Marine Genomics in the Era of High-Resolution Sequencing: Exploring PacBio, 10x Genomics, and Hi-C Applications

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國立臺灣海洋大學
2024.12.10.

Services & Prices

Sample QC

- Quantification
- Electropherogram
- Pippin Size Selection
- Purification

Illumina System

- Illumina Library Prep
- Illumina HiSeq 2500 Sequencing
- Illumina MiSeq Sequencing
- Illumina NextSeq 2000 Sequencing
- Illumina iSeq 100 Sequencing

PacBio System

- PacBio Sequel Library Prep
- PacBio Sequel Sequencing

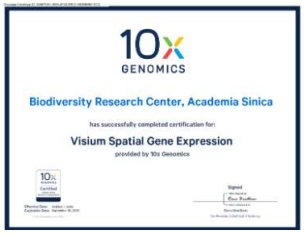
Oxford Nanopore System

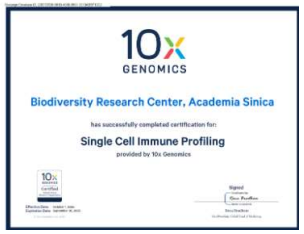
- ONT Library Prep
- GridION Sequencing

10x Genomics

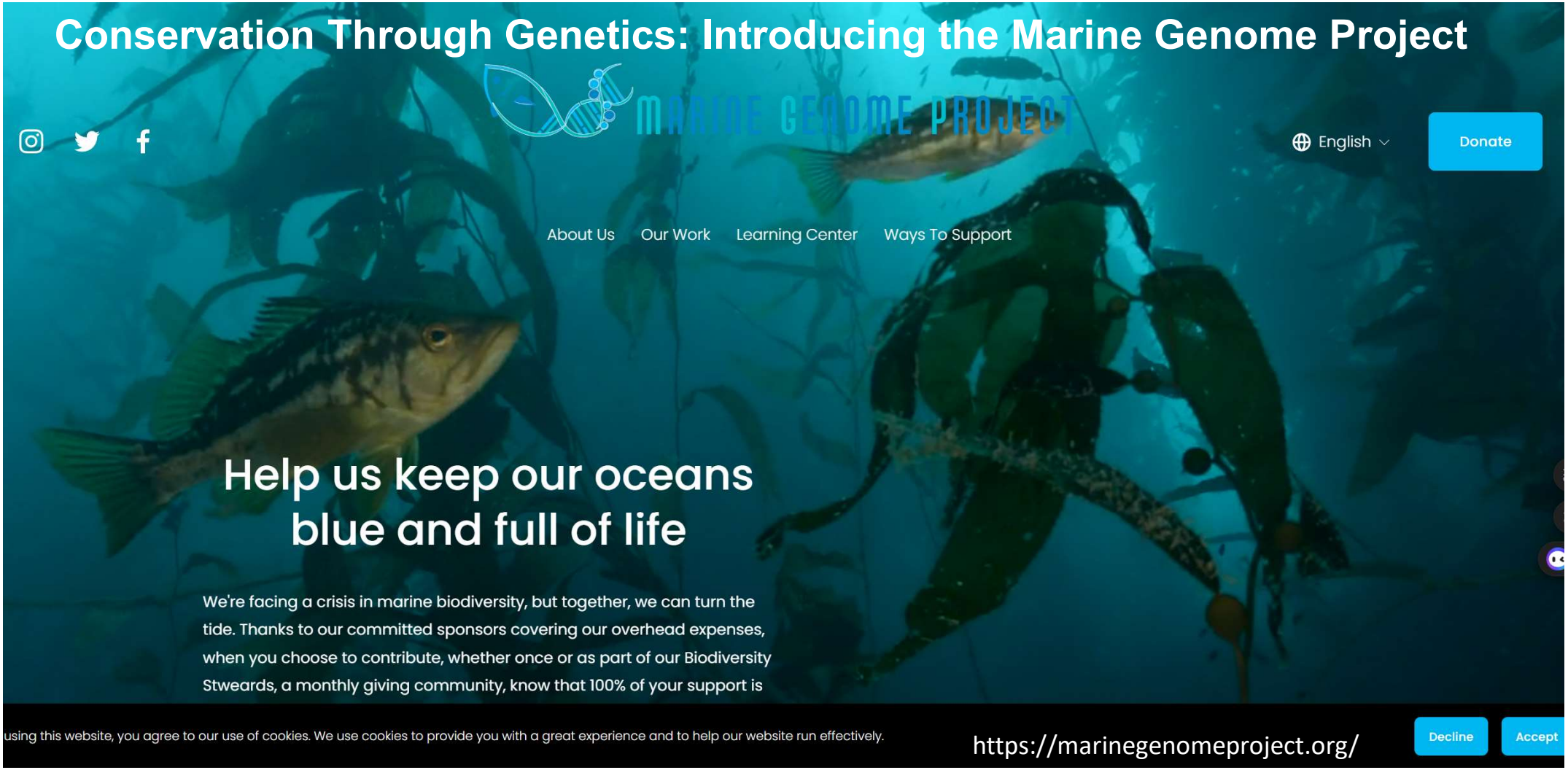
- 10x Genomics – Spatial Transcriptome (Visium)
- 10x Genomics – Single Cell (VDJ, ATAC)

10x GENOMICS Certified Service Provider





Marine Genome Project: ~ 2/3 marine species are at risk of extinction · Join us in collecting DNA from 1000 thousand species by 2029 to protect our ocean.



Key Objectives of Marine Genome Projects:

1. **Biodiversity Understanding**: genome builds, genetic diversity, traits for env. adaptation
2. **Ecological Insights**: species intrxn, nutrient cycles, ecosystem dynamics, unique genes for marine traits
3. **Conservation Applications**: identify endangered species, conservation strategy, pop. monitoring
4. **Biotechnological Potential**: Exploring marine species for bioactive compounds, enzymes, or materials useful in medicine, industry, or bioengineering.
5. **Climate Change Studies**: mechanisms to adapt to changing environmental conditions

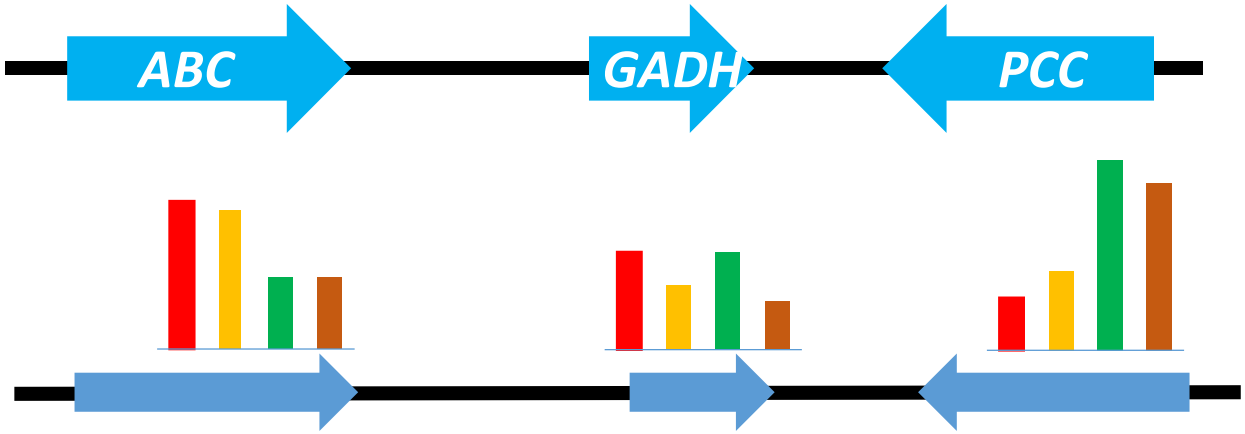
Tools and Technologies Used:

- **PacBio and Hi-C Sequencing**: Enables chromosome-level assembly and resolution of complex genomes.
- **Metagenomics**: Investigating microbial communities and their roles in marine ecosystems.
- **Functional Genomics**: Linking genes to traits and ecological functions.

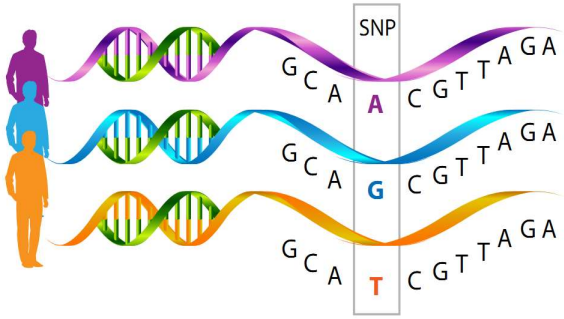
Conservation Through Genetics: Introducing the Marine Genome Project

What can we learn from meta/genome?

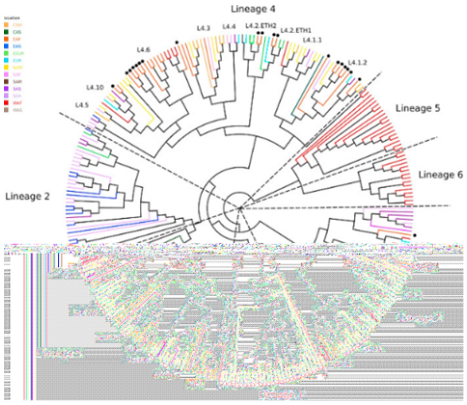
1. Assembly
2. Gene prediction
3. Functional annotation
4. Expression profiling



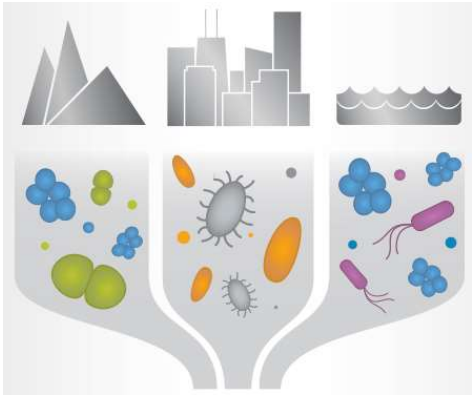
5. Genome variants

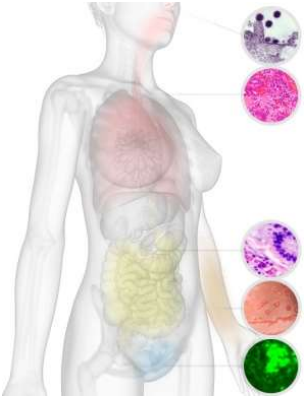


6. Population Genomics Cohort



7. Metagenome/microbiota





NGS High Throughput Genomics Core at BRCAS
新世代基因體定序核心實驗室

Project Consultation:

1. **Defining your research goals:** refine your research questions to ensure the chosen sequencing approach delivers the necessary insights.
2. **Sample preparation recommendations:** Whether it's DNA, RNA, or tissue, find optimal prep methods to maximize data quality and yield.
3. **Platform selection:** With Illumina, PacBio, and Nanopore, choose the platform that best fits your budget, desired read length, and target genome complexity.
4. **Experimental design:** Optimize your library prep, sequencing depth, and replication strategy for reliable and statistically robust results.

*** Advanced applications:**

1. **3D genome analysis:** Delve into the spatial organization of chromosomes and explore higher-order genome structure for a deeper understanding of gene regulation and function.
2. **Single-cell RNA-seq:** Unravel cellular heterogeneity and explore gene expression at the individual cell level, revealing hidden cell populations and dynamic transcriptional landscapes.
3. **Spatial transcriptomics:** Map gene expression patterns across tissues and organs, providing a powerful tool for studying development, disease, and complex biological processes.

Key technologies:

1. PacBio HiFi:
   - De novo assembly
   - Haplotype phasing
   - Metagenome assembly
   - Transcrtipome Isoseq: full-length Isoform sequencing
   - High-resolution profiling: taxonomy, CRISPR-screening
2. 10x Genomics:
   - Single-cell: RNA-seq, ATAC-seq, immune repertoire
   - Spatial RNA-seq
   - HT in-situ hybridization
3. 3D genome: Hi-C, microC, Hi-ChIP, meta-HiC
   - Hi-C: genome scaffolding, chrmosome phasing
   - Micro-C: ehromatin dynamics: chromatin interactions
   - Hi-ChIP: epigenetic regulation by ChIP-seq+Hi-C
4. Complementary applications:
   - Single-cell/Spatial IsoSeq
   - Single-cell Hi-C

Marine Genomics: important considerations

1. Biological questions in the genome perspectives:
   - Genome assembly, functional annotation
   - re-sequencing: qualitative vs quantitative
2. Sample nature:
   - Single genome, population, mixed culture, community, symbionts
   - Source amount, purity
3. Genome nature:
   - size, purity, heterozygosity, ploidy
4. Choice of HTS :
   - LR vs SR: assembly, phasing, isoforms, paralogues, repeats
   - Data scale: qualitative vs quantitative
5. Sequencing and Bioinformatics approaches:
   - Shotgun vs target enrichment, capture vs amplicon
   - Assembly vs Database mapping
   - Transcriptome database, isoform discovery
   - Expression profiling

Key technologies:

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Current Major NGS & TGS Platforms

Sanger

Dideoxy terminator

Illumina

Reversible terminator

HiSeq, MiSeq, NovaSeq

PacBio

SMRT

Sequel IIe

Oxford NanoPore

MinION

GrinION

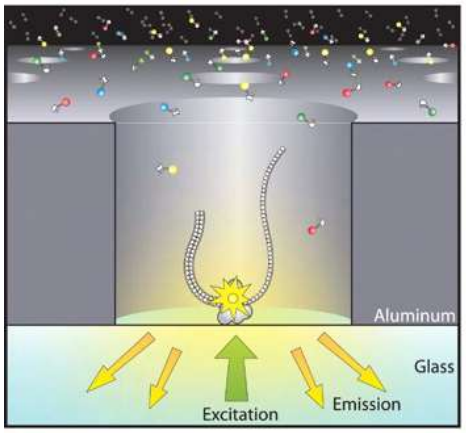
P2 Solo

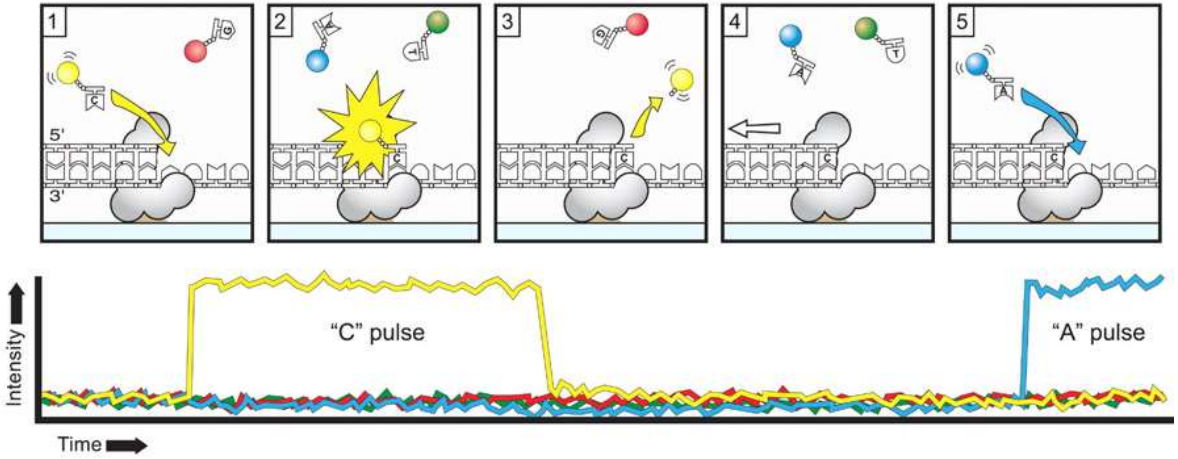
PromethION

ElementBio

Aviti24

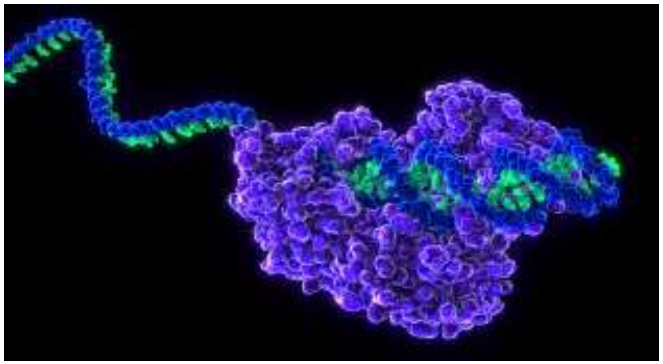
PacBio: Single-molecule fluorescent signals

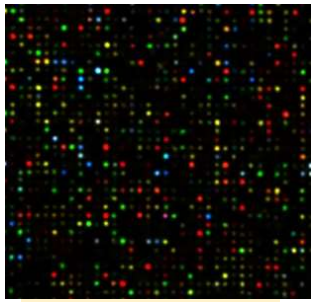




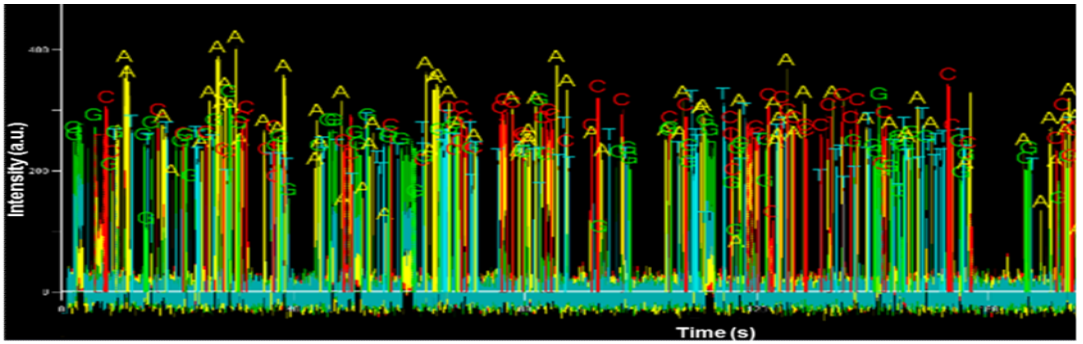
- **Single Molecular Real Time (SMRT) real-time technology**
- ZMW (zero-mode waveguides), a 100-nm hole with DNA/Polymerase complex immobilized at the bottom; recording fluorescence released from P-dNTP upon incorporation

PacBio – HiFi CCS (circular consensus seq.)

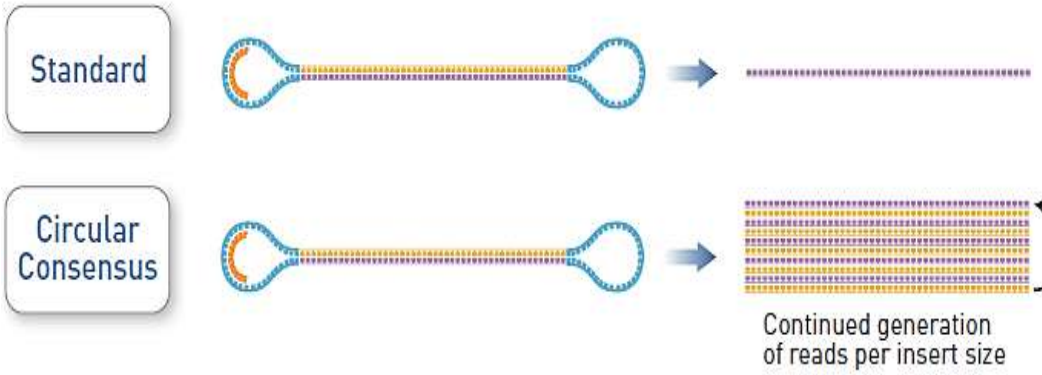


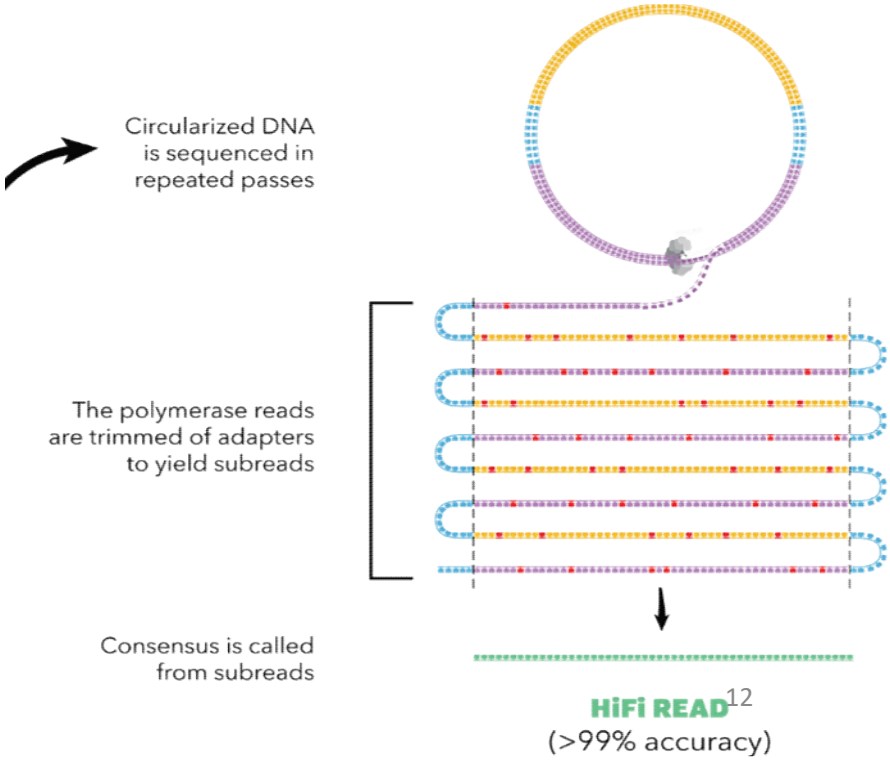


Movie trace

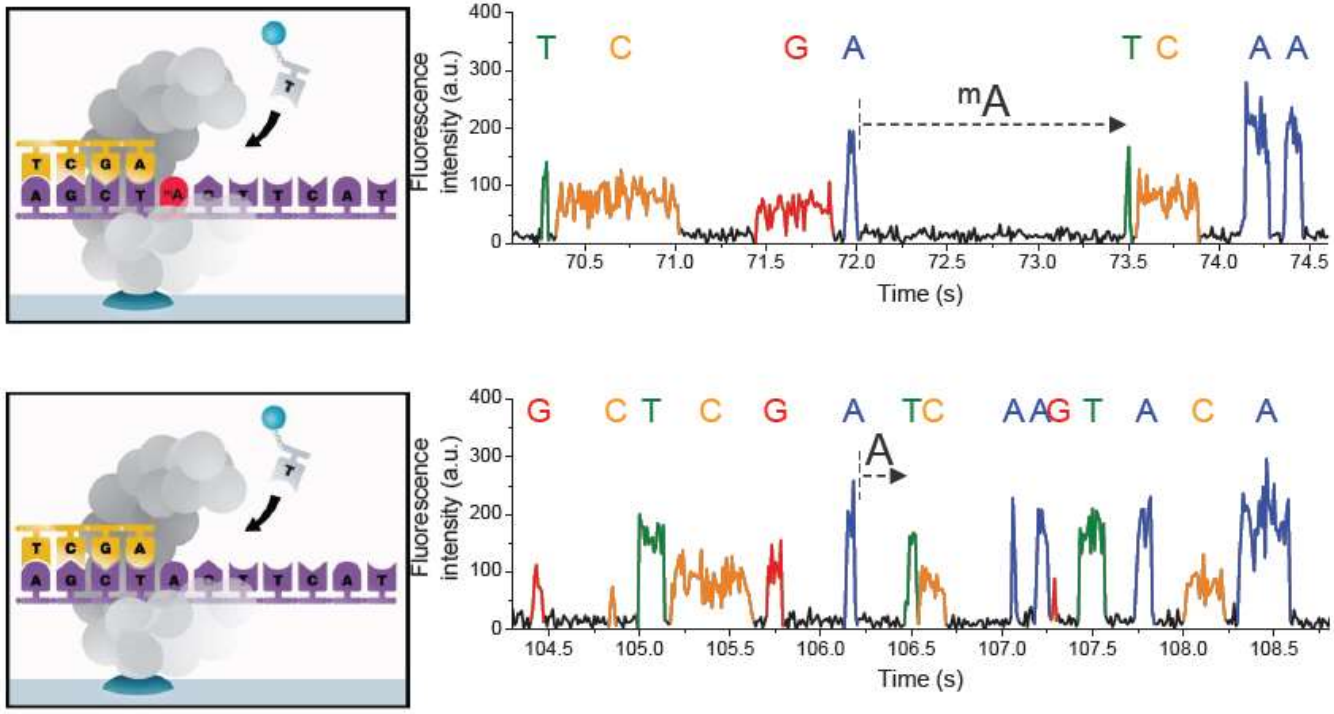


CCS length: avg. 1-15kb, max. >100kb
Throughput: 10-30 Gb; Accuracy: 99.9% (10X)

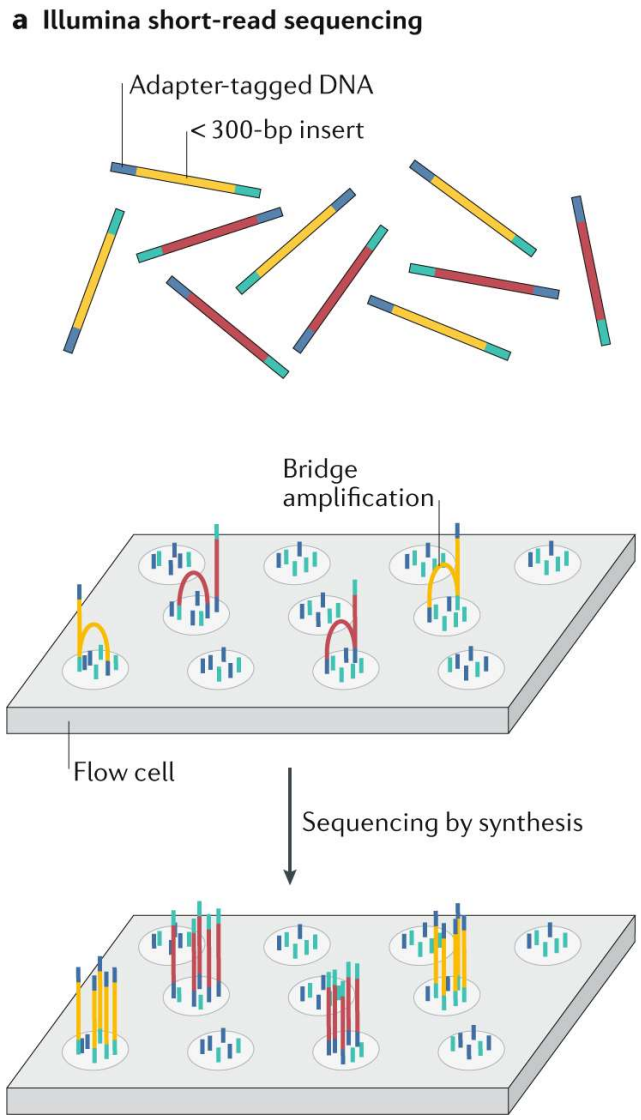


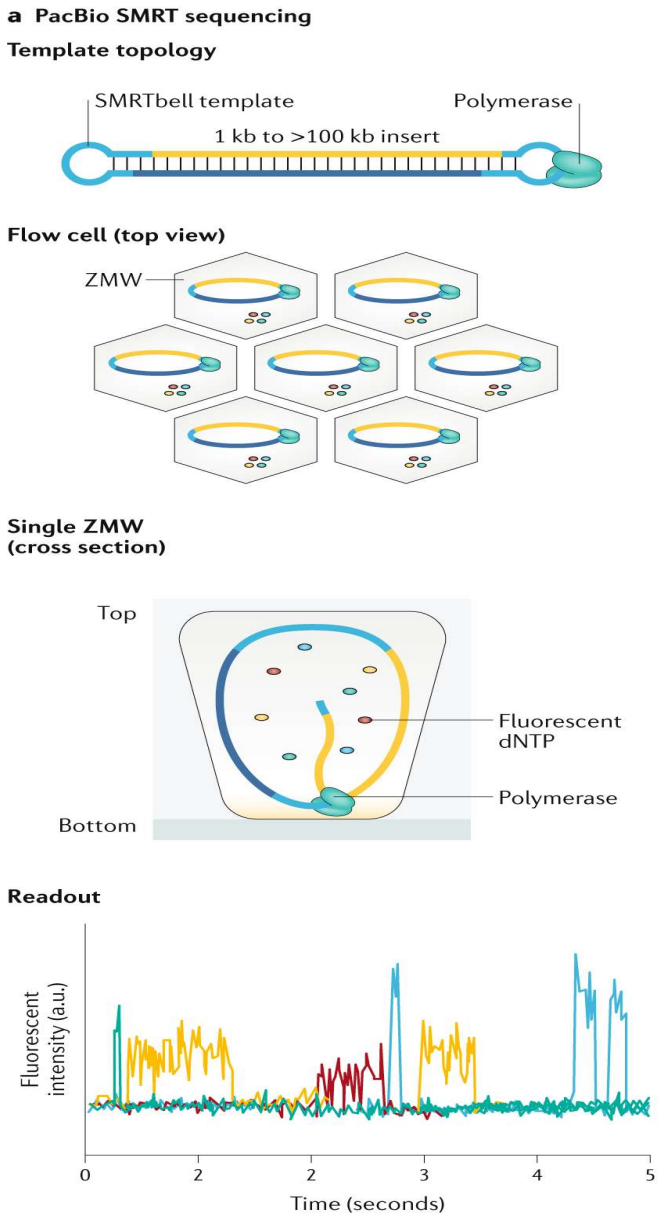


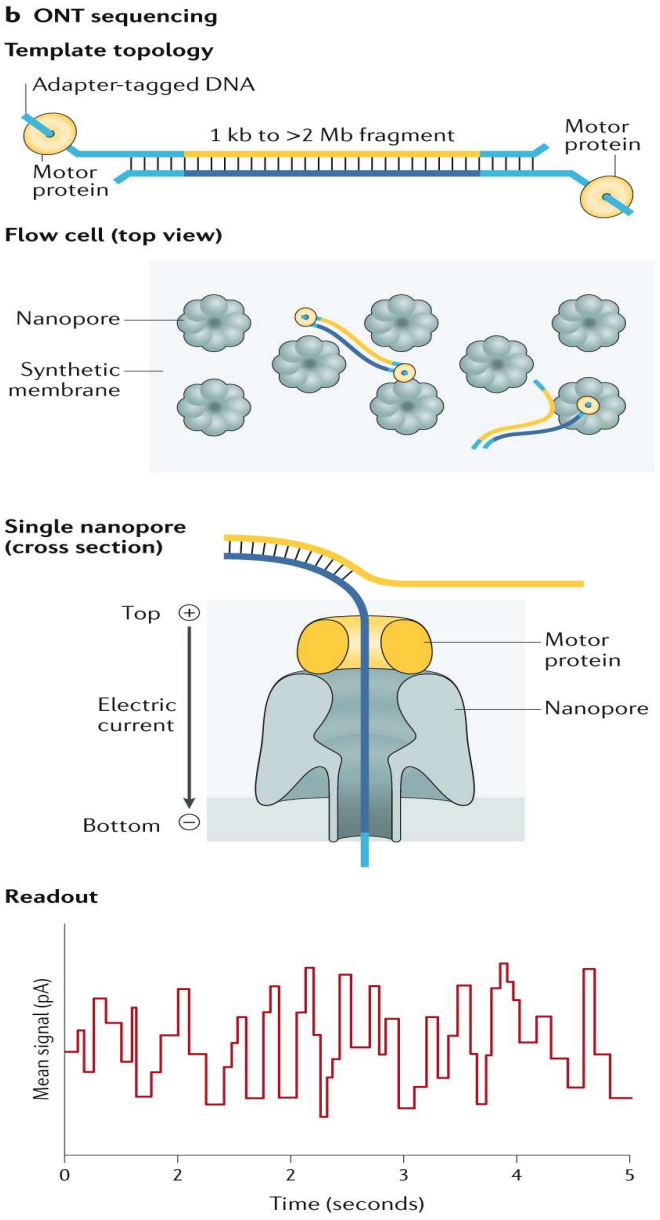
Key Feature: Kinetic Information



- Differentiation between modified and non-modified bases
  - Epigenetics, DNA damage, New, novel modifications
- Direct observation (*e.g.* no bisulfite)







Nat Rev Genet **21,** 597–614 (2020).
https://doi.org/10.1038/s41576-020-0236-

Sample & Library QC

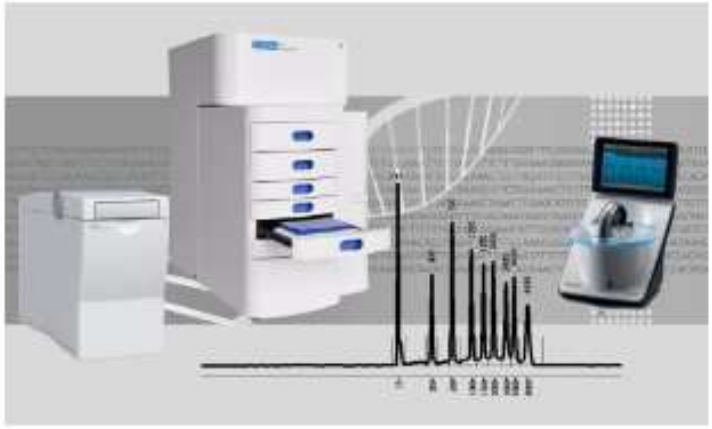
1. DNA
2. RNA

- NanoDrop
- Gel check
- BioAnalyzer or Fragment Analyzer
- Qubit quantification
- qPCR

Sequencer

Shearing
Barcoding
Gel sizing
qPCR

QC Related



NanoDrop
Qubit
BioA/FA

Library Prep Related

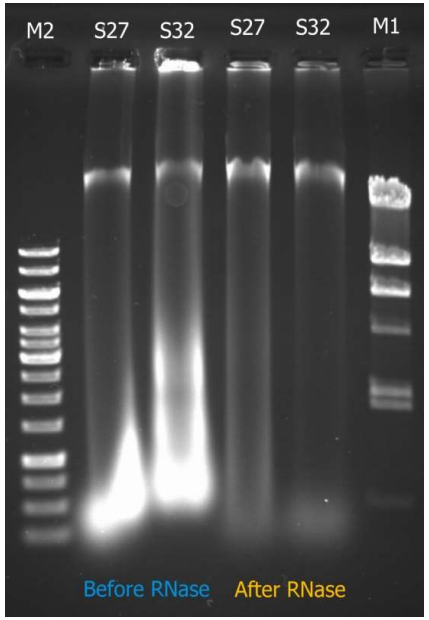
Shearing
Barcoding
Gel sizing
qPCR

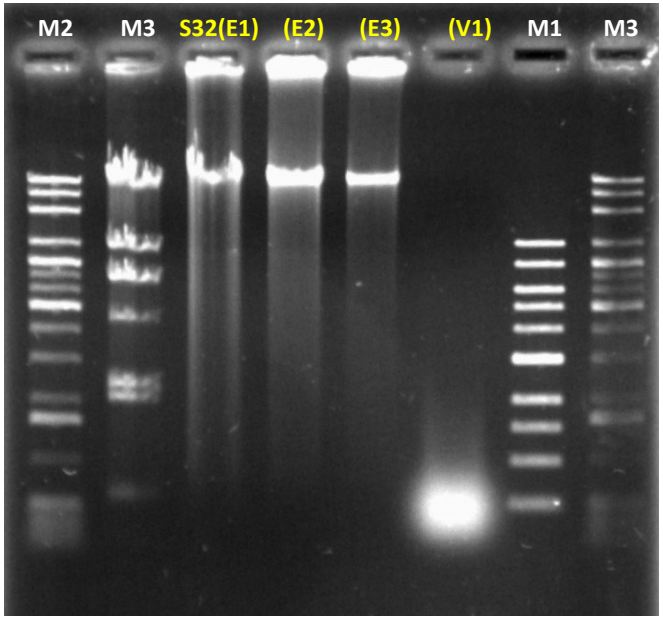
10x Related

Single-cell

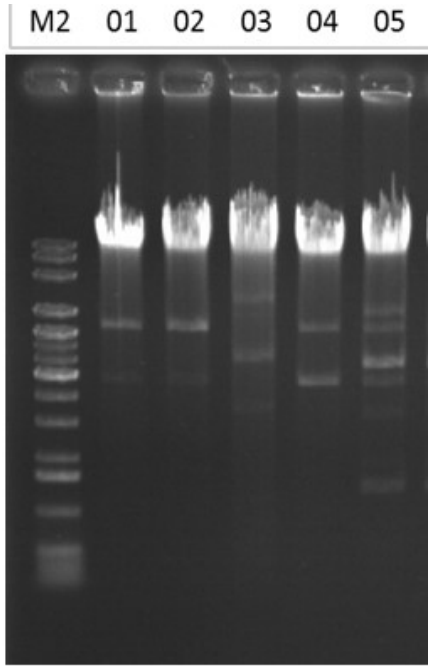
Spatial

Genomic DNA +RNase treatment



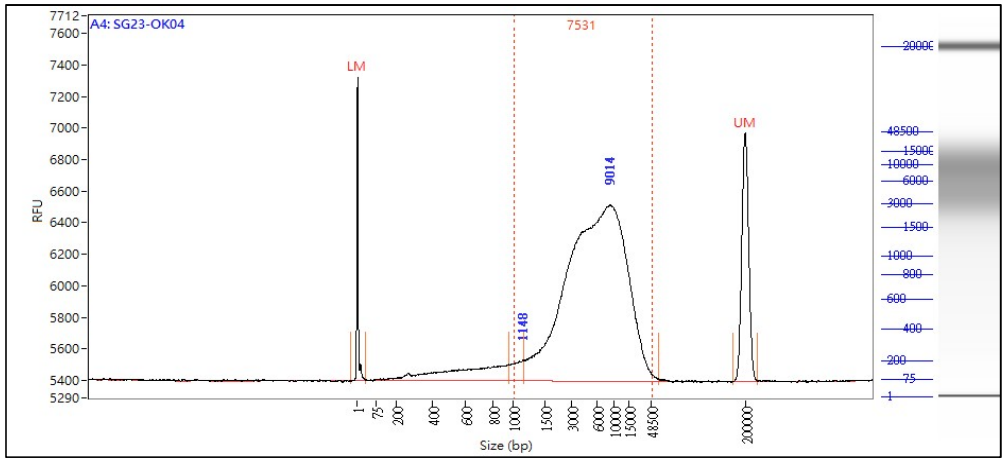


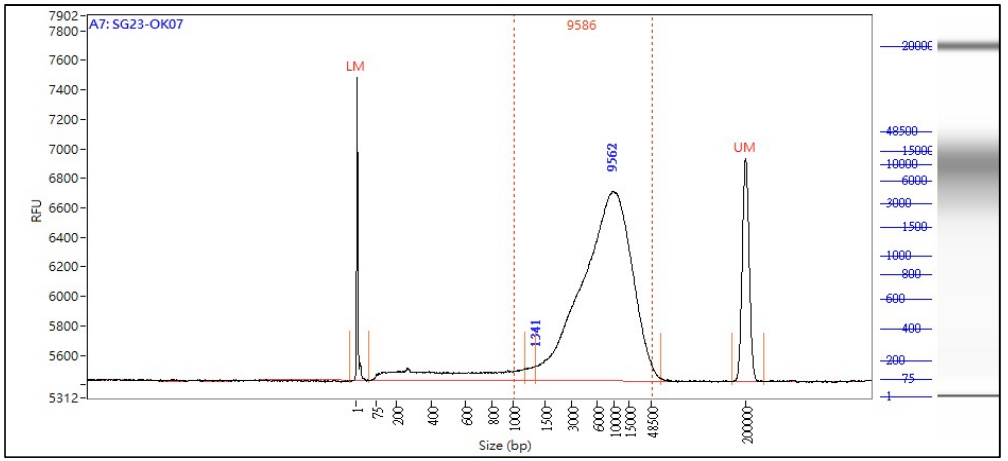
Chr+Plasmids



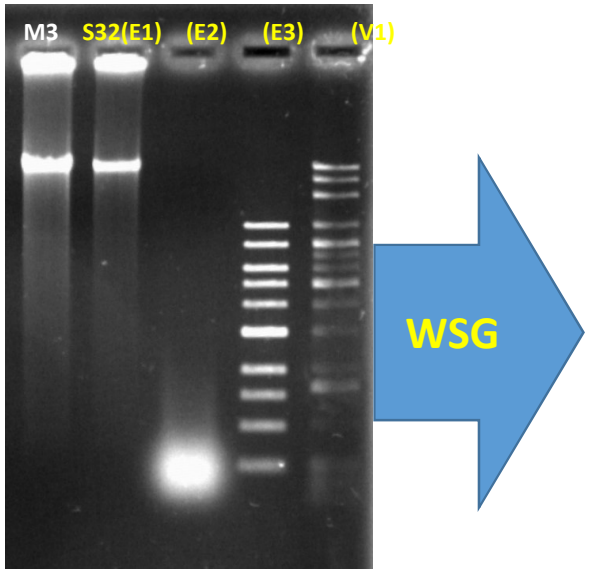
Fecal DNA QC: bead-beating extraction

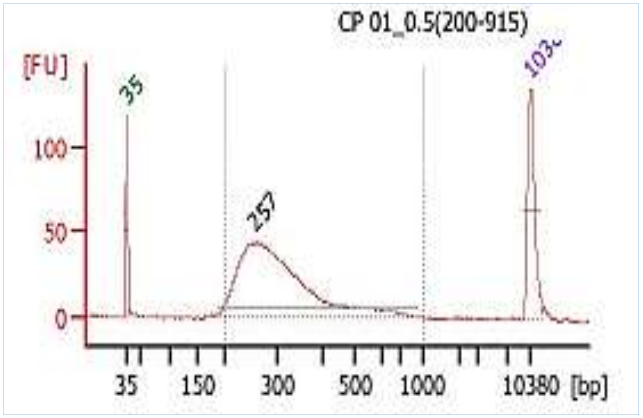
High yield, fragmented with peak @ major 7-10 kb



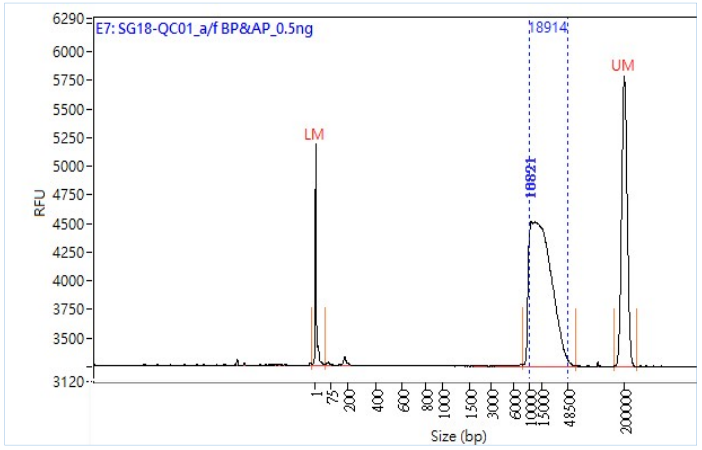


Whole Shotgun Metagenome





Illumina: 200-700bps



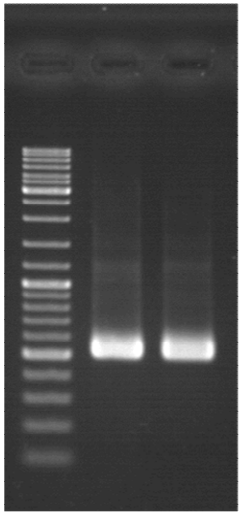
PacBio:
20~>80kb (extra long shotgun)
6~20kb (multiplexed bacteria)

Target Amplicon Metagenome

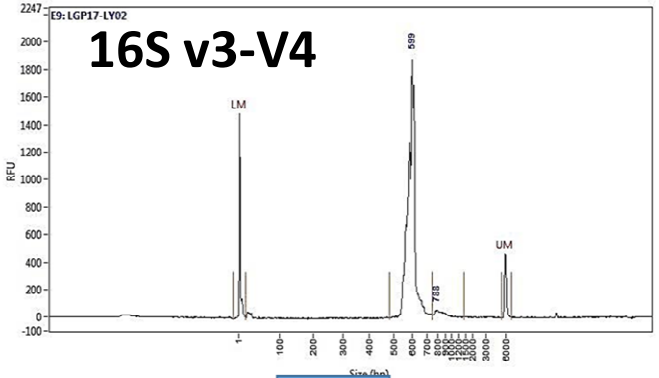
- **Bacteria**
- **Eukaryotic microbes**

Considerations:

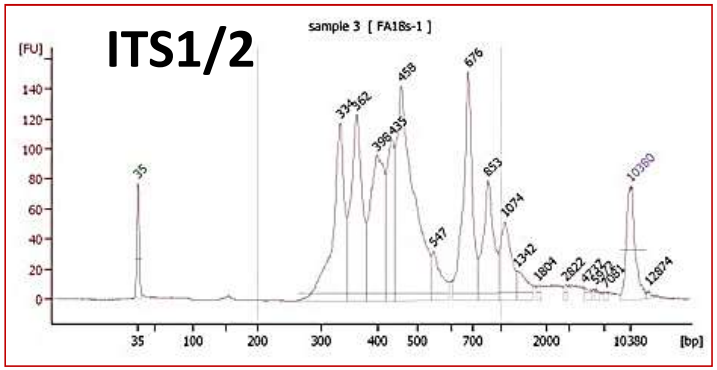
- Amplicon size
- PCR indexing approach
- Amplification bias
- Read length & Accuracy

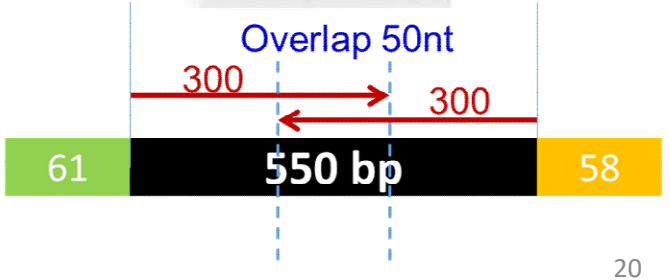


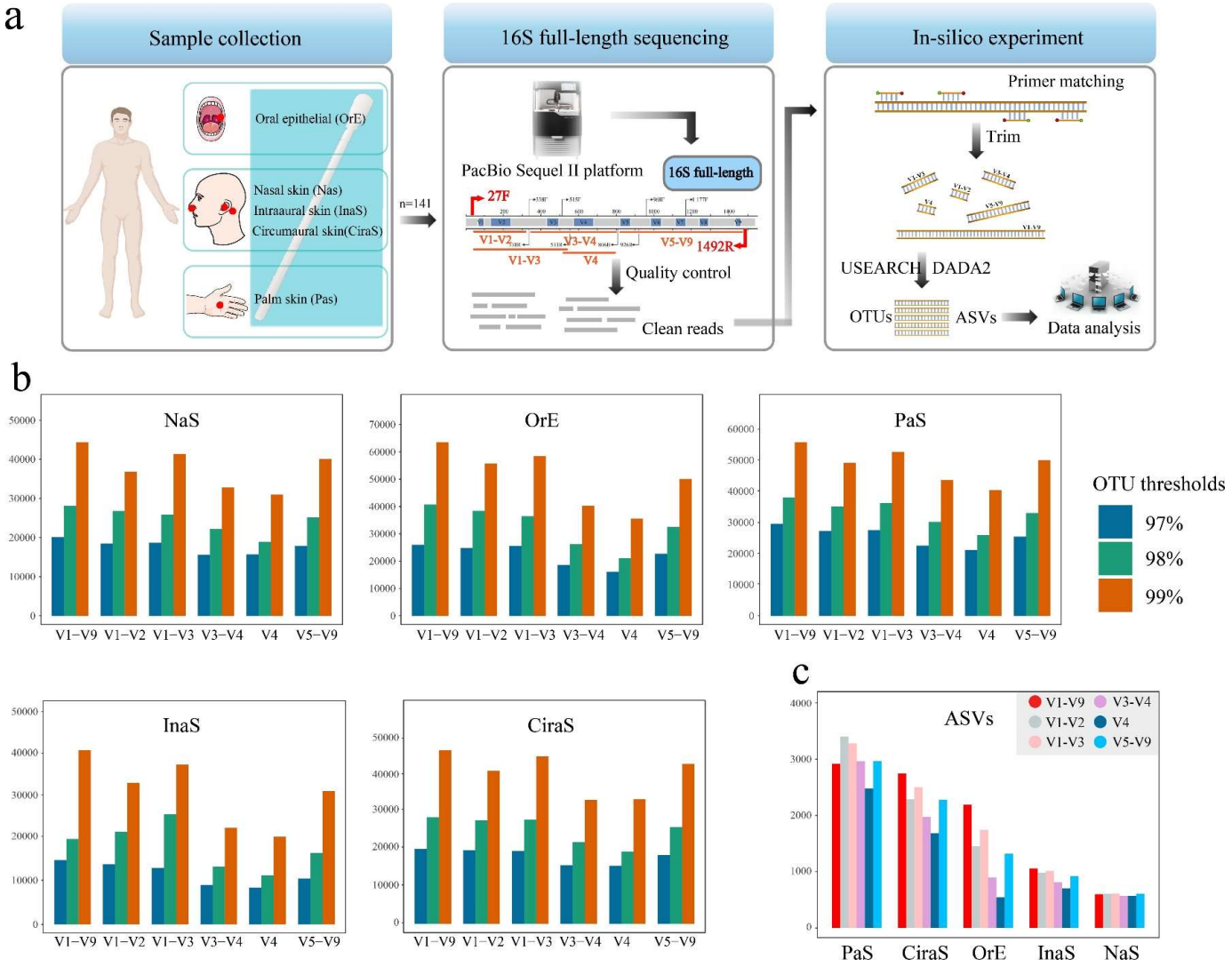
Indexing PCR



SEQ







Comparison of the full-length sequence and sub-regions of 16S rRNA gene for skin microbiome profiling.
https://doi.org/10.1128/msystems.00399-24

PacBio Kinnex concatenation:

- 12-mer arrays of FL-16S units (19kb HiFi reads)
- Sequel IIe: supporting ~50k reads/384-plex

RNA integrity & purity

BioAnalyzer RNA ladder

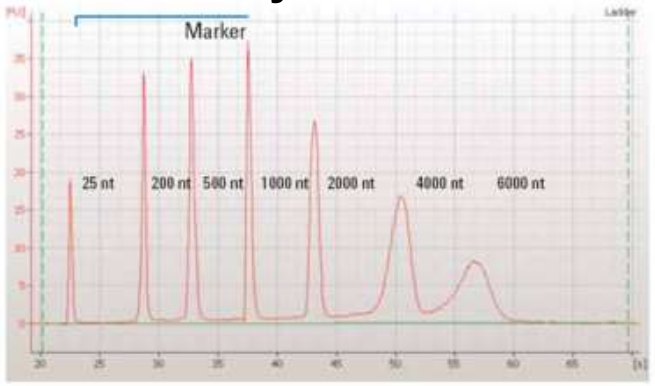
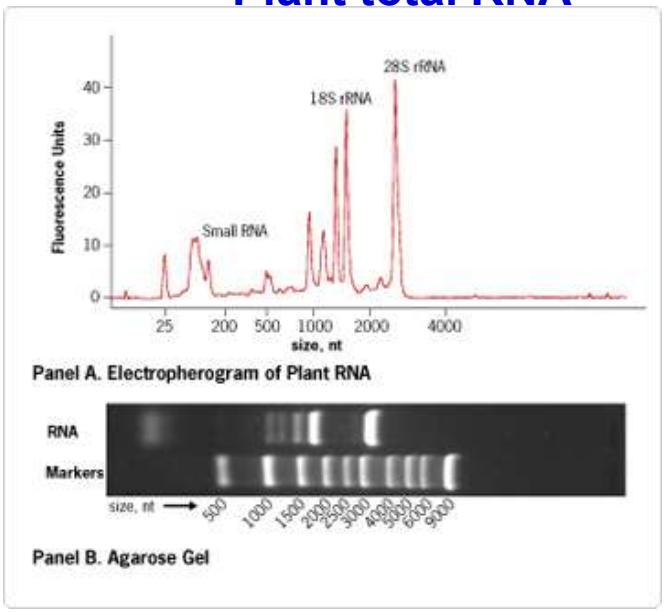
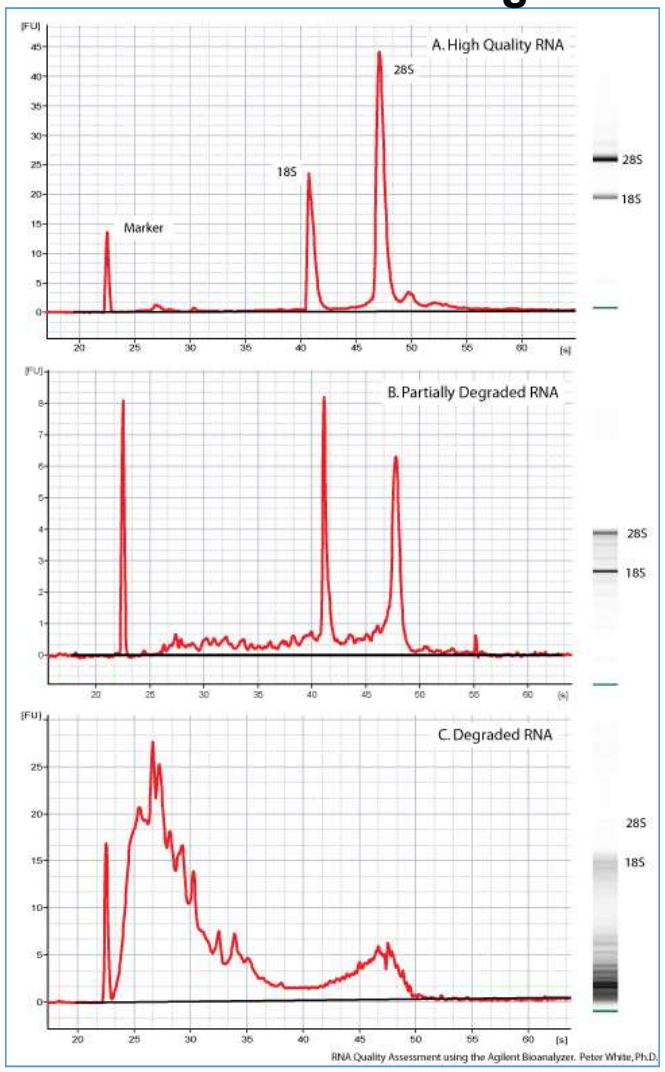


Figure 1 RNA 6000 Nano ladder

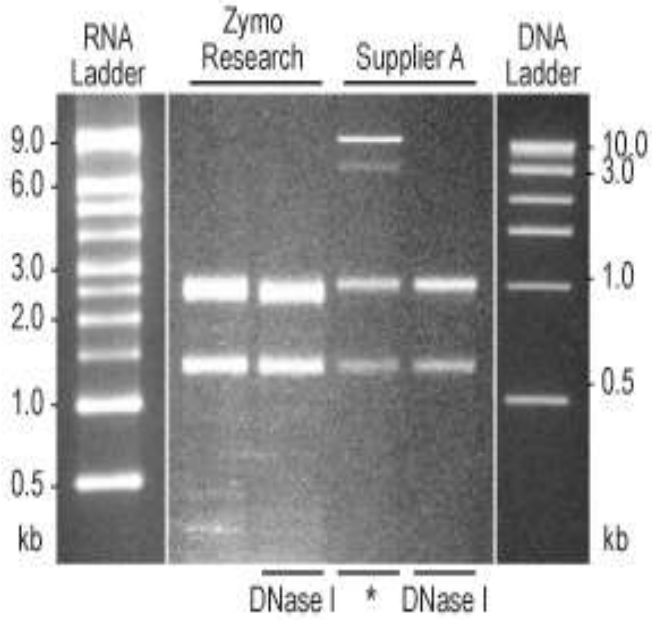
Plant total RNA



Human RNA – various degradation



RNA: +/- DNaseI treatment



Current NGS Platforms & Features

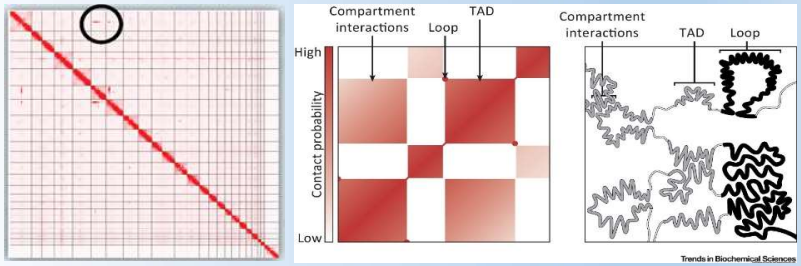


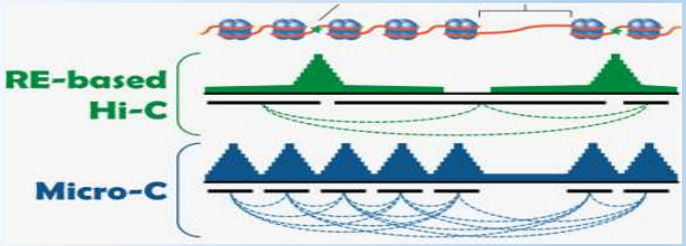


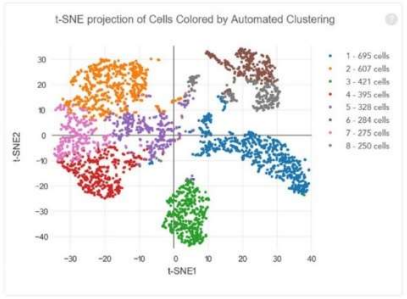
	Illumina NextSeq 2000	Illumina MiSeq	Element BioSci. Aviti24	PacBio Sequel, Sequel II	Oxford ONT GridION, P2 Solo
Chemistry	Cyclic reversible terminator Of amplified DNA clusters		RCA-based polony, ABC Avidite chemistry	SMRT-tech; DNA polymerization	Electrical current passing through a nanopore channel
Chip format			Dual flwocells		
Output/run	P2: 120Gb P3: 330 Gb	up to 15 Gb	P2: 120Gb P3: 330 Gb	Current: 5-30 Gb	Current: 5-30 Gb
Read length	**PE 50-300 nt**	**PE 50-300 nt**	**PE 50-150 nt**	**1-20 kb (max>100kb)**	**1-50 kb (max>200kb)**
# Fragments /lane	400 M (P2) 1100 M (P3)	12-15 M (v2) 20-25M (v3)	1000 M (Std) 2000 M (HT)	350-700 K / SMRT cell	30-300 K / chip
Data quality	Q30 bases >75% Tolerate homopolymer; sensitive to high GC		PE150: Q30 bases >90%	Raw 85-89%; HiFi ~99.9%; Random homopolymeric errors; tolerate high GC%	Raw 80~94%; Systematic homopolymeric errors; tolerate high GC%
Application	De novo assembly; Re-sequencing; Single-cell sequencing	De novo assembly; Re-sequencing; amplicon	WGS and RNA-seq; Single-cell sequencing, Teton spatial seq.	Genome assembly; structural variation; phasing; Iso-Seq	Genome assembly; structural variation; phasing; RNA/DNA-seq

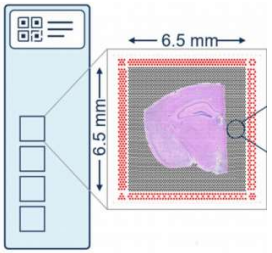
Current Platforms

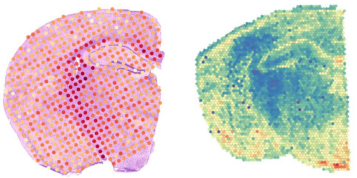
3D-Genomes











10x Genomics: Single-Cell & Spatial seq

Countess

Chromium X

CytAssist

EVOS

MiSeq NextSeq2000

ElemBio Aviti24

PacBio Sequel & SQIIe

Nanopore GridION Promethion P2 Solo

Advanced HTS Applications

1. Genome approaches:

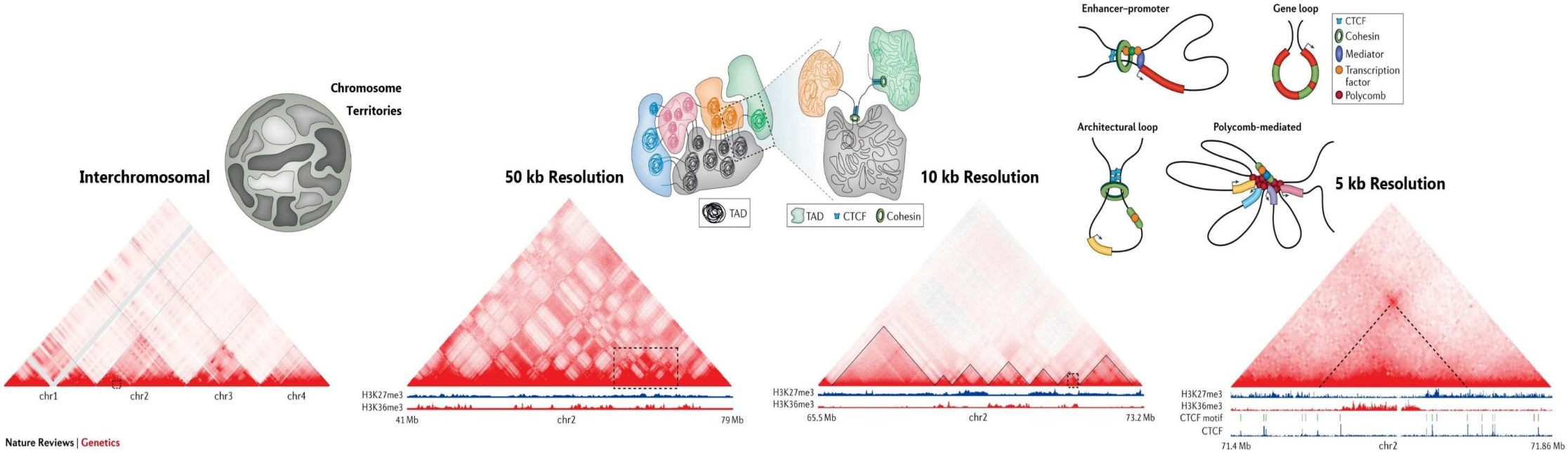
- hybrid NGS (Illumina + 3rd-Gen)
- 3D genomes (chromatin interaction)

2. Multidimensional studies:

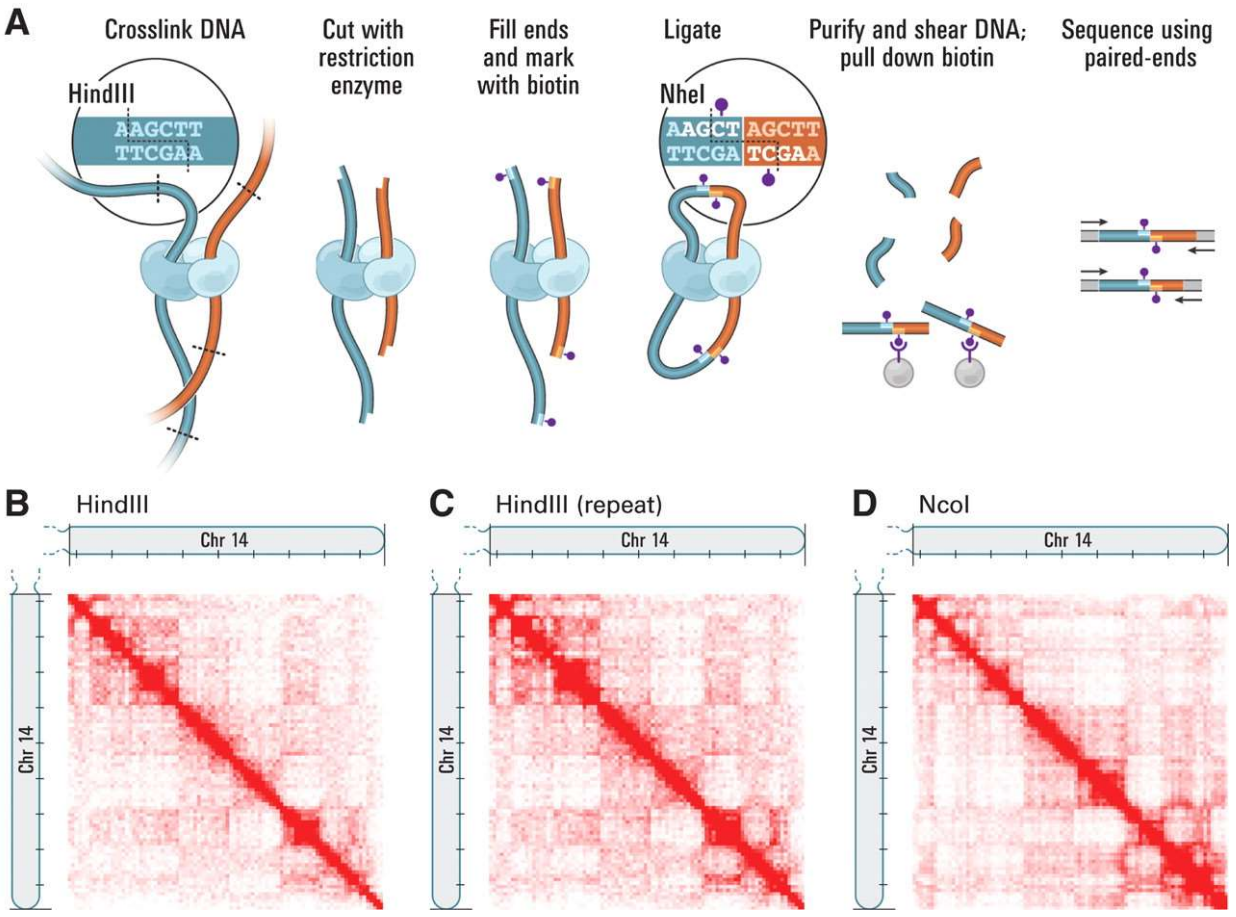
- Single-cell analyses: cell lineage, immuno repertoire
- Spatial transcrtipome

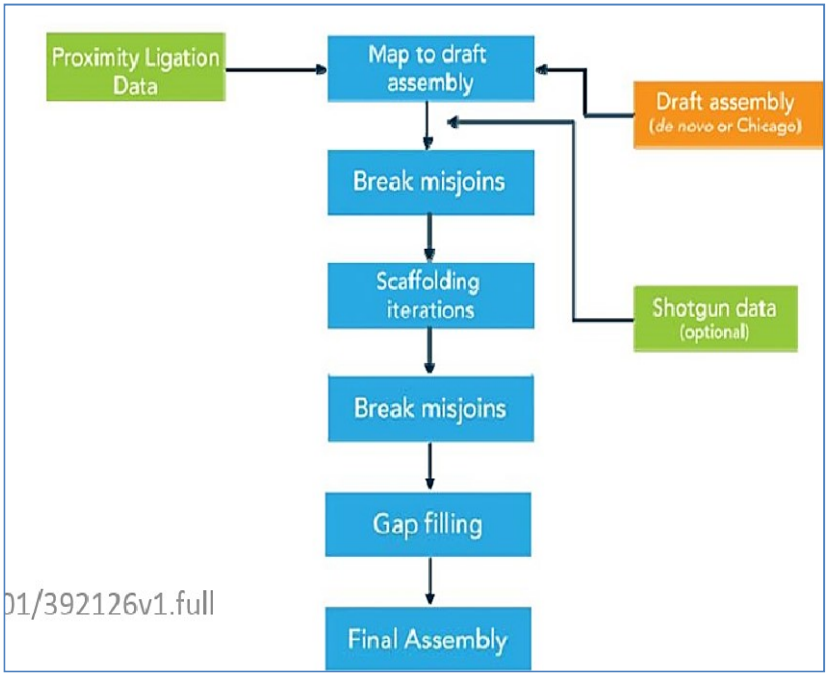
Hierarchical Organization of Chromatin Structure

https://doi.org/10.1038/nrg.2016.112



Hi-C/Omni-C: Genome scaffolding w/ chromatin proximity ligation





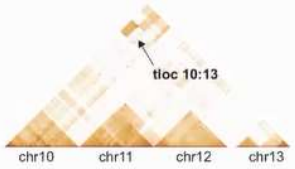
Have applied on plants, insects, and animals.

Science. 2009 Oct 9; 326(5950): 289–293.

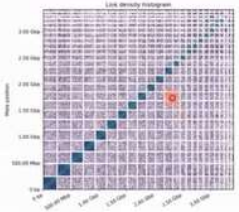
Rich Data Type Enables Multiple Applications

Sample → Proximity Ligation → Sequence Data

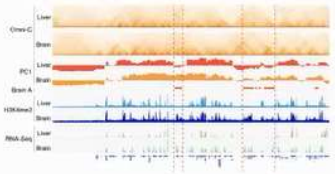
- SV Detection
- SNP & InDel Genotyping
- Genome Assembly
- Haplotype Phasing
- Chromatin Conformation



Library Type	True Positive	False Positive	False Negative	Sensitivity	Precision
Shotgun	2,696,291	9,270	6,814	99.7%	99.7%
Omni-C	2,666,339	20,081	36,766	98.6%	99.3%
RE Based Hi-C	2,387,235	33,554	315,870	88.3%	98.6%

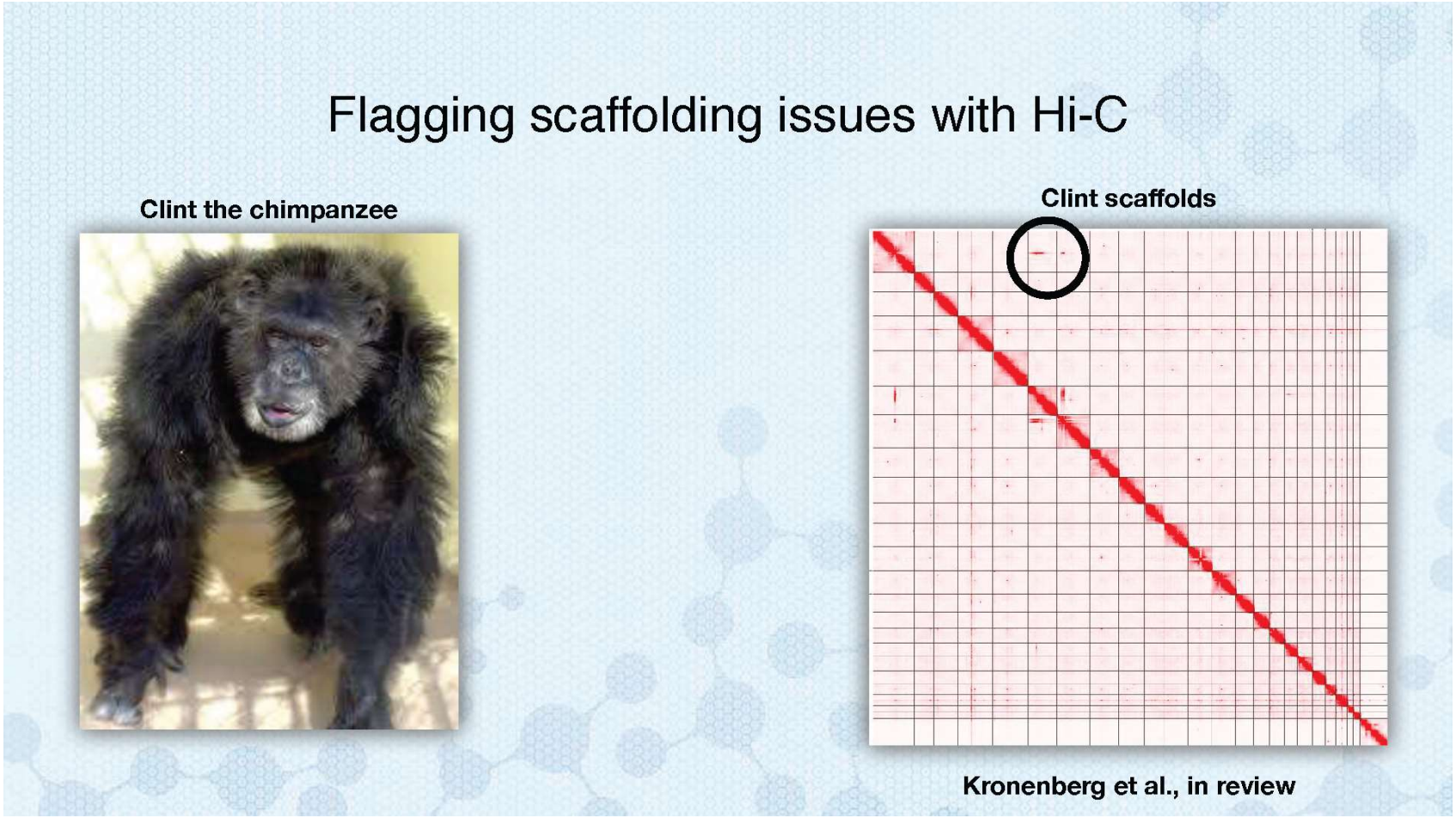


Library Type	#Variants Phased	% Heterozygous SNPs phased	Switch Error Rate	#Chromosomes phased end to end
Shotgun	2,229,492	81.44%	0.0035	0
Omni-C	2,299,248	84%	0.0100	23
RE Based Hi-C	1,986,467	72.6%	0.0357	23



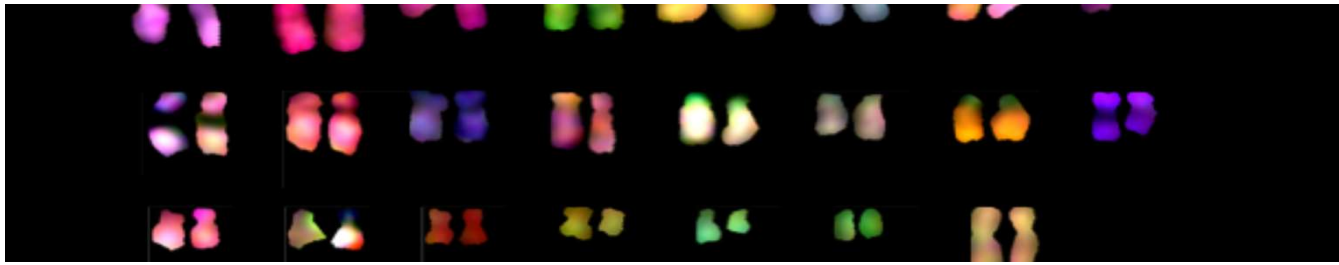
https://dovetailgenomics.com/

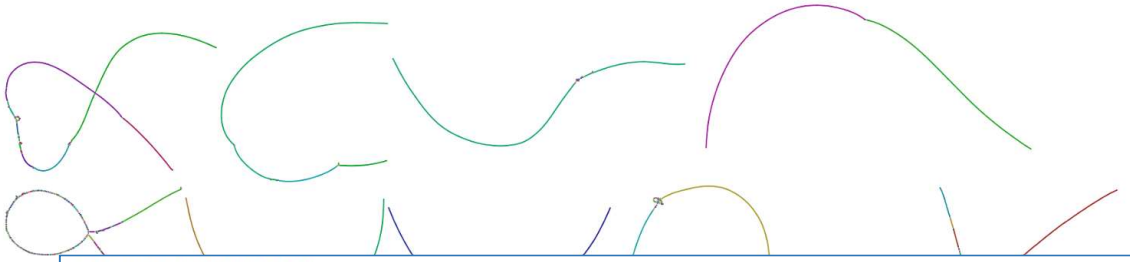
Hi-C: long range SV; detect assembly error



https://youtu.be/uzINKcj-p78

Beyond GRCh38:
T2T genome assembly v1.0





- T2T v1.0: using long-read PacBio and Oxford Nanopore techs
- contain >100Mb novel seq compare to GRCh38
- *Complete* human chr

- Chr X & Chr8: ONT UL backbone; PacBio + ILMN polishing
- Stepwise:
  1. PacBio HiFi reads: construct highly accurate assembly grap
  2. resolve structural ambiguity using Nanopore UL reads
  3. Define complete chr by taking consensus of HiFi reads fron
  4. Nanopore patches several GA-rich repeat gaps in PacBio as
  5. Correct SV and SNP errors with all reads (DeepVariant and
  6. Final polished genome accuracy: error <E10-6
  7. 23 Chr (no Y), 1 Mito.: 3,045,441,522 bp
  8. (only the 5 rDNA arrays remain unfinished: near-identical t

T2T-CHM13v2.0

Description: T2T CHM13v2.0 Telomere-to-Telomere assembly of the CHM13 cell line, with chrY from NA24385
Organism name: Homo sapiens (human)
BioSample: SAMN03255769
BioProject: PRJNA559484
Submitter: T2T Consortium
Date: 2022/01/24
Synonyms: hs1
Assembly level: Complete Genome
Genome representation: full
GenBank assembly accession: GCA_009914755.4 (latest)
RefSeq assembly accession: GCF_009914755.1 (latest)
RefSeq assembly and GenBank assembly identical: no (hide details)
- Only in GenBank: chromosome MT (in non-nuclear assembly-unit)
- Data displayed for RefSeq version

Expected final version: no
Genome coverage: 30x

IDs: 11828891 [UID] 31127148 [GenBank] 31865168 [RefSeq]

24 chr: 22 autosome+XY

https://genomeinformatics.github.io/CHM13v1/

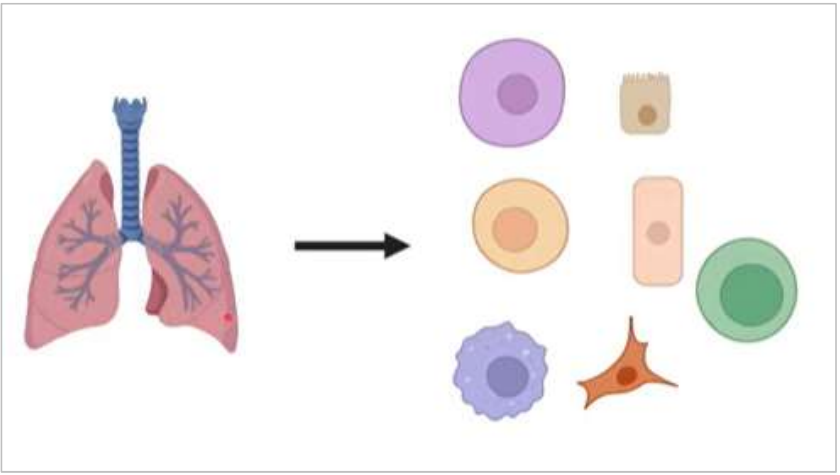
Advanced NGS Applications

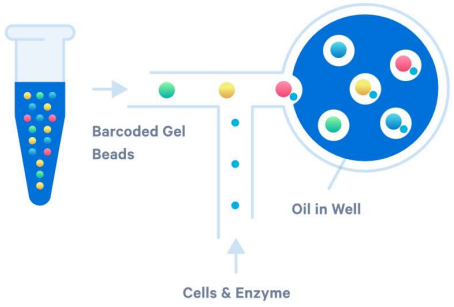
1. Genome approaches:

- hybrid NGS (Illumina + 3rd-Gen)
- Hi-C scaffolding (high-order chromatin)
- BioNano (optical mapping)

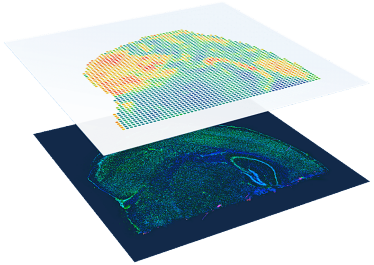
2. Multidimensional studies:

- Single-cell analyses: cell lineage, immuno repertoire
- Spatial transcriptome





10x GENOMICS Certified Service Provider

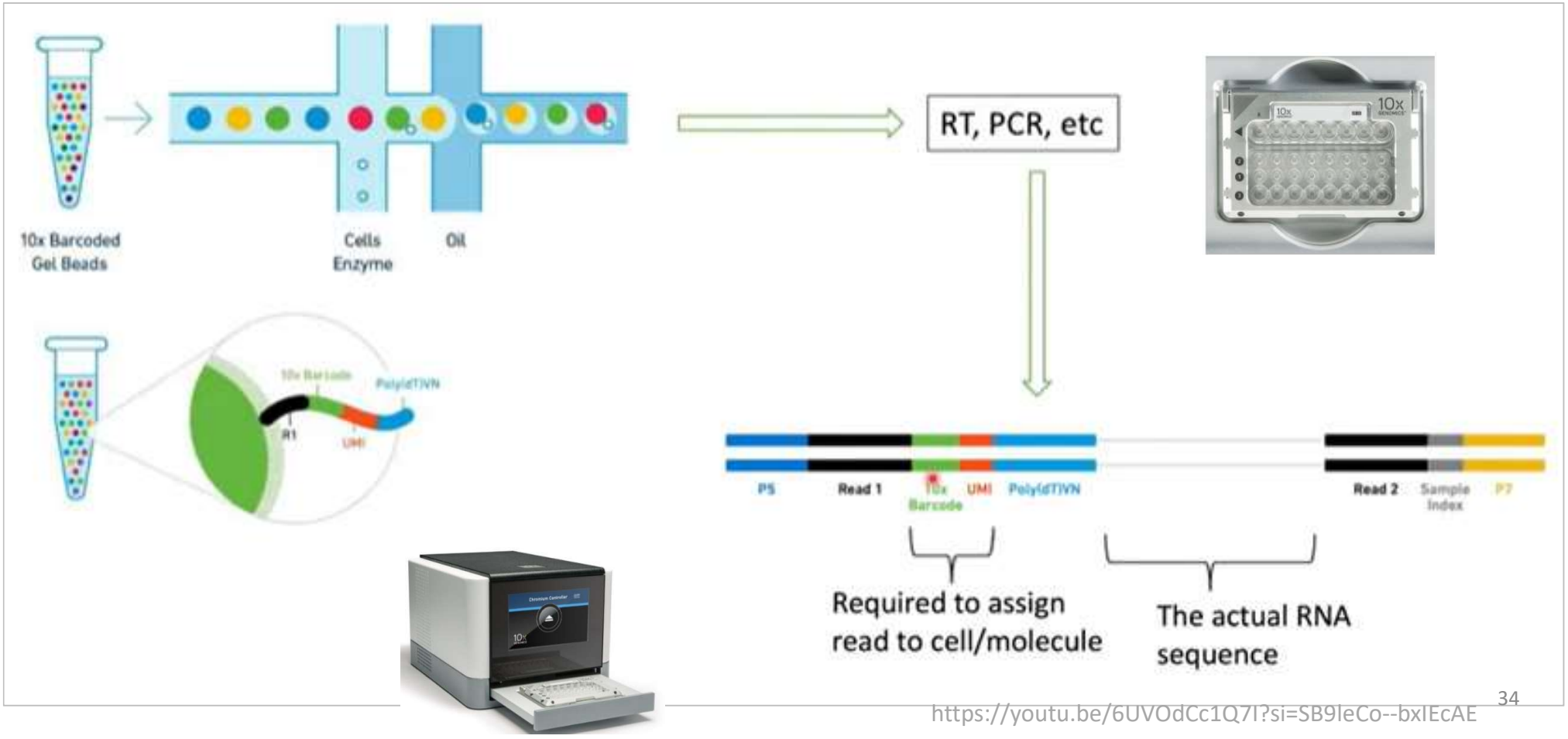


	10x Single Cell
(X-CSC)	10x Chromium 3′ Single Cell RNA Prep
	Single Cell Chip G
(X-VDJ)	10x Chromium VDJ 5′ Single Cell RNA Prep
	Single Cell Chip K
(X-ATAC)	10x ATAC-seq prep (Chromatin accessibility)

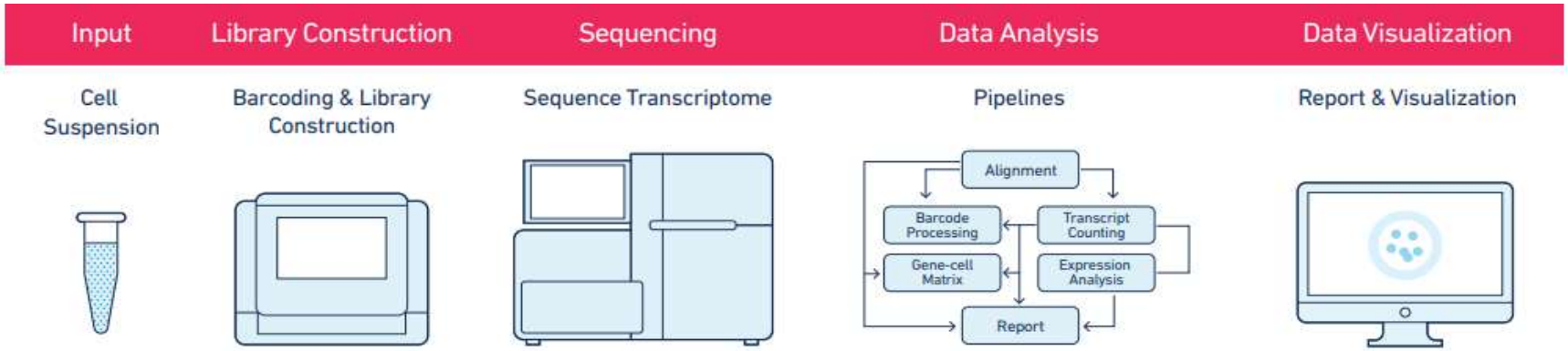
	10x Visium
(X-VTO)	10x Visium Tissue Optimization (per slide)
(X-VGE)	10x Visium Gene Expression (per sample)
(XFRZN)	Fresh Frozen Tissue Preparation
(XSLD1)	Cryosectioning for Fresh Frozen Tissue
(XRNA1)	RNA Extraction
(X-VFP)	10x Visium Gene Expression for FFPE (per sample)
(XSLD2)	Sectioning for FFPE Tissue
(XRNA2)	FFPE RNA Extraction

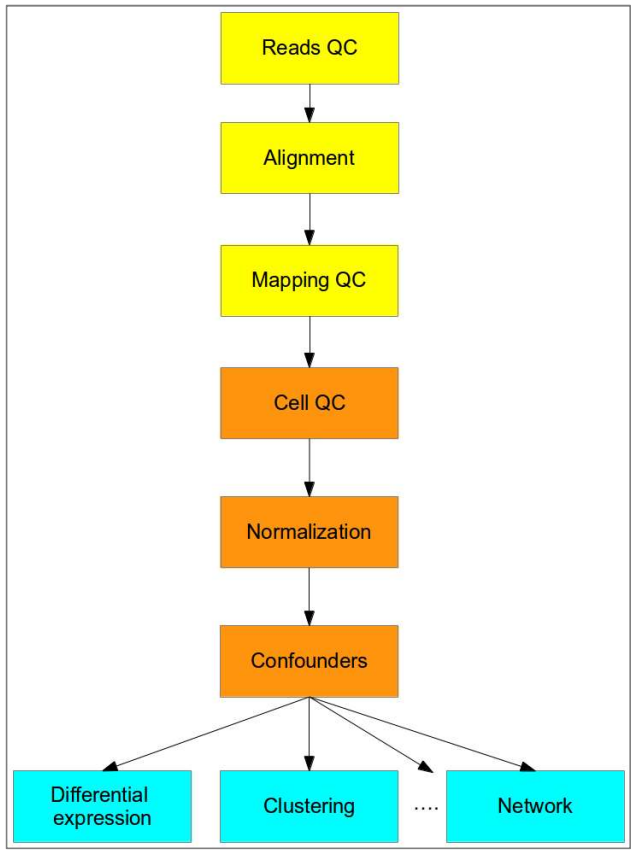
10x Genomics Chromium : barcoded gel beads for single cells

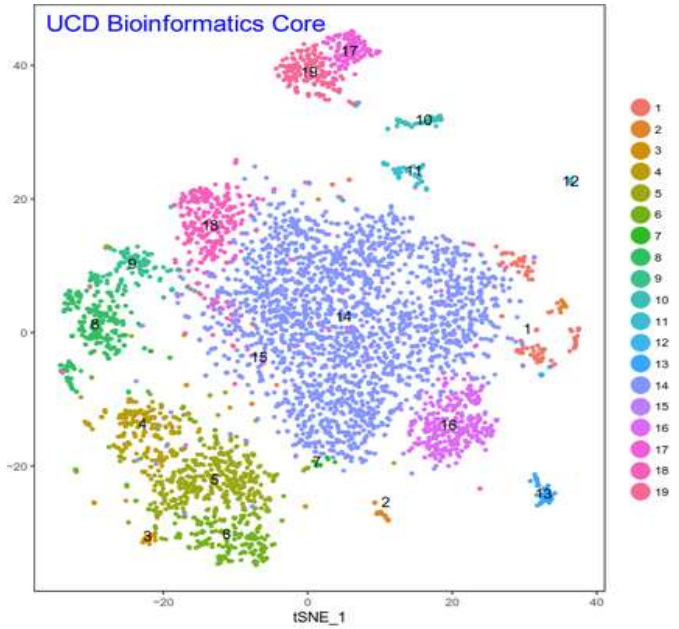
Single-cell embedding

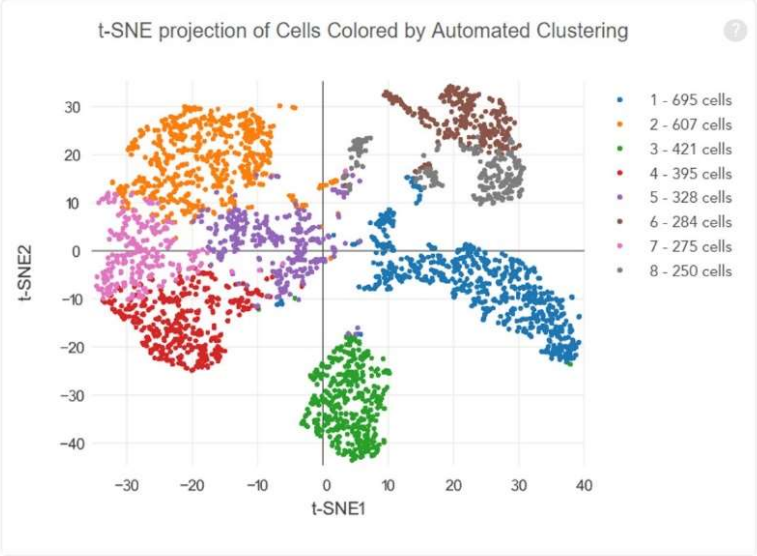


https://youtu.be/6UVOdCc1Q7I?si=SB9leCo--bxIEcAE





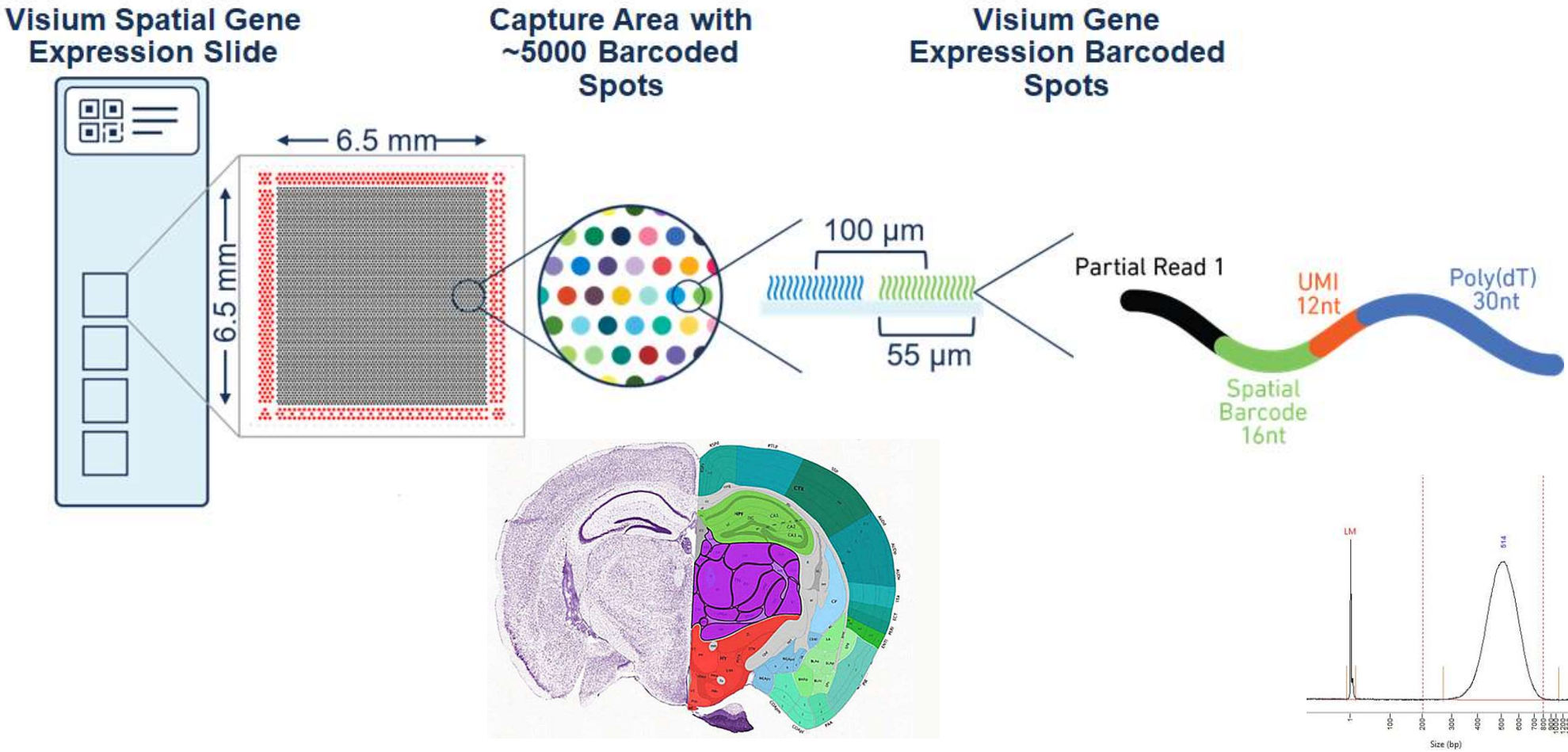




https://dnatech.genomecenter.ucdavis.edu/single-cell-analyses/

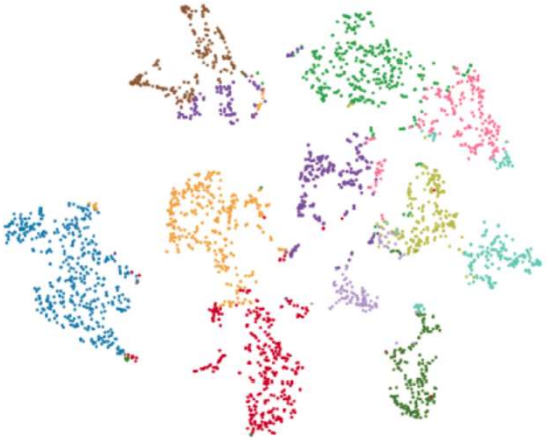
10x Genomics:
Spatial Transcriptome

10x GENOMICS | Certified Service Provider

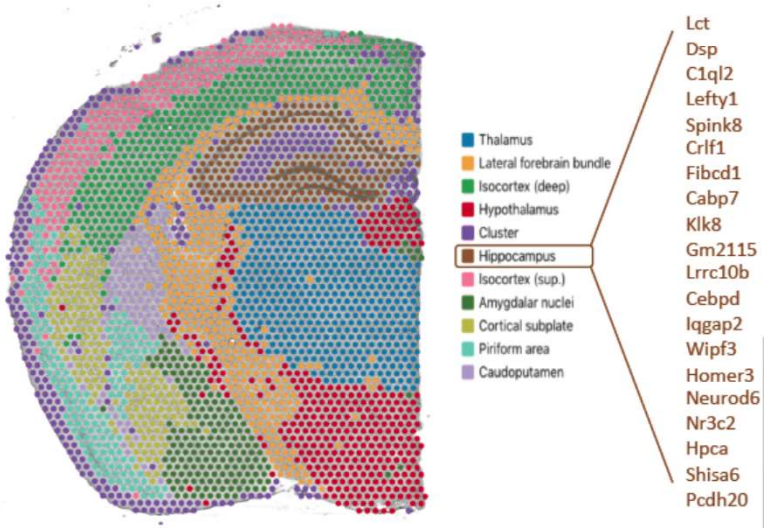


Cluster or Image Driven Analysis of Spatial Data

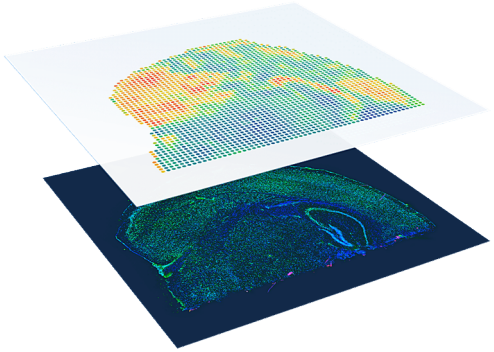
Start With the Gene Expression Data or microscopy images of the same section

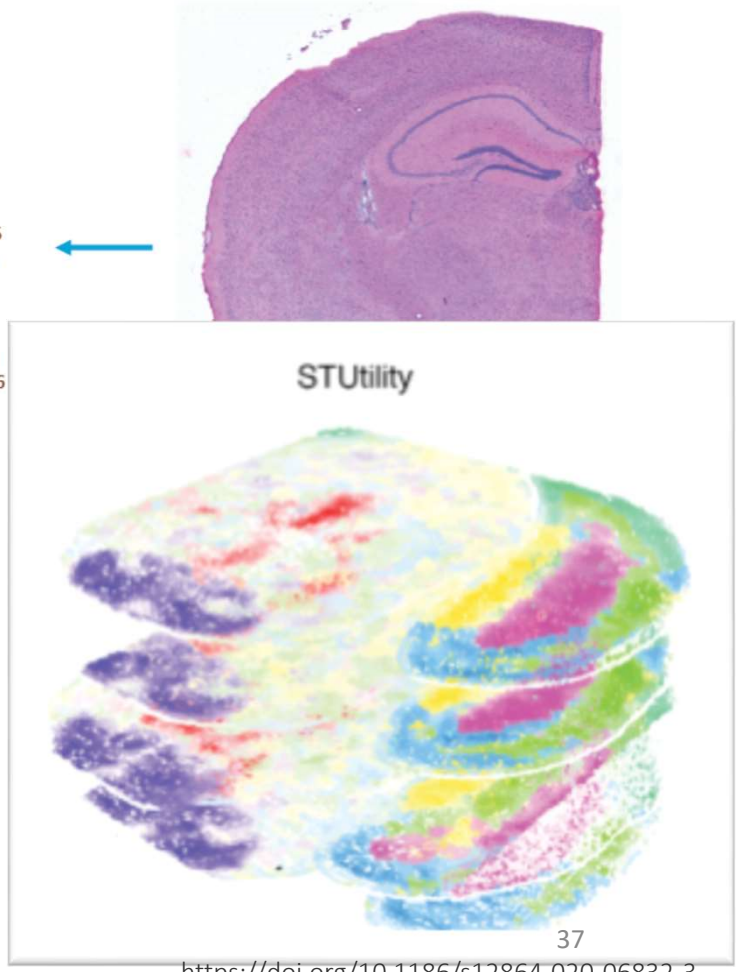


Clustering/classification of glass slide spots based on their gene expression profile



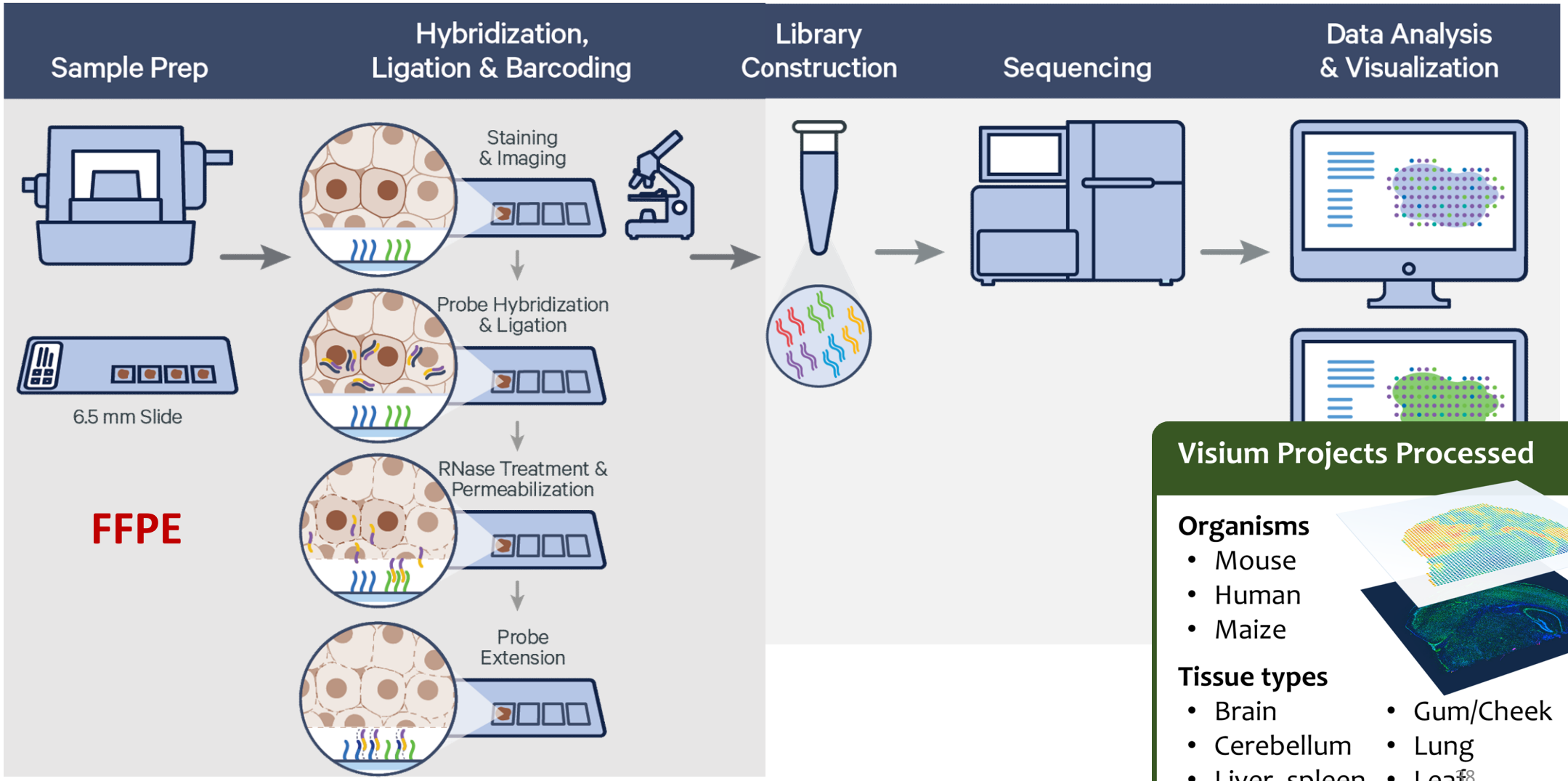
Annotation directly on H&E image





https://doi.org/10.1186/s12864-020-06832-3

Exploring Spatial Transcriptomics with 10x Genomics Visium



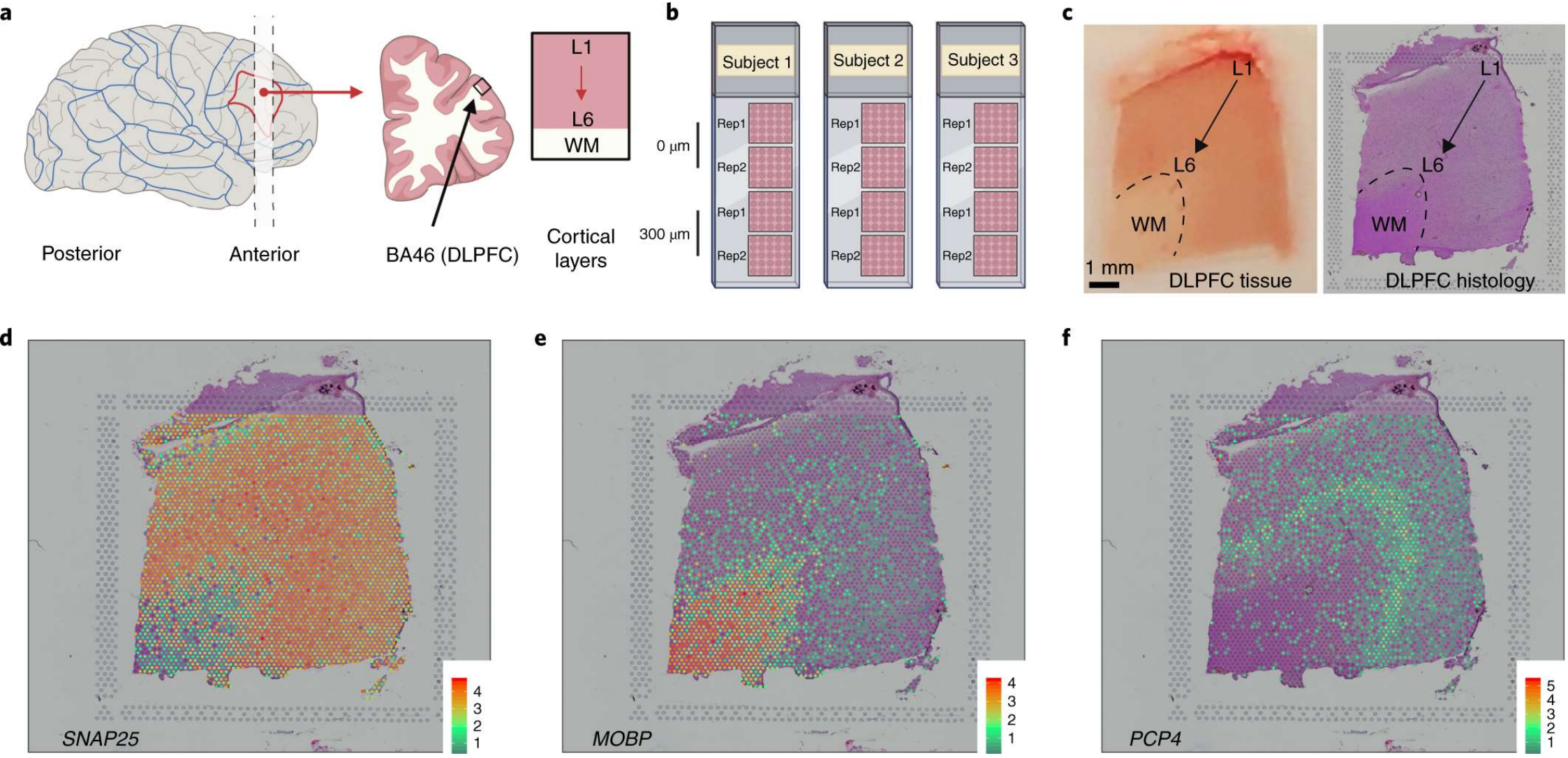
Visium Projects Processed

Organisms

- Mouse
- Human
- Maize

Tissue types

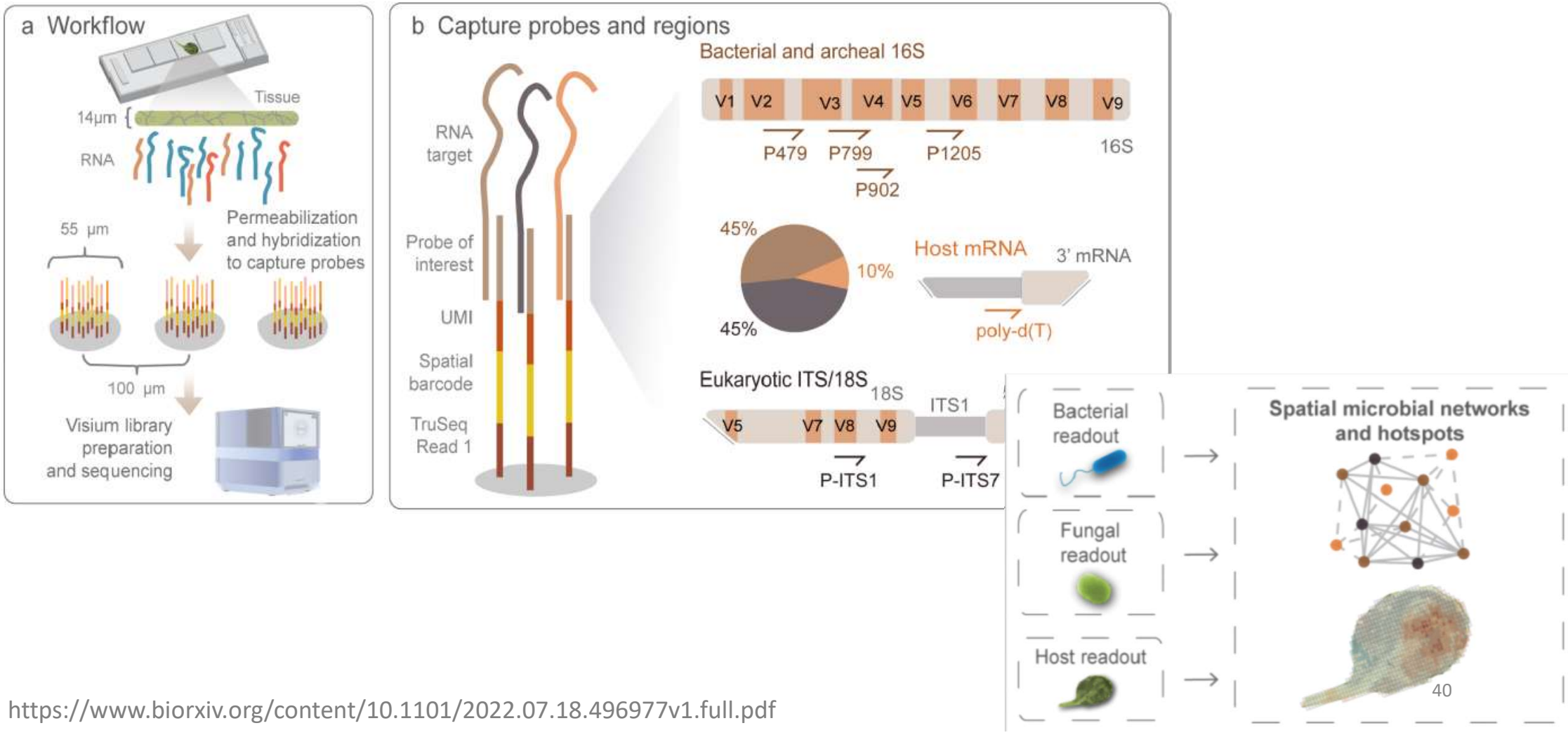
- Brain
- Cerebellum
- Liver, spleen
- Gum/Cheek
- Lung
- Leaf



Maynard, K.R., Collado-Torres, L., Weber, L.M. et al. Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex. Nat Neurosci 24, 425–436 (2021). https://doi.org/10.1038/s41593-020-00787-0

Spatial metaTranscriptomics (SmT):

a sequencing-based approach that leverages 16S/18S/ITS/poly-d(T) multimodal arrays for simultaneous host transcriptome- and microbiome-wide characterization of tissues at 55-µm resolution.



https://www.biorxiv.org/content/10.1101/2022.07.18.496977v1.full.pdf

Advanced HTS Applications

Case Studies

PacBio + Hi-C

* Advanced Sequencing Genome Technologies

➢PacBio Sequencing:

- Utilizes single-molecule real-time (SMRT) sequencing to produce long reads, enhancing the resolution of repetitive regions and structural variants.

➢Hi-C Technology:

- Captures three-dimensional chromatin interactions to assist in scaffolding contigs into chromosome-level assemblies.

*Applications in Marine Genomics

➢Chromosome-Level Genome Assemblies:

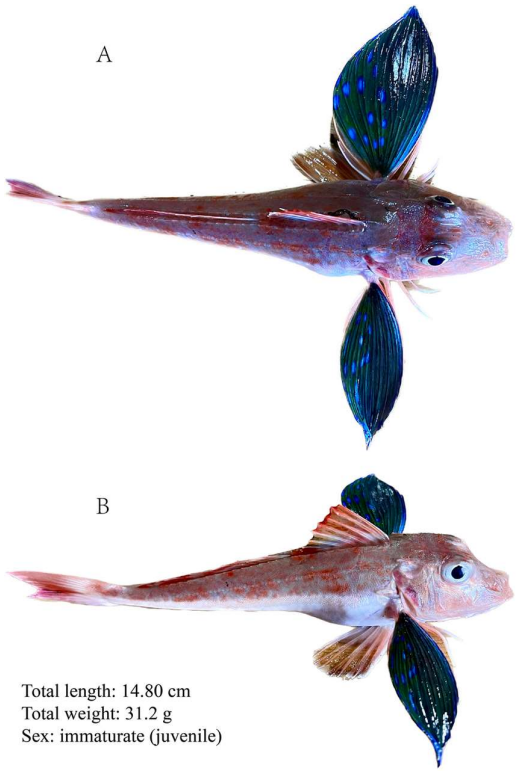
- Integration of PacBio and Hi-C has enabled the assembly of high-quality genomes in various marine species, providing insights into their genetic makeup.

➢Comparative Genomics:

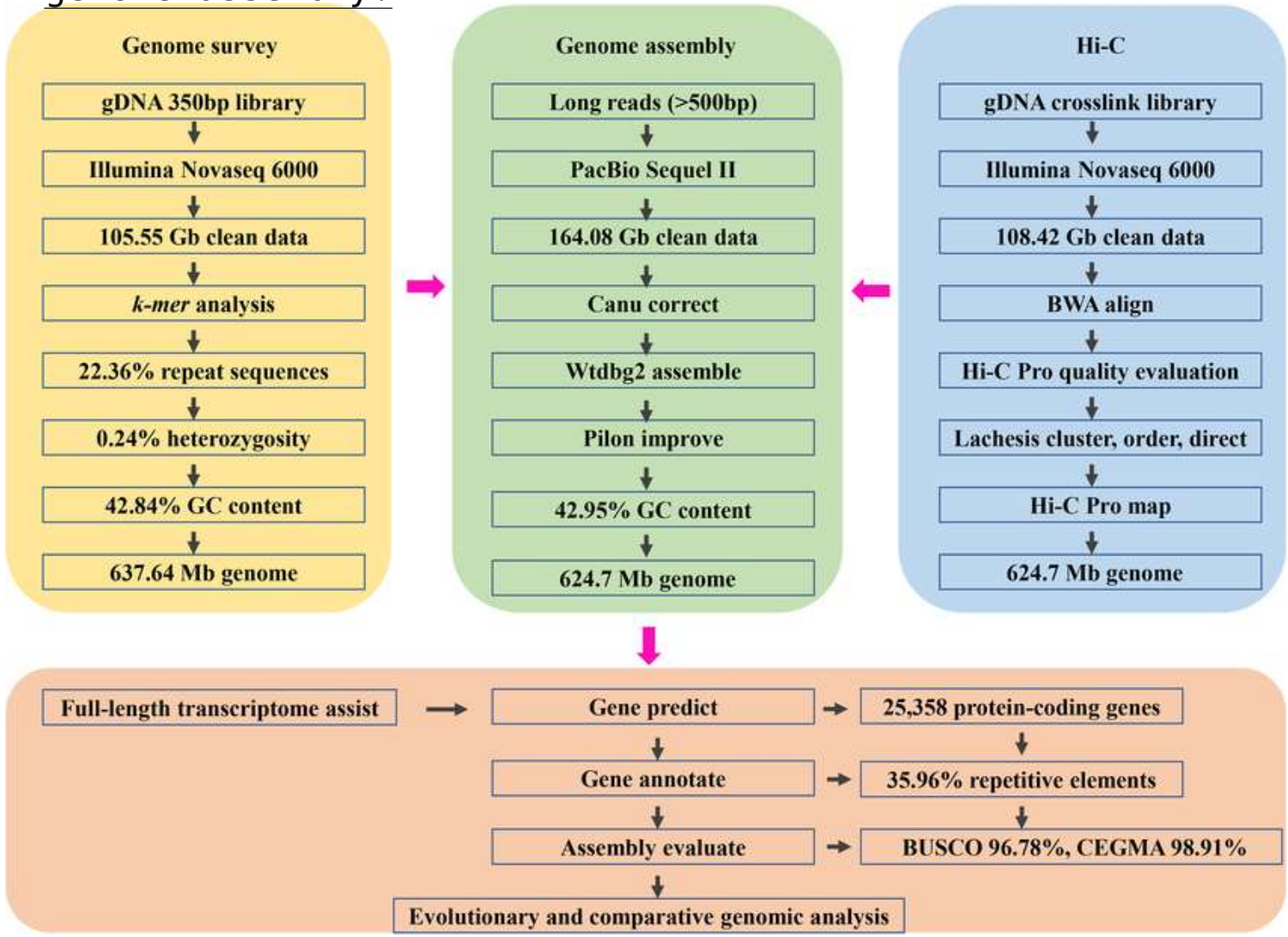
- Facilitates the comparison of genomic structures across species, aiding in the study of evolutionary relationships and adaptation mechanisms.

➢Functional Genomics:

- Assists in the accurate annotation of genes, including those involved in unique marine adaptations and metabolic pathways.



The pipelines overview of C. spinosus chromosome-level genome assembly.

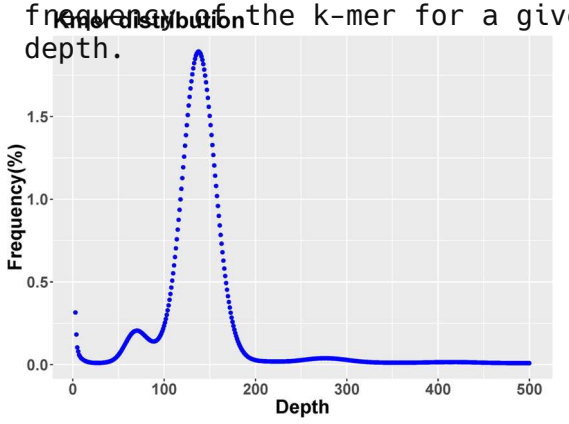


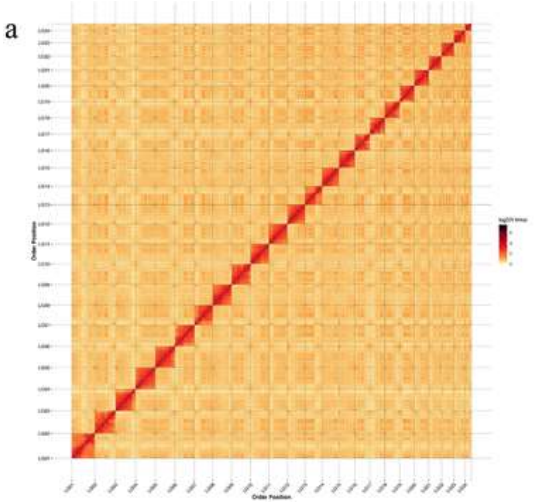
Chromosome-level genome assembly of largemouth bass (Micropterus salmoides) using PacBio and Hi-C technologies

August 2022 · Scientific Data · 9(1)
DOI: 10.1038/s41597-022-01601-1

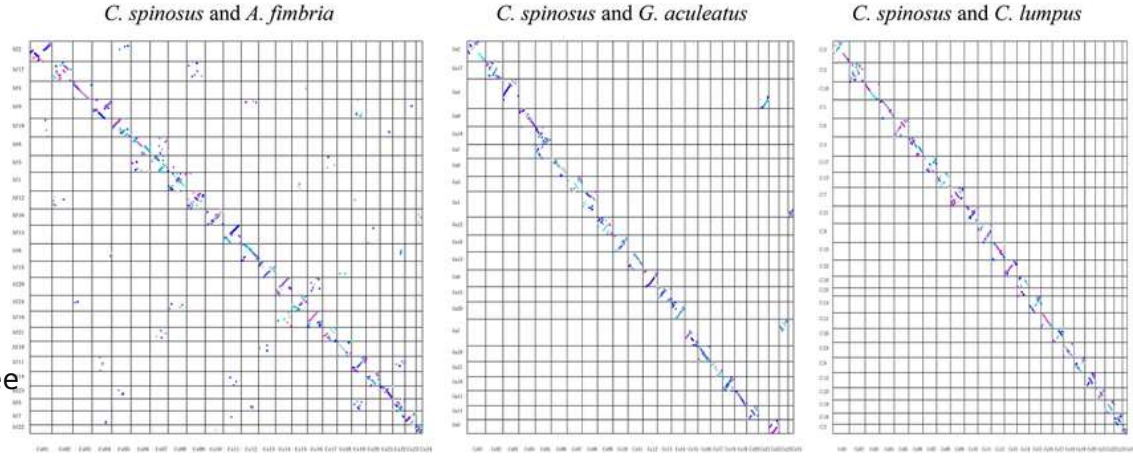
大口黑鱸

21-mer frequency distribution in C. spinosus genome. The X-axis is the k-mer depth, and Y-axis represents the frequency of the k-mer for a given depth.





Characteristics of the C. spinosus genome. (a) Hi-C intra-chromosomal contact map of the C. spinosus genome assembly. (b) Circos plot of the C. spinosus genome assembly.



A dot plot depicting the collinearity relationships among C. spinosus and its three closest species (Anoplopoma fimbria, Gasterosteus aculeatus, Cyclopterus lumpus).

Chromosome-Level Genome Assembly and Comparative Genomic Analysis of the Barbel Chub (*Squaliobarbus curriculus*) by Integration of PacBio Sequencing and Hi-C Technology

鰱魚

single live adult female fish

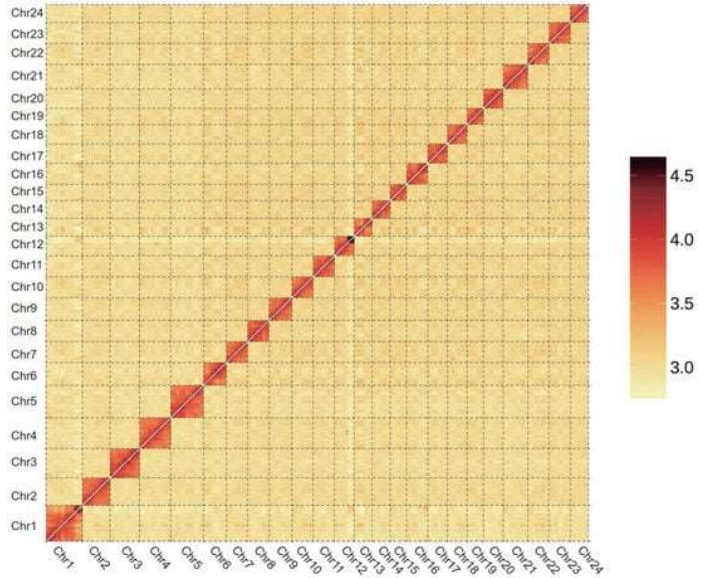


Table 1. Statistics for the sequencing data of *S. curriculus* genome.

Pair-End Libraries	Library Size (bp)	Sequencing Platform	Total Data (Gb)	Sequence Coverage (×)
Illumina reads	350	Illumina NovaSeq−6000	43.88	47.40
PacBio reads	20,000	PacBio Sequel II	155.34	167.82
Hi-C reads	350	Illumina NovaSeq−6000	145.69	157.39
Transcriptome	350	Illumina NovaSeq−6000	39.78	42.97
Total			384.69	415.58

Note: Sequence coverage was calculated using an estimated genome size of 925.66 Mb.

Table 2. BUSCO analysis result of the *S. curriculus* genome.

Statistics	Number of Genes	Percentage (%)
Complete BUSCOs	2511	97.10%
Complete and single-copy BUSCOs	2441	94.40%
Complete Duplicated BUSCOs	70	2.70%
Fragmented BUSCOs	44	1.70%
Missing BUSCOs	31	1.20%
Total BUSCO groups searched	2586	100%

Chromosome-Level Genome Assembly and Comparative Genomic Analysis of the Barbel Chub (*Squaliobarbus curriculus*) by Integration of PacBio Sequencing and Hi-C Technology

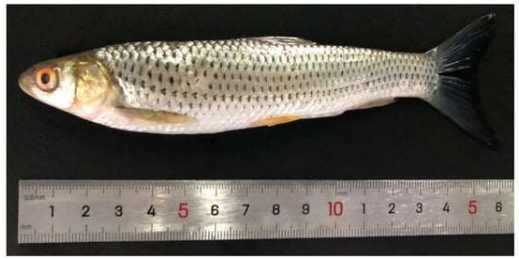
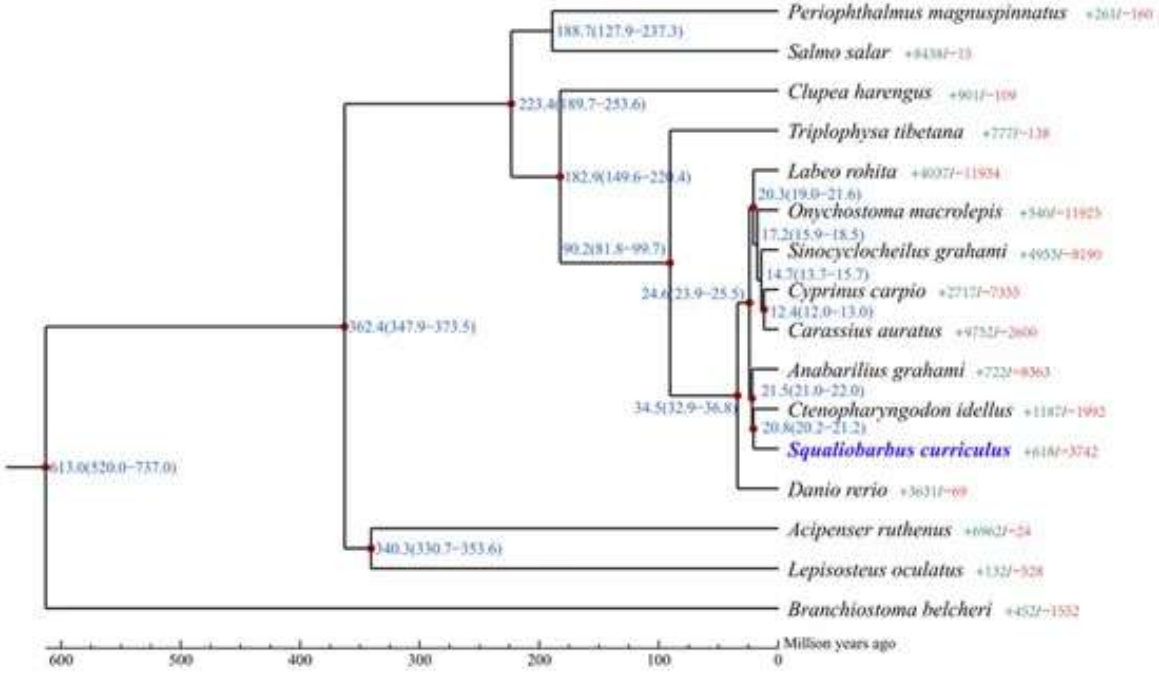


Table 4. Summary of functional annotations for predicted genes of *S. curriculus* genome.

Annotation Database	Number of Annotated Genes	Percentage (%)
Swissprot	21,440	83.20
Nr	24,328	94.40
KEGG	21,328	82.70
InterPro	22,481	87.20
GO	16,145	62.60
Pfam	20,160	78.20
Annotated	24,402	94.70
Unannotated	1377	5.30
Total	25,779	-

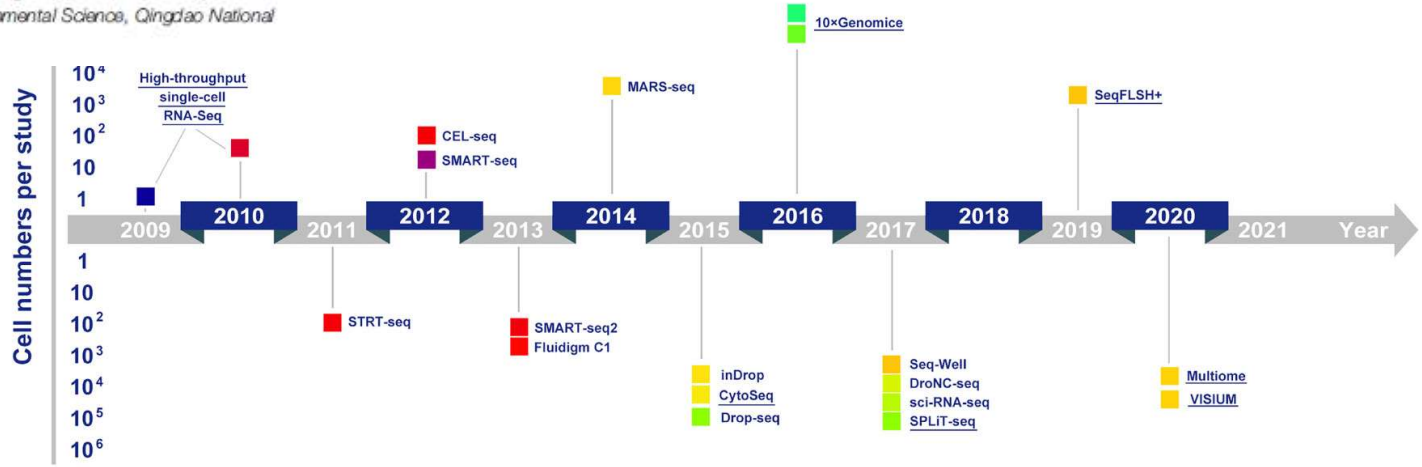


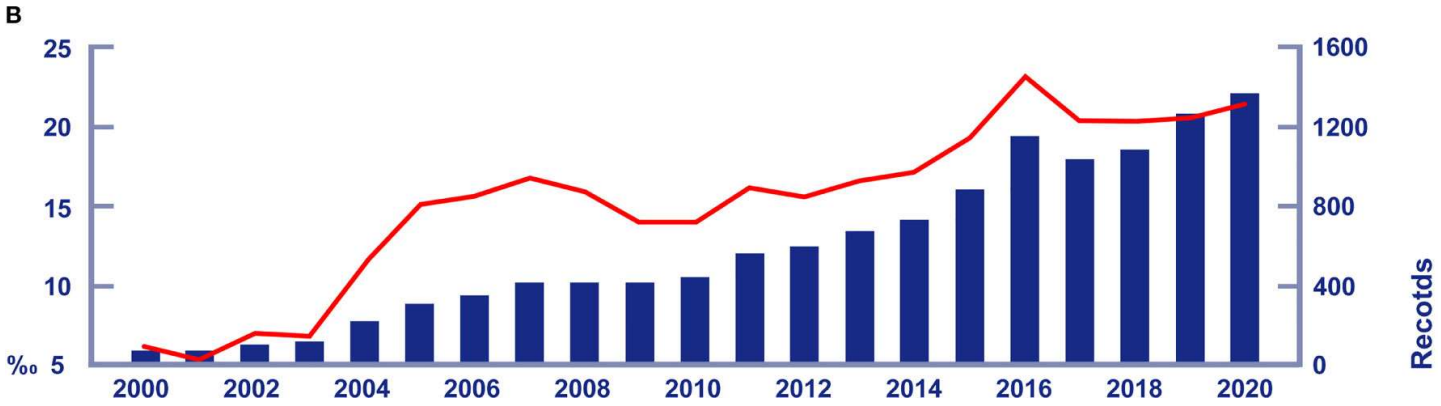
scRNA for marine species

Single-Cell Sequencing on Marine Life: Application and Future Development

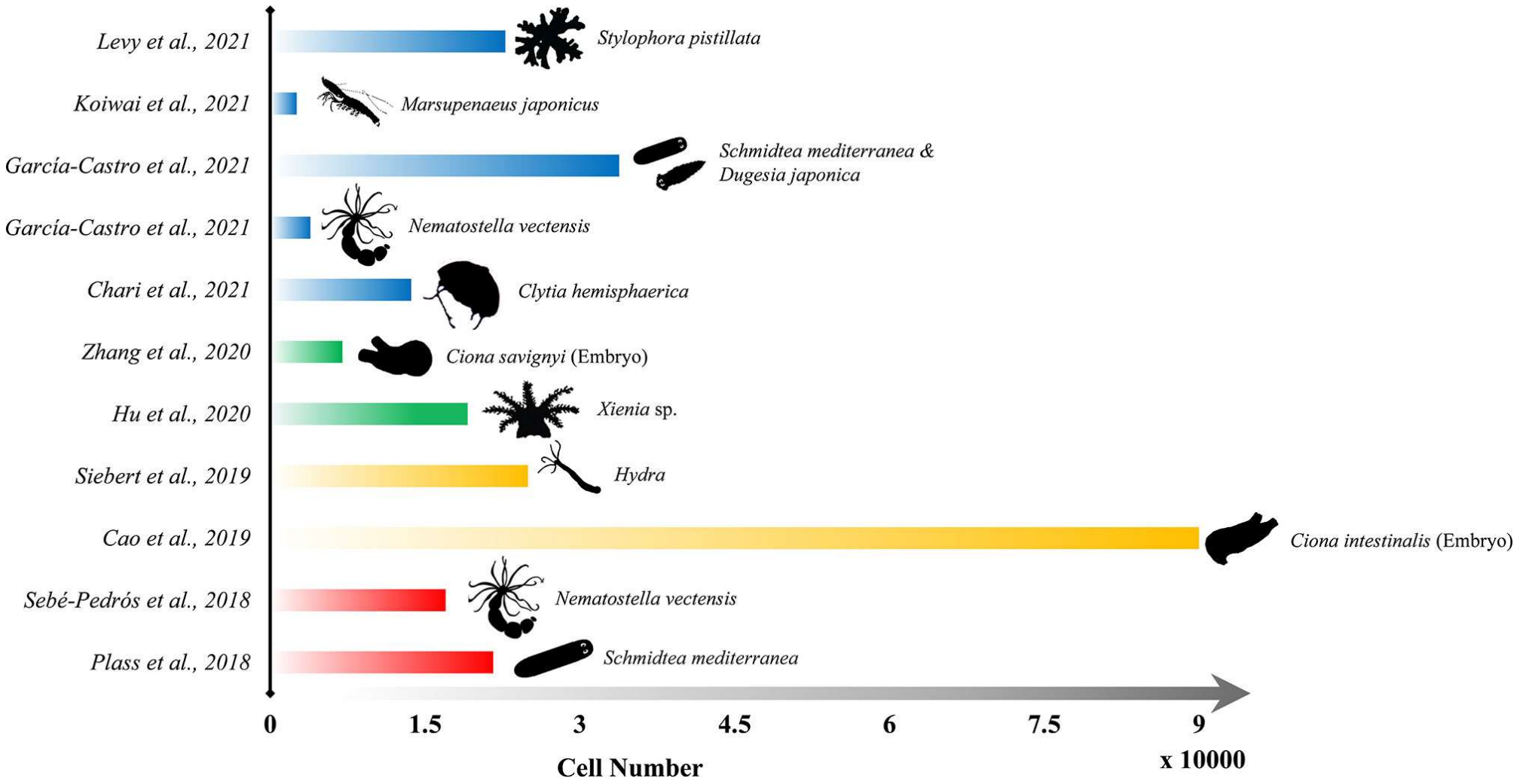
Jing Li[1,2†], Hao Wang[1†] and Chaolun Li[1,2,3]*

[1] Center of Deep-Sea Research, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China, [2] University of Chinese Academy of Sciences, Beijing, China, [3] Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China





2022 | https://doi.org/10.3389/fmars.2022.906267

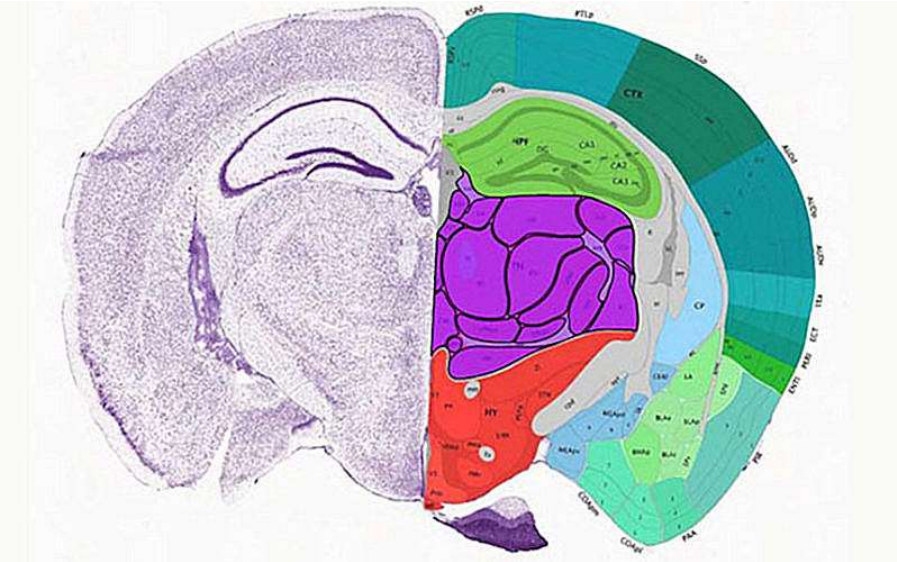


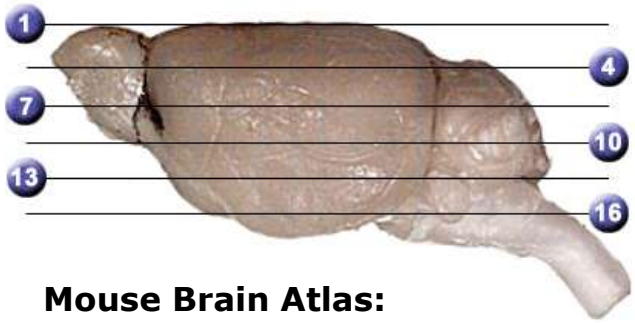
2022 | https://doi.org/10.3389/fmars.2022.906267

Spatial Transcriptome

Dissection direction: Top/Down, Front/Back, Left/Right

ALLEN BRAIN ATLAS

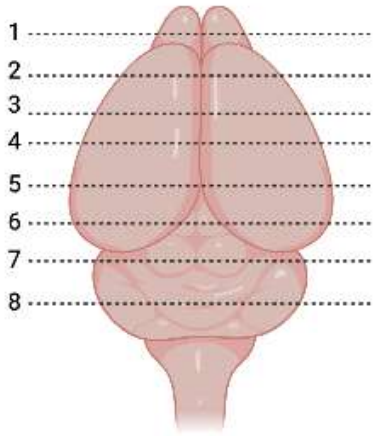




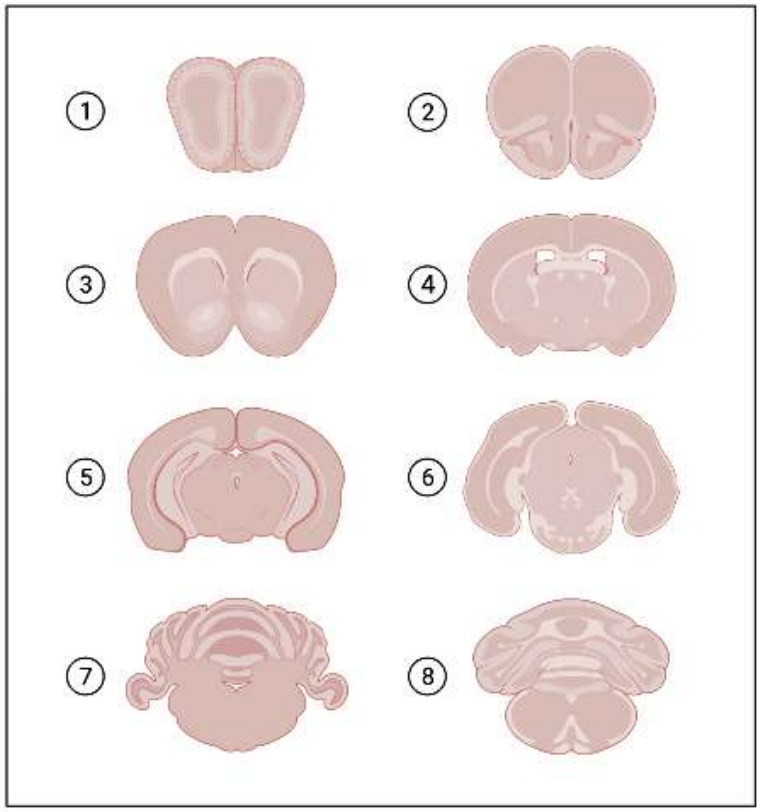
Mouse Brain Atlas:
C57BL/6J Horizontal

https://www.mbl.org/atlas232/atlas232_frame.html

Mouse Brain Anatomy
Brain Cross-sections



Dorsal view



Coronal brain slices

https://app.biorender.com/biorender-templates/t-60db3df7297d8a00a410d7be-mouse-brain-anatomy-coronal-sections

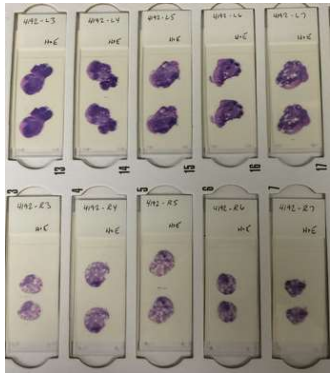
Complete setup for spatial transcriptome workflow

1. OCT embedding

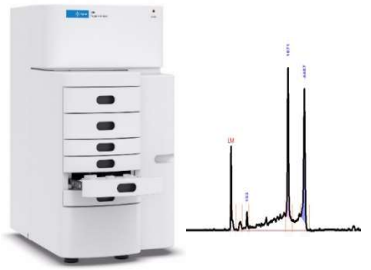
2. Cryosectioning

3. H&E staining

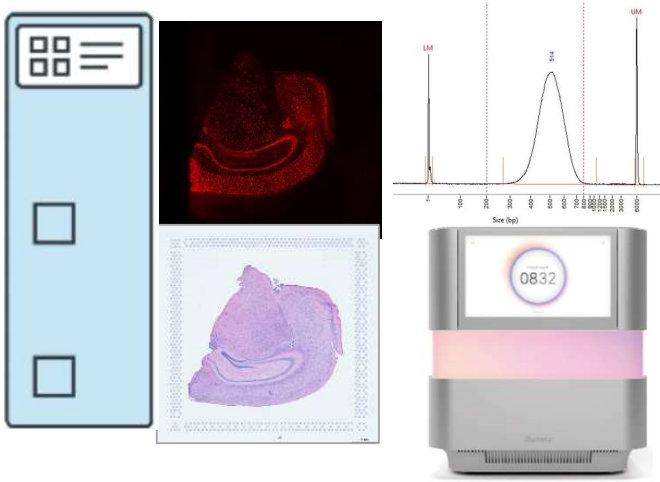
4. Tissue catalog



5. RNA QC

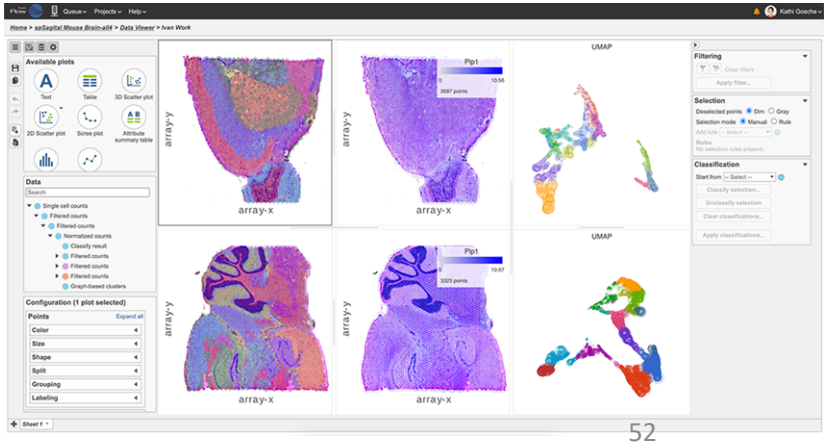


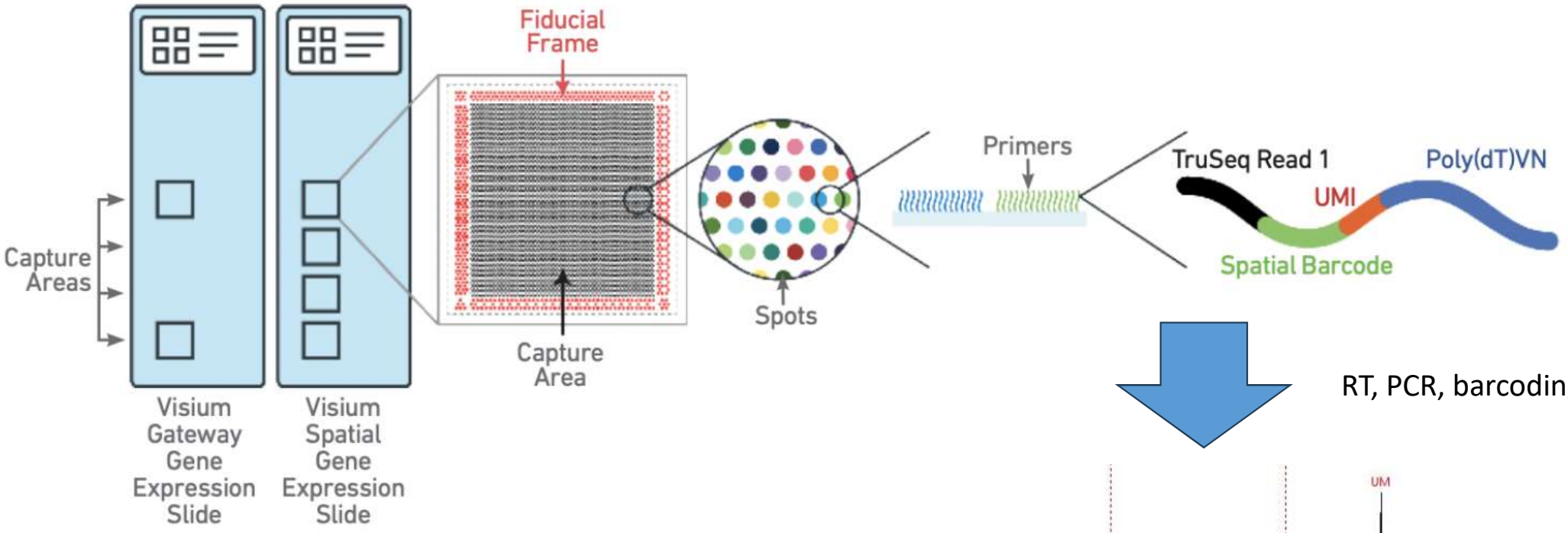
6. Visium prep & Sequencing



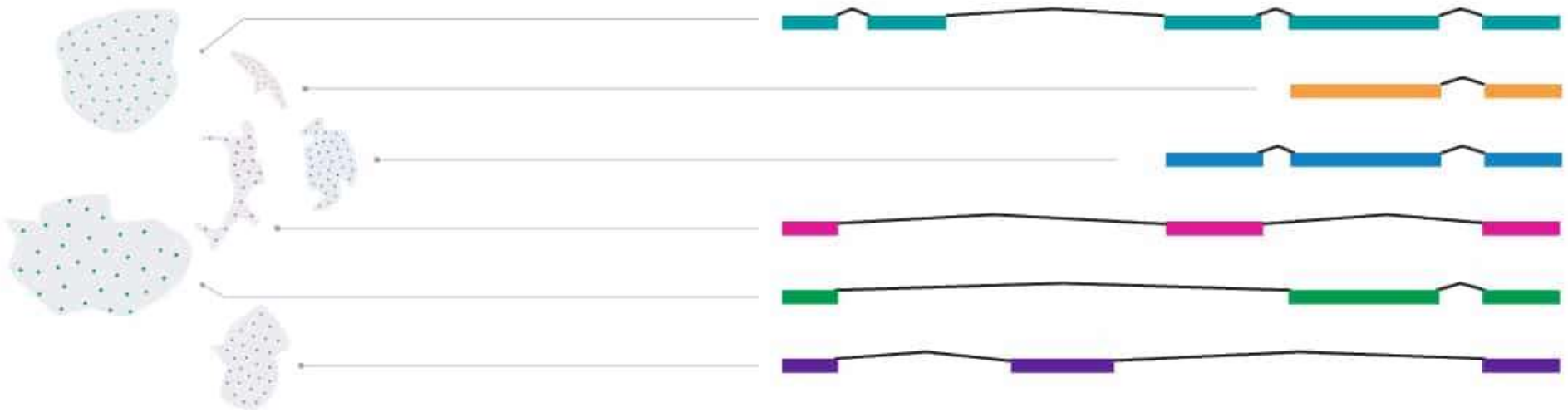
7. Fluorescent imaging

8. Data analysis with GUI interface




Capture Areas
Visium Gateway Gene Expression Slide
Visium Spatial Gene Expression Slide
Fiducial Frame
Capture Area
Spots
Primers
TruSeq Read 1
Poly(dT)VN
UMI
Spatial Barcode
RT, PCR, barcoding
LM
514
UM
Size (bp)

"…Detection of 214,516 unique isoforms covering 22,391 genes, 72.6% of the isoforms are novel."

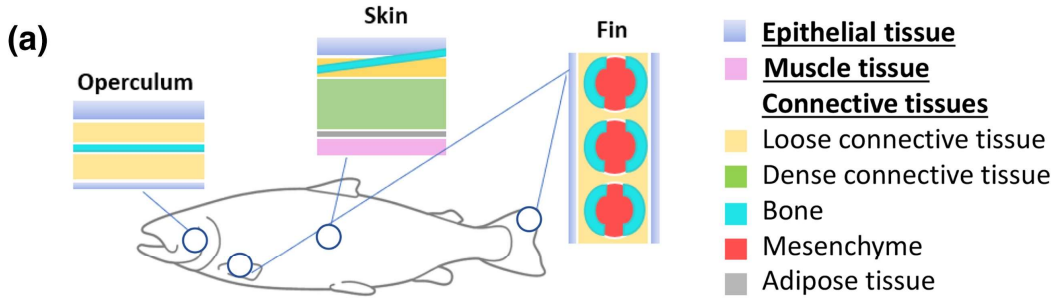


Cell-type-specificity of isoform diversity in the developing human neocortex informs mechanisms of neurodevelopmental disorders

doi: https://doi.org/10.1101/2023.03.25.534016

https://www.pacb.com/blog/the-hifi-difference-a-better-cell-atlas-with-full-length-isoform-sequencing/

Spatial Transcriptomics of Atlantic Salmon Skin



Transcriptomic landscape of Atlantic salmon (*Salmo salar* L.) skin

Lene R. Sveen,[1,*] Nicholas Robinson,[1,2] Aleksei Krasnov,[1] Rose Ruiz Daniels,[3] Marianne Vaada
Elisabeth Ytteborg,[1] Diego Robledo,[3] Sarah Salisbury,[3] Binyam Dagnachew,[1] Carlo C. Lazado

[1]Nofima, Fish Health, Tromsø NO-9291, Norway
[2]School of BioSciences, The University of Melbourne, Melbourne 3010, Australia
[3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh EH25 9RG, UK

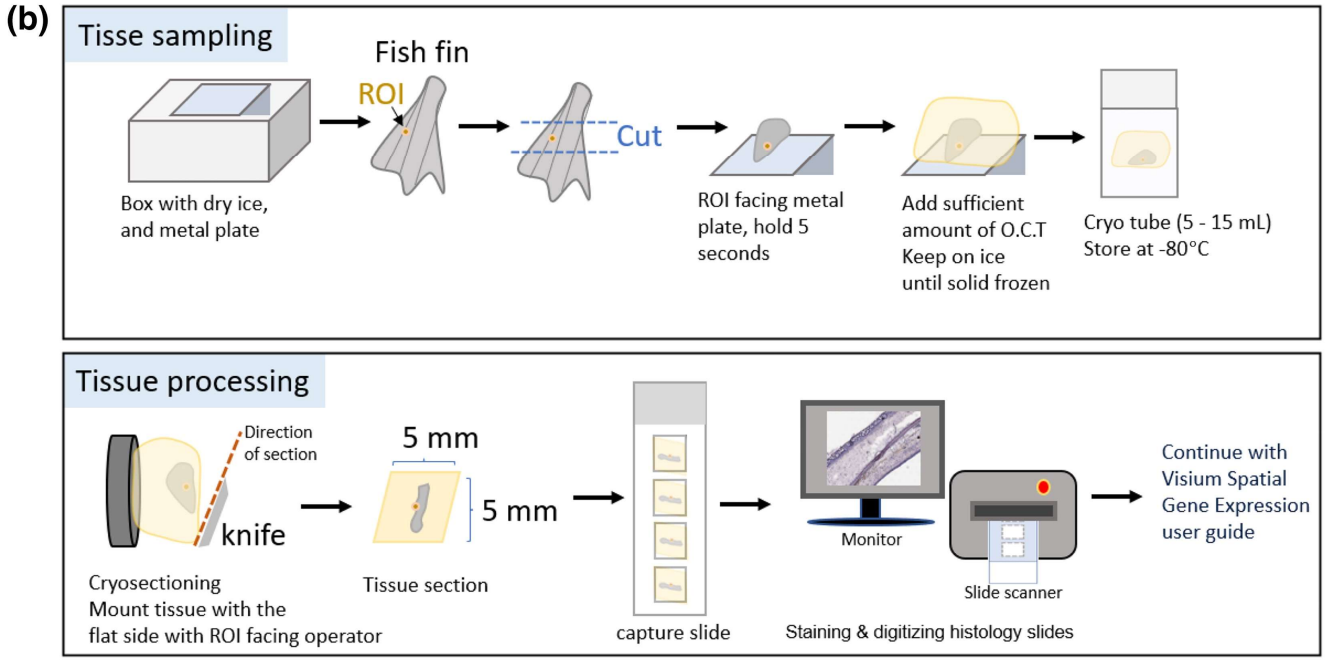


Fig 1: Overview of the Study: showing tissue sampling sites and workflow for cryo-sectioning and Visium slide preparation.

Background:

1. First spatial transcriptomic atlas of Atlantic salmon skin.
2. Explores four tissue sites: operculum, lateral line, pectoral fin, and caudal fin.

Technology and Methodology:

1. Utilized 10x Genomics Visium platform for spatial transcriptomics.
2. Achieved high-resolution mapping with 80,000+ transcripts and ~30,000 genes per sample.

G3 Genes|Genomes|Genetics, Volume 13, Issue 11, November 2023, jkad215, https://doi.org/10.1093/g3journal/jkad215

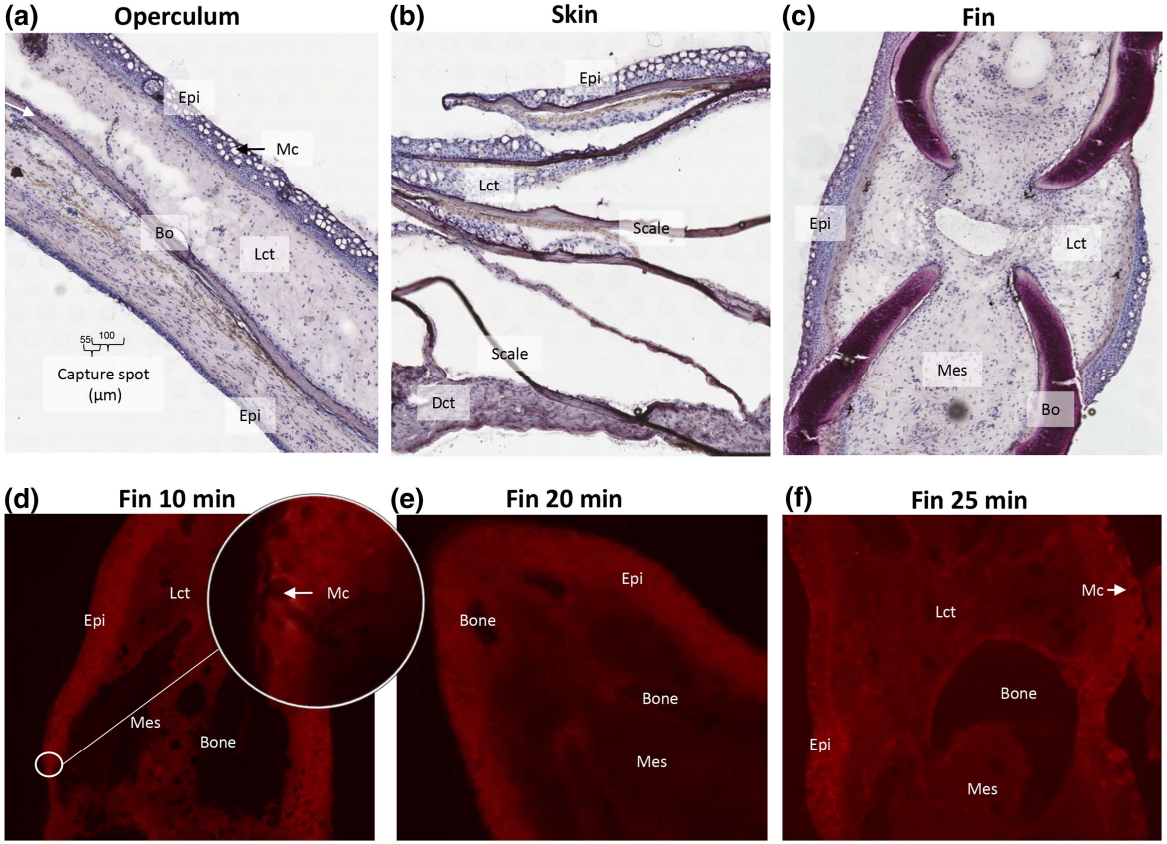


Fig. 2. Tissue sections and permeabilization time

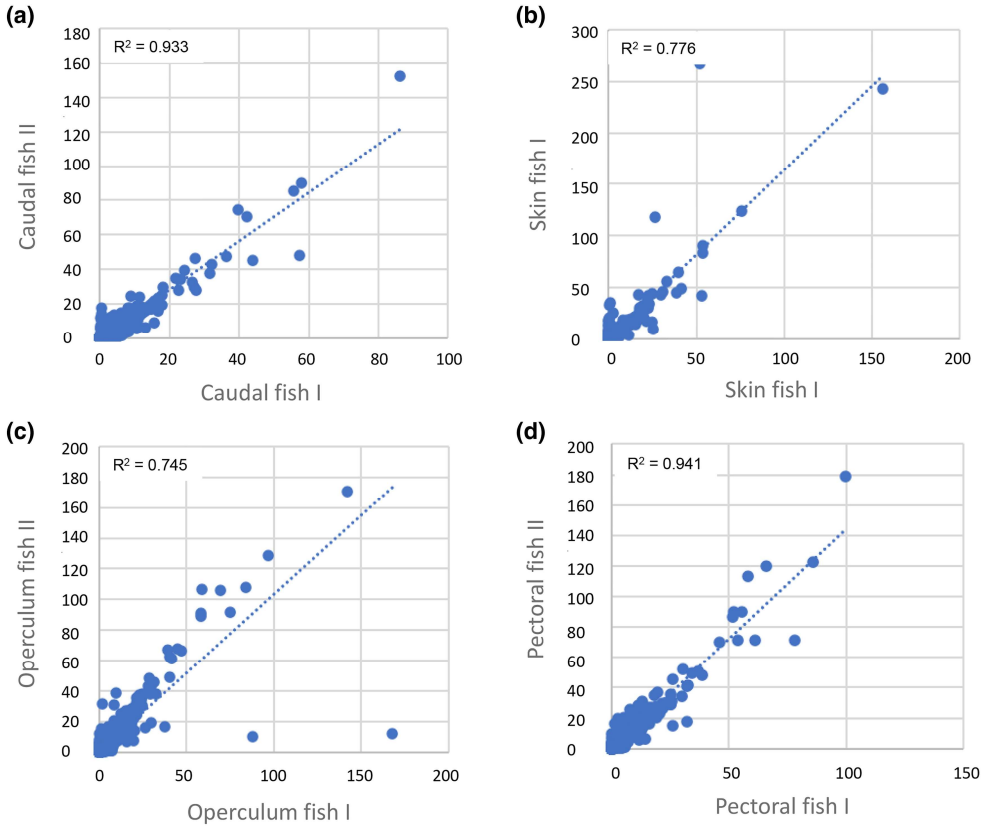
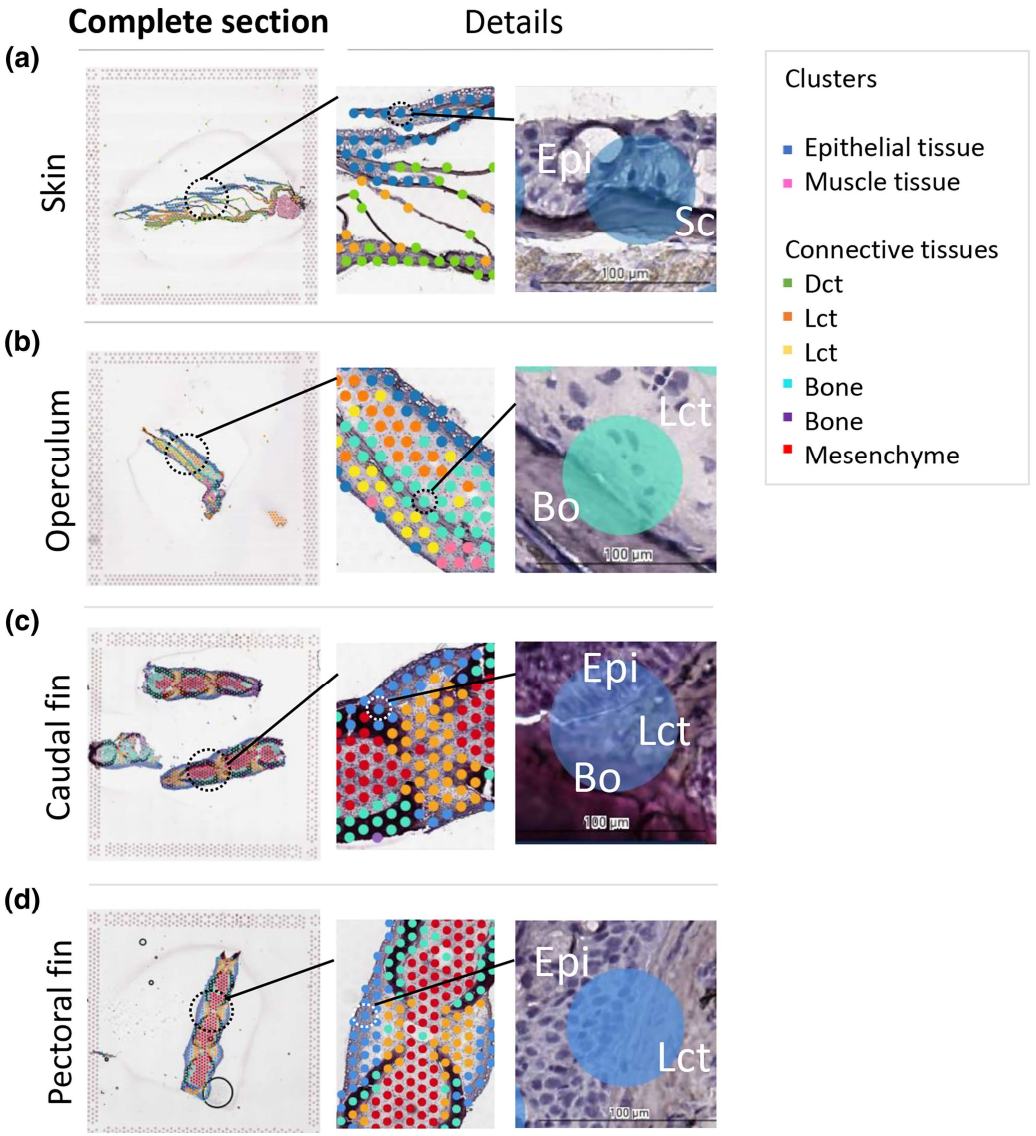


Fig. 3. Normalized gene counts for Fish I and Fish II.

G3 Genes|Genomes|Genetics, Volume 13, Issue 11, November 2023, jkad215, https://doi.org/10.1093/g3journal/jkad215

Clustering of Tissue Types



Key Results:

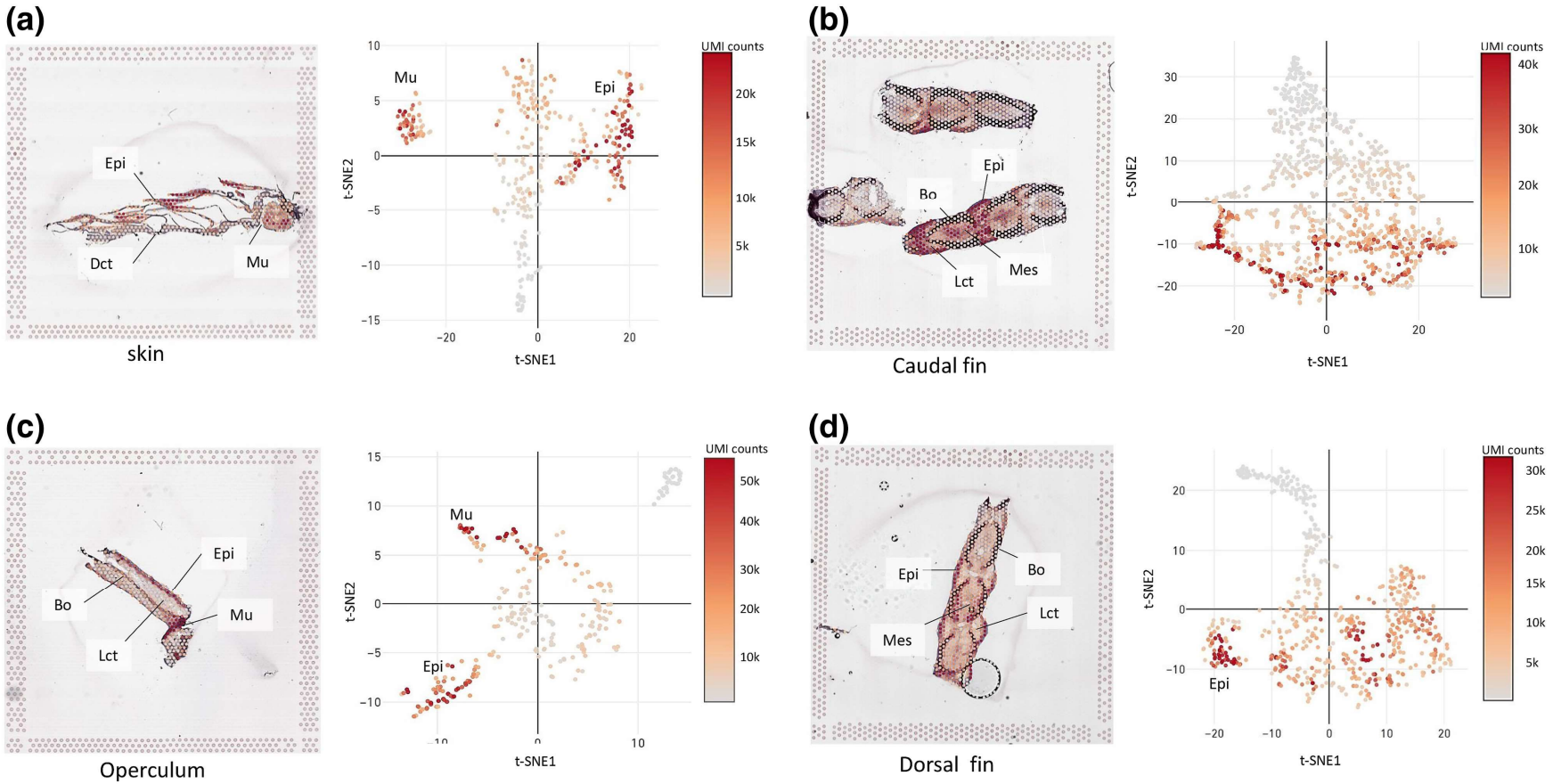
1. Epithelial tissues showed the highest transcript counts.
2. Graph-based clustering revealed spatial domains for epithelial, connective, and bone tissues.
3. Collagen type I and keratin proteins are dominant in skin structure.

Findings:

1. Spatial clustering aligned well with histological identification.

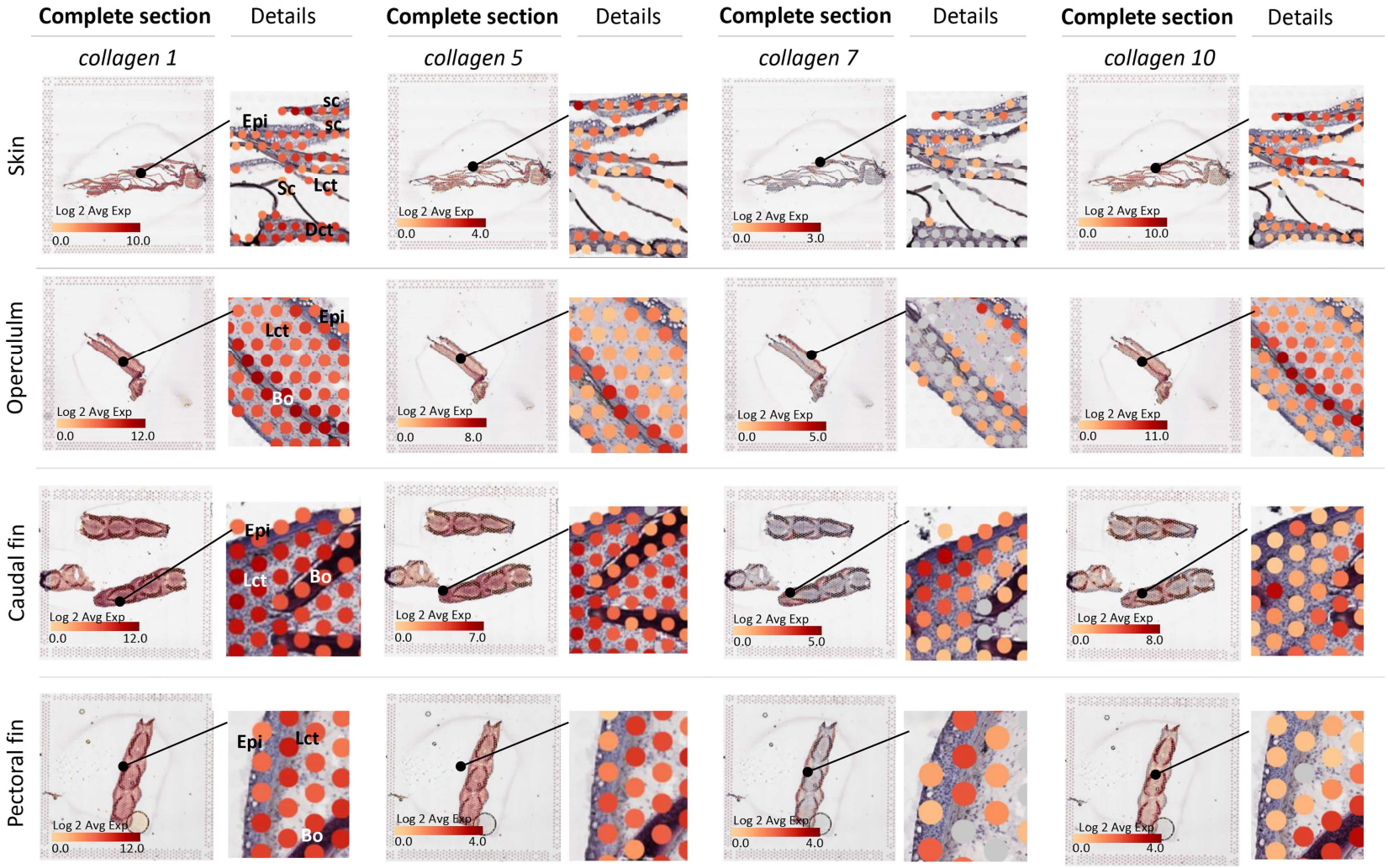
Fig 5: Tissue Clustering and Spatial Domains, showing graph-based clustering and spatial domains in tissue sections.
a) Skin, b) operculum, c) caudal fin, and d) pectoral

Fig. 4. UMI counts in tissue from Fish I. a) Skin, b) caudal fin, c) operculum, and d) pectoral fin. For each sample, ...



G3 Genes|Genomes|Genetics, Volume 13, Issue 11, November 2023, jkad215, https://doi.org/10.1093/g3journal/jkad215

Gene Markers and Tissue Specificity



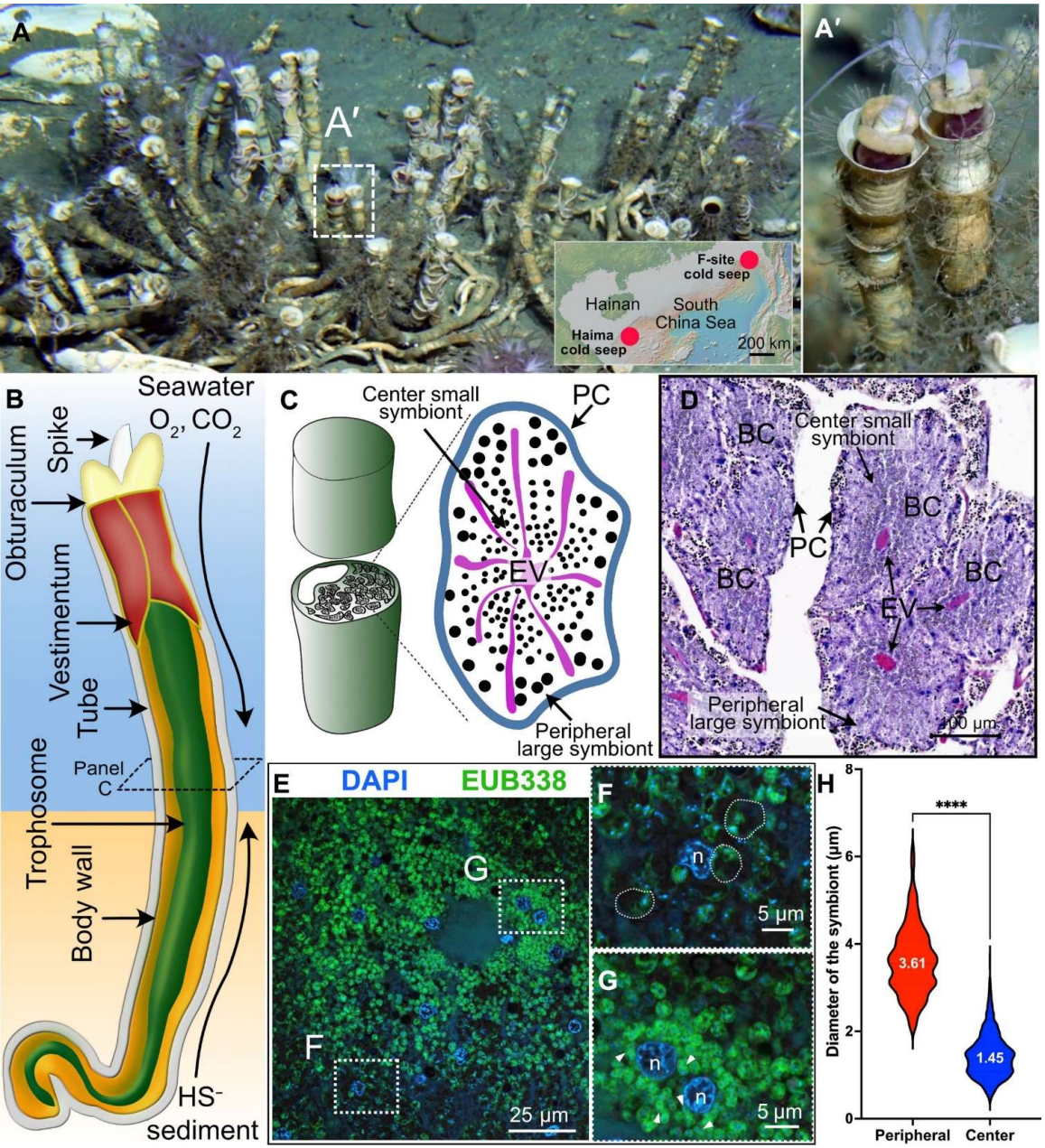
Key Insights:

1. Identified gene markers for epithelial (e.g., claudin 1), bone (e.g., collagen type 10), and mesenchymal tissues.
2. Collagen and keratin expression patterns highlight tissue specialization.
3. Supports applications in aquaculture for understanding disease and improving fish health.

Concluding Statement:

1. Spatial transcriptomics provides a molecular toolbox for tissue health management.

Fig. 7: Gene Marker Insights, showing gene expression of collagen types 1, 5, 7, and 10 across tissue sections.



Host-Symbiont Interactions in Deep-Sea Tubeworms

1.Background:

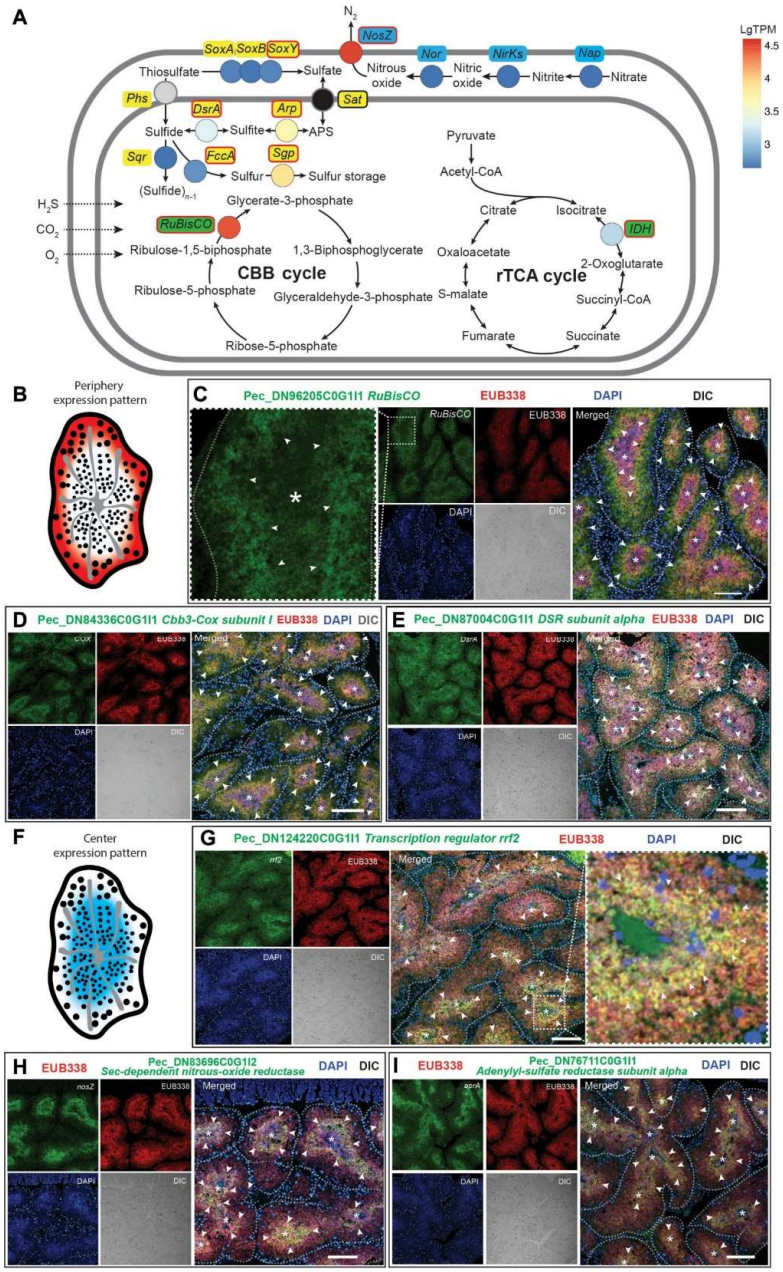
1. Vestimentiferan tubeworms (Paraescarpia echinospica thrive in chemosynthetic ecosystems, relying on endosymbiotic bacteria.
2. Investigation of host-symbiont molecular interactions using single-cell RNA sequencing.

2.Approach:

1. Development of deep-sea in situ fixation for single-cel transcriptomics.
2. Analyzed trophosome tissues for spatially distinct symbiotic roles.

Figure:

Include **Figure 1**, showcasing the tubeworm's habitat, trophosome structure, and symbiont distribution.



Slide 2: Spatial Microniches and Symbiont Roles

Title: *Metabolic Microniches in Trophosome Lobules*

Content:

1.Key Findings:

1. Two distinct metabolic niches identified in the trophosome:
    1. **Periphery:** Aerobic respiration, active chemosynthesis, and symbiont digestion.
    2. **Center:** Anaerobic denitrification, nitrogen waste management.

2.Implications:

1. Spatial segregation enables efficient metabolic specialization.

Figure:

Include **Figure 5**, showing gene expression in peripheral and central symbiont subpopulations.

Considerations for 10x Genomics Projects

Single-cell and Spatial transcriptome profiling:

- Questions and Expectations
- Input materials
- Experiments
- Command lines/bioinformatics
- Parameter tunning
- Output validation & evaluation
- Reject / Adjust / Accept

Conclusion and Key Takeaways

1. Best practices:
   - Personal & Lab hygiene
   - Filter tips
   - Gamma-radiated plastic consumables (better than autoclave)
2. DNA extraction:
   - Know your sample nature
   - Try a few methods and check with **QC**: **Yield, Purity, Integrity!**
   - Avoid cross-contamination
3. Choose Seq platform:
   - Read length: Short-read vs Long-read
   - Read depth: metagenome vs enriched culture
   - Assembly vs target-seq
4. Amplicon generation:
   - Target selection
   - Primer design
   - PCR optimization
   - Amplicon purification (optional)
5. Data processing:
   - Data Quality assessment
   - Q trimming/filtering