

Customer results: Scaling single cell RNA sequencing organoid studies.

Benefits

- Single cell resolution in each organoid
 - Load 500–60,000 cells per HIVE CLX™ collector¹
 - No specialized instruments needed
- Store organoid suspensions before sequencing
 - No sample damage from cryopreservation²
 - Align endpoints for staggered cultures
- Minimize technical variability
 - Process all samples on the same day
 - Extend your budget & collect more data

Complete the whole picture for organoid biology


- Cutting edge transcriptomics data supports immunostaining, functional assays, etc.
- Capture cell variability between organoids

Single cell sequencing can examine biological variability between organoids

Organoids connect different cell types in a three-dimensional network that resembles the source organ. Brain organoids generally contain neurons and glial cells but can be tuned to model specific brain regions to capture cell dynamics with sufficient biological complexity to study neuroinflammation & other brain diseases. The HIVE Single Cell RNA-seq solution helps researchers scale organoid studies to tease out biological diversity from technical variability.

Researchers at the AIBN (Australian Institute for Bioengineering & Nanotechnology, University of Queensland) chose the HIVE™ Single Cell Solution to analyze transcriptomes of different cell types within brain organoids. Across months of culture, they processed organoids & froze cell-loaded HIVEs collectors to consolidate their RNAseq, reducing technical variability in their data & immediately yielding growth trajectories for their models.

For research use only. Not for use in diagnostic procedures.



Study parameters

Brain organoid production

- Cultured organoids at the Wolvetang Lab at the AIBN³
- Harvested four organoids (Org A-D) after 50 days
 - Imaged via brightfield microscopy
 - Dissociated with papain for 25 minutes⁴
 - Measured viability via flow cytometry (DRAQ7)⁵

HIVE processing and single cell RNAseq

- Loaded 30,000 live cells with the HIVE V1 protocol⁶
- Stored HIVE collectors at -20°C for ~20 days to batch library prep
- Sequenced on a NextSeq 2000⁷ with 300M reads/HIVE
- Data analyzed by the Mar Group at the AIBN⁸

The HIVE reveals organoid consistency

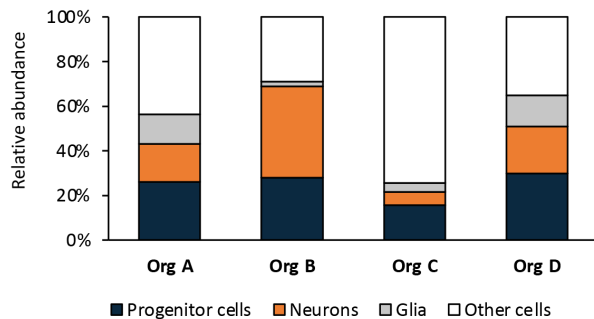
Success right away, for every organoid

- Harvested single cells from Org A-D with >95% viability
- Sequenced ~6,000 high quality cells/organoid
- <10% mitochondrial reads in all cases

New discoveries in brain organoid consistency

- Single organoids can be sequenced without pooling
- Biological replicates of organoids cluster consistently
- All four brain organoid samples share similar levels of neural progenitors, neurons, & glia

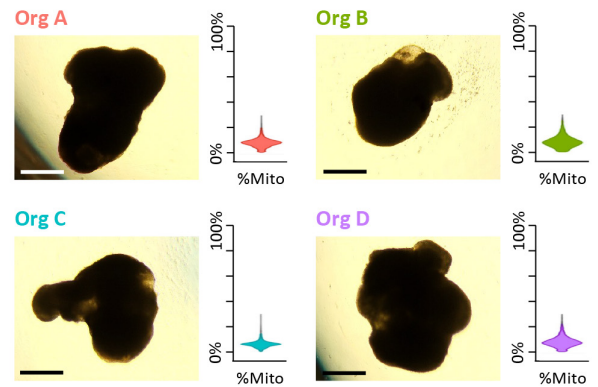
Variability of cell types between brain organoids



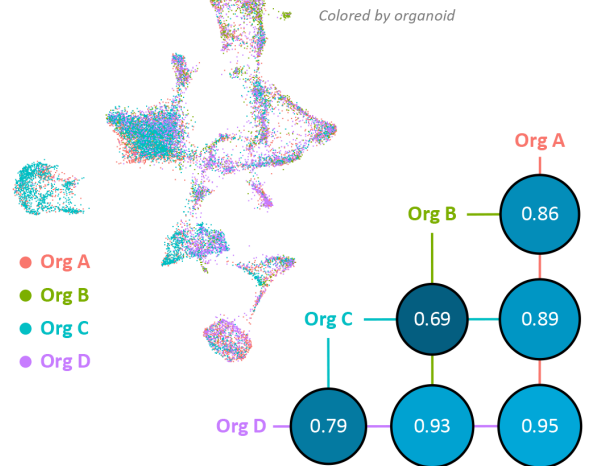
Organoid characterization

| Sample | Size (mm) [†] | Viability | Mito Reads [‡] |
|--------|------------------------|-----------|-------------------------|
| Org A | 2.0-3.2 | 97.5% | 8.0% |
| Org B | 2.2-3.2 | 97.8% | 7.9% |
| Org C | 1.8-2.6 | 95.6% | 6.0% |
| Org D | 2.5-3.1 | 98.1% | 7.1% |

†Measured via particle analysis (ellipsoid fit) in ImageJ
 ‡Reported median number of mitochondrial reads



UMAP plots



Correlation plots for gene expression

Data include 500 highly variable genes expressed by all organoids

References

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5. PhenoVue™ DRAQ7™ Dead Cell Nuclear Stain—Overview. Revvity, Inc. <https://www.perkinelmer.com/product/phenovue-draq7-1000-cp171>
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8. Mar Group. Australian Institute for Bioengineering & Nanotechnology, University of Queensland. <https://aibn.uq.edu.au/mar>



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