SINGLE CELL DISCOVERIES

Single-cell sequencing for cell and gene therapy

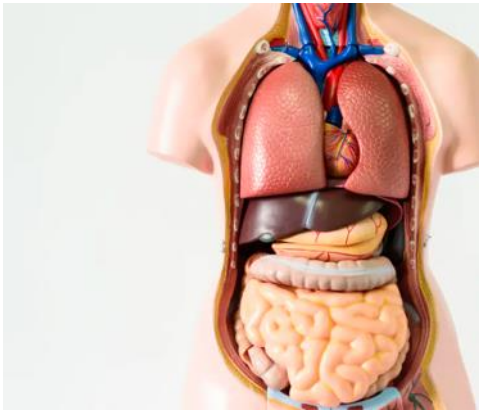
From vector design to product characterization

CGTP EUROPE, OCTOBER 23RD, 2025

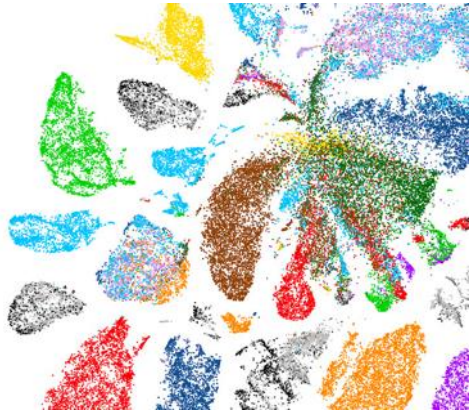
MAURO MURARO

CO-FOUNDER & CEO

Single-cell sequencing: biology at true resolution



Classical view



Single-cell view

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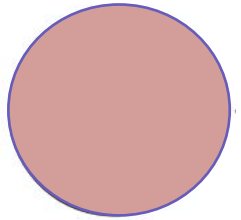
Why is a single-cell view important?



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Adapted from Shalek & Benson, *Sci. Transl. Med.* 2017

Single Cell Discoveries supports clients with end-to-end projects

- We've been developing (scRNA-)sequencing methods since **2012** and supporting clients since **2015**
- Team of 35 operating from **purpose-built labs and offices** of 1200 m^2 in Utrecht, the Netherlands
- We offer a combination of **services & assay development** for biotech, pharma, and universities globally.
- Several technologies invented by SCD team and **uniquely offered at SCD.**
- **Fast TaT** and **high quality**

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10x GENOMICS Certified Service Provider

REGISTERED scientist.com SUPPLIER

SCALE biosciences Service and Technology Endorsed Provider

BioSkryb GENOMICS Certified Service Provider

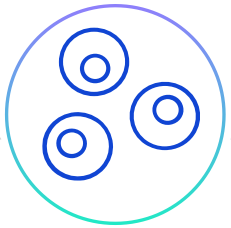
PARSE BIOSCIENCES CERTIFIED SERVICE PROVIDER

Different biological questions require different solutions

SORT & VASA-seq

FACS & plate-based

- Highly versatile, complex data (12K genes/cell)
- Total RNA and full length



scRNA seq high throughput

Up to Millions of cells

- *10x Genomics* 3' & 5', Flex, CITE-seq, V(D)J
- *ScaleBio* RNA seq, CRIPSR enrichment kit
- *Parsebio* Evercode WT

Bulk RNA seq

Ideal for compound screening

- Low input possible
- High-throughput Discovery-Seq
- 3' and full-length RNA

Spatial transcriptomics

Unbiased Whole-Transcriptome Mapping of Tissue Architecture

- *10x Genomics* Visium HD
- Integrated single-cell & spatial transcriptomics from the same sample

Single-Cell sequencing in drug development

Target Identification

- Find new targets by analyzing cell type-specific response to treatment.

Target Validation & Mechanism of Action

- By studying drug treatment and dose effects

Mechanism of Disease & Biomarker Discovery

- Identify diseased or non-responder cell states

Lead Identification & Optimization

- Measure transgene incorporation.

Preclinical

- Compare model organisms with patient samples.

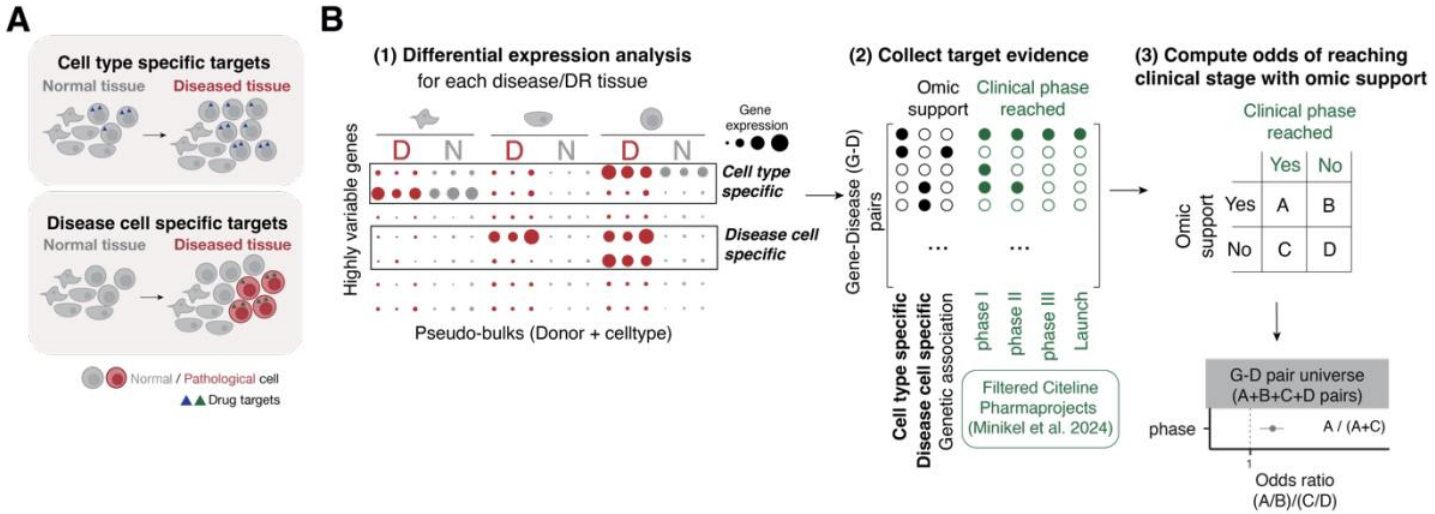
Clinical Trials

- Stratify responders and non-responders

Single-cell sequencing across the drug development pipeline

- It has historically been used for target identification, validation.
- Increasing use in biomarker discovery and exploratory clinical trials.
- Has specific use cases in Cell & Gene Therapy.
- Predicted to improve clinical trial success

Better chance to reach Phase III with single-cell data



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Dann et al, MedRxiv 2024 (Sanger, Cambridge, Stanford Ensocell, Scailyte, GSK, Sanofi)

Single-cell sequencing use cases in CAR-T therapy

Characterization

- Identify T-cell subtypes
- Quantify CAR heterogeneity
- Reveal mechanisms of potency, persistence, toxicity

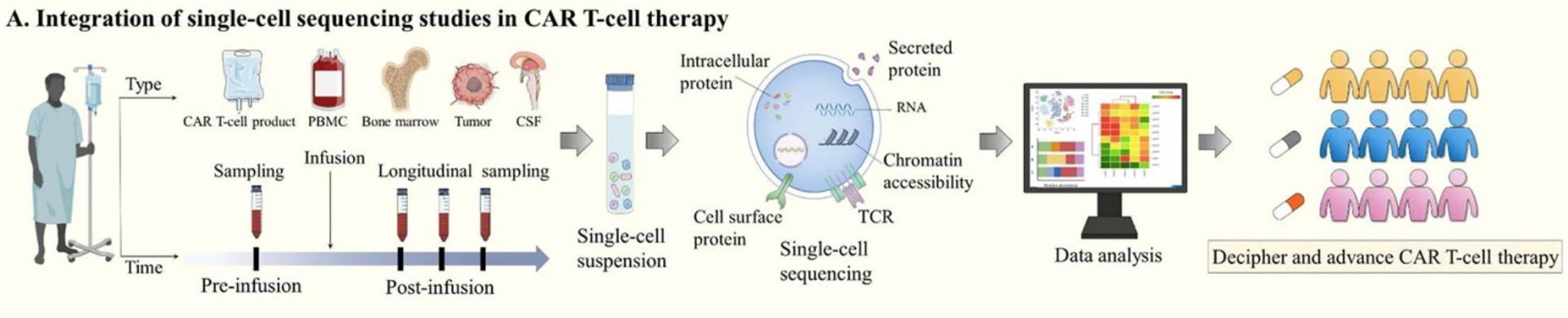
Development

- Validate construct and vector design
- Monitor T-cell state evolution during expansion
- Identify predictive efficacy & persistence markers

QC & Batch Release

- Assess lot-to-lot consistency & composition
- Detect rare undesired populations
- Build reference single-cell batch fingerprints

Huang et al. Mol Can. 2023



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Using scRNA-seq to find predictors of long persistence.

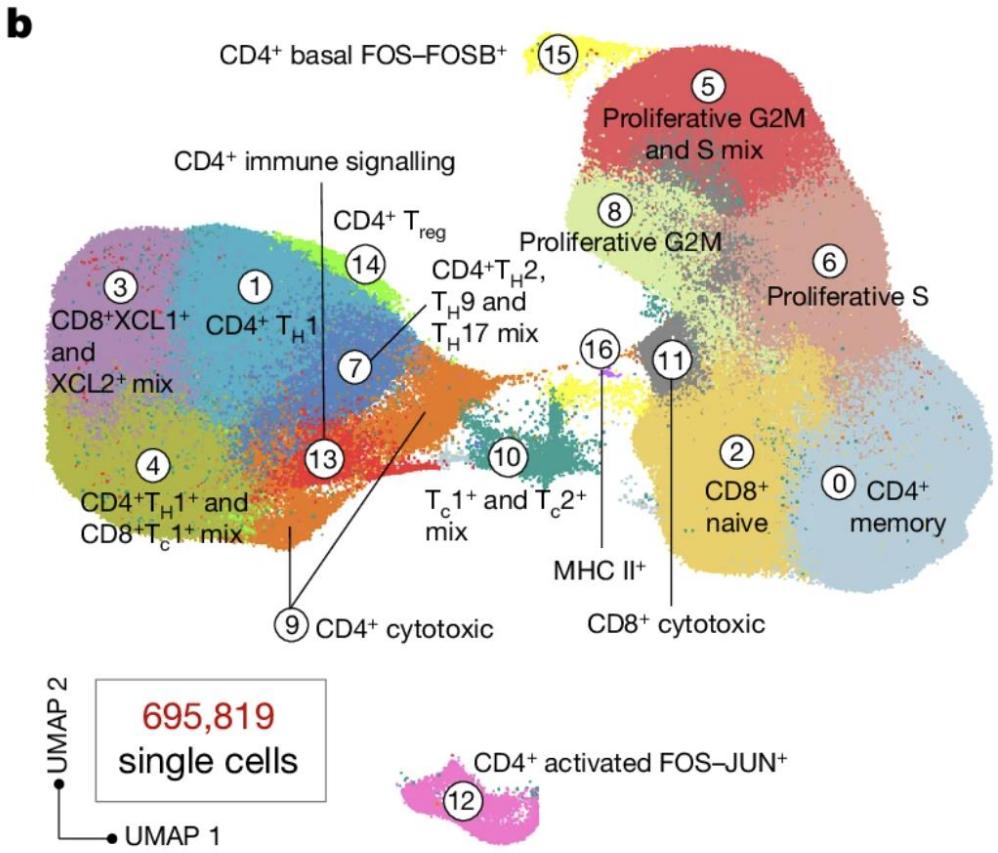
Article

Single-cell CAR T atlas reveals type 2 function in 8-year leukaemia remission

https://doi.org/10.1038/s41586-024-07762-w
Received: 28 May 2023
Accepted: 27 June 2024
Published online: 25 September 2024
Open access

Zhiliang Bai[1,16], Bing Feng[2,3,16], Susan E. McClory[4,5], Beatriz Coutinho de Oliveira[6], Caroline Diorio[4,5], Céline Gregoire[6], Bo Tao[7], Luojia Yang[8], Ziran Zhao[6], Lei Peng[8], Giacomo Sferruzza[8], Liqun Zhou[8], Xiaolei Zhou[2,3], Jessica Kerr[6], Alev Baysoy[1], Graham Su[1], Mingyu Yang[1], Pablo G. Camara[9], Sidi Chen[8], Li Tang[2,3] ✉, Carl H. June[10,11,12] ✉, J. Joseph Melenhorst[6] ✉, Stephan A. Grupp[4,5] ✉ & Rong Fan[1,7,13,14,15] ✉

- Large study of 700K infusion-product CAR-T cells from 82 pediatric ALL patients.
- Identify functional signature in the infusion product is associated with ultra-long persistence
- adding IL-4 during manufacturing improved fitness in in-vivo models.



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scRNA-seq use cases in Gene Therapy

Vector Design & Tropism

- Screen variants for cell-type tropism
- Detect off-target transduction
- Combine RNA + DNA to quantify vector integration and expression

Dose & Toxicity Assessment

- Map biodistribution across tissues
- Profile immune responses at single-cell resolution
- Detect off-target edits via single-cell DNA/RNA sequencing

QC & Batch Release

- Link transduction profiles to potency outcomes
- Detect residual producer or off-target cell types
- Support regulatory comparability and release documentation

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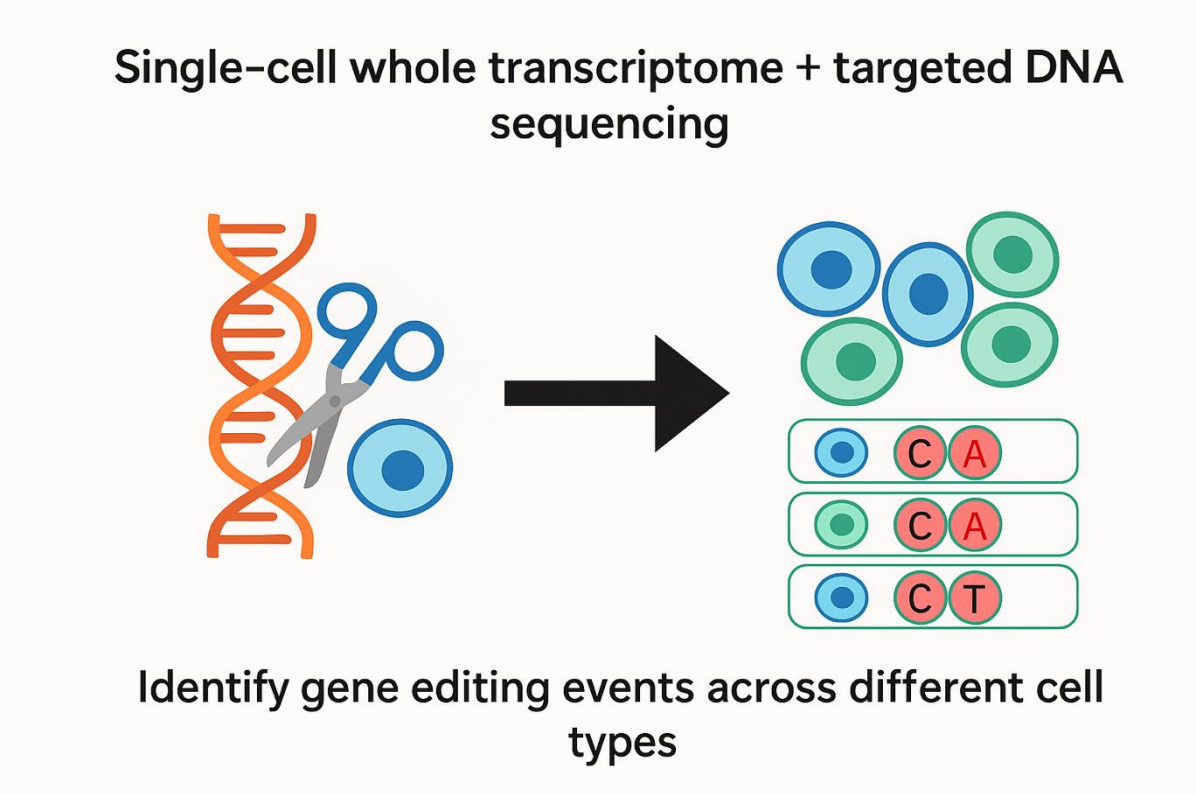
Case study 1: Exclude gene editing in germline

- Gene editing tested in mice & NHP
- What is % of affected cells in each cell type?
- Is germline (ovary cells) excluded?
- Custom transcriptome + targeted DNA sequencing to map gene editing events across cell types

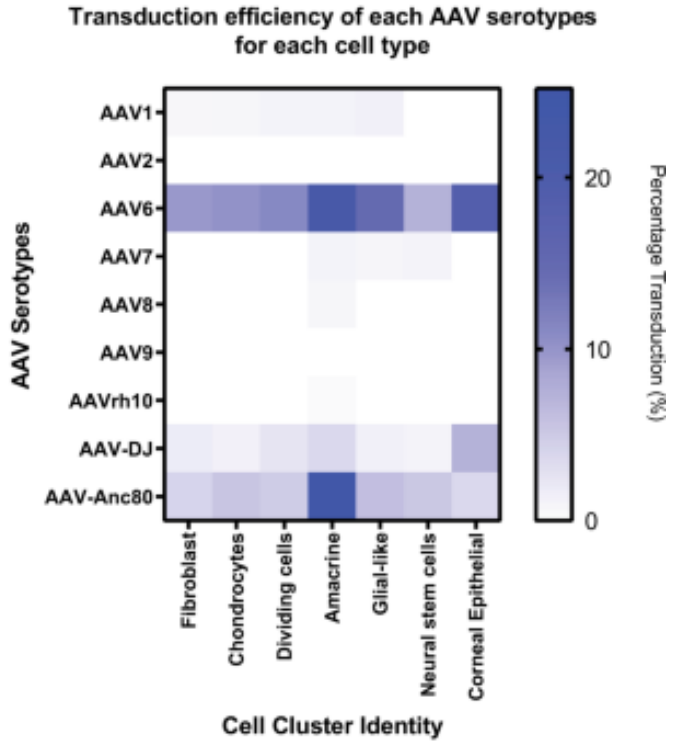


Case study 2: AAV lead optimization in ocular & brain tissue

In what cell types are AAV transgenes expressed?

- Map viral vectors across cell types in the brain
- Isolate nuclei from frozen rodent & NHP tissue
- Viral barcode + transcriptome from single cells
- Targeted amplification of lowly expressed transgenes

AAV distribution in ocular cells



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Keng, C.T., Guo, K., Liu, YC. et al. Multiplex viral tropism assay in complex cell populations with single-cell resolution. Gene Ther 29, 555–565 (2022). https://doi.org/10.1038/s41434-022-00360-3

The challenges using scRNA-seq from a regulatory standpoint

Data & Technical Complexity

- Zero inflation and dropouts
- Integration of different datasets hard
- High dimensionality: 20 samples = 4.8 Billion datapoints!

Process

- Cell dissociation / freezing alters profile
- High depth needed for rare events
- Batch effects reduce lot-to-lot comparability

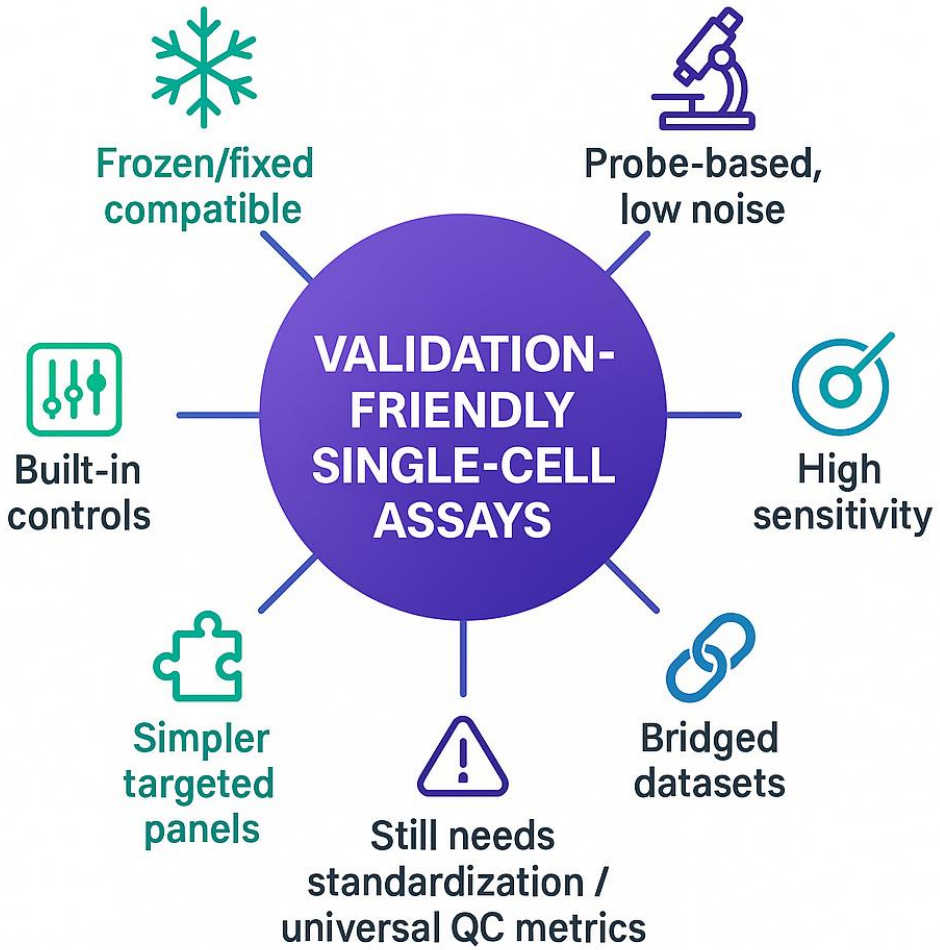
Regulatory & QC gaps

- No accepted, standardized QC
- Reproducibility and audit trails hard
- No standardized analysis pipelines or platforms – we don't even agree on what a cell type is

	CELL_000001	CELL_000002	CELL_000003	CELL_000004	CELL_000005	CELL_000006	C
ENSG00000272758	0	0	0	2	0	0	
ENSG00000154678	0	0	0	1	0	1	
ENSG00000148737	0	0	0	1	3	2	
ENSG00000196968	0	0	0	1	2	2	
ENSG00000134297	0	0	0	1	1	1	
...	...	...	...	...	...	...	
ENSG00000237289	0	1	0	0	0	0	
ENSG00000238098	0	0	0	0	1	0	
ENSG00000133433	0	0	0	0	0	1	
ENSG00000054219	1	0	0	0	0	0	
ENSG00000137691	0	3	0	0	0	0	

16468 rows × 902 columns

Key developments to make scRNA-seq go beyond discovery & development



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Summary & Outlook

- scRNA-seq is a rapidly growing and extremely powerful technology
- Mostly used in discovery & development
- This will change
- Assays are hard to validate & data is complex
- Adaptations are possible (targeted panels, built in controls)
- scRNA-seq will **complement** existing assays, not replace them.

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When to consider scRNA-seq

- When products are variable from batch to batch due to cell type composition.
- When small subpopulations or rare events can cause failure.
- To understand off-target effects.
- When MoA of different treatment outcomes is still unclear

Questions?
Reach out!
m.muraro@scdiscoveries.com

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Why **Work With Us?**

Experienced service provider
Sequenced millions of cells & thousands of samples across 40 organisms.

Single-cell experts
35 PhD/MS-level scientists, 300 publications by our team in scRNA-seq.

Bespoke solutions
Only CRO dedicated to single-cell and transcriptomics services & methods development.

Fit-for-purpose technologies
Proprietary VASA, SORT, Biodistribution, and Discovery-seq solutions. Certified provider for 10x, Parse Bio, Scale Bio and Bioskryb

Trusted Industry Leaders
Core group of scientists working on single-cell sequencing for 10 years.

Data analysis and integrity
Nextflow tower, HPC and AWS integrated pipelines for rapid analysis. Data consulting to support discovery.

End-to-end workflow
From experimental design to sequencing and exploratory data analysis, we think along with you to provide solutions to your research questions.

Highly accurate and reliable
A quality control environment characterized by meticulous attention to detail and outstanding Q30 and client satisfaction scores.

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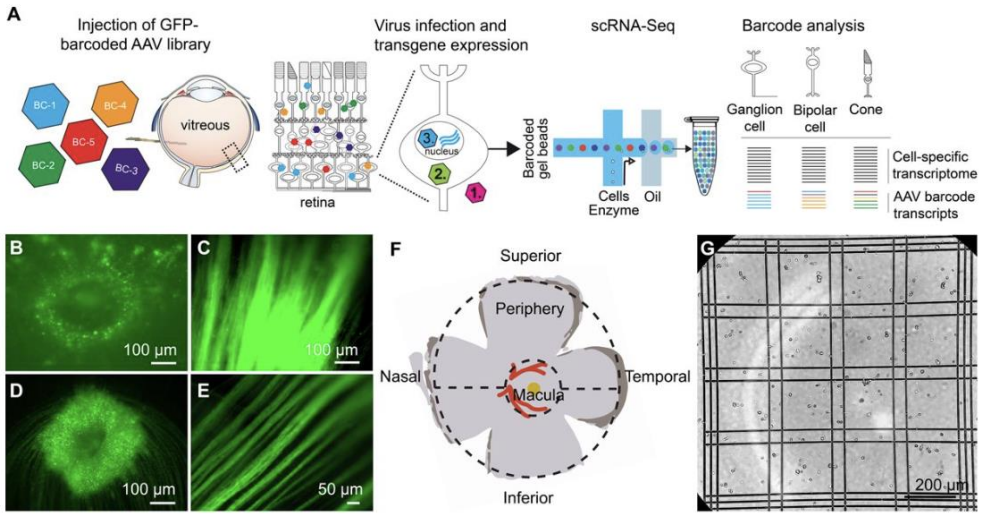
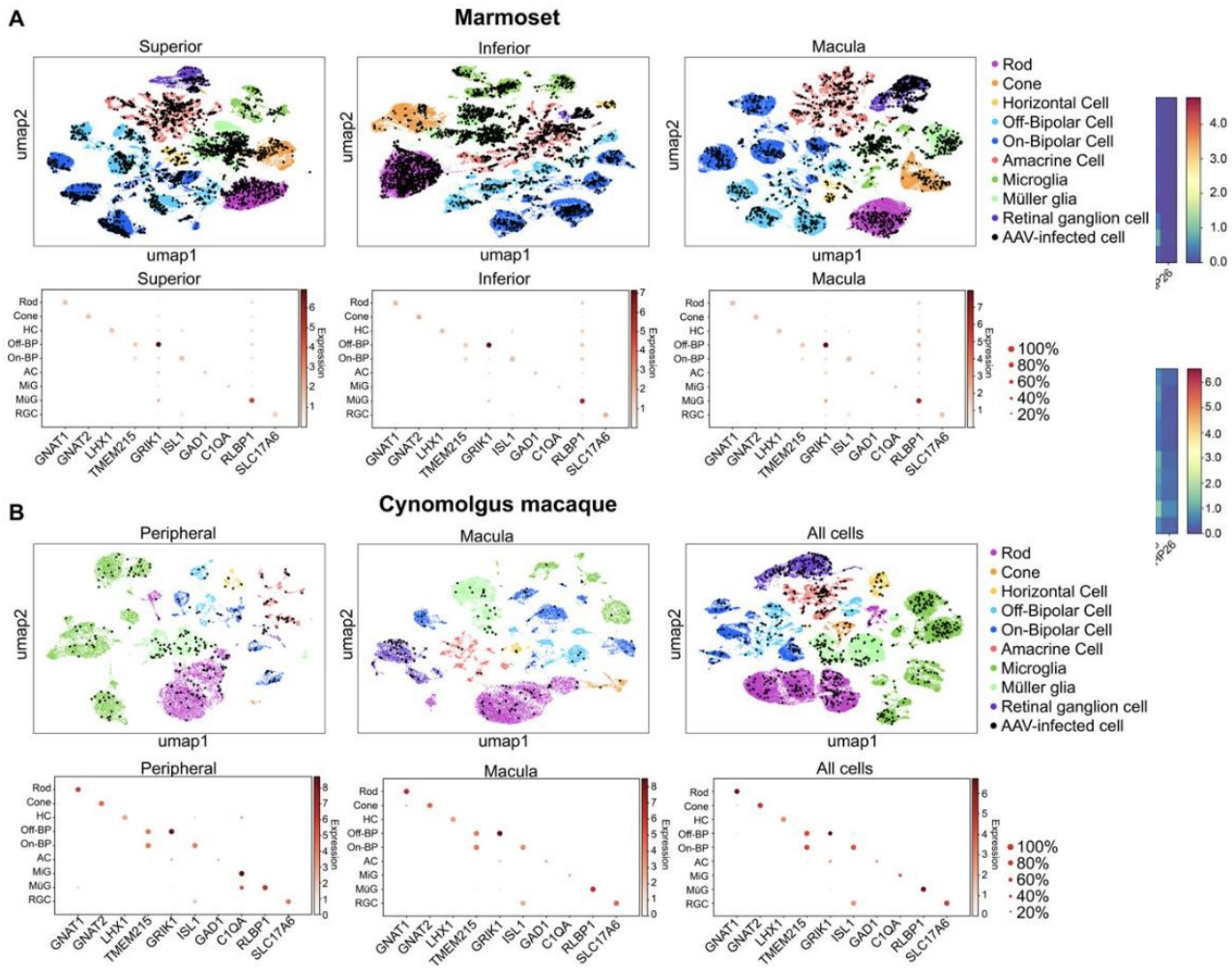
AAV Capsid design





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Ozturk et al. Elife; 2021

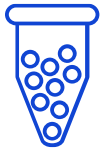
How our assays work together to speed up your research

Biological question

1

Profile drug response across treatments

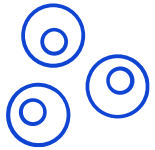
Discovery-seq Bulk RNA-seq



2

Discovery-seq/ RNA-seq to screen hundreds to thousands of conditions

ScRNA-seq high-throughput



3

Analyze dozen samples (100.000 cells/sample)

Data Analysis

4

Clustering analysis to find new cell type / gene signature

Targeted enrichment

5

FACS enrichment of cell population or targeted amplification of gene(s) in question

VASA-seq ScRNA-seq

6

Profile subpopulation with VASA-seq to discover new targets and MoA

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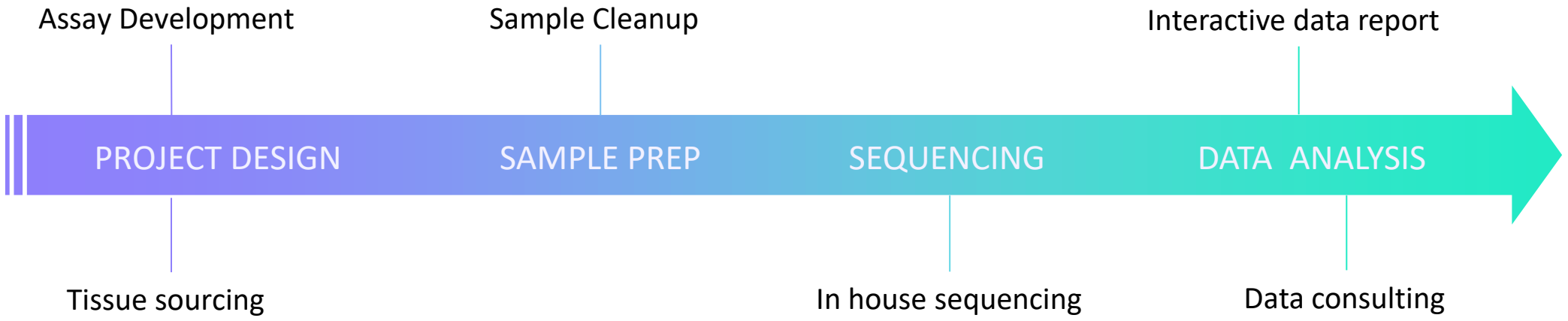
How we work

Expertise since inception

Tailored innovations

Trusted Partner



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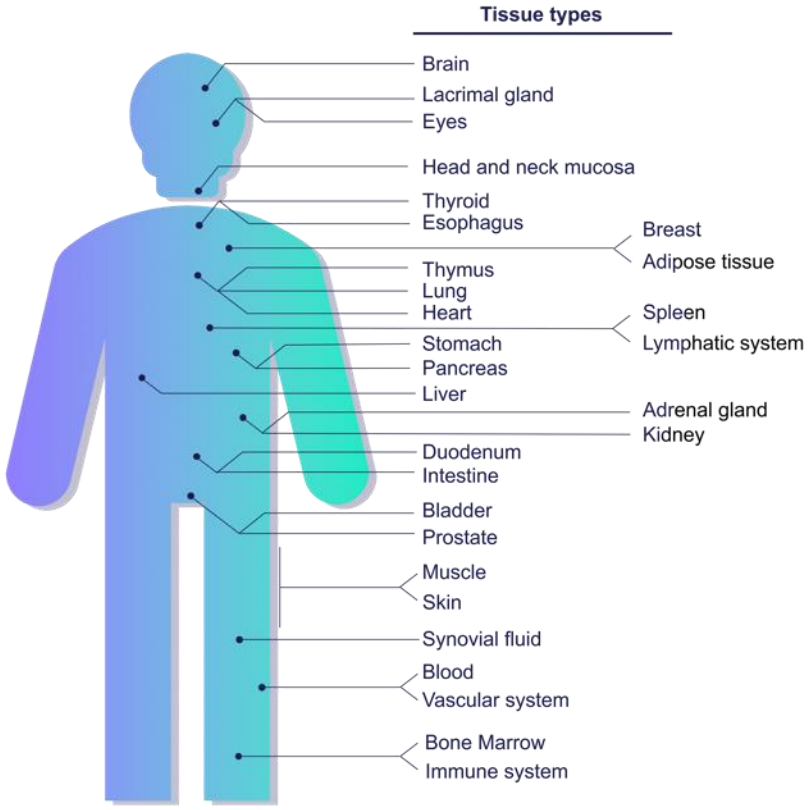
Experience with a variety of sample & tissue types

Tissue types



Sample types

- Over 30 tissues & 40 organisms
- Including primary tumors, organoids & brain
- We can work from snap-frozen or fixed cells or tissue

Xenografts	**Virus**	**Primary tissue**	**Cell cultures**	**iPSCs**
Tumor samples	**Cardio myocytes**	**Organoids**	**Gastruloids**	**Single nuclei**

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