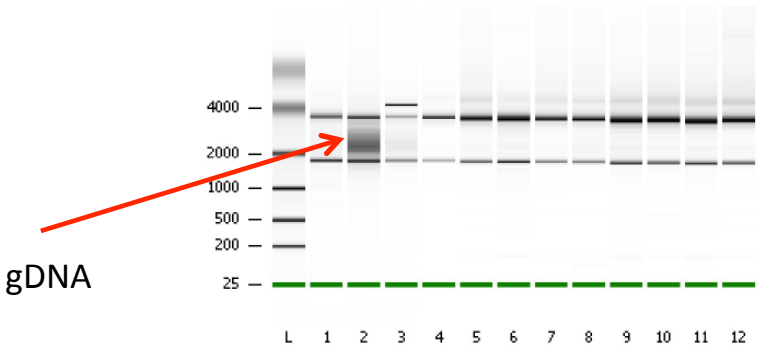
Quality Control for RNA

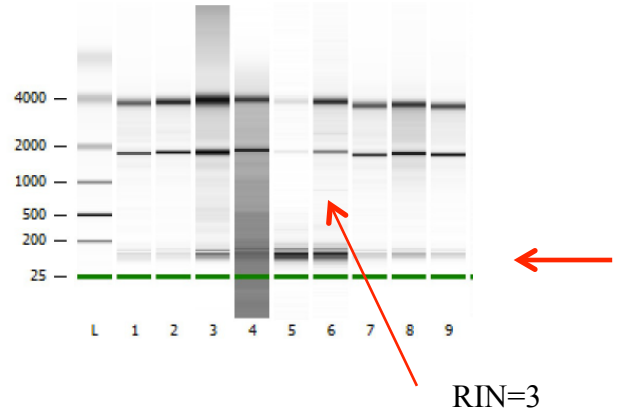
Transcriptome analysis



RIN number (RNA integrity number)

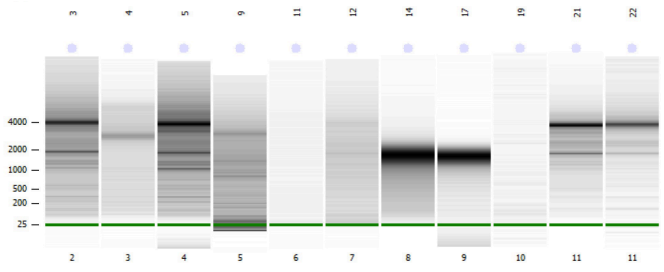
250ng RNA, RIN >- 8





Total stranded RNA prep

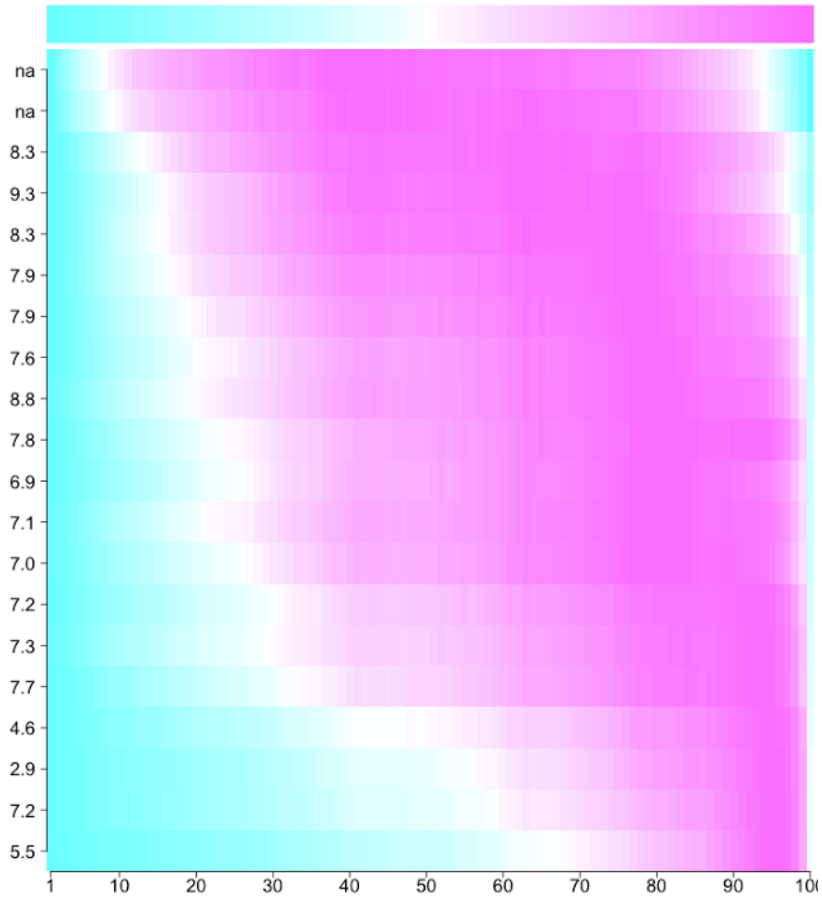
Requires 500ng RNA, RIN >- 2



Effect of RIN on gene body counts

Gene body coverage heat map

RIN



Gene body coverage (5’ to 3’)

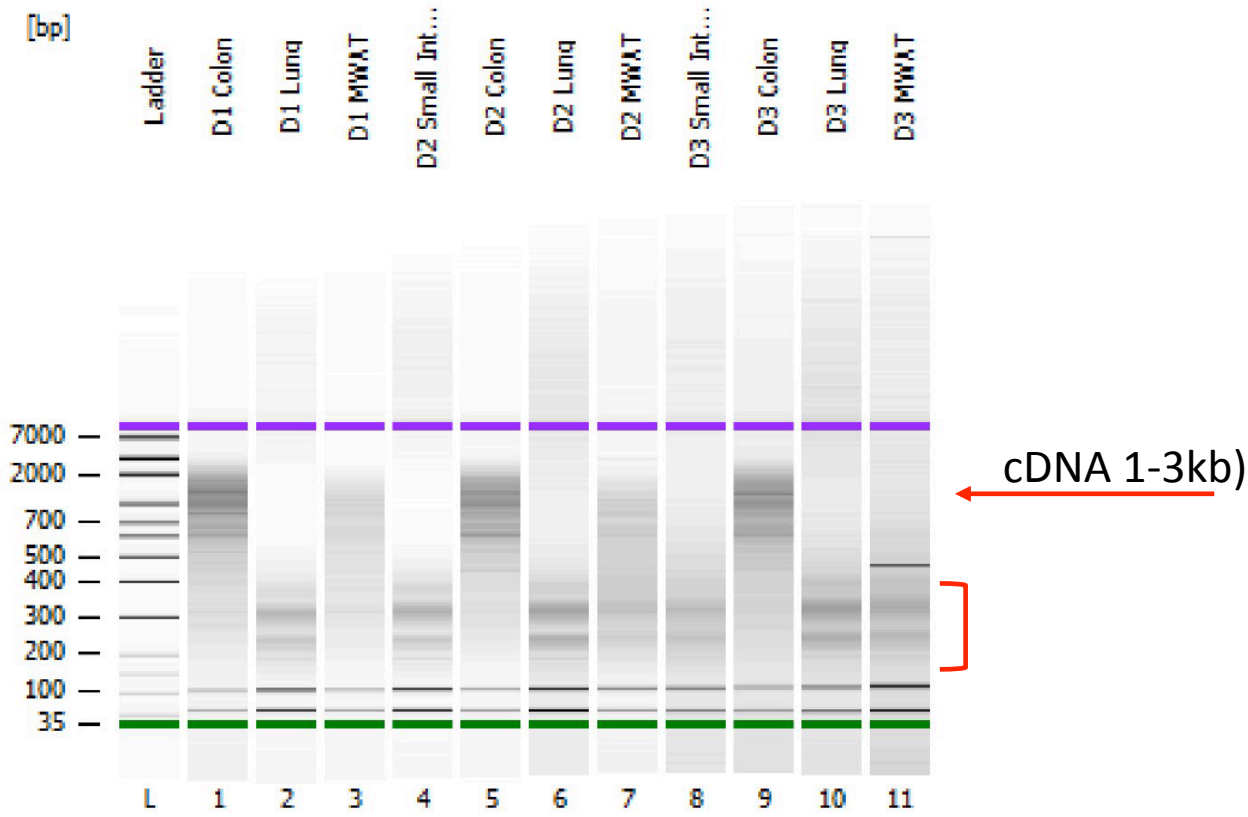
Piali Mukherjee

Quality Control for RNA (low input)

UltraLowInput RNAseq (SMART technology)

Requires 1-10ng RNA, RIN >- 8; or 10-500 cells

Quality control is done after cDNA preparation



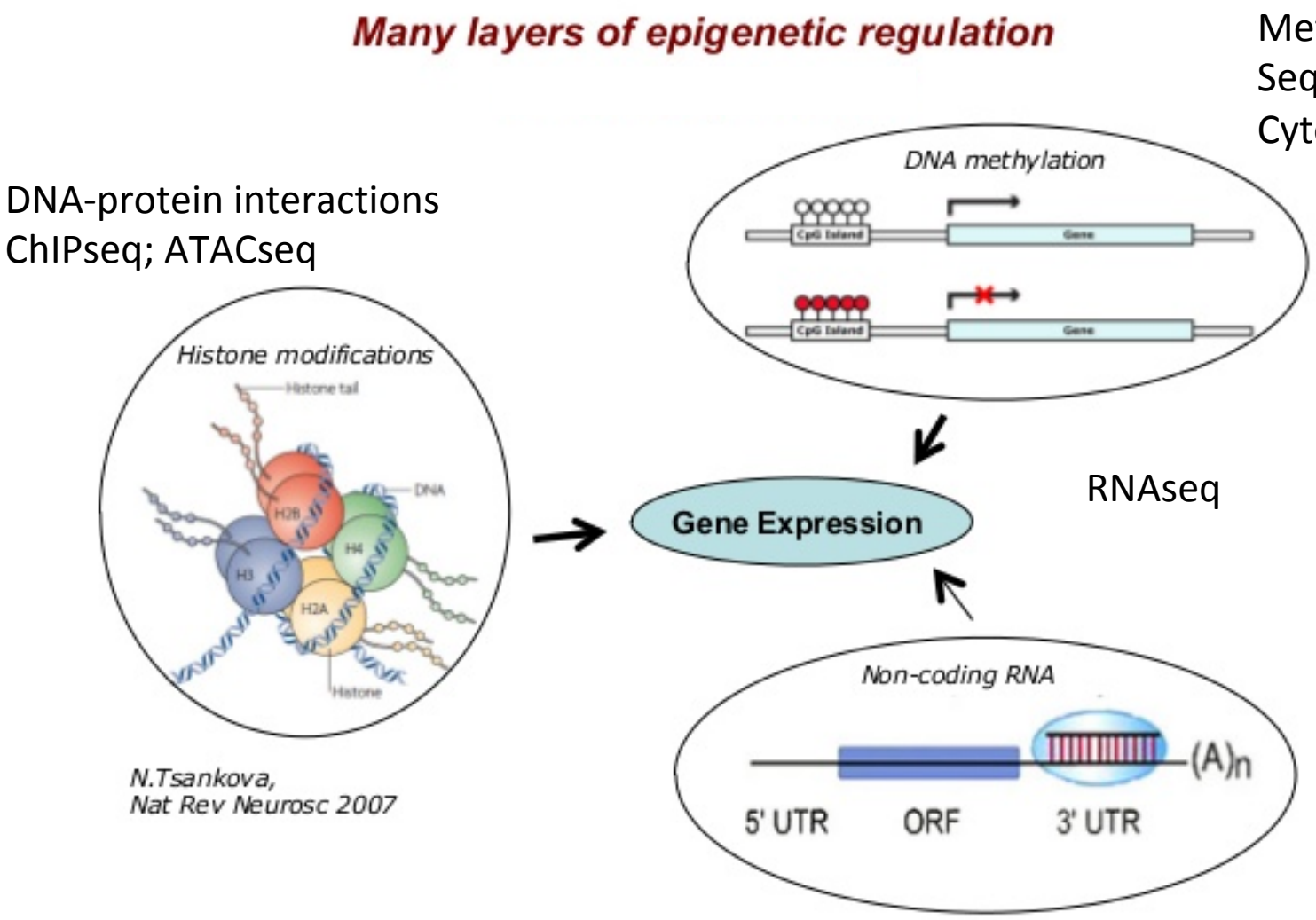
Genome vs Epigenome

Genetic information- DNA (**constant**)

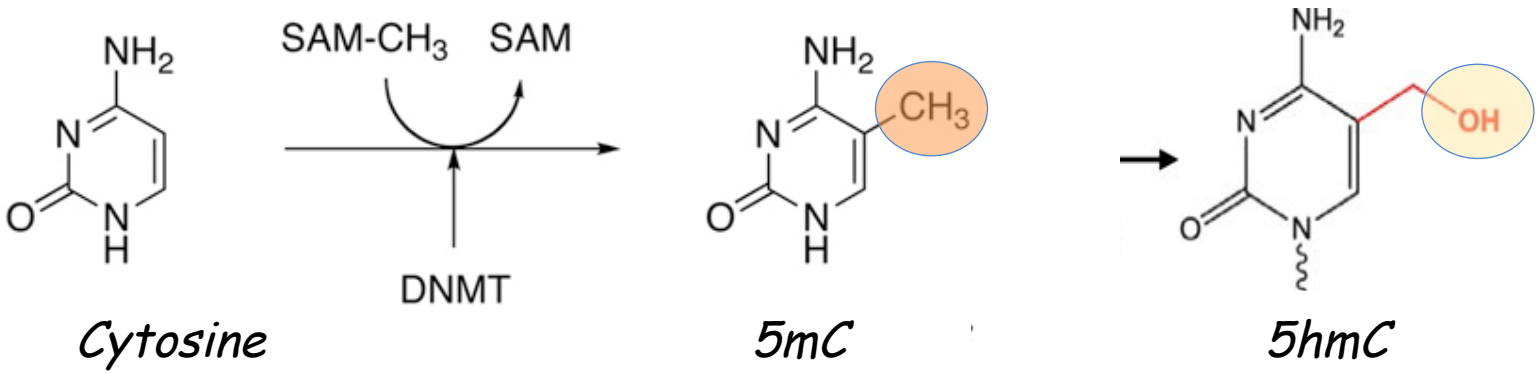
Epi-genetic information

The Epigenetic code, which modifies gene expression, has **plasticity** and can be **reprogrammed**

Epigenetic code affects gene expression



A modified C cannot be identified by sequencing by synthesis methods

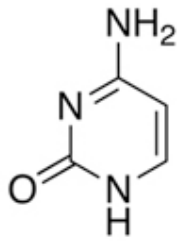


Cytosine *5mC* *5hmC*

Sequencing by synthesis depends on a DNA polymerase, C methylated C and all oxidative products will be amplified as a G

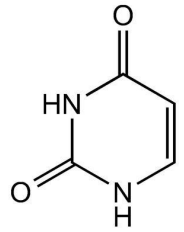
Methylation sequencing refers to a chemical step prior to library preparation (Bisulfite conversion - BS)

Step 1: conversion of Cytosine to Uracyl



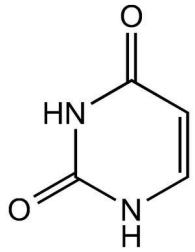
Cytosine

Deamination by bisulfite treatment



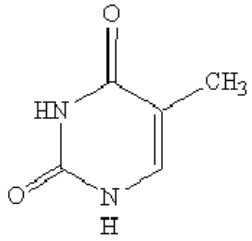
Uracil

Step 2: PCR amplification



Uracil

PCR amplification results in every C converted to a T



Thymine

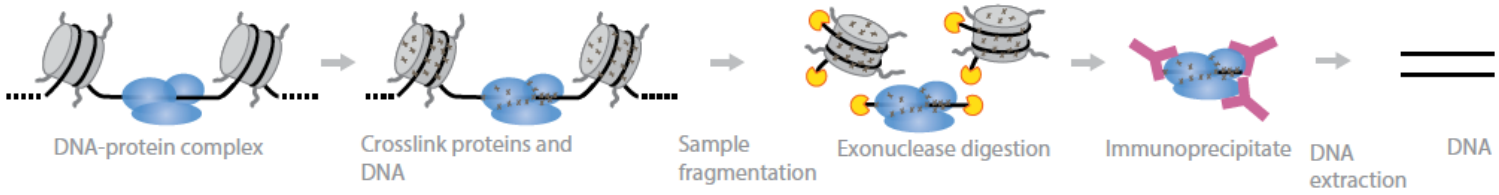
This chemical step preserves both 5mC and 5hmC, which are about 1% of the total Cs
It reduces the complexity of the genome (mostly) to a 3-letter code

Quality Control of DNA

2. Genome wide mapping of DNA binding proteins

- Chromatin Immunoprecipitation.

Researcher

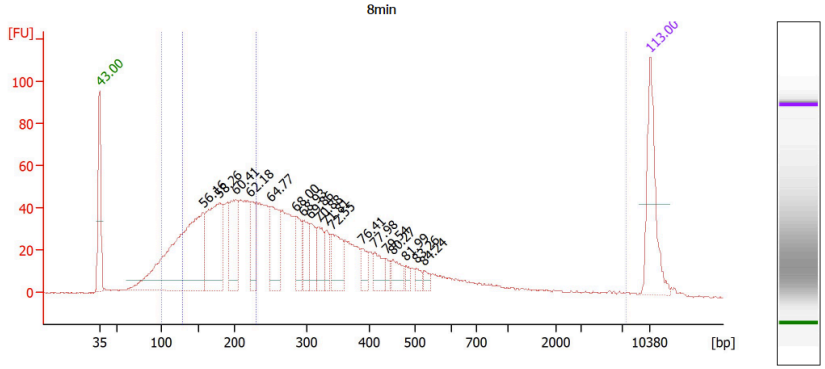


Core:

Input requirements

- a minimum of 11ng of sonicated ChIP DNA at ≤ 1ng/ul, determined by Qubit

- 10% of total material must be in the size range of the DNA fraction required for library preparation (130-230bp)

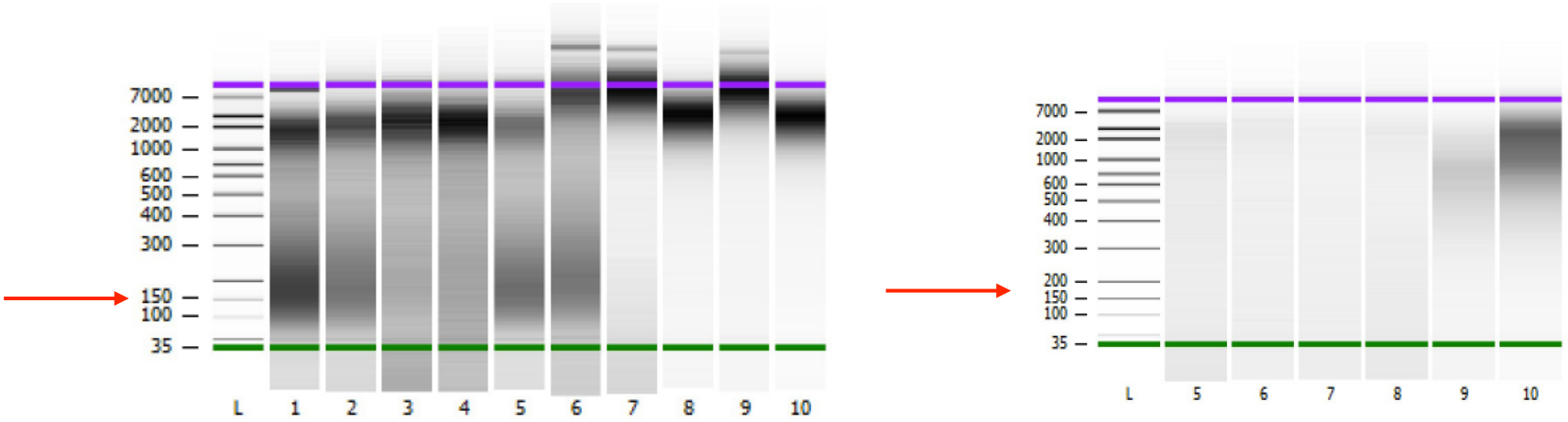


Region table for sample 4 : 8min

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]
100	7,500	1,795.4	95	390
130	230	675.3	36	182

Samples submitted most often look like this

Agilent Bioanalyser trace

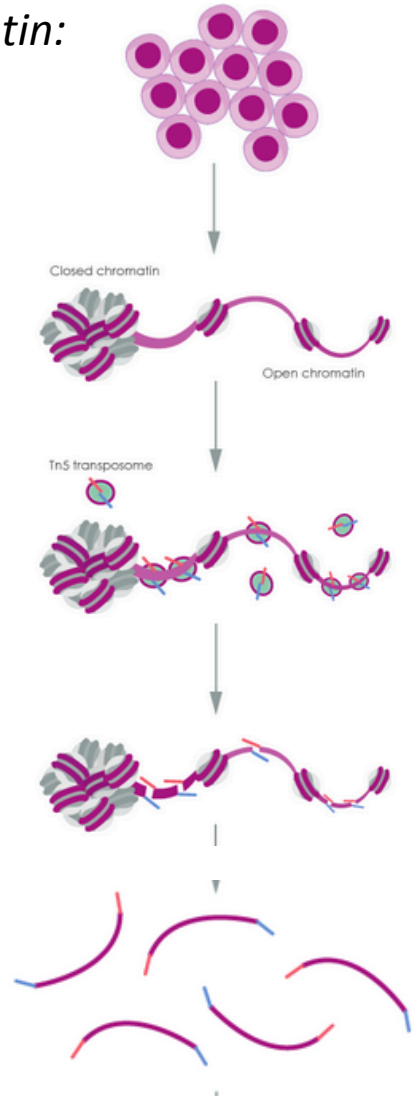


Epigenomics Core

Quality control of cells

Functional state of chromatin:
Assay for transposase-accessible chromatin: ATACseq

Technique was published by Greenleaf's lab in 2013



1. 90% live cells in suspension

2. Prepare nuclei (50,000 cells)

3. Add Tagmentase

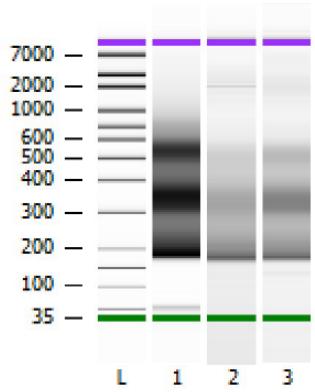
4. Short **barcoded** fragments are released by tagmentation

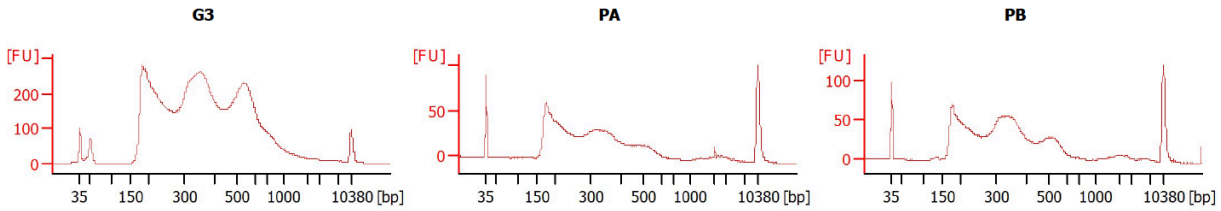
5. PCR amplify and Sequence

Quality control of ATACseq libraries is at the sequencing stage!

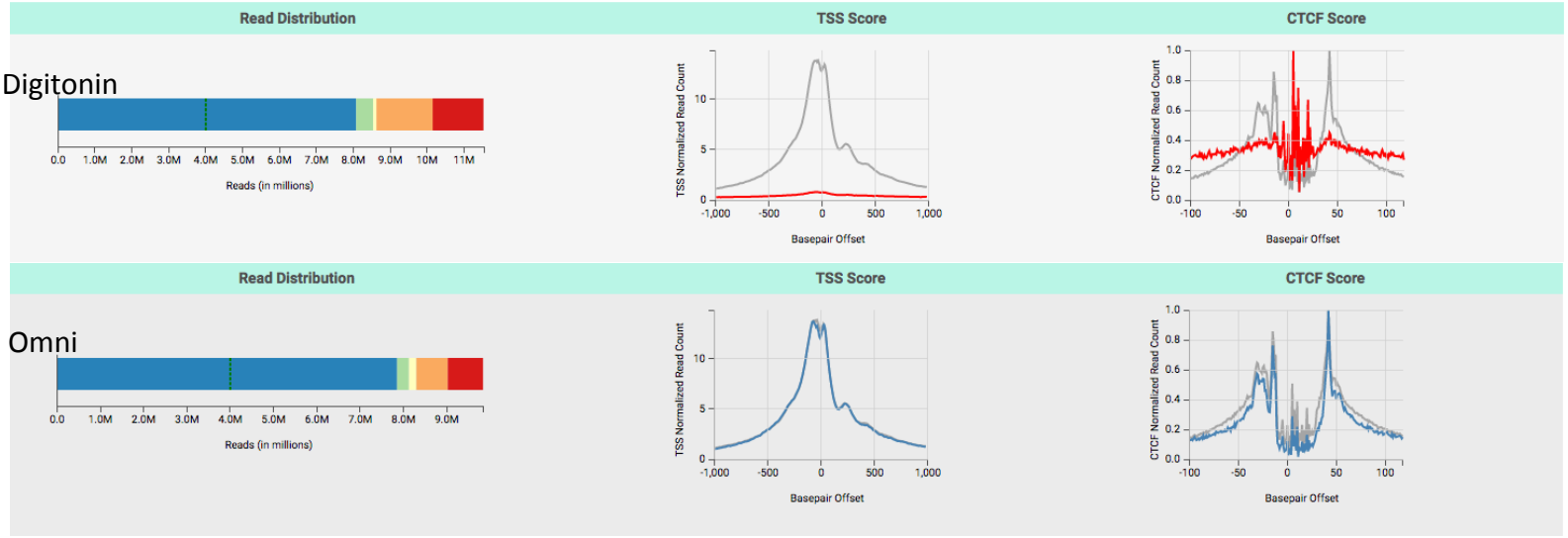
Bio

Bioanalyzer QC





QC using Epinomics website (*www.epinomics.co/)*Greenleaf initiative)



Collaboration Elemento, Ashley Doane

Illumina NGS workflow

- **Good quality nucleic acid**: DNA and RNA
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  - Total RNA, immunoprecipitated RNA, RNA from FFPE (degraded)

- **Library preparation**: (from DNA)
  - random fragmentation of DNA, addition of 5' and 3' adapters

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- **Sequencing**:
  - proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands.

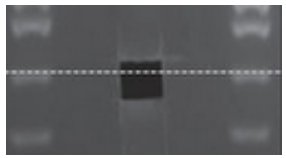
Library preparation

1. Shear substrate to ~200bp

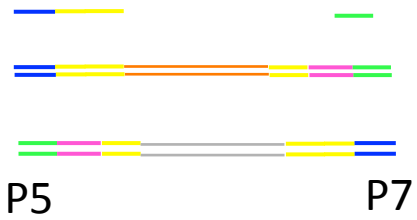
2. End repair, A-tailing

3. Adapter ligation

4. Size selection of DNA from 250-350bp
(original DNA ~130-230bp)

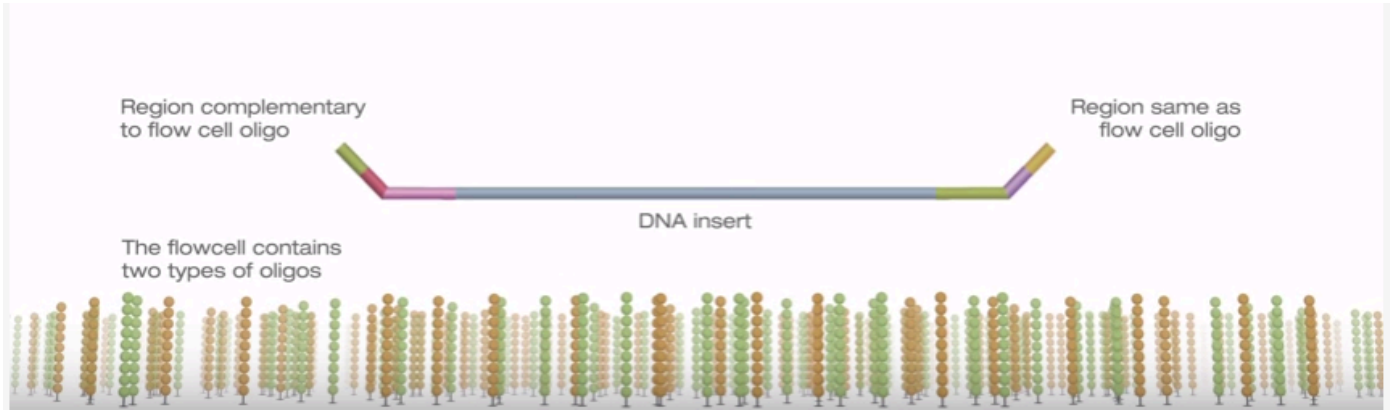


5. PCR amplification



Library preparation

- **Library preparation**:
  - fragmentation of DNA
  - addition of 5' and 3' adapters (will tether the DNA to the Illumina flowcell)
  - PCR amplification if needed.
  - 5' end has a UNIVERSAL primer

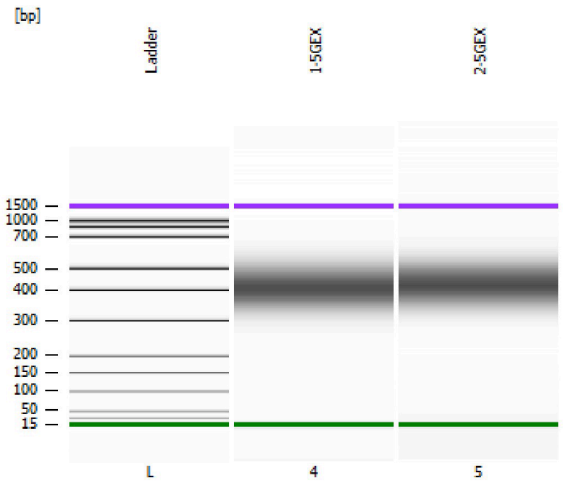


Flow cell

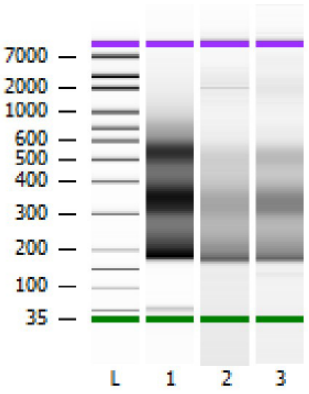
Quality Control of Library preparation

Before sequencing, libraries are analyzed for quantity and quality

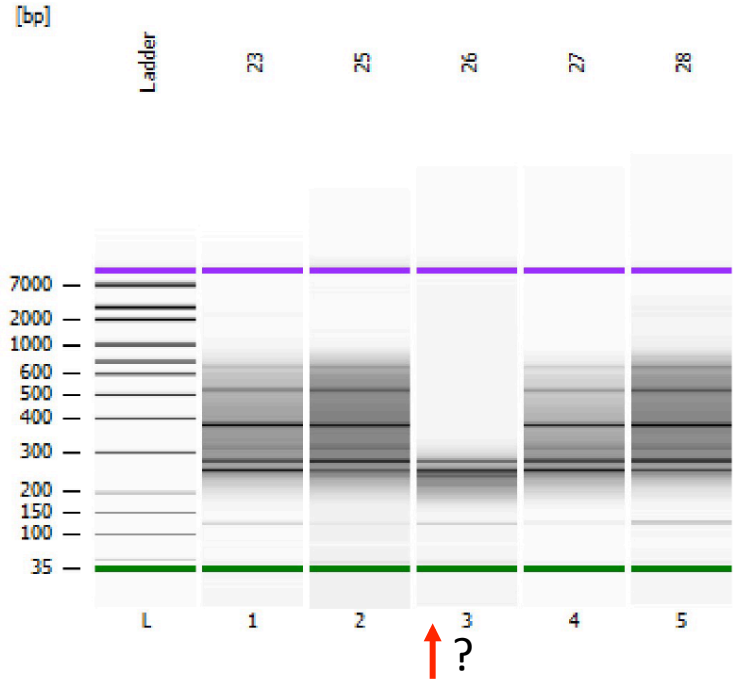
RNAseq libraries



ATACseq libraries



36 gDNA for methylation sequencing (RRBS) , all passed DNA QC …



Illumina NGS workflow

- **Good quality nucleic acid**: DNA and RNA
  - DNA eukaryotic or prokaryotic, DNA methylation, from immunoprecipitations, from ATACseq, from Hi-C
  - Total RNA, immunoprecipitated RNA, RNA from FFPE (degraded)

- **Library preparation**: (from DNA)
  - random fragmentation of DNA, addition of 5' and 3' adapters

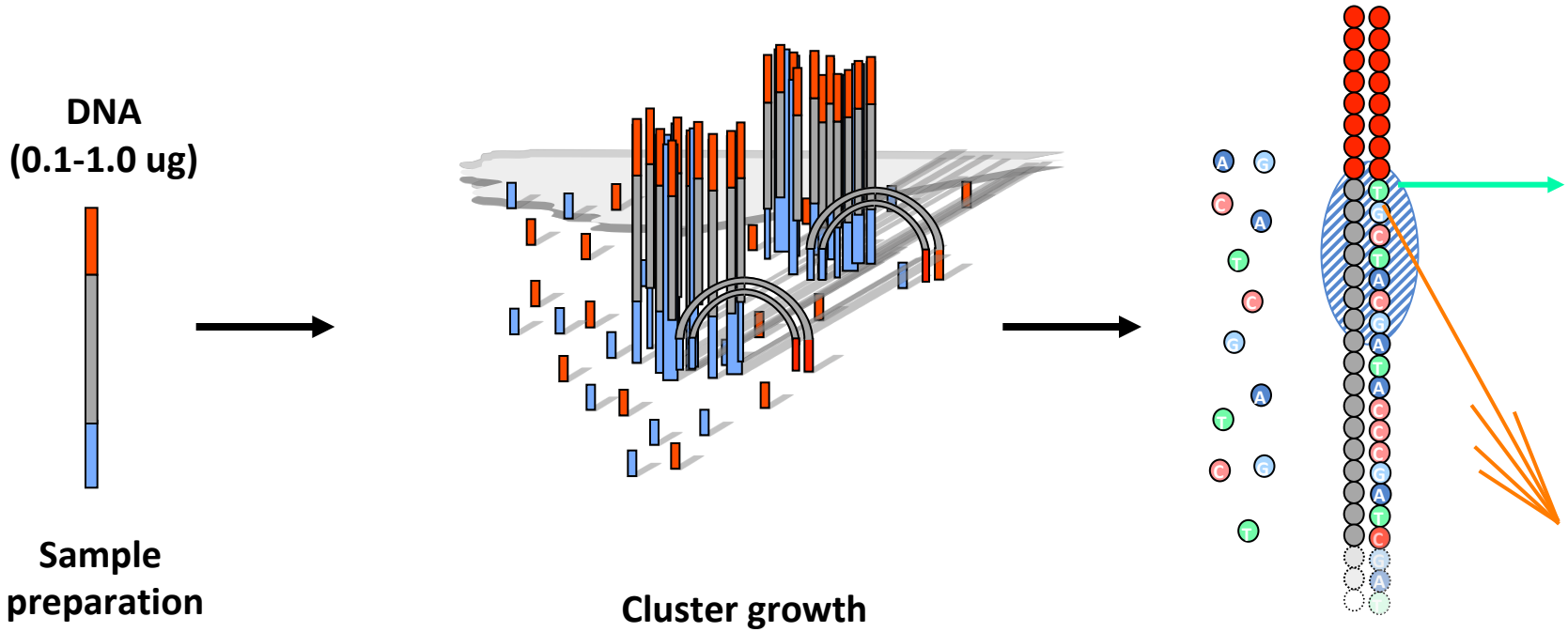
  (which will allow binding to the Illumina flowcell) and then undergo PCR amplification if needed.

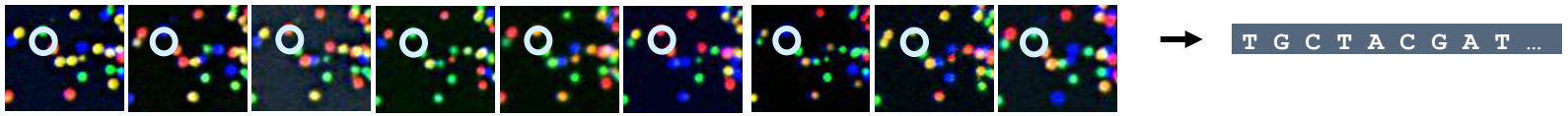
Sequencing indexing strategy must be thought out before the libraries are made

- **Cluster Generation**:
  - library is loaded into an Illumina flowcell, DNA fragments are captured on a lawn of surface bound oligos complementary to the library adapters. Fragments are amplified into clonal clusters through bridge amplification.

- **Sequencing**:
  - proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands.

Illumina Clustering and Sequencing



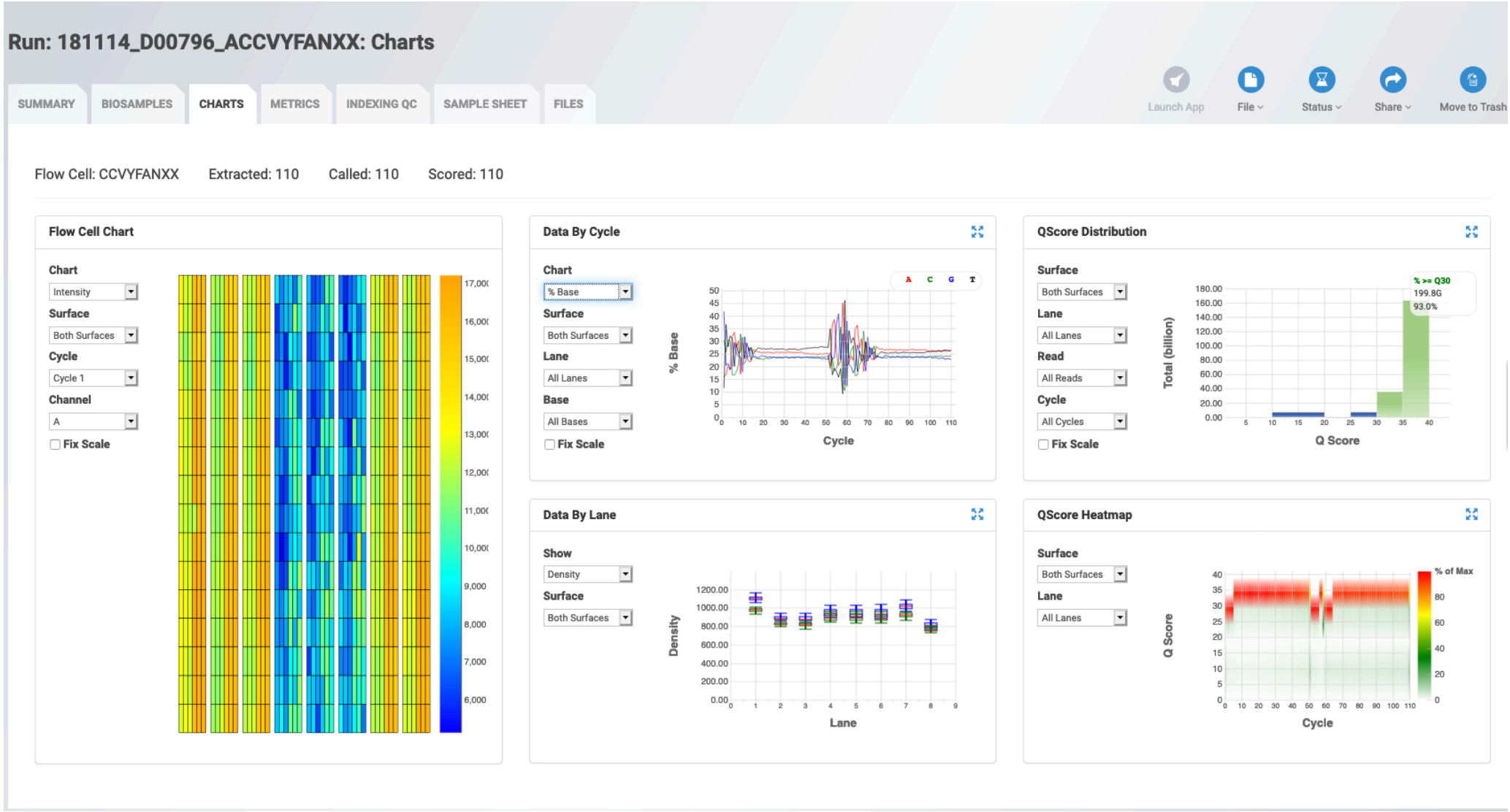


http://seqanswers.com/forums/showthread.php?t=21

http://www.illumina.com/technology/sequencing_technology.ilmn

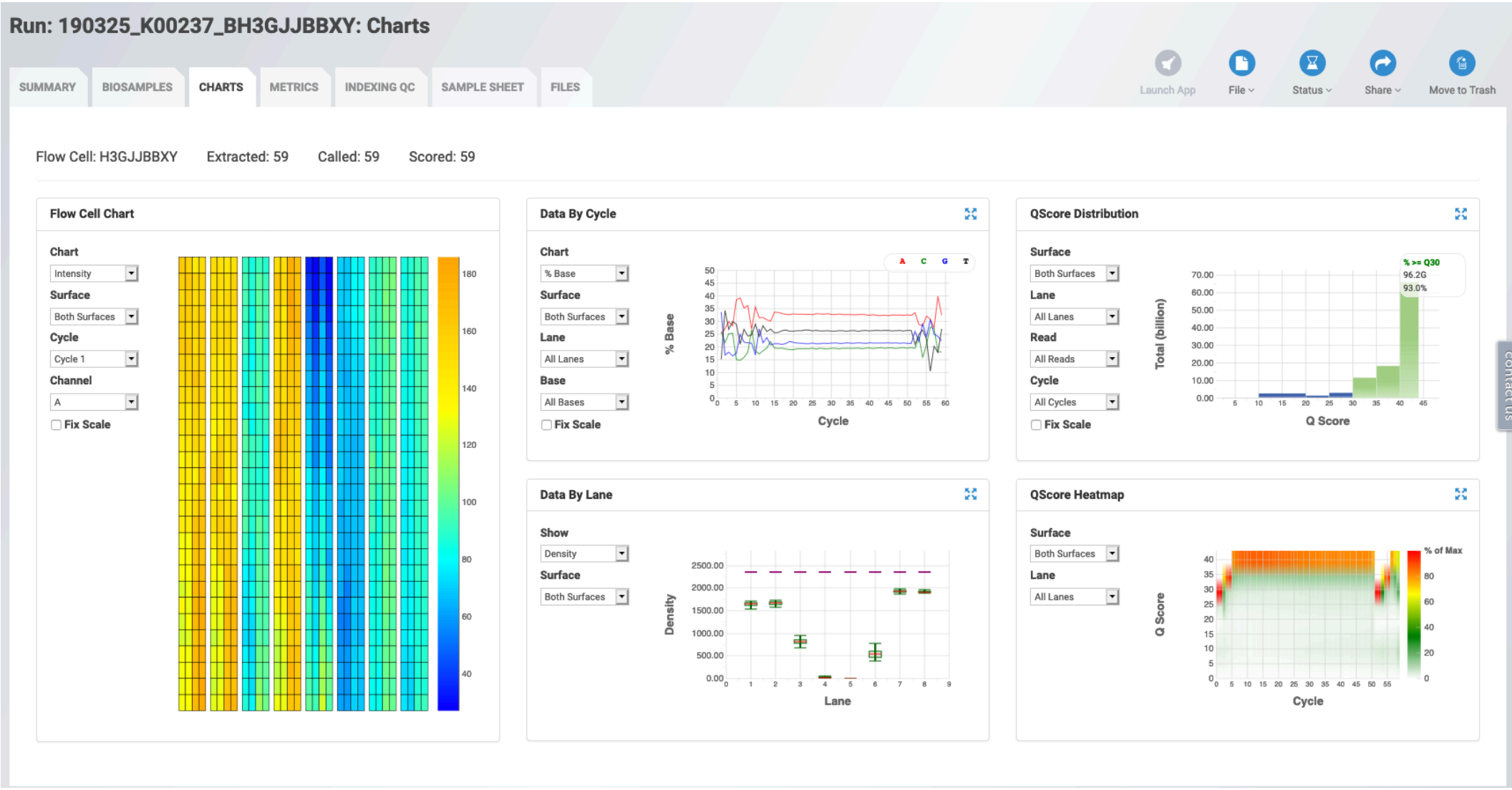
Sequencing run quality control

This is a good run:

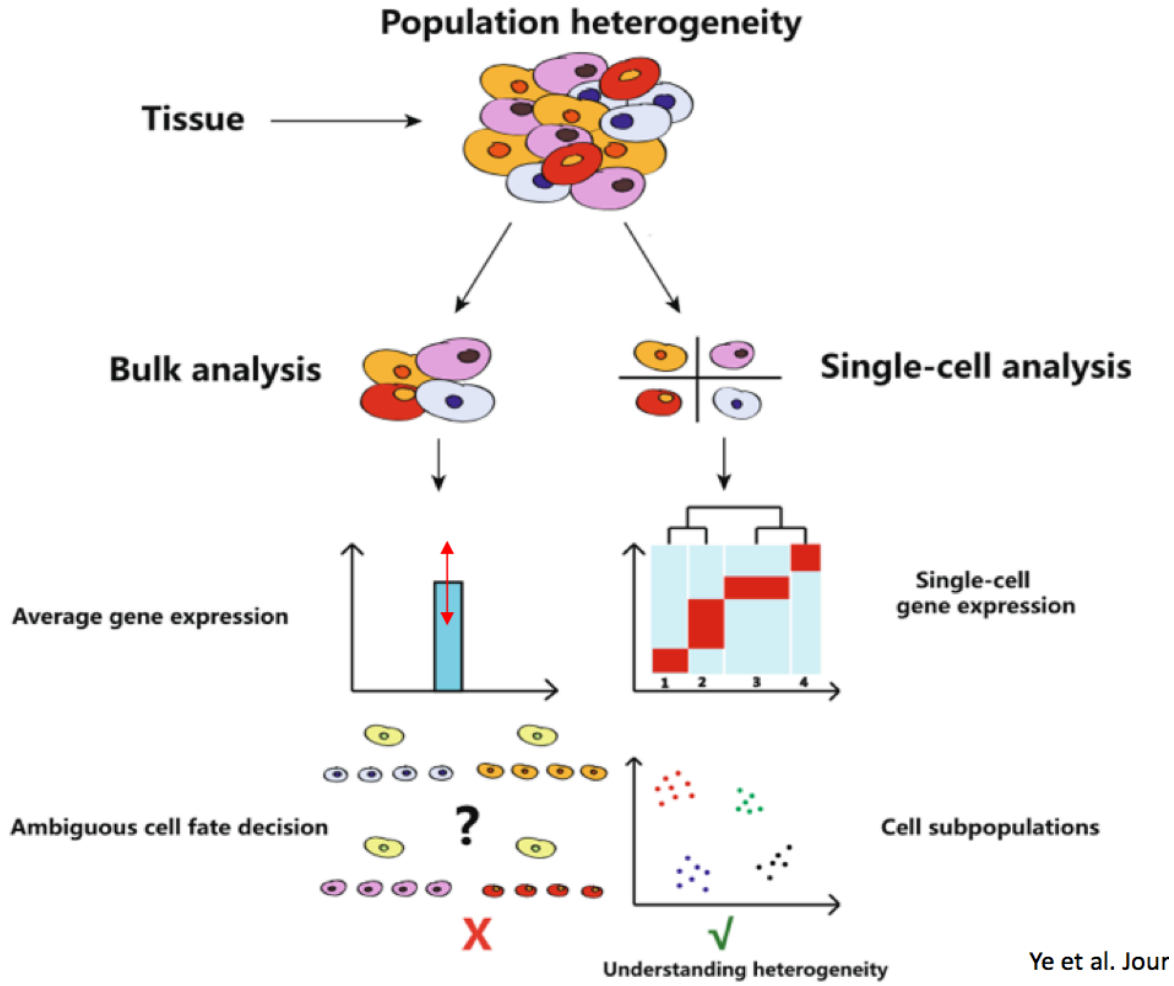


Sequencing run quality control

Some lanes on this run need to be repeated



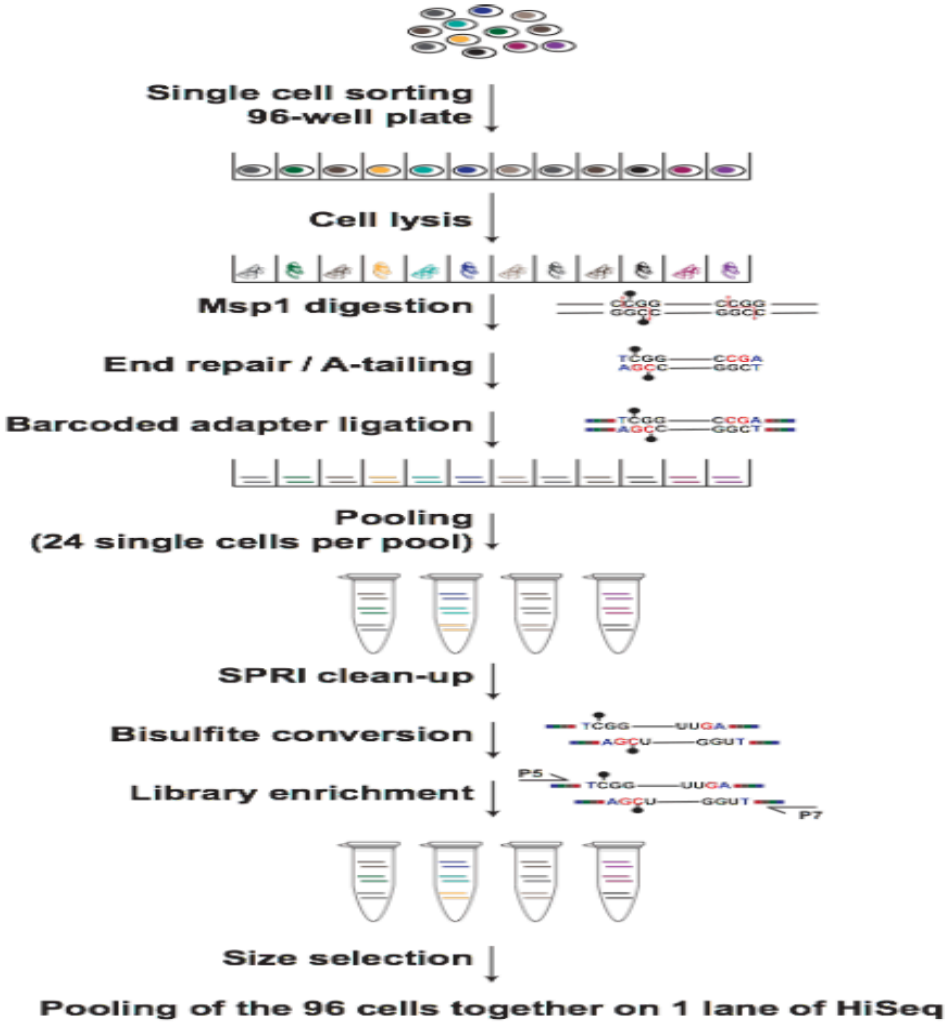
Bulk vs Single Cell Genomics



Ye et al., J. Hematology Onc. 2017

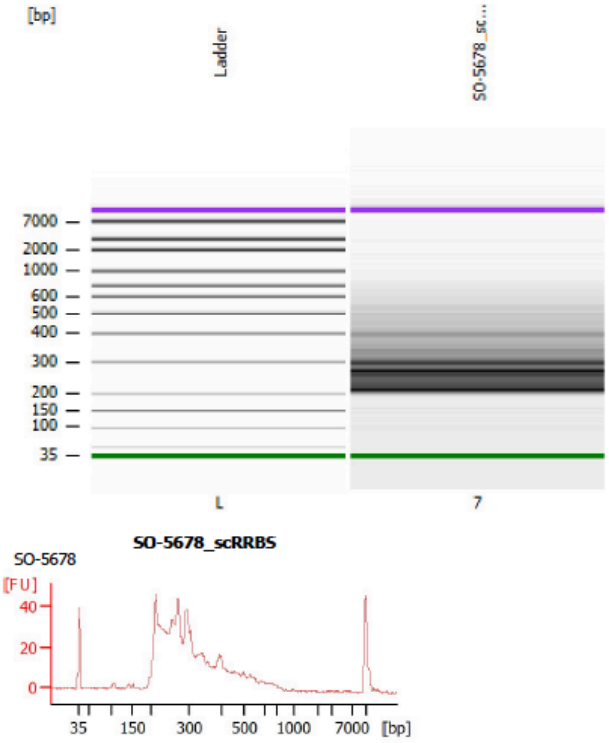
Single cells methylation sequencing (RRBS)

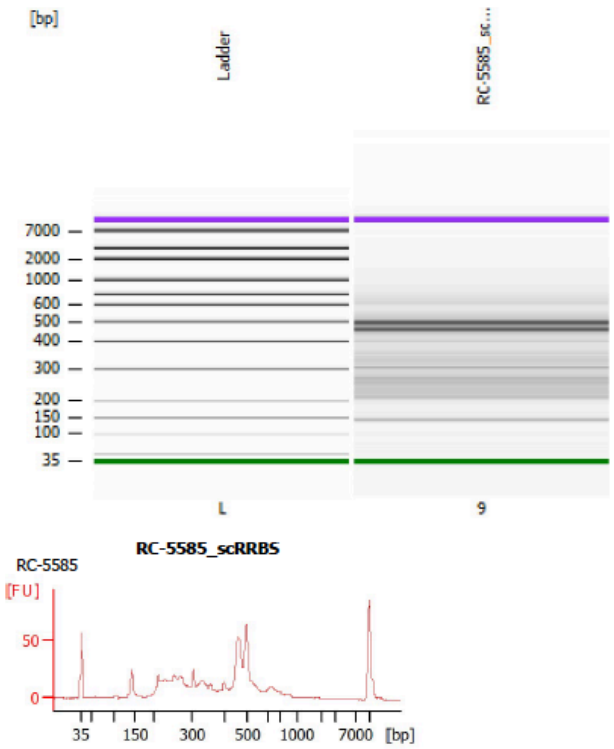
Manual, 96 cells at a time



Single Cell Quality Control of Library preparation

But, for single cell …

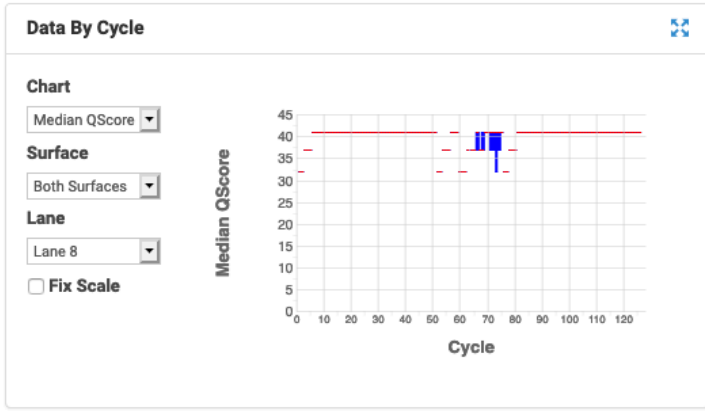


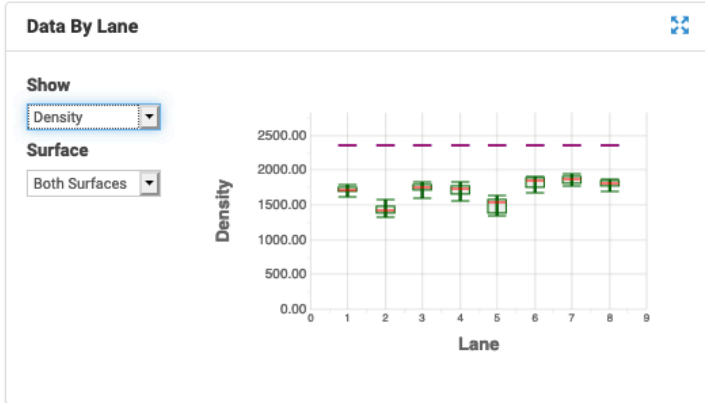


Single Cell Quality Control of Sequencing Run

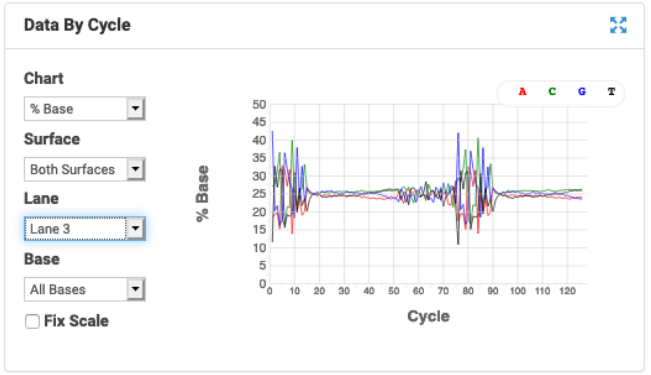
But, for single cell …

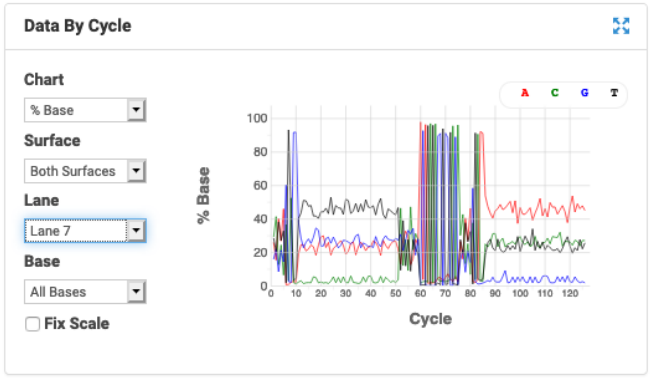
Sequencing QC

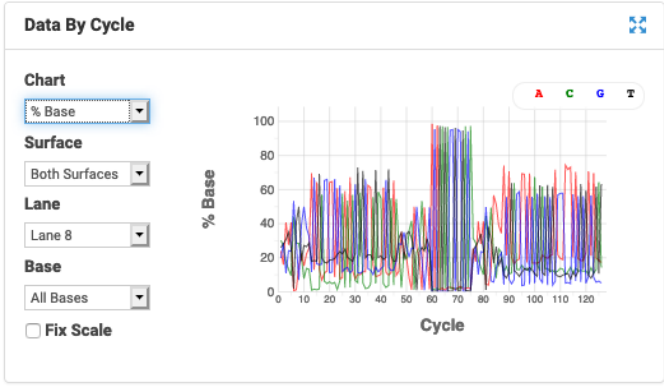




Base Composition





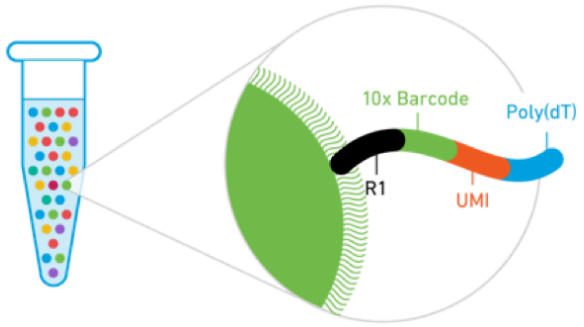


Single cell transcriptomics

10 X Chromium Platform:
Single Cell 3’ Digital Expression 500-10,000 cells

3 Components:

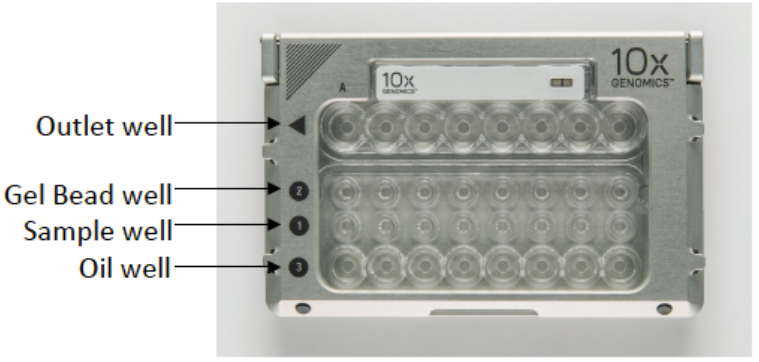
1. Gel beads with 750,000 unique barcodes



2. Cell suspension with RT reagents

3. Partitioning oil

Single-use microfluidics chip



Single Cell transcriptomics

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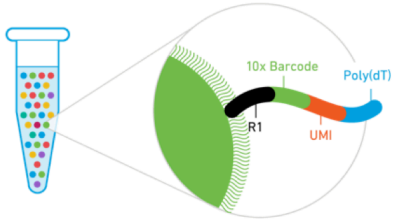
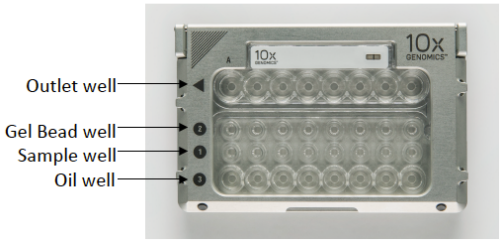
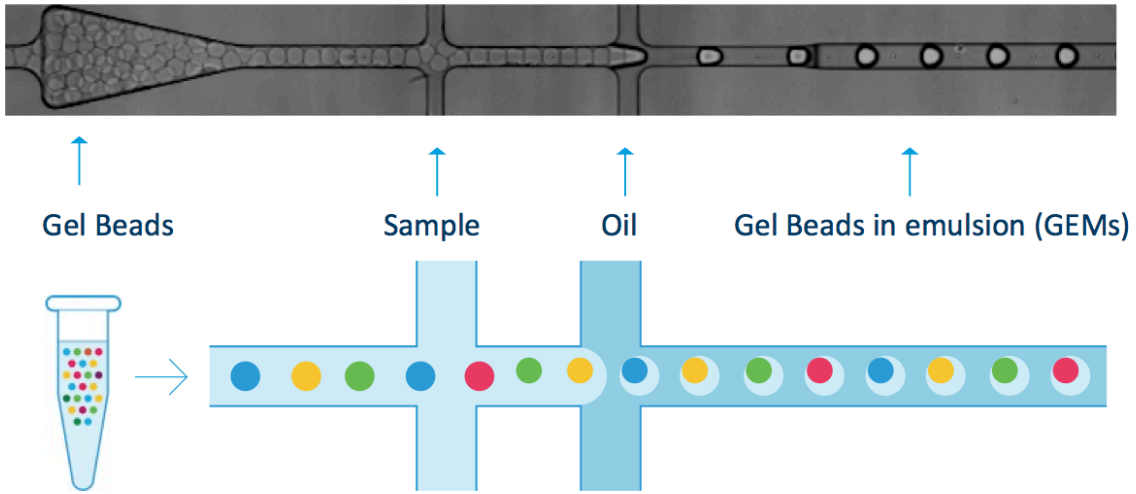


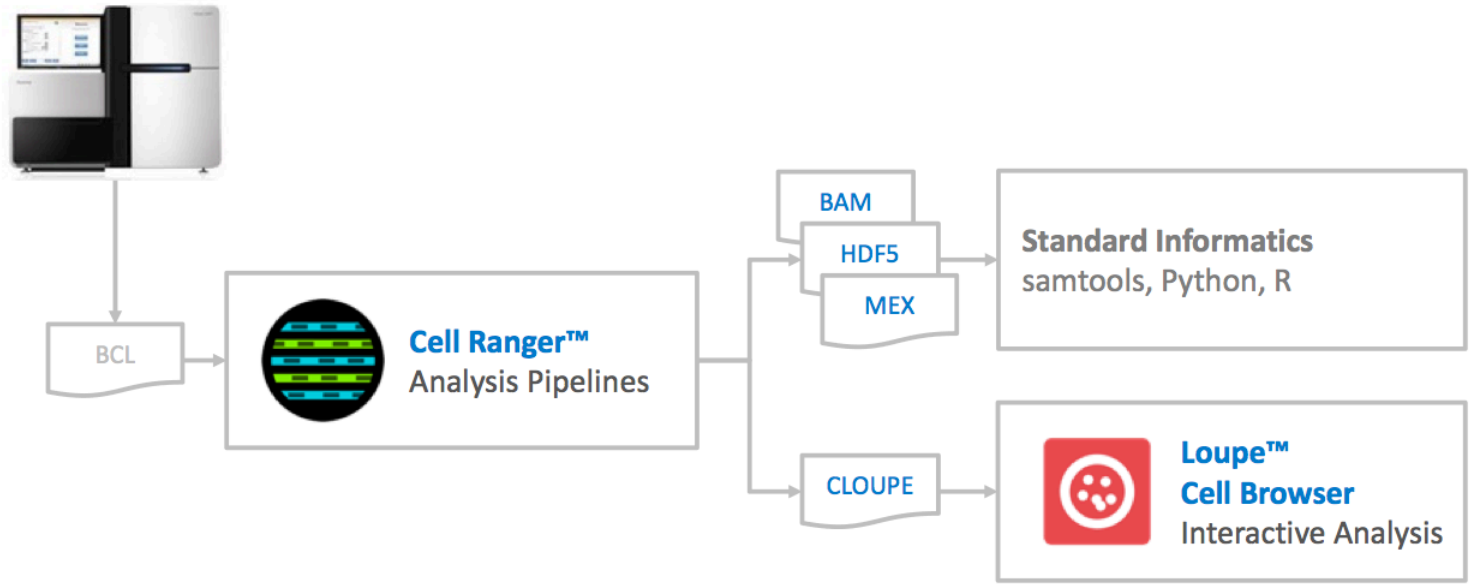
Single-use microfluidics chip





Chromium platform, Bioinformatics Support

- Sequence Chromium libraries
- Cell Ranger™ pipeline converts sequence data to single cell gene expression profiles
- Loupe™ Cell Browser enables interactive analysis



10 X Chromium Platform:
Single Cell 3’ Digital Expression

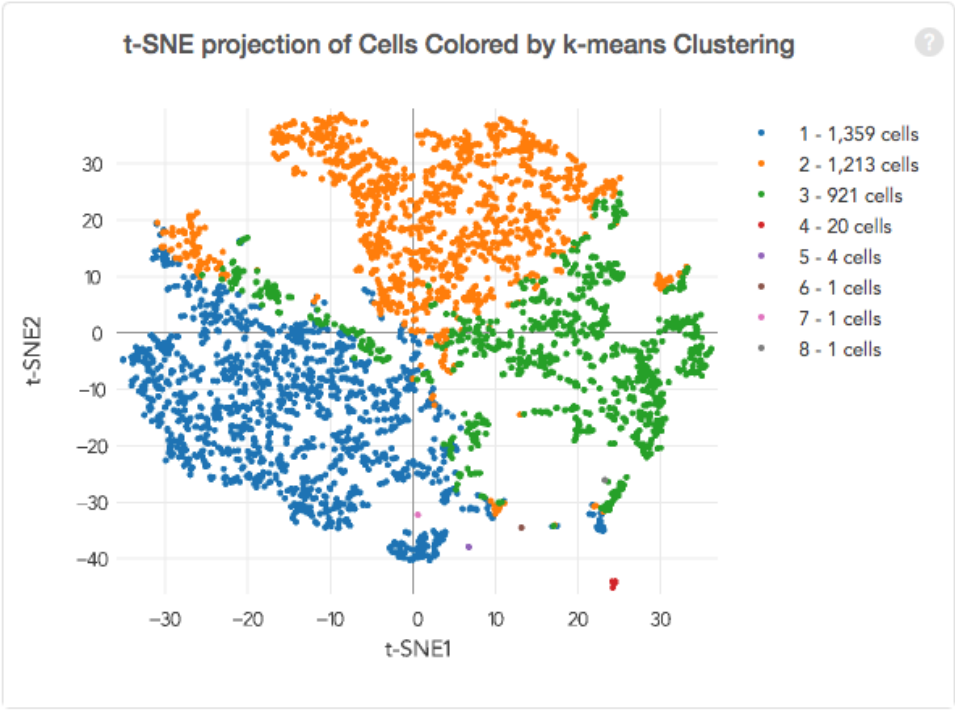
Cell Ranger analysis pipeline

Estimated Number of Cells
3,520

Mean Reads per Cell	Median Genes per Cell
41,267	2,151

Sequencing

Number of Reads	145,260,501
Valid Barcodes	98.3%
Reads Mapped Confidently to Transcriptome	55.5%
Reads Mapped Confidently to Exonic Regions	58.6%
Reads Mapped Confidently to Intronic Regions	15.3%
Reads Mapped Confidently to Intergenic Regions	3.4%
Sequencing Saturation	61.4%
Q30 Bases in Barcode	95.9%
Q30 Bases in RNA Read	87.5%
Q30 Bases in Sample Index	95.6%
Q30 Bases in UMI	96.4%



10 X Chromium Platform:
Single Cell 3’ Digital Expression

Sometimes, it fails …

Estimated Number of Cells
3,574

Mean Reads per Cell
48,598

Median Genes per Cell
3,255

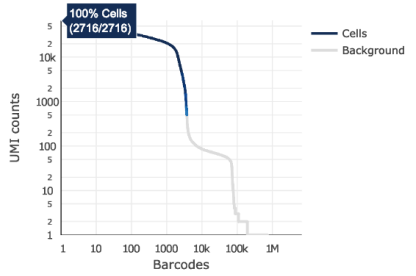
Sequencing

Number of Reads	173,689,296
Valid Barcodes	98.5%
Sequencing Saturation	46.1%
Q30 Bases in Barcode	98.9%
Q30 Bases in RNA Read	61.3%
Q30 Bases in Sample Index	95.5%
Q30 Bases in UMI	98.7%

Mapping

Reads Mapped to Genome	90.7%
Reads Mapped Confidently to Genome	88.0%
Reads Mapped Confidently to Intergenic Regions	4.8%
Reads Mapped Confidently to Intronic Regions	19.2%
Reads Mapped Confidently to Exonic Regions	64.1%
Reads Mapped Confidently to Transcriptome	61.4%
Reads Mapped Antisense to Gene	0.6%

Cells



Estimated Number of Cells	3,574
Fraction Reads in Cells	90.9%
Mean Reads per Cell	48,598
Median Genes per Cell	3,255
Total Genes Detected	21,018
Median UMI Counts per Cell	14,527

Sample

Name	1412A
Description	
Transcriptome	hg19
Chemistry	Single Cell 3' v3
Cell Ranger Version	3.0.0

Estimated Number of Cells
184

Mean Reads per Cell
959,423

Median Genes per Cell
1,856

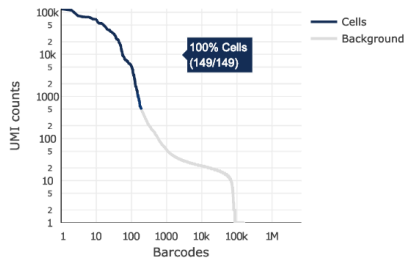
Sequencing

Number of Reads	176,533,869
Valid Barcodes	98.6%
Sequencing Saturation	95.8%
Q30 Bases in Barcode	98.5%
Q30 Bases in RNA Read	61.7%
Q30 Bases in Sample Index	95.4%
Q30 Bases in UMI	98.1%

Mapping

Reads Mapped to Genome	92.1%
Reads Mapped Confidently to Genome	88.4%
Reads Mapped Confidently to Intergenic Regions	5.3%
Reads Mapped Confidently to Intronic Regions	19.3%
Reads Mapped Confidently to Exonic Regions	63.7%
Reads Mapped Confidently to Transcriptome	61.1%
Reads Mapped Antisense to Gene	0.7%

Cells



Estimated Number of Cells	184
Fraction Reads in Cells	83.7%
Mean Reads per Cell	959,423
Median Genes per Cell	1,856
Total Genes Detected	16,925
Median UMI Counts per Cell	5,787

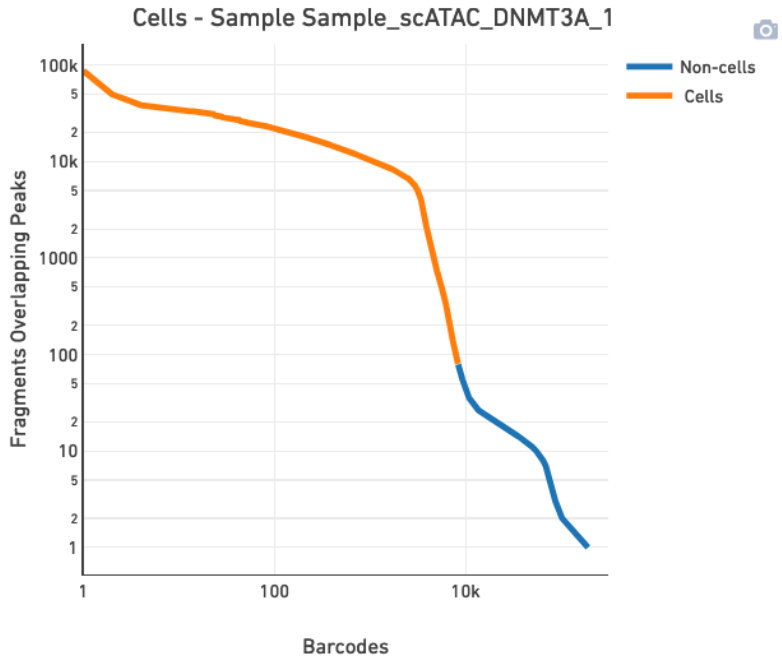
Sample

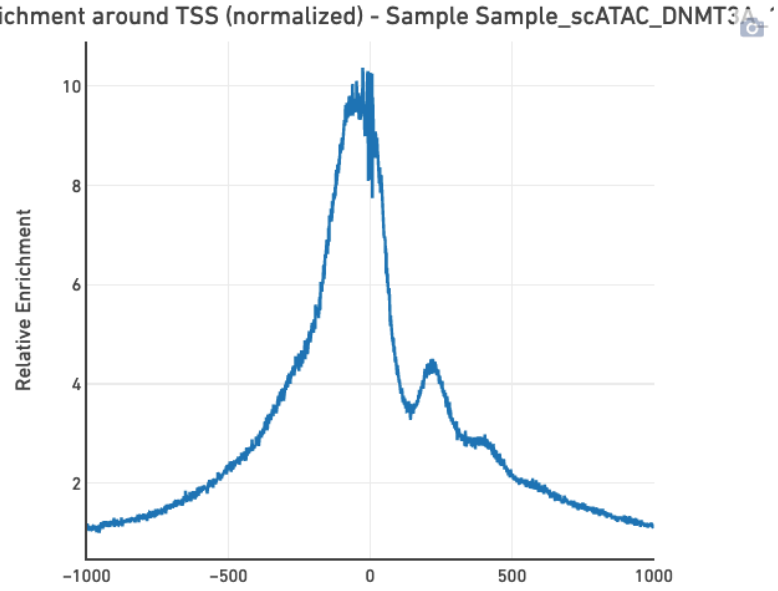
Name	1429A
Description	
Transcriptome	hg19
Chemistry	Single Cell 3' v3
Cell Ranger Version	3.0.0

10 X Chromium Platform:
Single Cell ATACseq

Cells

Estimated number of cells	8,352
Lower threshold on the number of fragments overlapping peaks per barcode to annotate barcode as cell	80.00
Median fragments per cell	2,380
Median fragments per non-cell barcode	1





Genomic analysis keep expanding, we keep expanding!

In development (collaboration with Landau's lab)

- Single cell methylation (RRBS)/RNAseq – single cell plate
- Single cell ATACseq/Rnaseq –single cell plate
- With 10x platform
    - Single cell expression with associated genotyping (paper re-submitted)

Testing commercial platforms

- Tapestri, Mission Bio –high throughput single cell DNA tumor variant phenotypes
- GeoMx DPS, Nanostring – spatial genomics –allows to detect both protein and RNA expression at a 10µM/100µM resolution

With Genomics Core

- Transition from HiSeq2500/4000 to NovaSeq

In spite of daily challenges, our core thrives!

Epigenomics Core

Home | Services | People | Resources

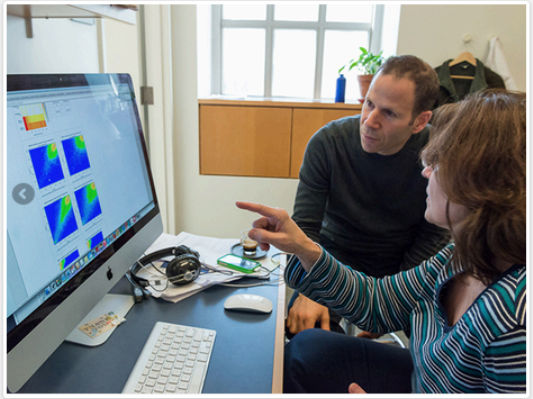
Overview

The Epigenomics Core Facility of Weill Cornell Medicine provides an array of epigenomics and bioinformatics research resources and services that include:

- **DNA methylation profiling** [More]
- **Protein-nucleic acid association** [More]
- **Single Cell Transcriptomics, Immune Profiling and Epigenomics** [More]
- **Long Range DNA Sequencing** [More]
- **Bioinformatics analysis** [More]

Core resources and services include sample preparation services and data generation on the Illumina HiSeq 2500, HiSeq 4000, NextSeq and MiSeq platforms.

Getting Started | Request Services | Pubshare | FAQ | Pricelist



Recent Publications

We require that core clients acknowledge the Epigenomics Core of Weill Cornell Medical College in publications and presentions enabled by our resources.

Check out our latest paper on the **comprehensive evaluation of four DNA methylation measurement platforms**:
Kacmarczyk T.J. et al. Epigenetics Chromatin. 2018. May 25;11(1):21. [PubMed]

- Moriyama, S. et al. **Science** 2018. 359(6379):1056-1061. [PubMed]
- Klose, C.S.N. et al. **Nature** 2017. 549, 282–286. [PubMed]
- Wang, H. et al. **Nature Commun.** 2017. 8(1):767. [PubMed]
- McIntyre, ABR. et al. **Genome Biol.** 2017. 18(1):182. [PubMed]
- Vu, LP., Pickering BF., Cheng Y. et al. **Nat Med.** 2017. Sept 18 EPub. [PubMed]
- Zhang, T. et al. **Blood Cancer J.** 2017. 7(9):e606. [PubMed]
- Xu, R. et al. **J Bone Miner Res.** 2017. 32(9):1811-1815. [PubMed]
- Glass, JL. et al. **Cancer Discov.** 2017. 7(8):868-883. [PubMed]
- Dhingra,P. et al. **Genome Biol.** 2017. 18(1):141. [PubMed]
- Murata, K. et al. **Immunity.** 2017. 47(1):66-79.e5. [PubMed]
- Loupasakis, K. et al. **PLoS One.** 2017. 12(7):e0179762. [PubMed]
- Kan, L. et al. **Nat Commun.** 2017. 8:15737. [PubMed]
- Kurt, IC. et al. **Cel Death Dis.** 2017. 8(6):e2897 [PubMed]
- Siqueira, LG. et al. **Biol Reprod.** 2017. 96(4):743-757. [PubMed]
- Anelli, V. et al. **Elife.** 2017. pii: e20728. [PubMed]

News

Sep 2018: In order to serve our scientific community more efficiently, all RNASeq, DNASeq and sequencing will be performed by the Weill Cornell Medicine Genomics Resources Core Facility (GRCF). **If you require these services** - please submit your samples directly to the GRCF. Please contact Dr. Jenny Xiang if you have any questions about these services.
If you have **ongoing/legacy projects**, questions or concerns, please contact us at epigenomicscore@med.cornell.edu
If you require an Epigenomics Core library preparation service, submit a request as before (see the Getting Started section in our Services page).

Aug 2018: The Epigenomics Core will be **discontinuing RNASeq, DNASeq and Sequencing Only services next month**. To continue availing of these services, please contact Dr. Jenny Xiang at the Genomics Resources Core Facility (GRCF). [GRCF Service Request Portal]

May 2018: Introducing **Microbiome Services** - officially launching June 2018! [Microbiome Core]

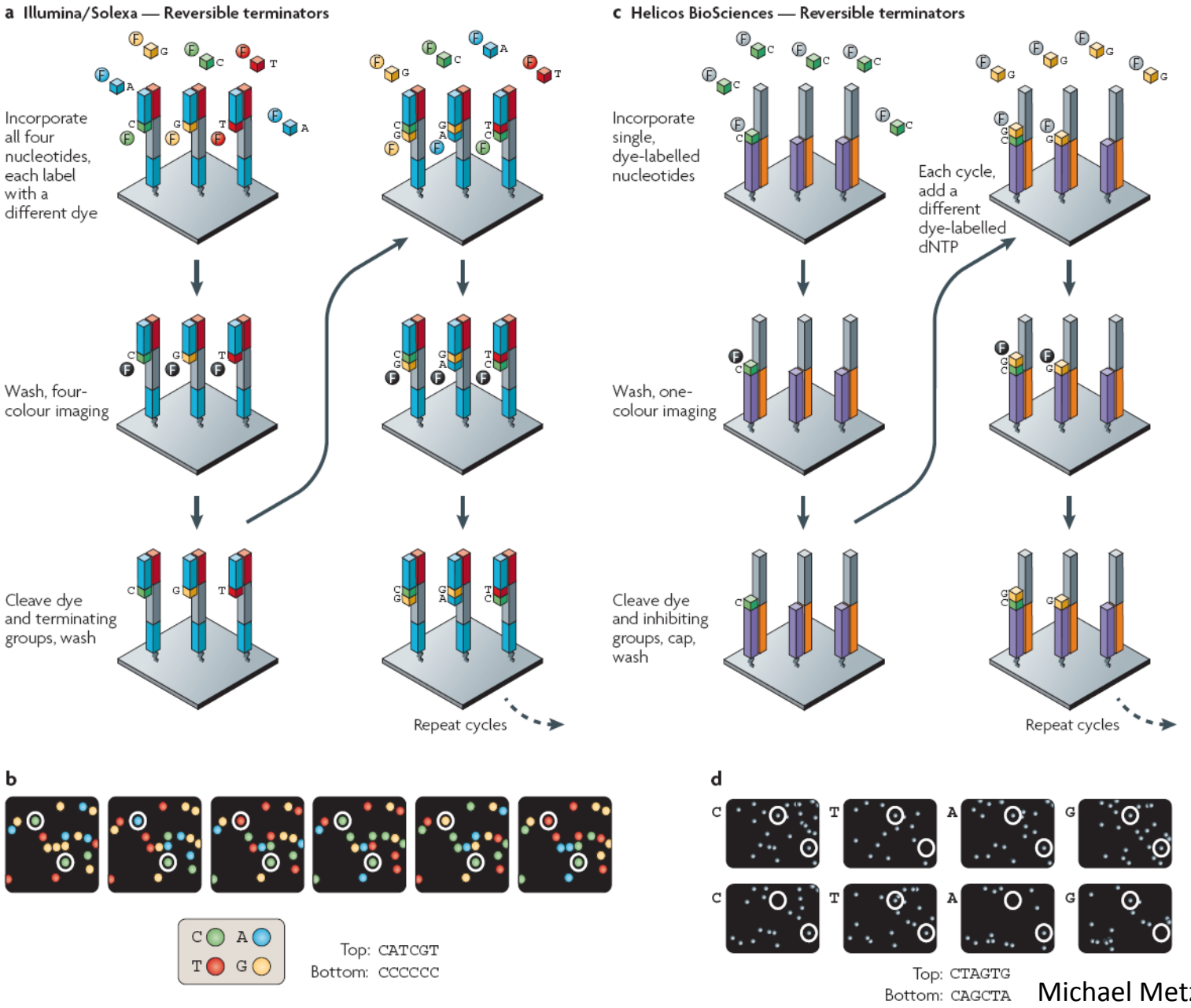
Tweets by @wcmcepigenomics

Epigenomics Core Retweeted

Doron Betel

THE END

Sequencing by Synthesis (SBS)



Michael Metzker, 2010

Now three kinds of chemistry

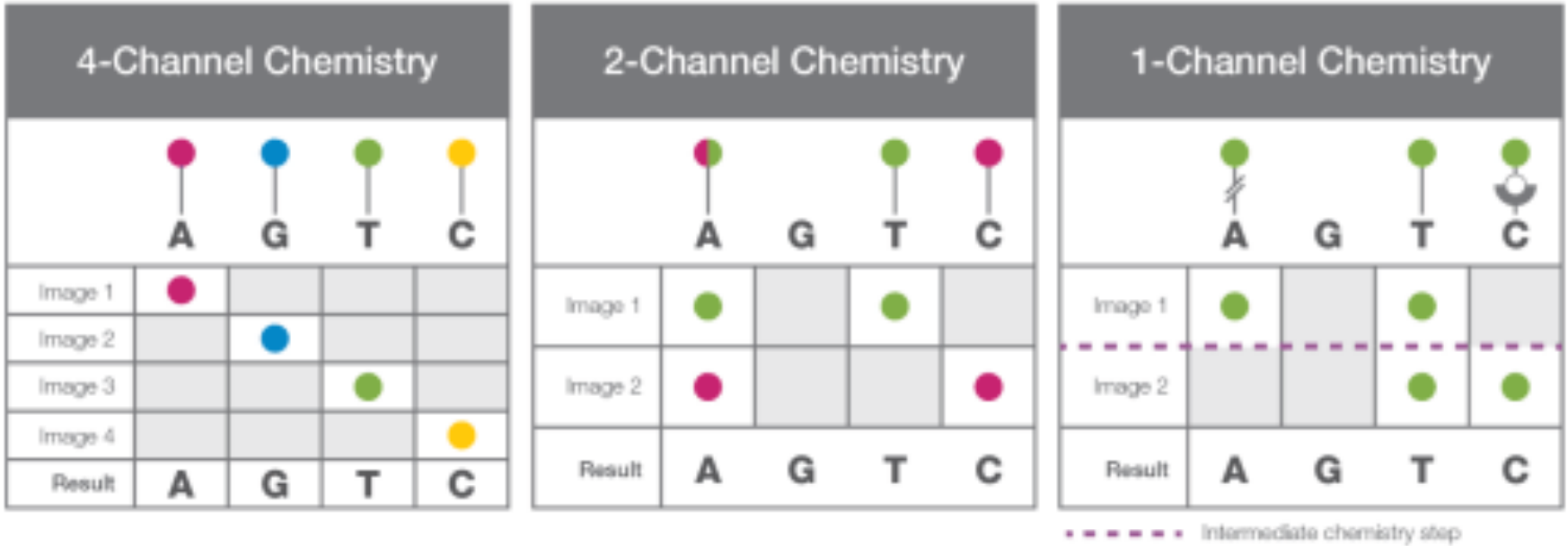
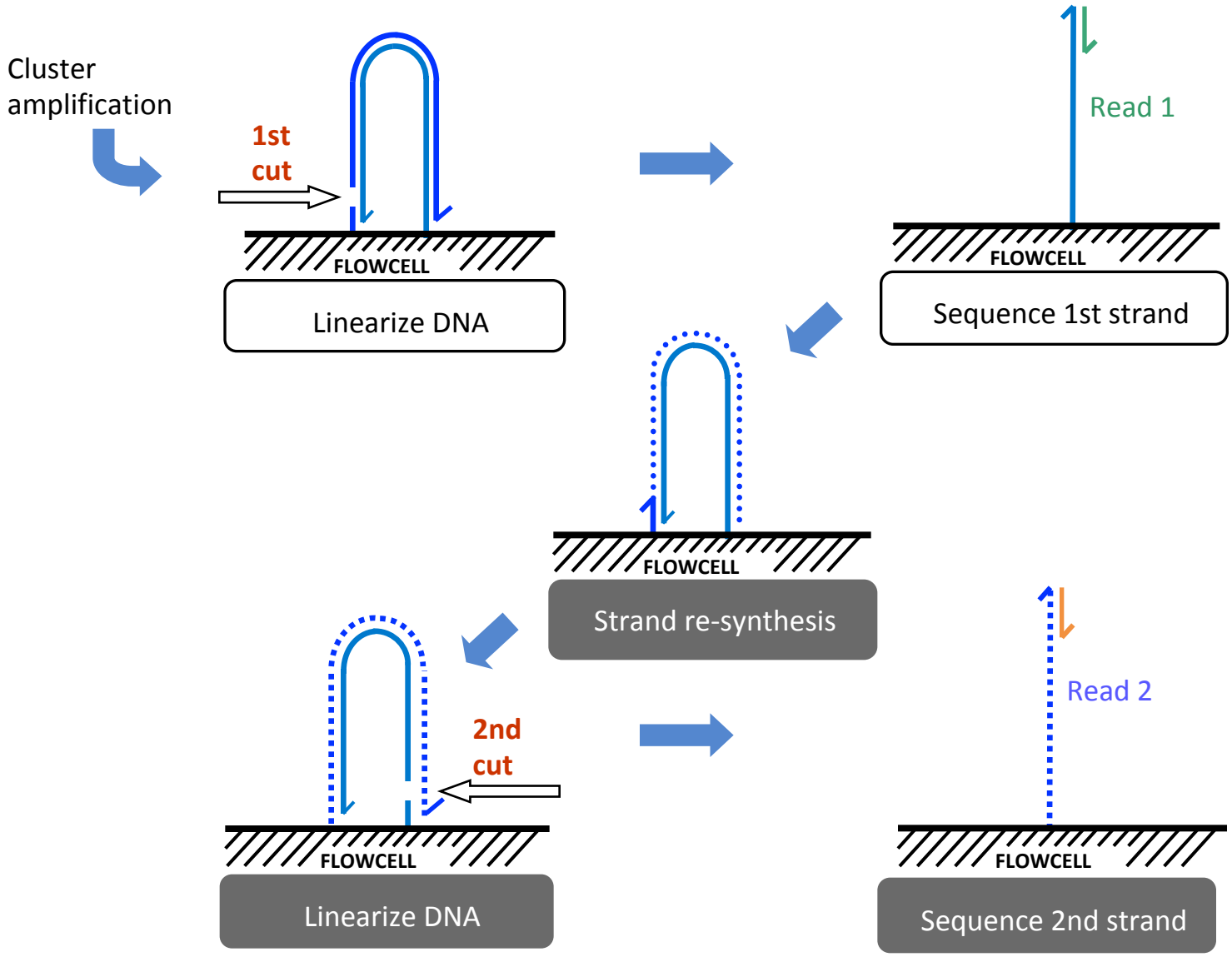
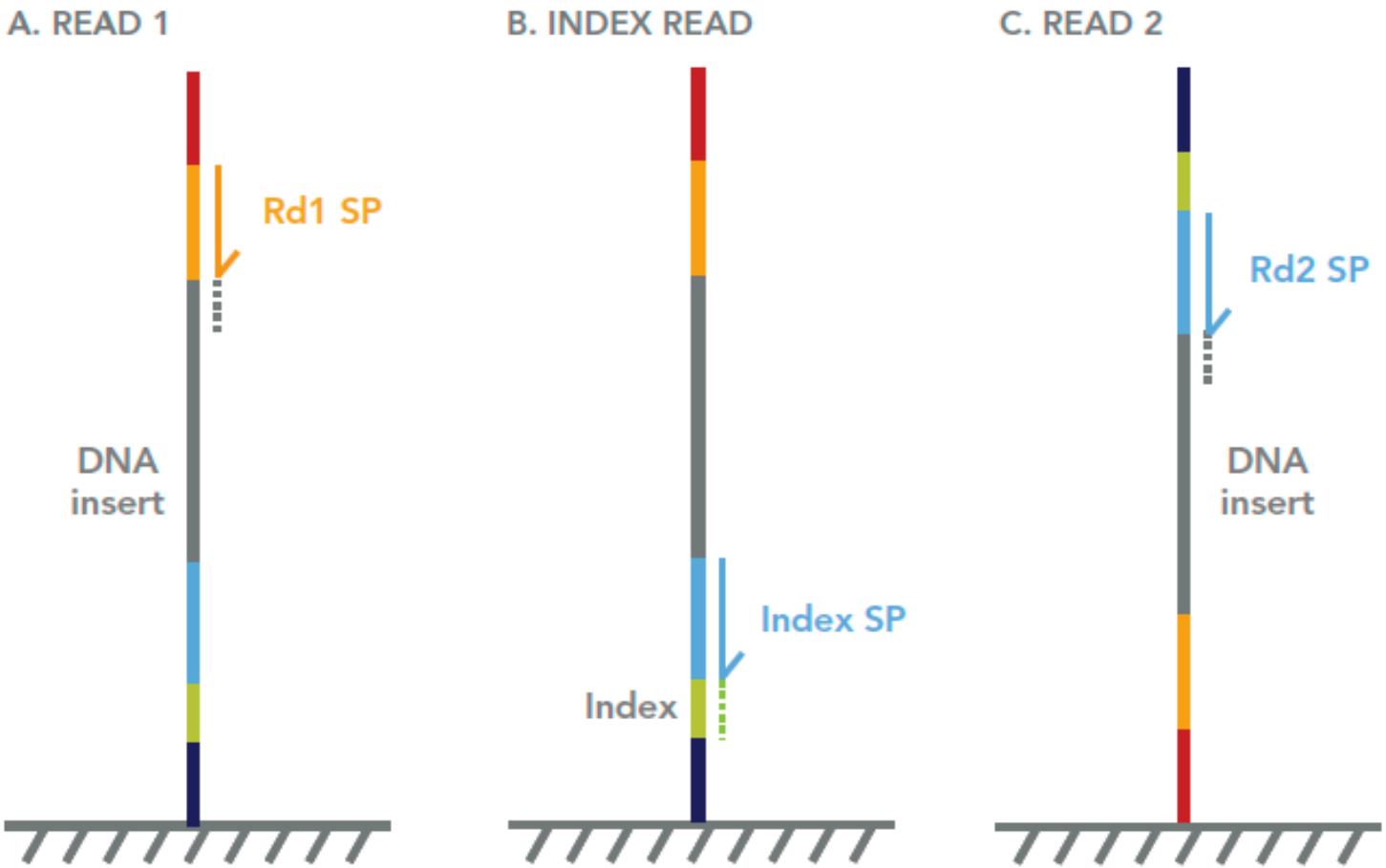


Figure 2: Four-, Two-, and One-Channel Chemistry—Four-channel chemistry uses a mixture of nucleotides labeled with four different fluorescent dyes. Two-channel chemistry uses two different fluorescent dyes, and one-channel chemistry uses only one dye. The images are processed by image analysis software to determine nucleotide identity.

Paired-End Sequencing allows for two looks at a sequence

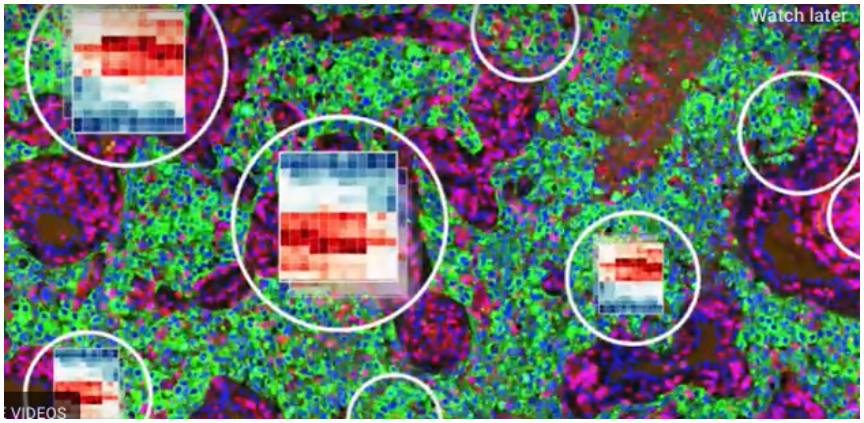


Indexed sequencing method is now standard for single and paired reads



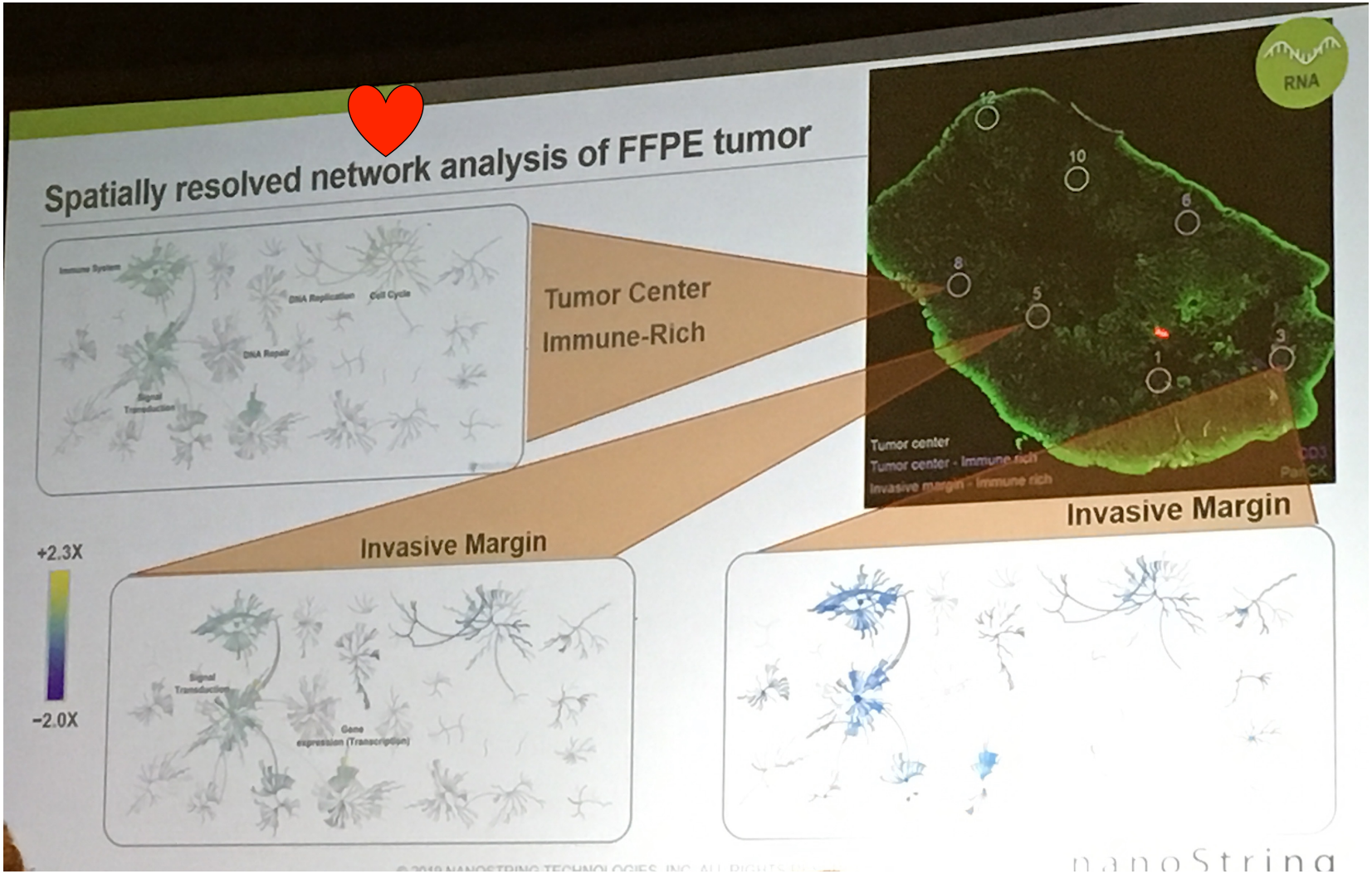
THE TOPIC: SPATIAL GENOMICS

KEEPING SPATIAL INFORMATION – SPATIAL GENOMICS

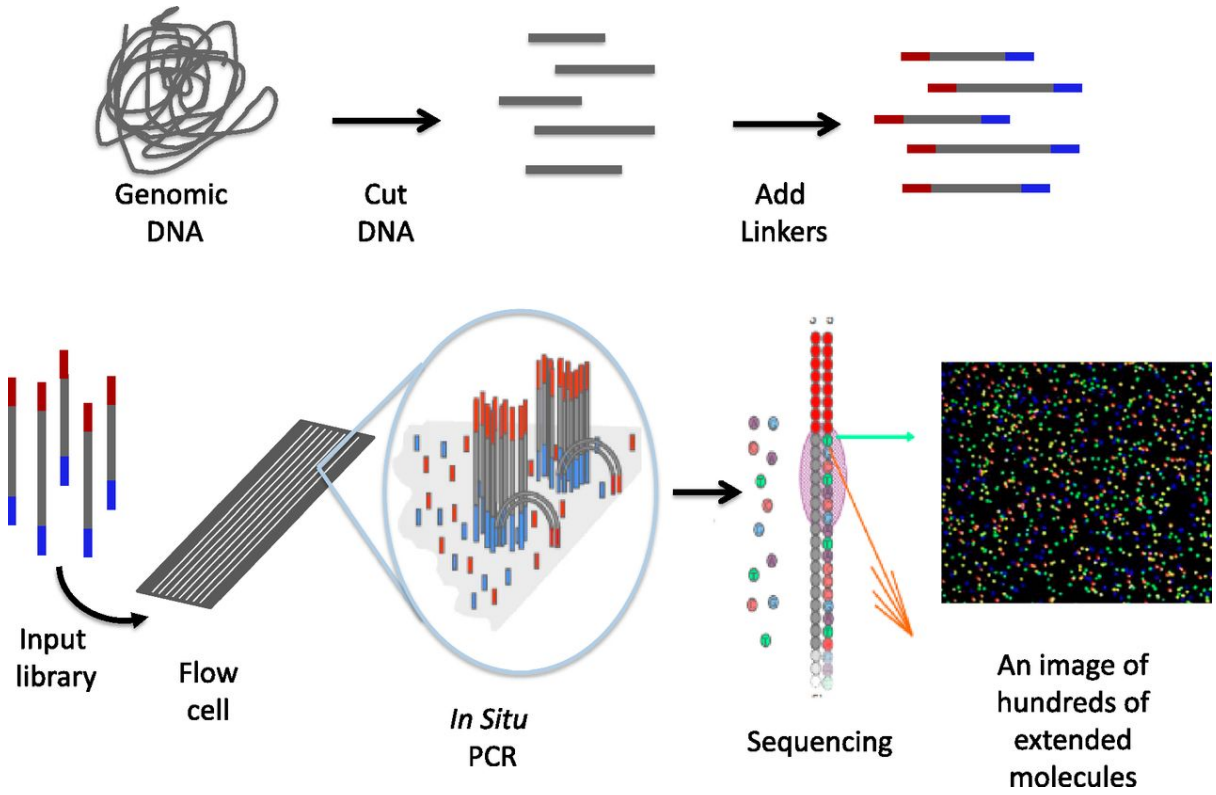


Weill Cornell Medicine

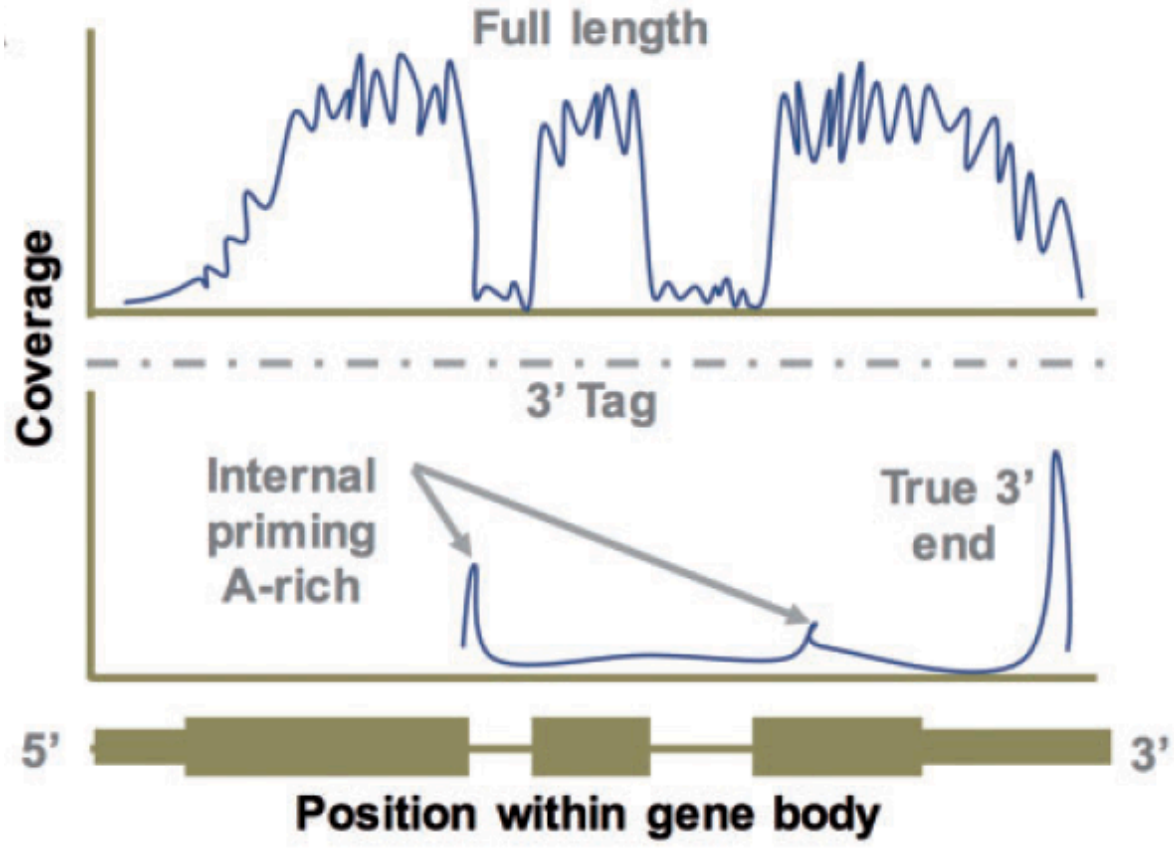
Spatial genomics – Network analysis



Weill Cornell Medicine


Genomic DNA
Cut DNA
Add Linkers
Input library
Flow cell
In Situ PCR
Sequencing
An image of hundreds of extended molecules

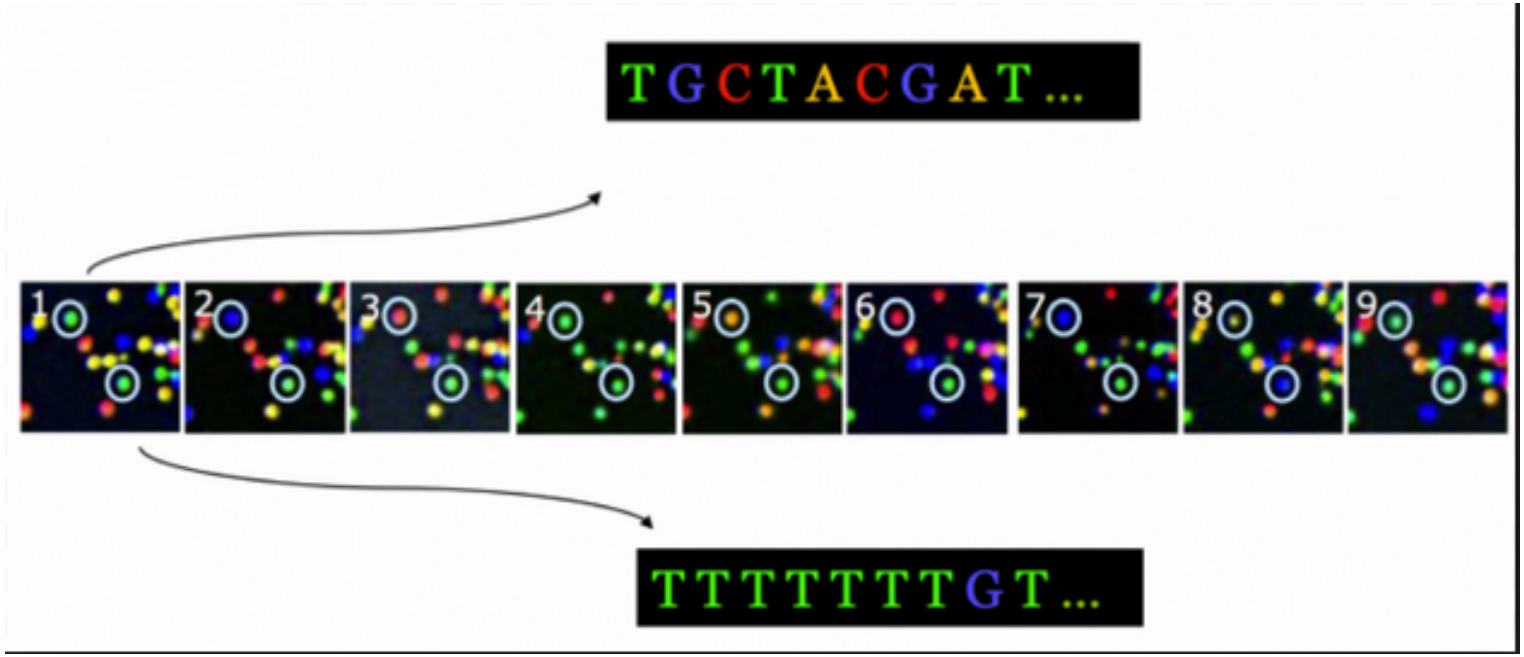
Single cell transcriptomics: full gene body coverage vs 3’ gene body coverage



SMARTseq/SMARTseq v2
C1 Fluidigm
NO UMI

Cell-seq, MARS-seq, Dropseq, InDrop, ddSeq, Chromium

Genomic Diversity and Index Diversity Matter!!!



- Algorithm that calls the base on a cluster is set up using the 1st 25 bases of the run.

These bases are expected to be color-balanced for A,G,C,T; if not the base call is incorrect

Index run also needs to be balanced

Sequencing Coverage

- Coverage (or depth):
  number of reads that are likely to be aligned at a given reference position

$$\textbf{Coverage} = \frac{\text{read length} \times \text{number of reads}}{\text{genome length (haploid)}}$$

- Coverage for Methylation sequencing of the human genome
  Using one lane of a HiSeq 2500 v4 chemistry

$$\frac{(100\text{bp}) \times (280\text{x}10^6)}{3\text{x}10^9} = \textbf{9.3X coverage}$$

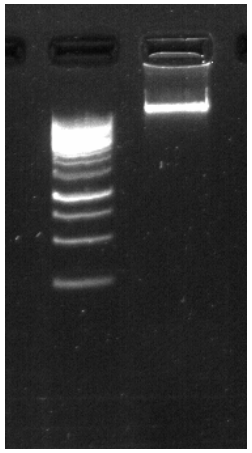
- Illumina's website has a coverage calculator app

http://support.illumina.com/downloadssequencing_coverage_calculator.html

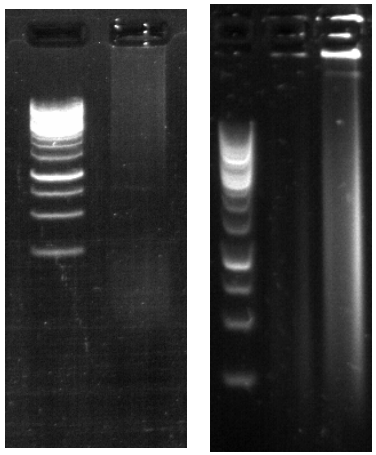
Genomic DNA sample QC: ~40kb, no RNA contamination

Using an agarose gel

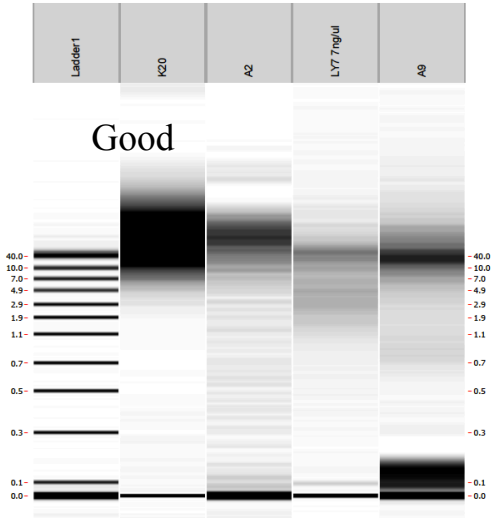
Good



Bad



Using the Perkin Elmer Labchip GX



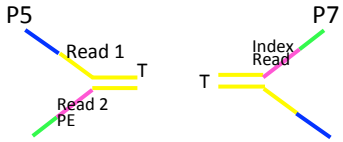
Enhanced Reduced Representation of Bisulfate Conversion (ERRBS)

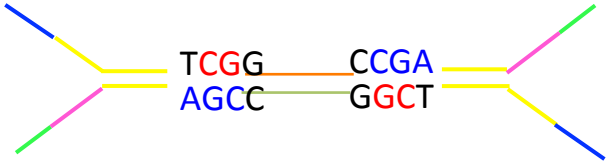
1. MspI digestion

2. End Repair, generates a de novo C, unmethylated and must be removed for data analysis

3. A-tail (for complementing the Illumina Adapter)

4. Adapter ligation (methylated version)

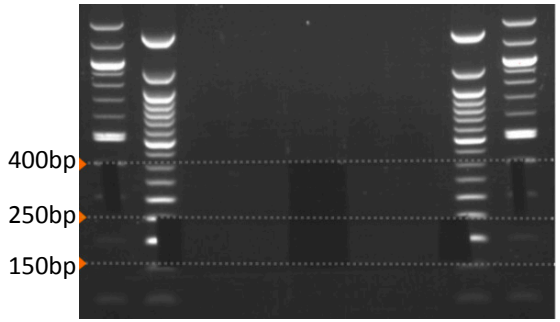




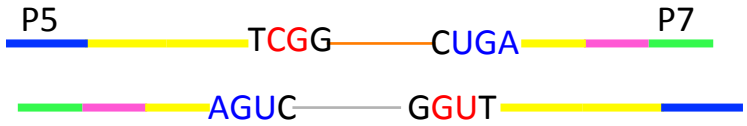
Enhanced Reduced Representation of Bisulfate Conversion (ERRBS)

5. Size selection (150-250bp and 250-400bp)
(DNA size 30-280bp)

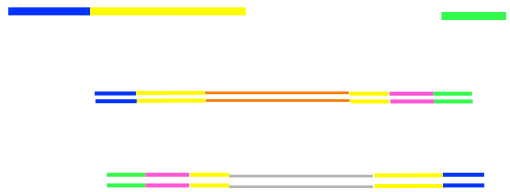
Two fractions are carried over for the rest of the preparation



6. Bisulfite conversion, all C are modified to U
DNA is no longer complementary ie single stranded

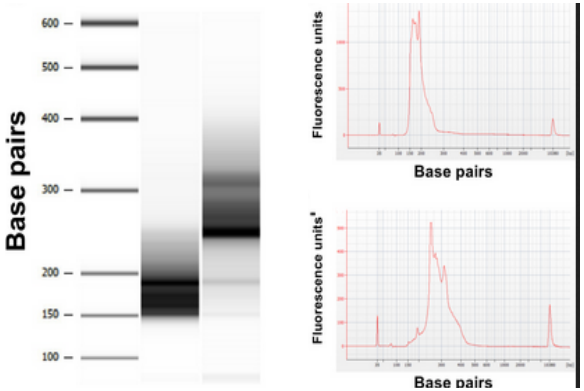


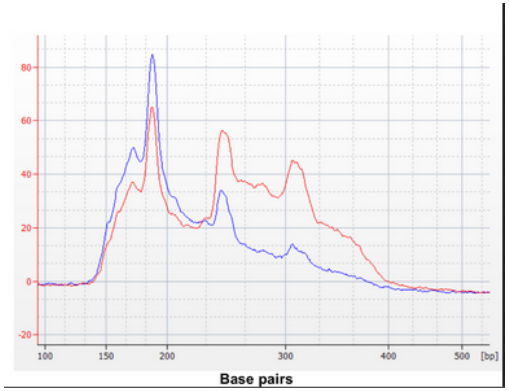
7. PCR amplification



Enhanced Reduced Representation of Bisulfate Conversion (ERRBS)

Library QC

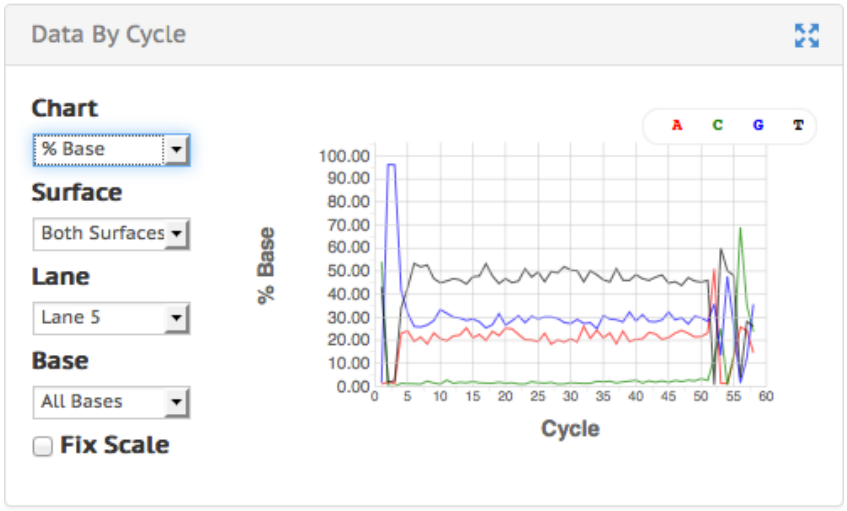


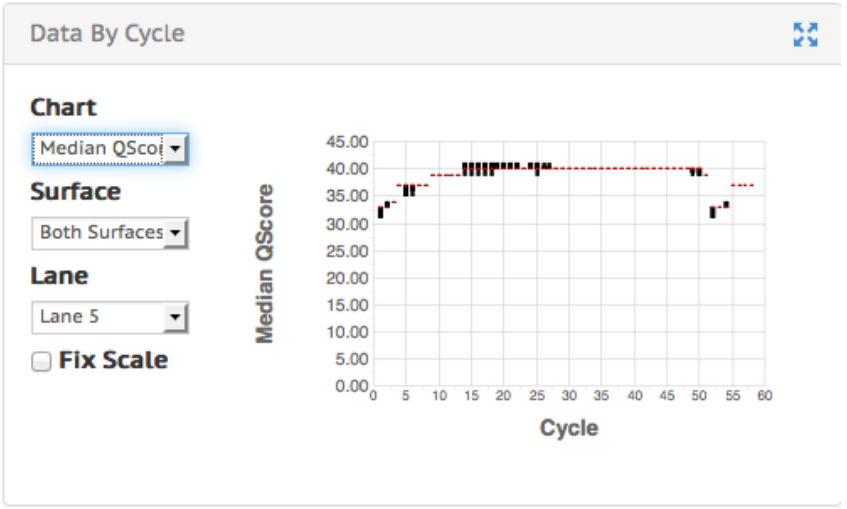


Library made from 2 independent PCR amplified products

Library made from size selection 150-400bp, one PCR amplification

Sequencing QC





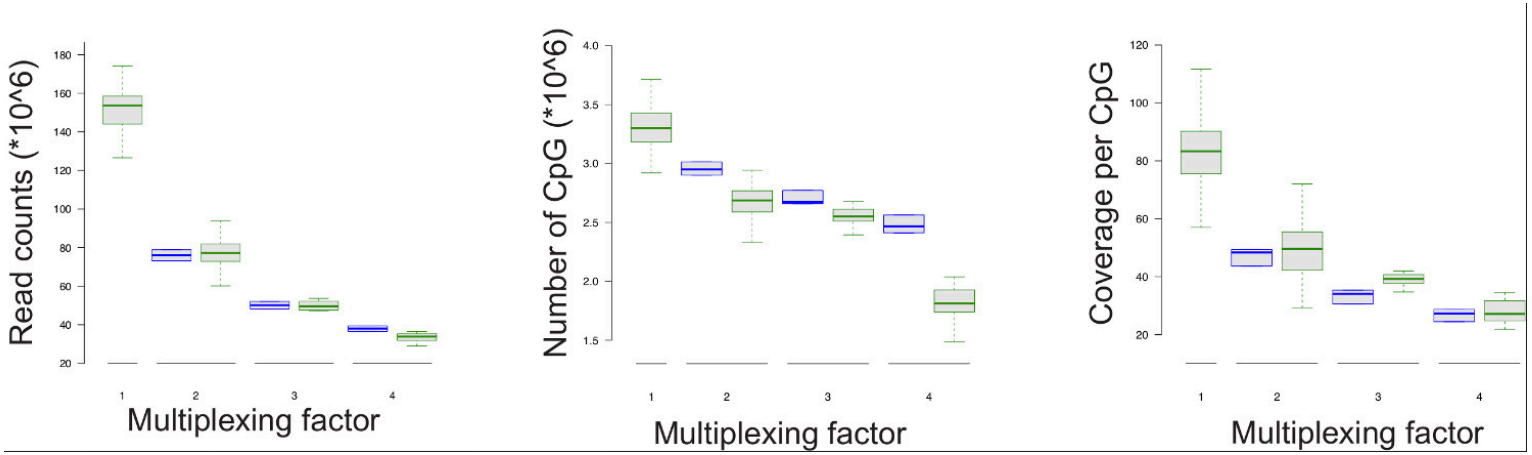
- Akalin A1, Garrett-Bakelman FE, Kormaksson M, Busuttil J, Zhang L, Khrebtukova I, Milne TA, Huang Y, Biswas D, Hess JL, Allis CD, Roeder RG, Valk PJ, Löwenberg B, Delwel R, Fernandez HF, Paietta E, Tallman MS, Schroth GP, Mason CE, Melnick A, Figueroa ME. Base-pair resolution DNA methylation sequencing reveals profoundly divergent epigenetic landscapes in acute myeloid leukemia. PLoS Genet. 2012;8(6):e1002781. doi: 10.1371/journal.pgen.1002781. Epub 2012 Jun 21.
- Garrett-Bakelman, F.E., Sheridan, C.K., Kacmarczyk, T.J., Ishii, J., Betel, D., Alonso, A., Mason, C.E., Figueroa, M.E., Melnick, A.M. Enhanced Reduced Representation Bisulfite Sequencing for Assessment of DNA Methylation at Base Pair Resolution. J. Vis. Exp. (96), e52246, doi:10.3791/52246 (2015).

Enhanced Reduced Representation of Bisulfate Conversion(ERRBS)

Sequencing depth

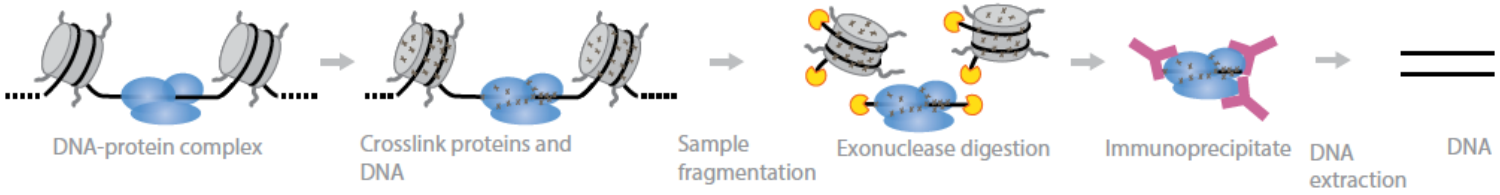
Sequencing recipe	Number of CpG covered (>10X)	Mean CpG coverage (>10X)
SR50	2,979,084	96.62
PE50	5,930,668	88.53
SR100	4,889,616	93.15
PE100	6,607,470	85.48

How does multiplexing affect ERRBS data?



ChIPseq

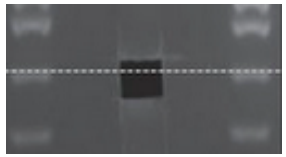
Chromatin Immunoprecipitation



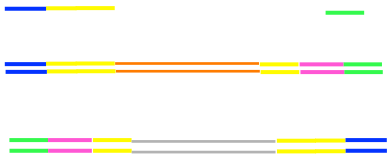
1. End repair, A-tailing

2. Adapter ligation

3. Size selection of DNA from 250-350bp
   (original DNA ~130-230bp)



4. PCR amplification



ChIPseq

Sample QC

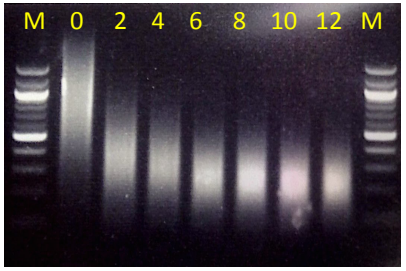
QC1: quantitation

a minimum of 11ng of ChIP DNA at ≤ 1ng/ul, determined by Qubit

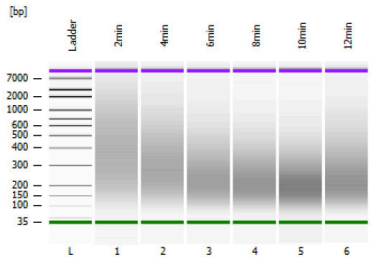
QC2: qualitative measurement

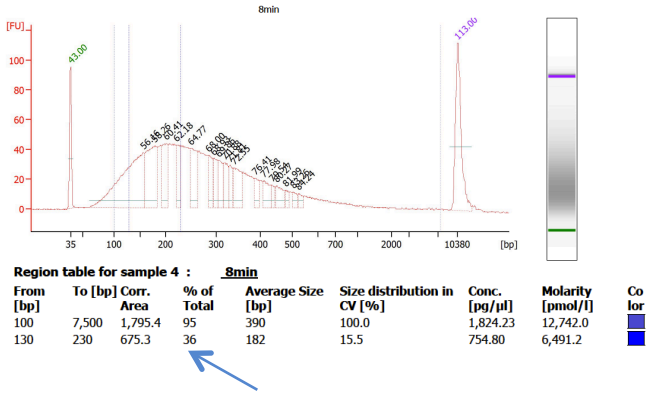
10% of total material must be in the size range of the DNA fraction required for library preparation (130-230bp)

1.5% agarose gel



BioAnalyzer High Sensitivity





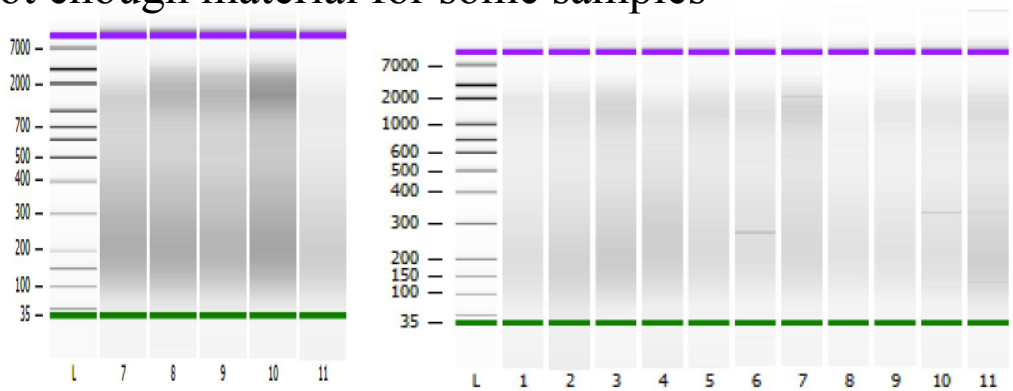
Region table for sample 4 : 8min

From [bp]	To [bp]	Corr. Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [pg/µl]	Molarity [pmol/l]	Color
100	7,500	1,795.4	95	390	100.0	1,824.23	12,742.0	
130	230	675.3	36	182	15.5	754.80	6,491.2	

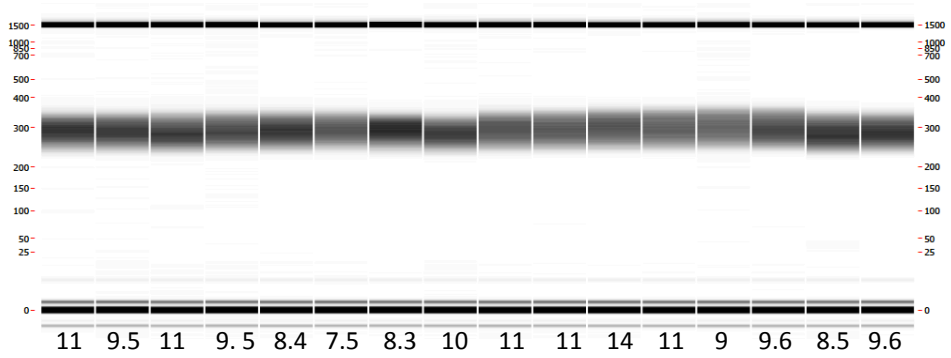
Typical ChIPseq project

QC2 BioA all samples >-10% within range

QC1 not enough material for some samples



Library QC



% duplication

Core: will send an email informing libraries are ready to be tested by qPCR

Fast QC report will give % duplication, which is one parameter to follow

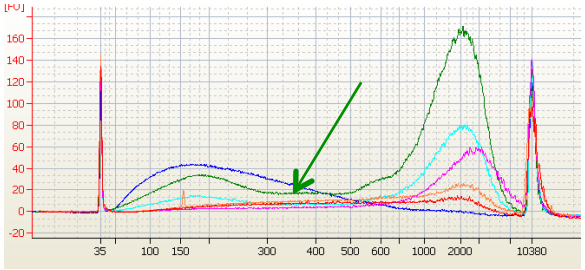
ChIPseq

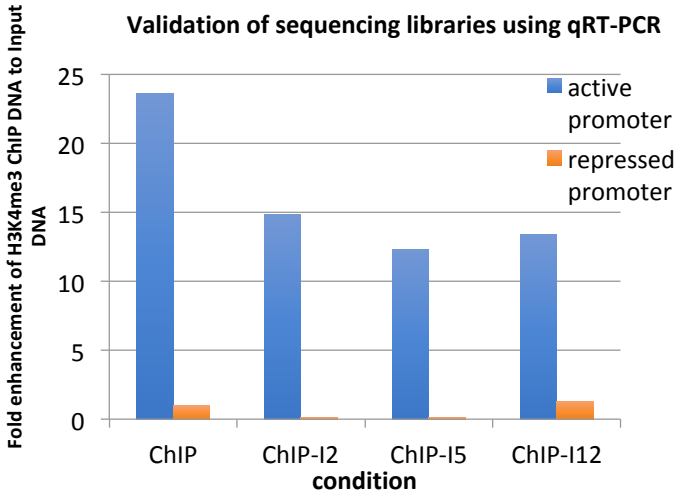
Sequencing depth:

depends on the antibody, number of binding sites, cell type, quality of the IP

Multiplexing of ChIP-Seq Samples in an Optimized Experimental Condition Has Minimal Impact on Peak Detection Thadeous J. Kacmarczyk, Caitlin Bourque, Xihui Zhang,Yanwen Jiang, Yariv Houvras,Alicia Alonso, and Doron Betel PLoS One. 2015 Jun 11;10(6):e0129350. doi: 10.1371/journal.pone.0129350. eCollection 2015.

QC2





Multiple libraries were made and sequenced on SR50 at different depths, from 181M to 27M

TruSeq mRNA prep

1. rRNA depletion: oligo dT hybridization, and purification

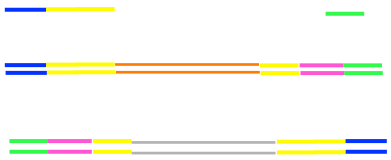
2. mRNA fragmentation: chemical cleavage (~150bp)

3. Random priming to obtain cDNA strand and dscDNA

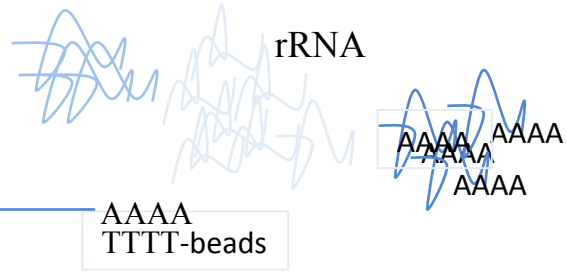
4. End repair, A-tailing

5. Adapter ligation

6. PCR amplification



TruSeq stranded mRNA prep



1. rRNA depletion: oligo dT hybridization, and purification

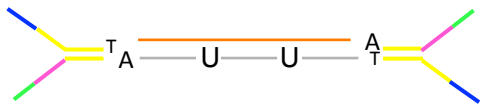
2. mRNA fragmentation: chemical cleavage (~150bp)

3. Random priming to obtain cDNA strand

4. dUTP incorporation on the dscDNA

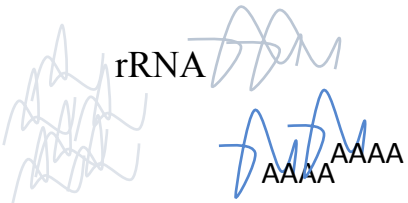
5. A-tailing

6. Adapter ligation



7. PCR amplification, removal of dUTP containing strand

TruSeq Total stranded RNA prep



1. **rRNA depletion: rRNA hybridization, and purification**

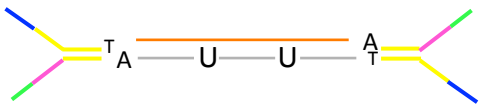
2. mRNA fragmentation: chemical cleavage (~150bp)

3. Random priming to obtain cDNA strand

4. **dUTP incorporation on the dscDNA**

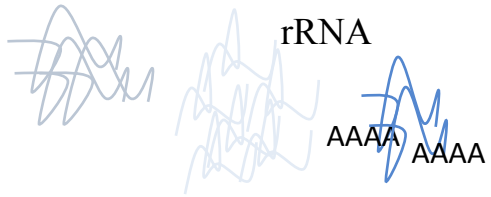
5. A-tailing

6. Adapter ligation

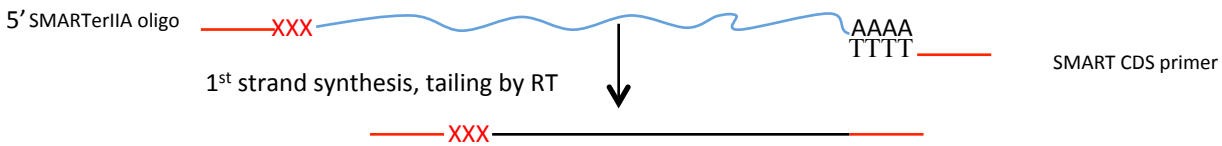


7. **PCR amplification, removal of dUTP containing strand**

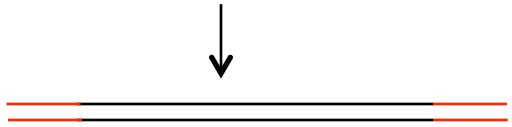
UltraLowInput RNAseq (SMART technology)
(Switching Mechanism At 5' End of RNA Transcript)

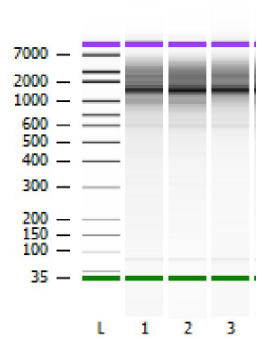


1. SMARTer Reverse transcriptase



2. PCR amplification from SMARTer primers (Full length dscDNA)





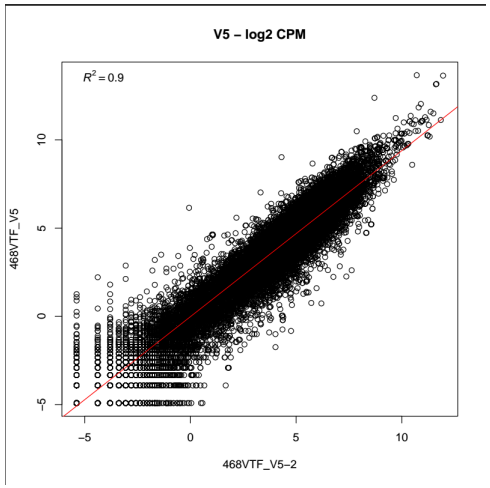
3. Sonication to ~200bp

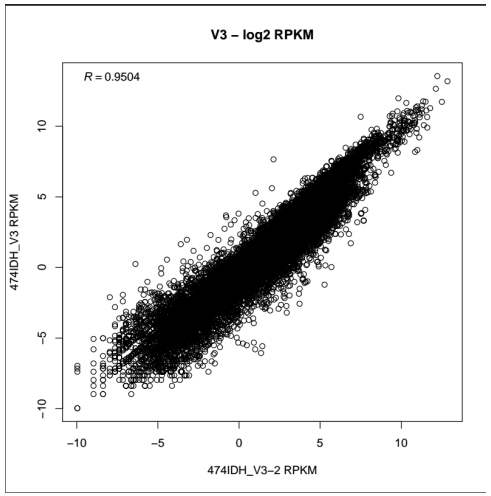
4. End repair, A-tailing, ligation of TruSeq adapters

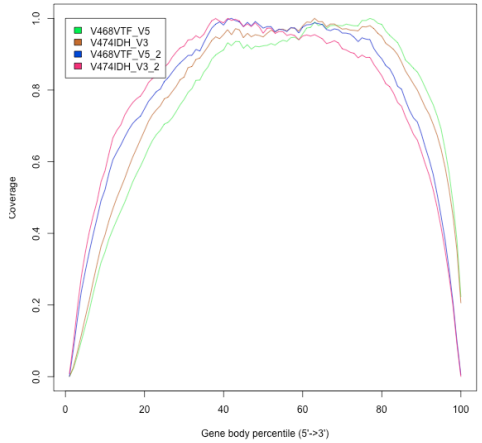
5. PCR amplification

Comparison between data generated by RNAseq and Ultra Low Input RNAseq

	468VTF_V5-2 (RNAseq)	468VTF-5 (ultralow)	474IDH_V3-2 (RNAseq)	474IDH_V3 (ultralow)
RIN	8.3	8.3	8.3	8.3
% duplication	48.22	45.95	47.99	44.53
% mapped reads	98.90	97.85	98.80	98.02
RefSeq IDs with > 10 counts	18602	17997	17868	17168
% rRNA in bam(RSeQC)	2.71	7.42	3.3	7.69



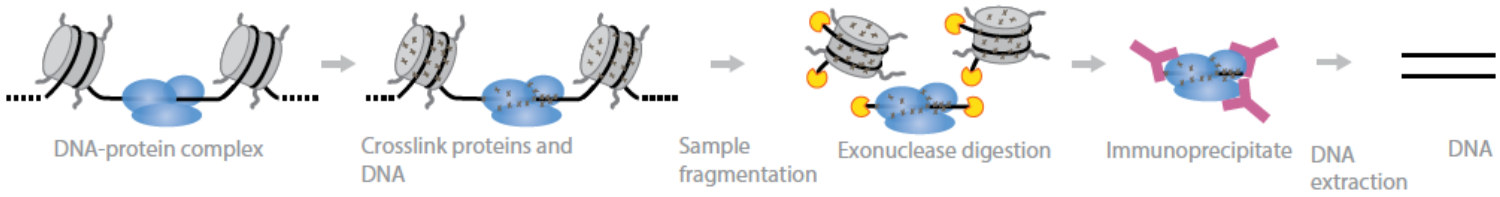




Xihui, Yushan, Piali

Quality Control of ChIP DNA

Chromatin Immunoprecipitation



QC1: quantitation

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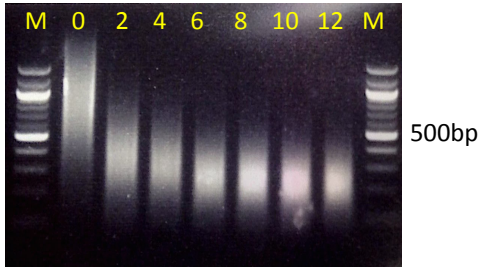
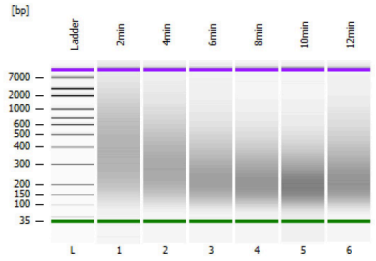
QC2: qualitative measurement

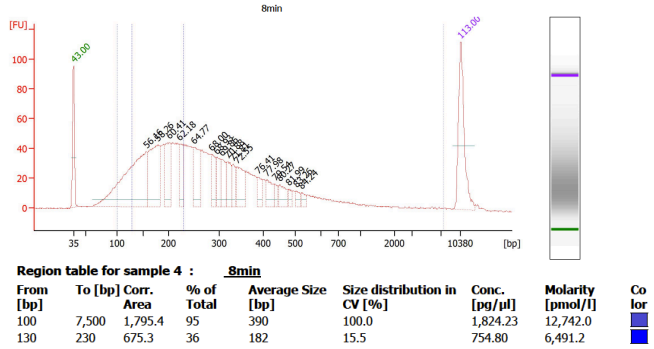
10% of total material must be in the size range of the DNA fraction required for library preparation (130-230bp)

1.5% agarose gel



BioAnalyzer High Sensitivity





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100	7,500	1,795.4	95	390	100.0	1,824.23	12,742.0	
130	230	675.3	36	182	15.5	754.80	6,491.2	

Epigenomics Core

Home | Services | People | Resources

(Epi)Genetic Profiling | Overview

Overview

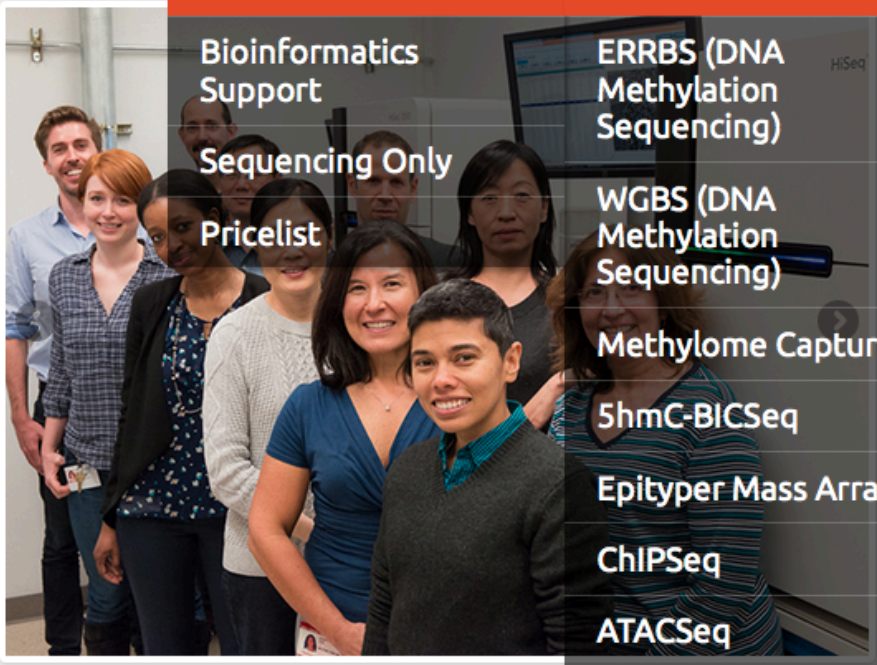
The Epigenomics Core Facility of Weill Cornell Medical College provides an array of epigenomics and bioinformatics research resources and services that include:

- **DNA methylation profiling** [More]
- **Protein-nucleic acid association** [ChIP-Seq]
- **Genome sequencing** [More]
- **Exome capture** [More]
- **RNA-seq** [More]
- **Bioinformatics analysis** [More]

Core resources and services include sample preparation services and data generation on the Illumina HiSeq 2500, MiSeq platforms and Sequenom MassArray platform.

Getting Started | iLab | Pubshare | FAQ

$ Pricelist



Bioinformatics Support

Sequencing Only

Pricelist

ERRBS (DNA Methylation Sequencing)

WGBS (DNA Methylation Sequencing)

Methylome Capture

5hmC-BICSeq

Epityper Mass Array

ChIPSeq

ATACSeq

Exome Capture

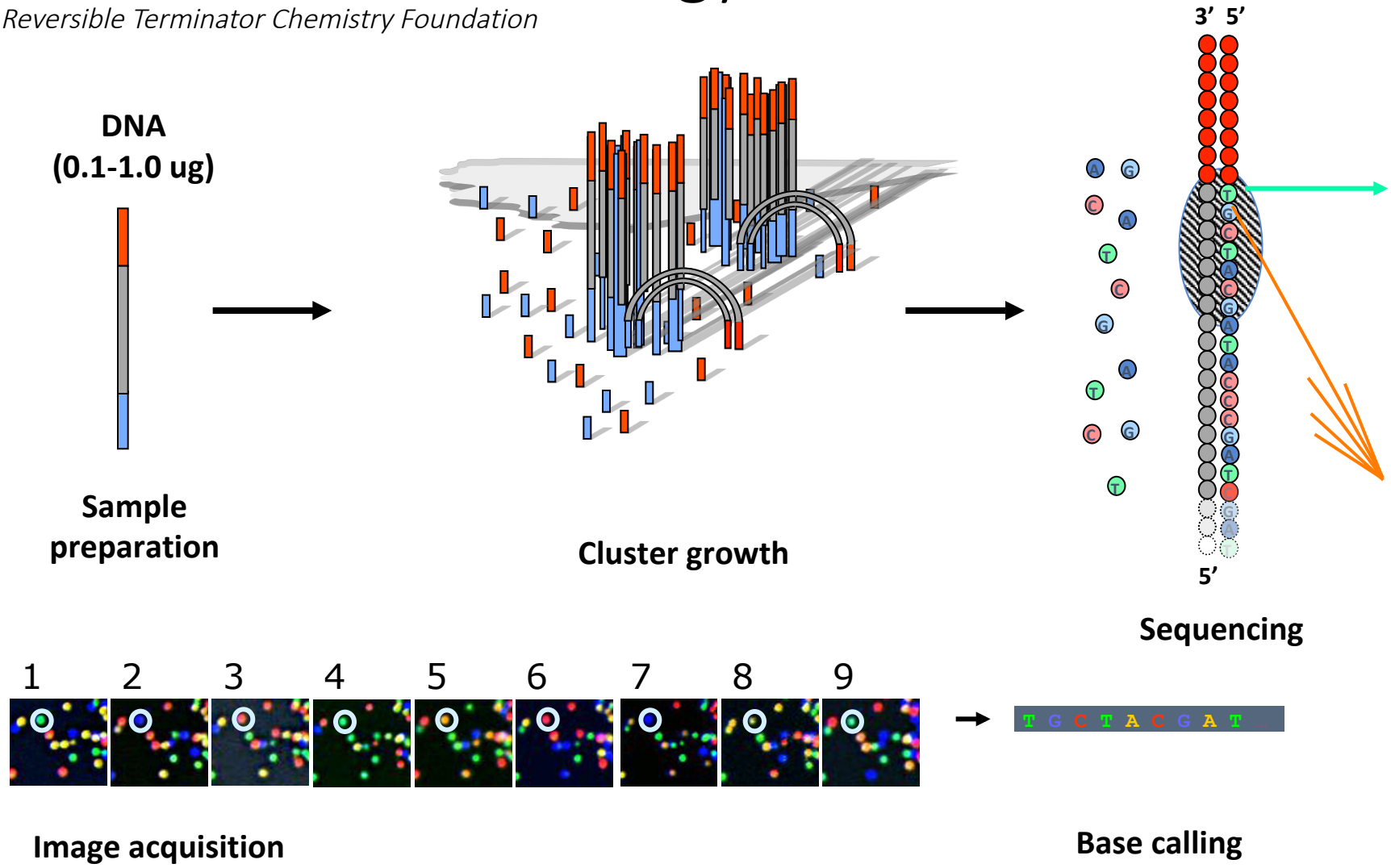
DNASeq

Targeted Resequencing

RNASeq

Illumina SBS Technology

Reversible Terminator Chemistry Foundation



http://www.illumina.com/technology/sequencing_technology.ilmn