

Genomics @ Pitt

An Overview (and history)

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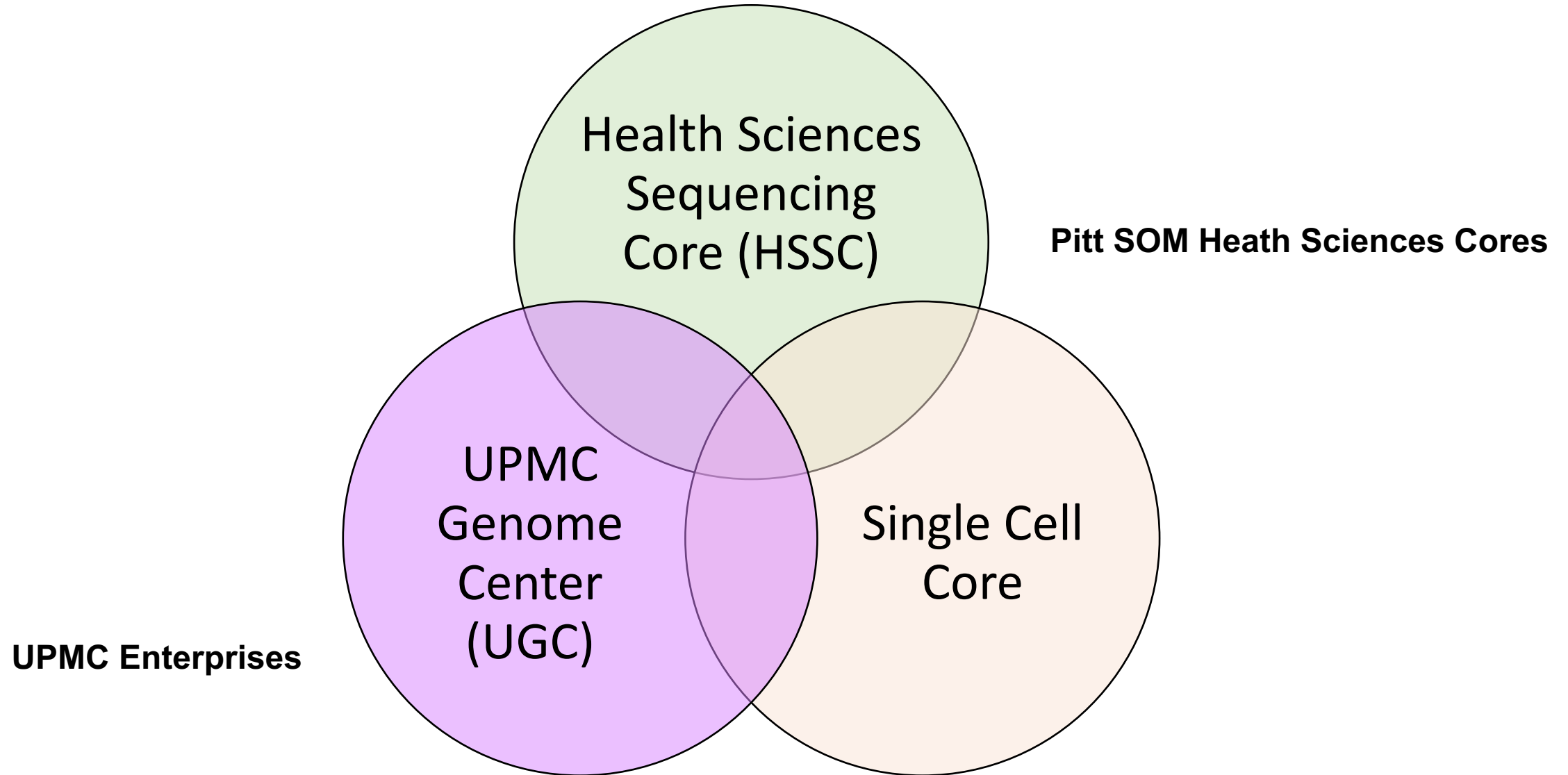


Genomics @ Pitt Landscape 2021

	Pitt SOM Health Sciences Cores			UPMC Enterprises
Facility Name	Genomics Research Center (GRC)	Health Sciences Sequencing Core (HSSC)	Single Cell Core	UPMC Genome Center (UGC)
Location	Oakland (Forbes)	UPMC Children's Hospital	Oakland BST South	Shadyside
Director	Dr. Janette Lamb	Dr. Amanda Poholek	Dr. Bob Lafyatis	Dr. Annerose Berndt
Established	>20 yrs	2016	2019	2017

Some integration from 2017 on
 -All sequencing projects at GRC were run
 and billed to HSSC

New Genomics @ Pitt Landscape

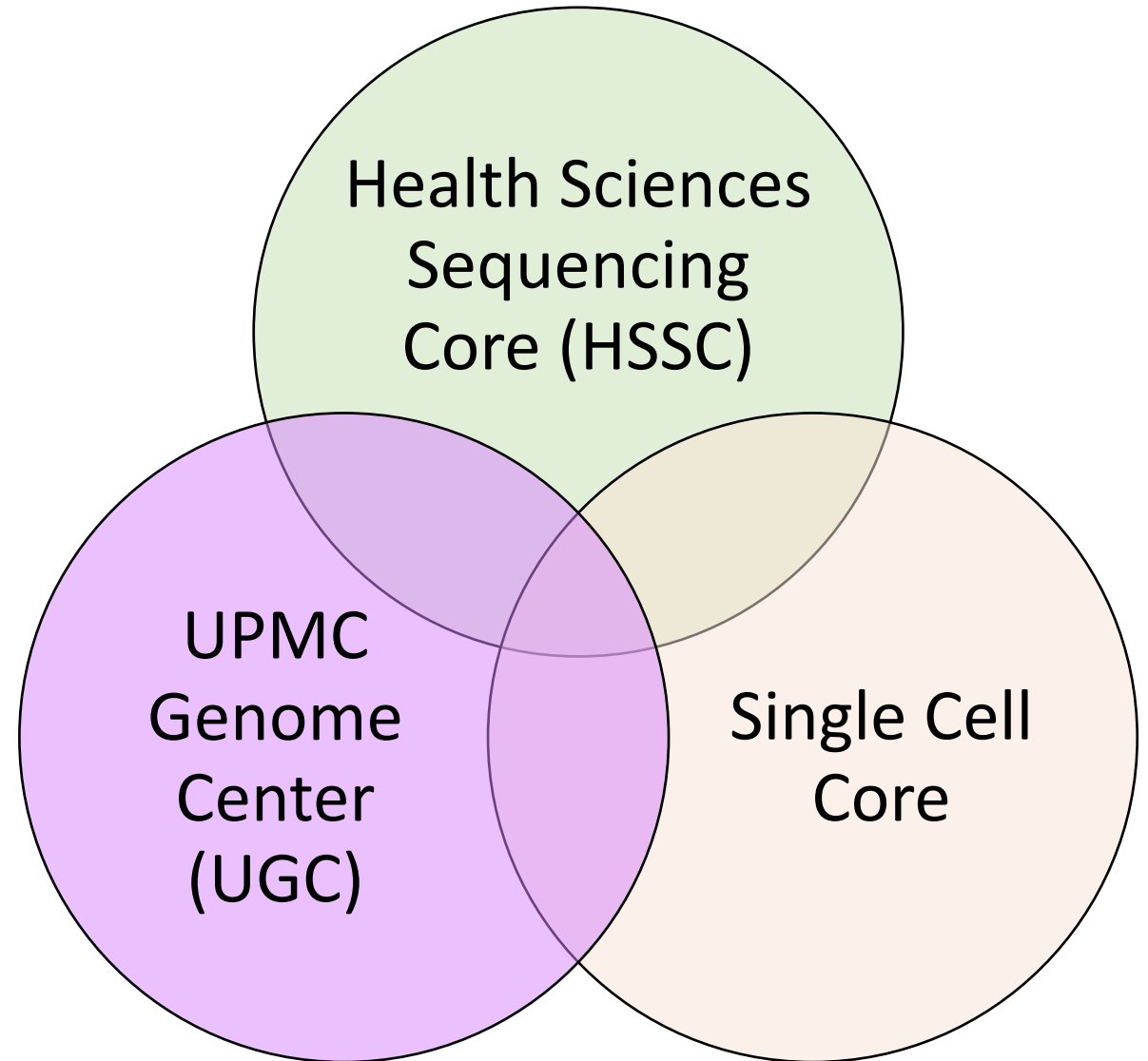


Genomics @ Pitt Landscape July 1 2022

Facility Name	Health Sciences Sequencing Core (HSSC)	Single Cell Core	UPMC Genome Center (UGC)
Location	UPMC Children's Hospital	Oakland BST South	Shadyside
Director	Dr. Amanda Poholek	Dr. Bob Lafyatis	Ed Smith/ Dan Bellissimo
Services	Bulk RNAseq (high and low input) ChIPseq ATACseq Mid-throughput pre-made libraries Visium Spatial Transcriptomics	scRNAseq scATACseq CITE-seq	Clinical Testing Services CLIA/CAP certified WGS WES Bulk RNAseq (high input) High-throughput pre-made libraries
Major Equipment	Nextseq 2000 (1.2B reads) Nextseq 500 (400M reads) MiSeq (long reads)	2 10X Chromium Controllers	5 Novaseq 6000 (10B reads)

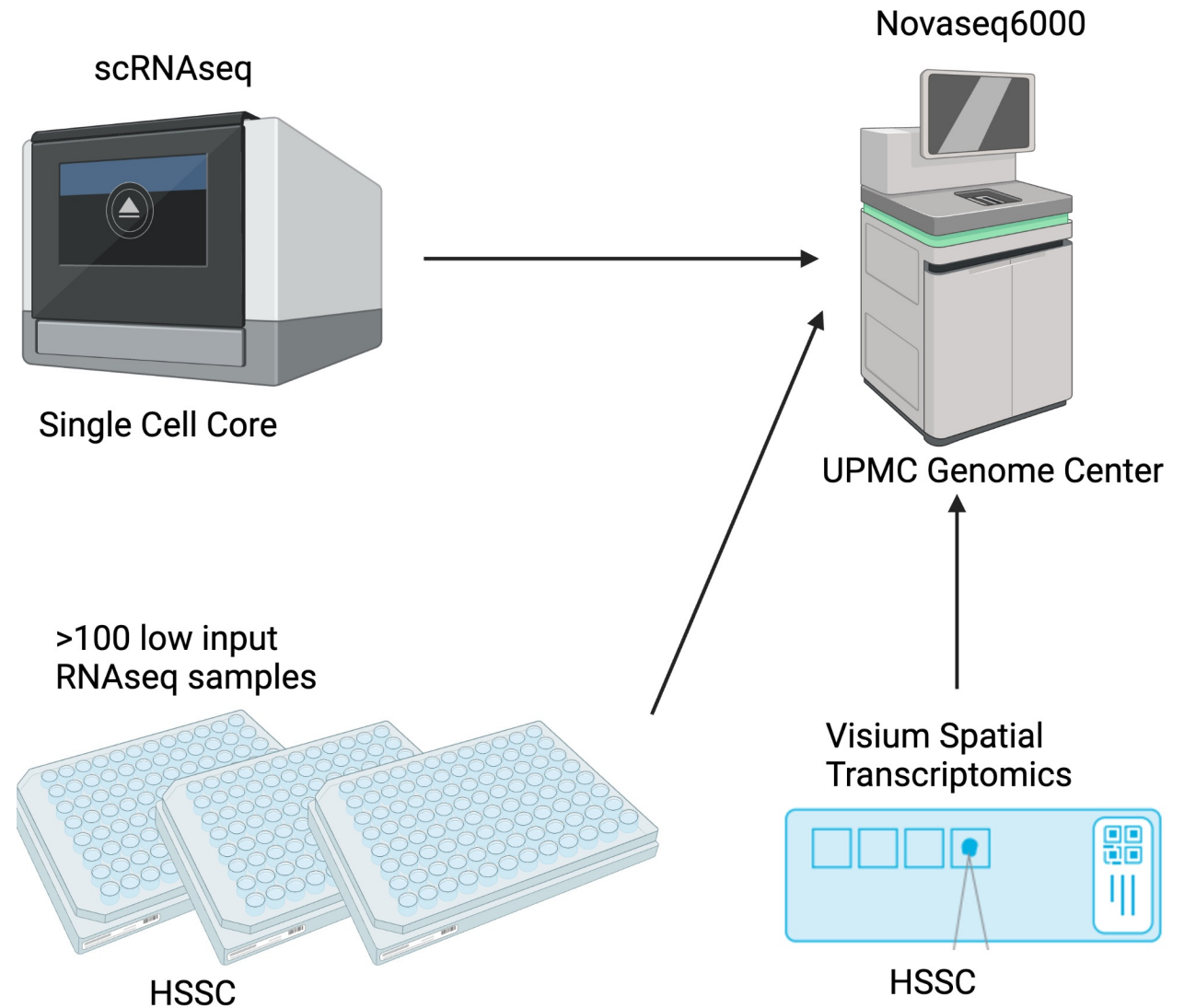
Working towards collaboration of three cores

- Biweekly meetings of three cores to discuss projects spanning cores, availability of machines, ongoing areas of integration
- Working to eliminate overlaps in services – each core has clear strengths, these should be further developed
- Implement single website that hosts all three core sites and clearly outlines contact, services, prices for all cores
- Implement a single Request for Services online form that will go to all cores and be answered by the core that provides appropriate service.



Common workflows across cores

- When needed, high capacity sequencing projects are more cost effective on a single Novaseq run than several Nextseq runs.
- For these projects, sequencing is performed at UGC to decrease costs to PI
- Increased collaboration through biweekly meetings should further help reduce costs by identifying projects that can be pooled and increased turnaround times



Health Sciences Sequencing Core (HSSC)

Staff

Director:

Amanda Poholek, PhD

Assistant Director:

William MacDonald, PhD

Senior Technicians:

Rania Elbakri

Bryan Thompson

Yvette Rhodes

Part time students:

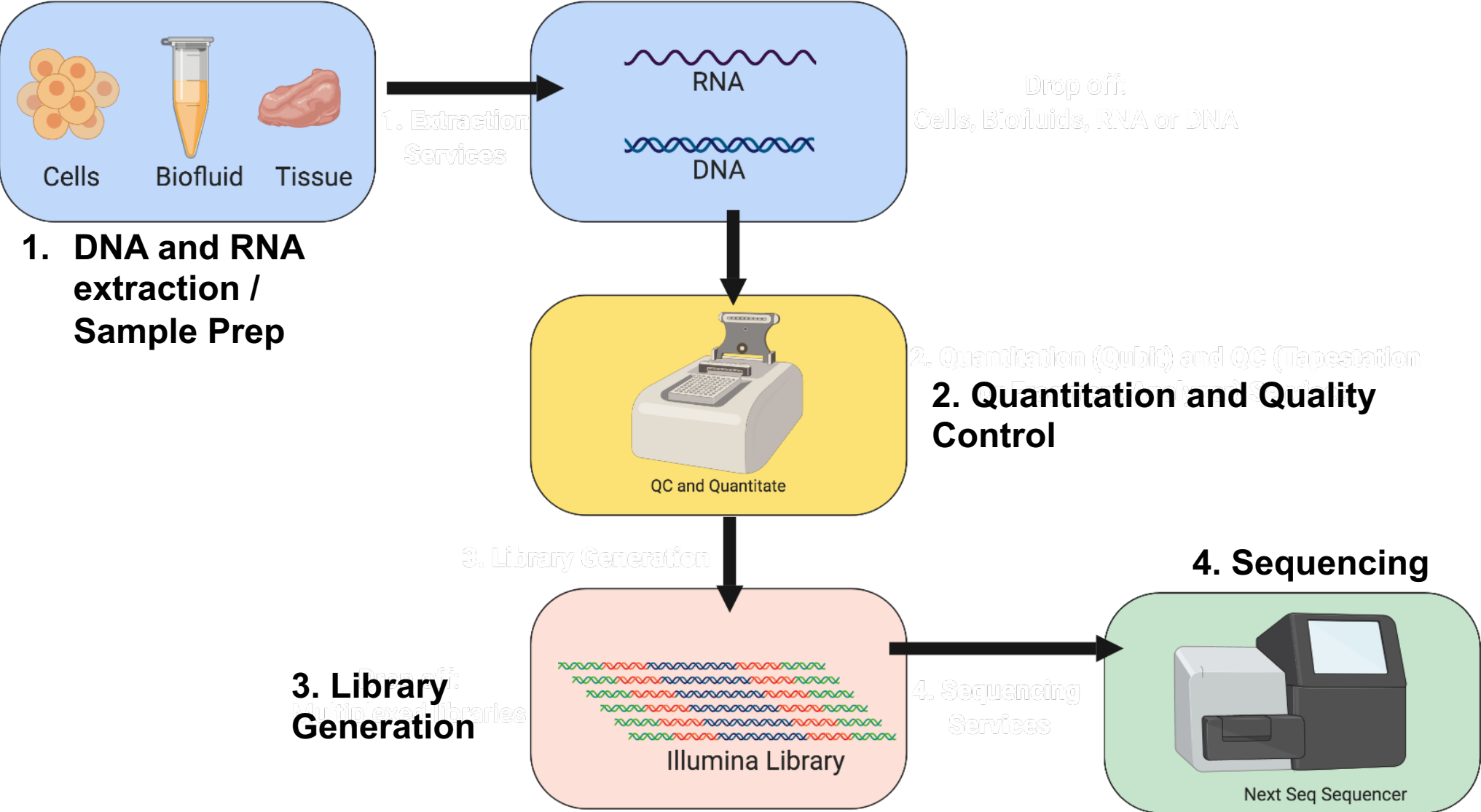
Rihanna Ericson

Location

- UPMC Children's Hospital of Pittsburgh
- Rangos Research Center
- Located in Rm. 8145, and Bay 15



Services Workflow



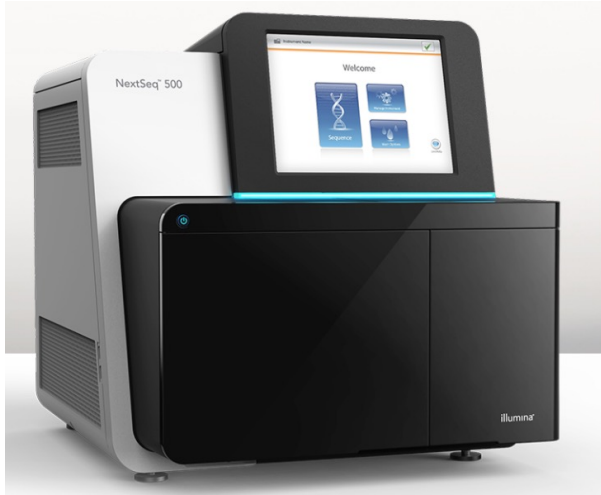
Key Services

- **DNA and RNA extraction/Sample Prep**
- **Quantitation and Quality Control**
(TapeStation/Fragment Analyzer/Qubit)
- **RNA-sequencing**
 - Standard input (TrueSeq mRNA and Total RNA)
 - Low Input (SMART-seq v4, SMART-Seq Stranded Total RNA)
 - RNA Exome (Access) for FFPE
 - Small RNA/MicroRNA
- **DNA-sequencing**
 - Nextera DNA XT
 - Illumina DNA prep (Nextera Flex)
 - DNA Exome
 - ChIP-seq
 - ATAC-seq
 - TruSight Oncology
- **Sequencing Services (Pre-pooled libraries)**
 - MiSeq
 - NextSeq 500
 - NextSeq 2000

New Services

- **10X Genomics Visium Spatial Gene Expression**
- **Droplet Digital PCR (QX200 Bio-rad)**

Instrumentation: Sequencers



Nextseq500 (2017)



Nextseq2000 (2021)



MiSeq

Max Output:	121 Gb	360 Gb	15 Gb
Max Reads per Run:	400 Million	1.2 Billion	25 Million
Max read length:	2 x 150bp	2 x 150bp	2 x 300bp
Run time:	11-29 hrs	11-48 hrs	4 - 56hrs

Instrumentation: Nucleic Acid quantitation and quality



Fragment Analyzer



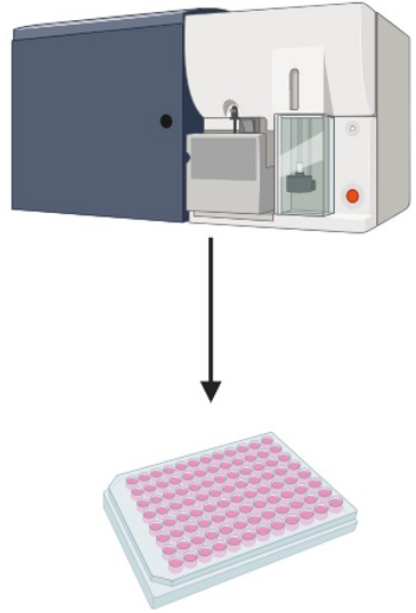
Tapestation 4150



Qubit Four/ Qubit Flex

Purpose:	Assess RNA/DNA Quality	Assess RNA/DNA Quality	Assess RNA/DNA Quantity
Max Sample #:	48	2-16	1-8

Impact Projects: RNAseq (Smart Seq2) on live sorted cells



Takara SMARTseq2

Samples: Low-input, live sorted cells

Challenge: Cell specific optimization of immune cells

- Optimization has been applied broadly to other groups

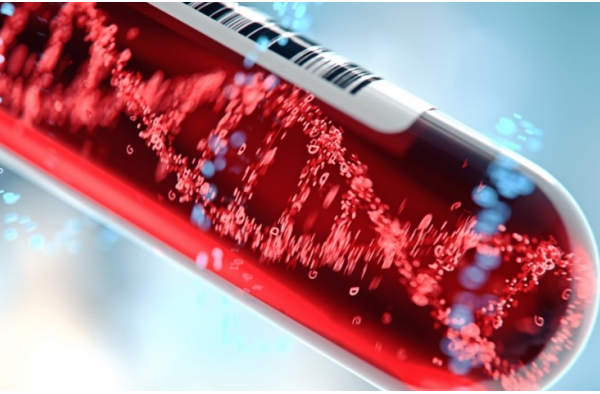
Impact:

- Minimize need for individual groups to independently optimize for cell type
- Increases efficiency and reduces failure rate
- Applied to non-immune cell scenarios
 - Single embryo collections (Y. Wu)
 - Patch-seq (G. Gonzalez Burgos)
 - Nuclei extraction from patch clamped neurons
 - International project w/ Canada and Germany



Impact Project: Targeted enrichment of cell free DNA

Adrian Lee, PhD & Steffi Oesterreich PhD



Samples: Human Cell Free DNA from blood

Challenge: “EnRich”: Targeted Enrichment of Esr1 gene

- Custom Oligo design for use with Illumina TruSight Sequencing Panel
- Developed in consultation with Illumina and IDT

Impact:

- Preliminary data contributed to a recently funded R01: *Estrogen receptor fusions genes as drivers of endocrine resistance in breast cancer*

Illumina TruSight Targeted
Panels for Translational
Research



Impact Project: Dual RNASeq for Transkingdom Interactome

Nader Shaikh, MD



Samples: Pediatric Nasal Swabs and Urine

- Suspected UTI

Challenge: Dual RNA-seq for human, bacterial and viral transcriptomes

- Piloted storage options and extraction methods for urine samples
- TruSeq RNA Access for urine samples (for human genome only)
- Total RNA & Ribo Zero Plus to remove human & bacterial rRNA
- Developing method for using urine samples with Takara SMART Stranded + CRISPR bacterial rRNA depletion (JumpCode Genomics, piloted in Lewis project)

Impact:

- Supporting international collaboration for bacterial & viral genome identification associated with UTI

Illumina RNA Access
Illumina TruSeq Total
Stranded



New to HSSC (from GRC): droplet digital PCR

QX200 Droplet Digital PCR System



- provides absolute quantification of target DNA or RNA molecules for EvaGreen or probe-based digital PCR applications.
- Most precise and sensitive digital PCR solution for a wide variety of applications
- **Applications:**
 - Cancer biomarker studies and CNV
 - Pathogen detection
 - Low abundance gene expression

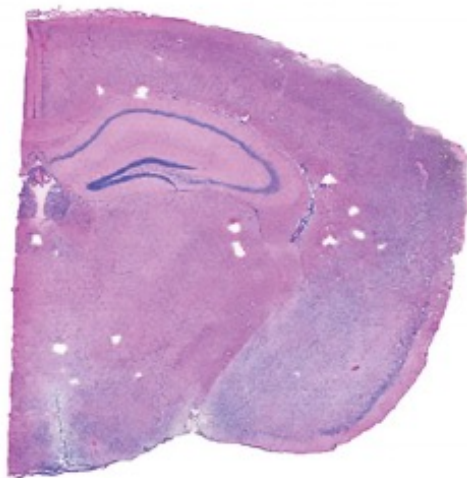
New technology: Visium Spatial Transcriptomics

10X GENOMICS

Visium Spatial Gene Expression Solution

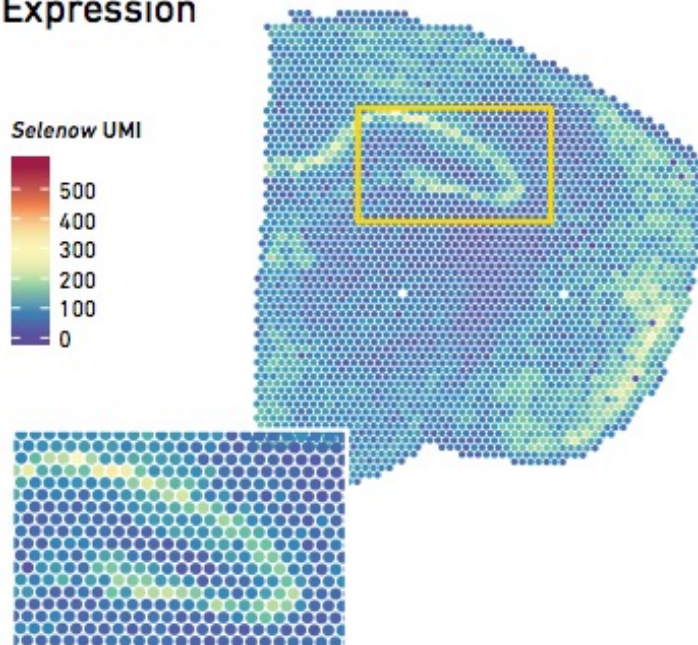
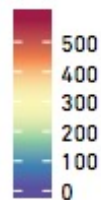
GENE EXPRESSION. VISUALIZED.

A
H&E Only



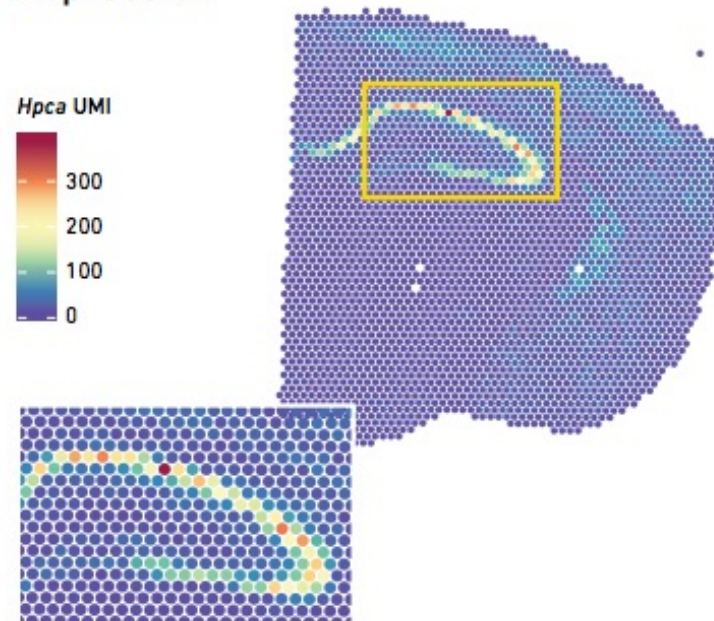
B
Selenow
Expression

Selenow UMI



C
Hpca
Expression

Hpca UMI



Workflow

Sample
prep

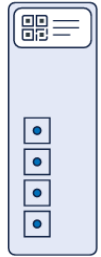
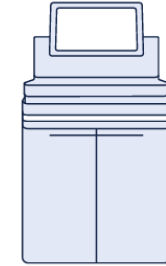
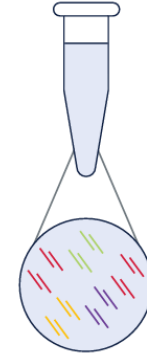
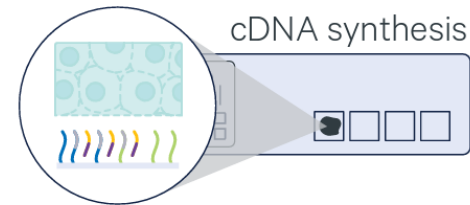
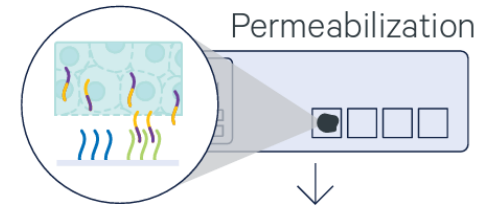
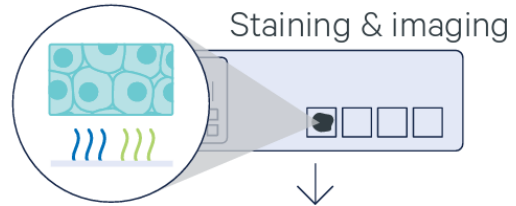
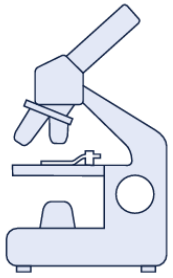
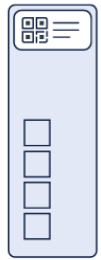
Imaging

Barcoding &
cDNA synthesis

Library
construction

Sequencing

Data
visualization



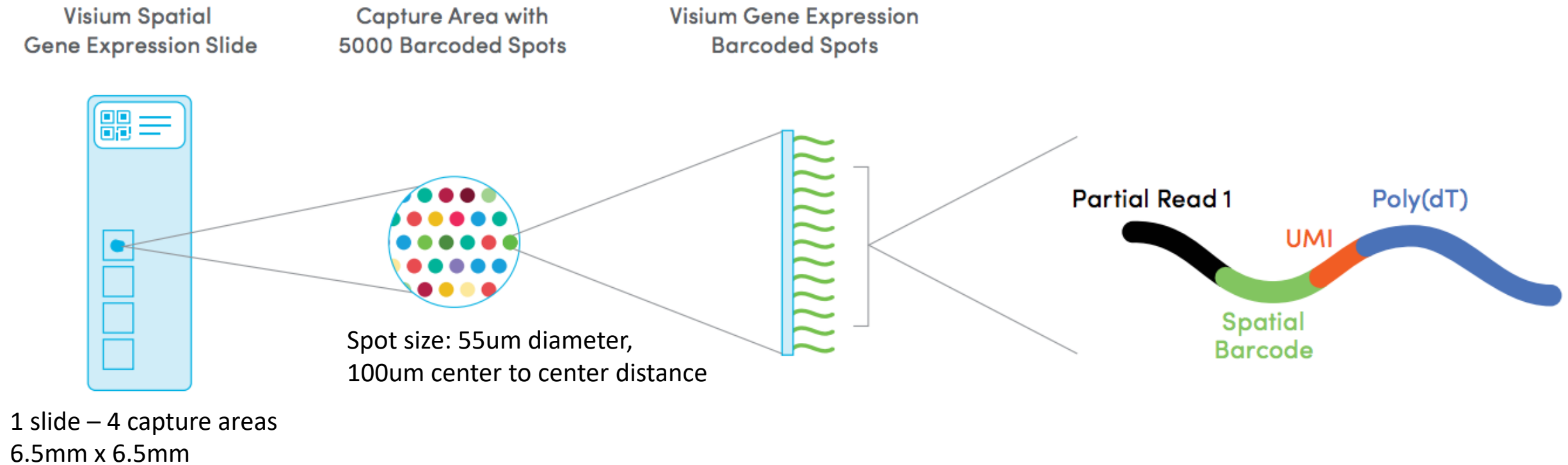
H&E for
tissue context



IF for protein
co-detection

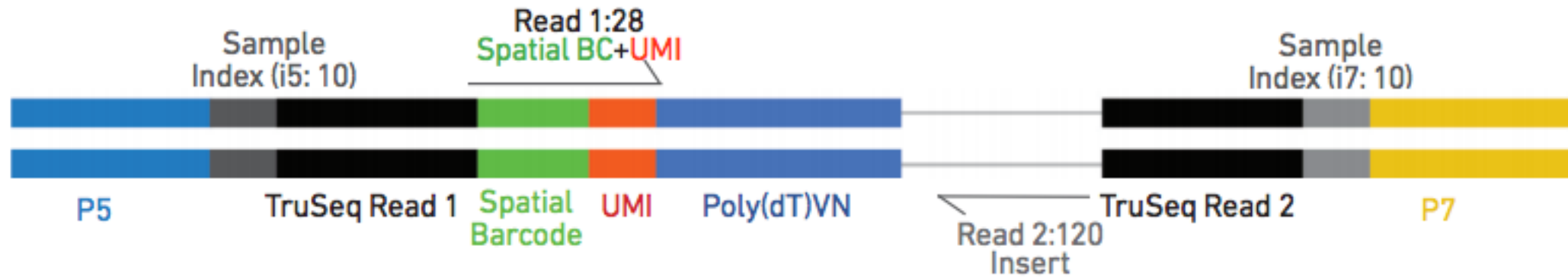


Technical details of 10X Visium Spatial Gene Expression



Key points and considerations – Sequencing

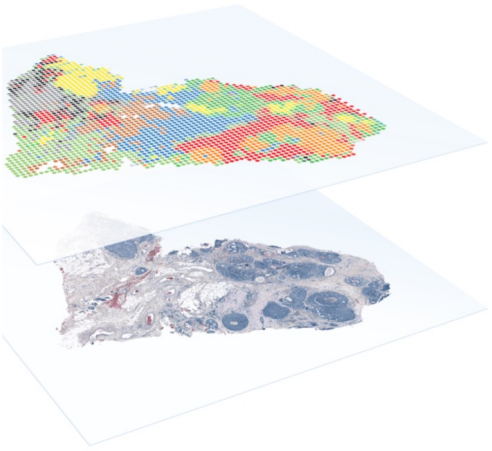
Visium Spatial Gene Expression Library



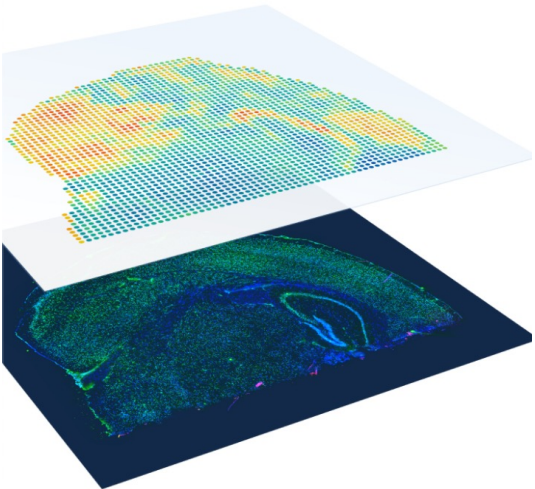
- Paired-end libraries after library preparation (dual index, read 1: 28 cycles, read 2: 120 cycles)
- Sequencing Depth: recommend 50,000 read pairs per **SPOT**
- **5,000 spots per capture area = 250 million reads per area assuming 100% coverage of area**
- The bigger the sample, the more expensive the sequencing

10X Visium Formats and Future

Current Available Formats



FFPE compatible
Targeted probe assay
(Human and Mouse only)



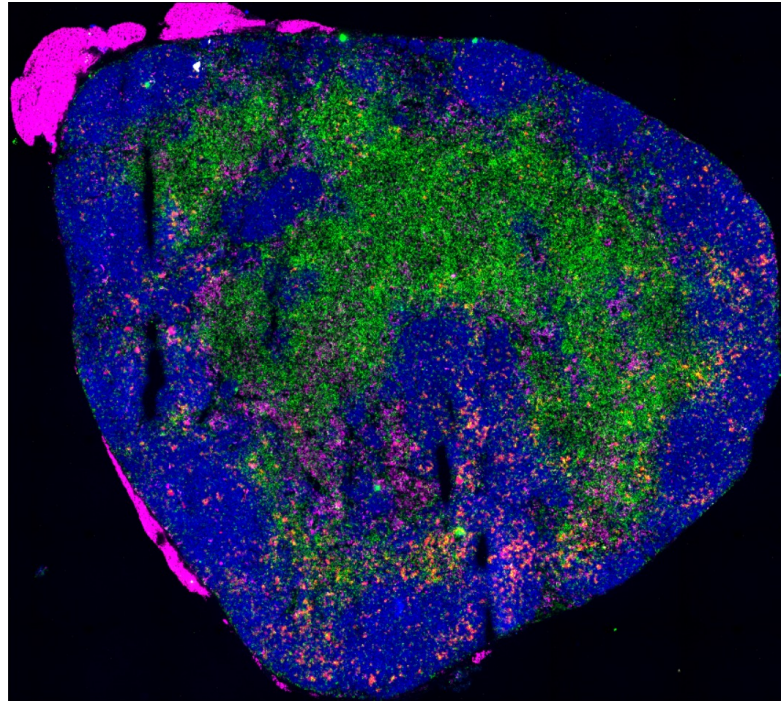
Immunofluorescence compatible
for protein detection

Future Plans/Innovations

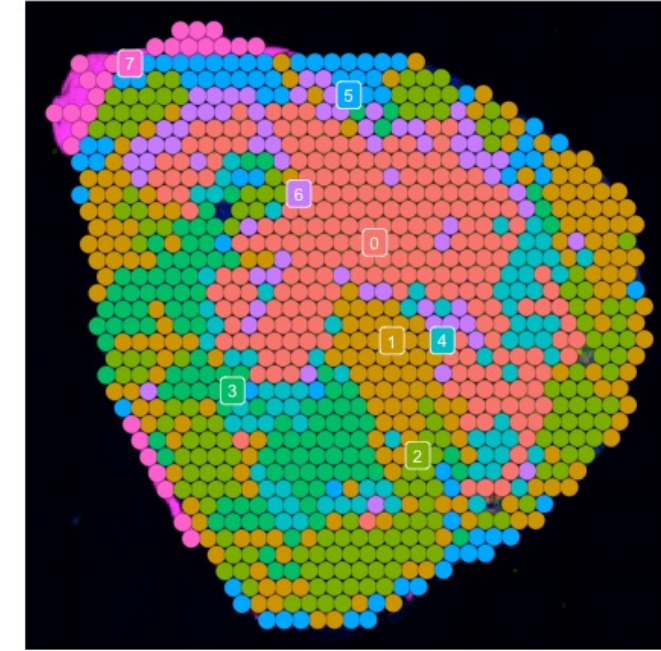
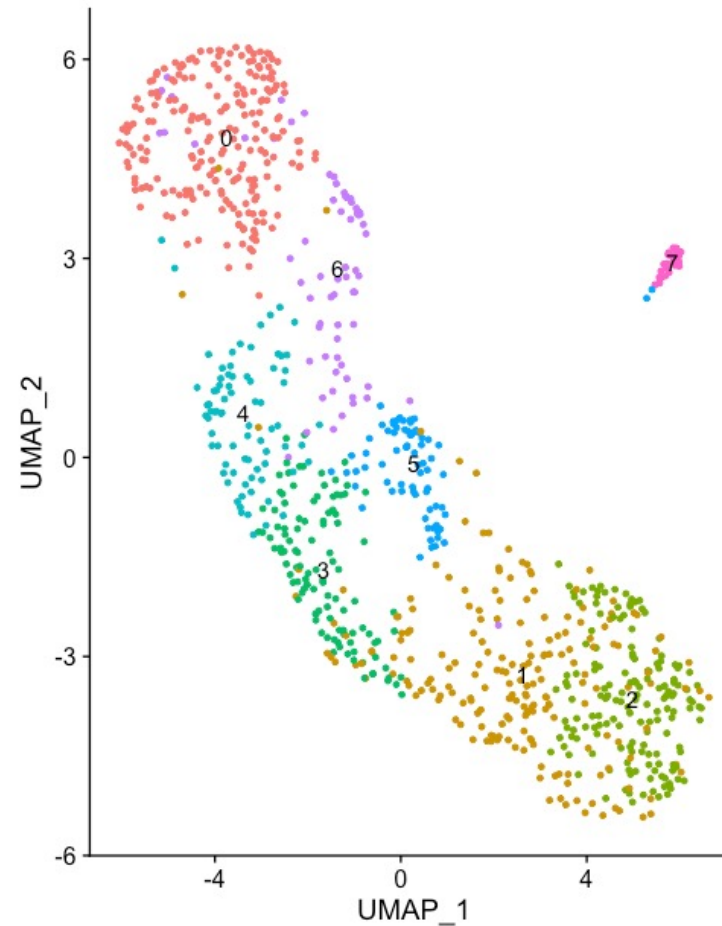
- Visium HD
 - Increased resolution (smaller spot size, more spots/area)
 - Millions of spots per tissue capture area
- Protein detection via barcodes (CITE-seq-like)
 - Increases number of proteins capable of detecting
- Larger capture areas (11mm x 11mm)
- Automation with CytAssist (purchasing now)
 - FFPE application only
 - Staining and imaging on normal glass slide
 - transfer of hybridized probes to Visium capture slide

IF Fresh Frozen Visium Pilot

Mouse LN post immunization
3 color immunofluorescence



B220 – B cells
CD4 – T cells
CD11c –
Dendritic cells



Ongoing or Completed Projects:

- R. Sindh – Human FFPE Liver
- M. Moulik– Mouse FFPE Heart
- K. Torok Lab - Human FFPE pediatric scleroderma skin
- Poholek Lab – FF Mouse Lymph Node
- S. Chan Lab – FF Rat Lung from model of fibrosis
- Fitz/Koldamova Lab – FF Mouse Brain from Alzheimer's model

Acknowledgments

HSSC staff

- Will MacDonald, PhD
- Rania Elbakri
- Bryan Thompson
- Yvette Rhodes
- Rhianna Ericson
- William Horne
- Jenny Hughson
- Annabel Fergeson

HSCRF Administration

- Paul Wood
- Tracy Crawford
- Jason Sun
- Amy Elliot
- Erin Laswell

UPMC Genome Center

- Ed Smith
- Dara Kozak
- Lyndsey Kelly
- Yinghong Pan

Single Cell Core

- Bob Lafyatis
- Nina Morse
- Tracy Tabib
- Heidi Monroe

Funding Sources:

Terry Dermody – Pediatrics

Adrian Lee – Institute for Precision Medicine

Anantha Shekhar - School of Medicine



University of Pittsburgh
School of Medicine

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UPMC Genome Center

<https://upmcgenomecenter.com/>

Single Cell Core

<https://singlecell.pitt.edu/>