



**Earlham
Institute**

Decoding Living Systems

**Vizgen
MERSCOPE™**

at the Earlham Institute

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The Vizgen MERSCOPE™ platform

The Vizgen MERSCOPE instrument uses the MERFISH technology, which is based on smFISH probe detection of RNA transcripts.

This is an imaging readout as opposed to other platforms which generate sequencing-type data. Unlike many spatial platforms currently available, the MERSCOPE enables the analysis of tissues from species other than mouse or human, with the additional benefit of resolution to a subcellular level.

We often work with non-model organisms, which is one of the reasons we chose the MERSCOPE platform.

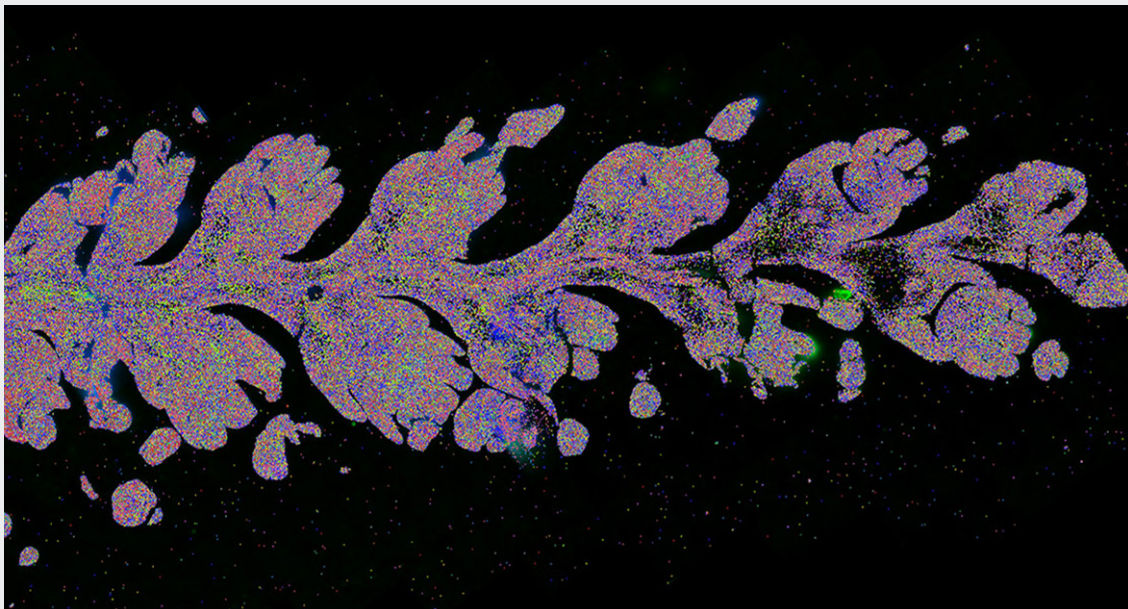
Each sample slide has a total imaging area of 1 cm². Multiple small samples can be placed within this region, allowing for replicates to be analysed on a single slide, optimizing both cost and efficiency.

The Earlham Institute is a **Certified Service Provider** for the MERSCOPE in situ Spatial Transcriptomics Workflow.

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Verification kits

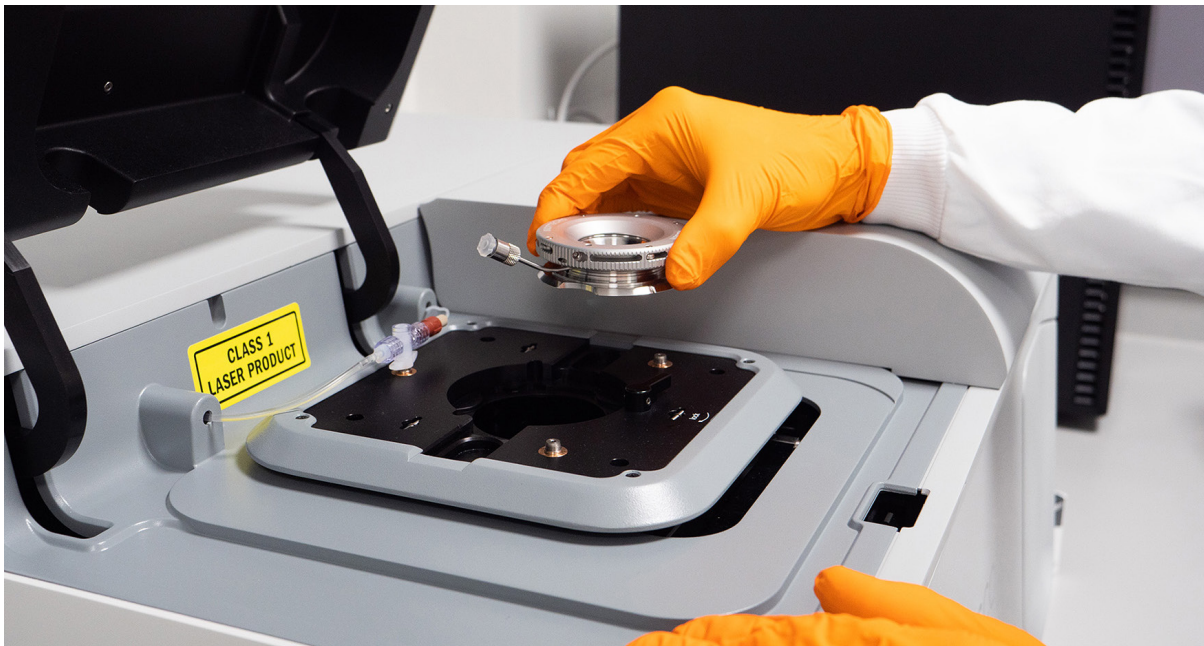
Verification kit workflows are used to be sure that the sectioned samples will work with the technology, before the purchase or use of the far-more expensive gene panels.

For new samples to the Earlham Institute, we will always factor verification into timescales and pricing as part of the project plan. Pre-made verification kits for mouse and human exist and can be purchased (and delivered) quickly from Vizgen.

The gene used in pre-made kits are housekeeping genes Elongation factor 2 (EEF2) in Homo sapiens and Elongation factor 2 (Eef2) in Mus musculus. The verification kit gene should be expressed highly in all cell types.

Custom verification kits are possible and can be produced by discussing this with the Vizgen sales team. Verification kits for protein co-detection are also available and will be required before progressing to full probe runs.

Please note: custom verification kits can take up to 4 weeks from design to delivery from Vizgen.



Gene panels and protein detection

Vizgen requires the use of gene panels to detect the genes of interest in your samples type.

Often, these panels are customised per project. Customised panels come with volume enough for 20 slide preparations.

We suggest that you consider this number as 15 slides to account for failed runs or sub-optimal tissue adherence issues.

Vizgen also produce predesigned 500 gene murine PanNeuro panel, human PanCancer and Immuno-Oncology panels which are cheaper than custom panels - <https://vizgen.com/predesigned-gene-panels> - and can be purchased in smaller volumes (for 4 samples for fixed or fresh frozen, 2 for FFPE or larger panels which accommodate 12 samples for fixed or fresh frozen, 6 for FFPE samples).

Gene panels can be different sizes: 140, 300, 500, 960. A small proportion of the panel numbers (20-40) are set aside as blank probes (controls). Customised protein detection kits are also possible for up to 6 proteins which are co-detected, 9 for plant users.

Gene panel design is processed through the Vizgen team. They will be able to utilise data such as RNASeq or scRNASeq, from the same tissue.

Expression data should be roughly balanced but important, highly expressing genes can use the auxillary channels. You should be aware that the more of these you have, the less proteins you are able to detect, as they are read using the same channels. Genes and proteins detected using the auxillary channels cannot be quantified. Detection will appear as more of a stain.

Gene panels do not need to be specific to one species or tissue type. Single cell data is not essential. There are advantages of having single cell data, as this will provide expression data per cell type and cell typing markers.

Probes have a requirement of a unique 200bp region. This means that most differential isoform usage studies are not an option using this technology. Generally, a list of gene names with their associated FPKM values as well as a transcriptome or genome reference is needed to order a panel.

Please note: panels take around **10 weeks** from design submission to delivery from Vizgen.



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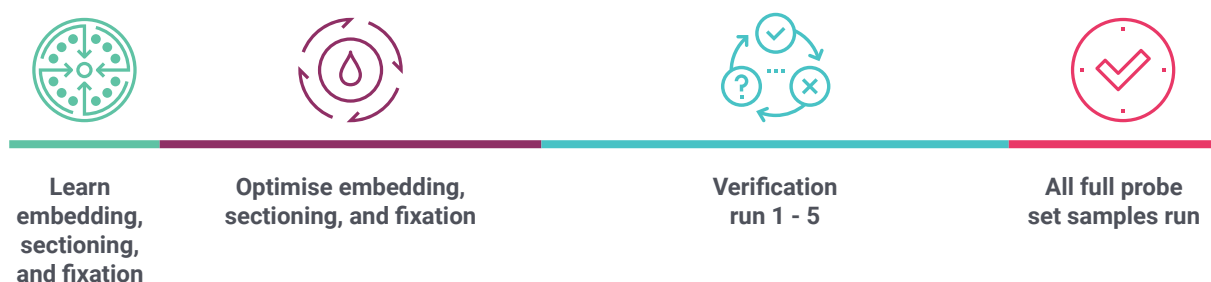


Cell boundary staining

Cell boundary staining kits come as standard with all Vizgen slide preparation kits.

They target 3 membrane components in mammalian cells. This is used for cell segmentation, required for single cell analysis. Cell boundary kits cannot be used on plant, fungi, or central nervous system cells. Cell segmentation is still possible using only DAPI nuclei staining, which is a standard.

The stages we would need to go through to process a new sample:



Sectioning thickness and general guidelines

The thickness of your section should be dictated by the diameter of the smallest cells in your tissue. We want to avoid overlapping or stacking of cells.

The MERSCOPE can accept sections of between 3-12um for fresh frozen or fixed frozen samples, 4-5um for FFPE samples. Sections should be well adhered in all regions of your tissue. Any areas that are not well adhered, cannot be focussed by the instrument resulting in loss of probe spot detection or blurriness of DAPI staining.

Sectioning guidelines for Vizgen samples can be found in MERSCOPE Resource Guide Histology Guide for Preparing Fresh and Fixed Frozen Tissue Samples for Experiments on the MERSCOPE® Platform Doc 91600129 Rev A and also MERSCOPE® User Guide Fresh and Fixed Frozen Tissue Sample Preparation 91600002 Rev E.

All Vizgen documents can be found here: <https://portal.vizgen.com/resources/user-guides>

FFPE sectioning guidelines can be found in MERSCOPE® User Guide Formalin-Fixed Paraffin-Embedded Tissue Sample Preparation 91600112 • Rev C.

Sample optimisation

If you are performing the sectioning yourself, this stage may be performed by you.

The tissue you would like to analyse will need to undergo protocol optimisation prior to full analysis using verification slides. This optimisation would normally involve the decision of whether the tissue requires fixation, getting the embedding right and practising sectioning.

Once this is good enough, the next stage we ask is that sections are used as input for RNA extractions.

We are looking for good length RNA fragment sizes therefore the RNA will need to be run on a gel-based QC platform for assessment. Please bear in mind, if you are planning to run tissue sections from multiple tissues types, each tissue type will need to be optimised for both sectioning, as well as the RNA quality. Once these checks are passed, we can proceed to verification.

A big issue with Vizgen MERSCOPE runs is tissue adherence. This needs to be optimised before verification kits are run. You should try this on a Vizgen slide to be sure that it will work on a run. Areas of lifting will cause out of focus regions where staining and probe spots cannot be resolved.

Verification

The verification slides use a single FISH probe to enable cost effective optimisation of tissue clearing, autofluorescence reduction and probe hybridisation so that the very expensive full gene panel runs have the best chance of success. Verification kits are also available for protein detection.

Once we are happy with the results of a verification run, we are able to proceed to full probe runs. This may require five verification runs, especially if the sample is tricky. Five samples should be factored into costings and timescales.

Full probe runs take longer to run, and will take significant time depending on the number of slides required by the users experimental needs. Generally, the hope is that if the verification kit has worked, which uses almost the same technology, the full probe runs should scale up.

Timescales

The instrument schedule can become quite busy, but we will provide an update on the next expected availability during the initial call.

Preparation of the verification slide takes approximately one week, from the sectioned slide stage to running and QC assessment. At this point, we will review our findings with you and provide feedback. This discussion will include our recommendations for further verification tests or confirmation of readiness to proceed with full probe runs.

Full probe runs take one week to prepare for loading. The data processing and transfers take a little longer. We suggest in total this comes to 1-2 weeks. Each slide is prepared and run individually, therefore, please account for this timescale with regard to the number of slides requested. We will aim to contact you as to how well the run performed.

Each gene panel purchased from Vizgen is enough for 20 slides but in reality, we suggest that we will only process 15 per gene panel as we need to account for failed runs, or samples.

We therefore ask a secondary gene panel is required for slide numbers greater than 15.

Data

Raw files constitute a significant portion of the total data generated by the Vizgen MERSCOPE platform. There is ongoing debate about whether retaining these raw files for each project is necessary or if they can be deleted after processing.

To ensure seamless data transfer, we require you to have adequate storage space available to accommodate all project files, including the sizable raw files.

Verification runs amass around 2Tb including raw files. Please allow for storage of 5 of these runs =12Tb.

Full probe runs can generate about 8Tb for a 300 gene panel run, this will be more for a 500 or 960 gene panel. Please allow for storage for your multiples of slide runs.

Large data storage at the Earlham Institute will accrue a significant cost over a short amount of time, therefore quick transfer is important.



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Data Visualisation software and post-processing

Vizgen use a software called Vizualizer which allows users to explore each area of the tissue as a microscopy image. It has many features such as the selection of the different probes to be visible or not, optimisation of staining etc.

It uses MERSCOPE generated files called VZGs which are mosaiced, stacked, zipped images.

It can be exported to files called HDM5s which are compatible with many downstream analyses such as Seurat or SCAMPI.

A note for plant and non-model sample type users: Without cell boundary staining, plant users are required to perform a python-based processing called Vizgen Post Processing in order to segment the image into cells.

For details, please see this link: <https://vizgen.com/vpt/>

The screenshot displays the Vizgen software interface. The main window shows a microscopy image of a plant tissue section with a red dashed line outlining a selected region. The interface includes a control panel on the right with the following elements:

- Visible Intensity Range: 1557-65535
- Color selection buttons: PolyT (white) and DAPI (dark blue)
- Total area: 98.36 mm²
- Region 3: 98.36 mm² (with a trash icon)

At the bottom of the image, there is a "Next" button. A status bar at the bottom of the image reads: "Region 3 is selected. Hold&Drag dots to change it location or start to draw a new region. Hide Done".

Pricing indications

The tables below provide a cost breakdown for custom gene panels based on panel size (140, 300, 500, or 960 genes/probes) and whether you opt to include Cell Boundary Stain Kits and Protein Stain Kits.

We ask customers to independently purchase their custom gene panels as well as the Cell Boundary and Protein Stain Kits (if these are included) from Vizgen directly, as they are specific to each project. Additionally, if your tissue is not from mouse or human, we require the purchase of a Custom Verification Kit.

The costs for “Verification Preps” and “Full Probe Preps” can be divided by the number of slides being run. For example:

- Running 3 verification slides incurs a prep cost of £3014.98 (£1004.99 x 3).
- Running 10 full probe slides incurs a prep cost of £38,495.95 (£3849.60 x 10).

Verification Slides		
Variable	Tissue sectioning optimisation (per tissue type)	£200.00
Variable	5 verification preps (variable, requirement to be agreed)	£5,024.97
Choose one	Custom Verification kit (5 samples worth) <i>Customer to purchase</i>	£670.00
	Sample Verification Kit (Human, 5 samples worth)	£450.00
	Sample Verification Kit (Mouse, 5 samples worth)	£450.00

Full gene panel slides		
Variable	20 full probe preps (experimental design requirement)	£76,991.90
Choose one <i>Customer to purchase</i>	Customised 140 Probe panel (20 samples worth)	£6,760.00
	Customised 300 Probe panel (20 samples worth)	£9,470.00
	Customised 500 Probe panel (20 samples worth)	£12,000.00
	Customised 1,000 Probe panel (20 samples worth)	£14,900.00
Optional	Cell Bounday Stain Kit	£3,480.00
Optional	Protein stain kits (per species or antibody stain)	£1,890.00

Prices are for fresh frozen tissue sections, additional costs are associated with FFPE sections.



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Example project

Based on: verification and 300 gene panel runs of Axolotl brain sections using 300 custom gene panel

Verification of 4 slides for non-model tissues		
Customer sectioned tissues (preferred option)	Tissue sectioning optimisation	£0.00
4 verification slides	4 verification preps	£4,019.98
4 verification slides	Custom verification kit (5 samples)	£670.00
Verification cost		£4,689.98

Full gene panel 8 slides		
Custom 300 probe runs	8 full probe preps	£30,031.52
<i>Customer to purchase</i>	Customised 300 Probe panel (20 samples worth)	£9,470.00
Full probe runs cost		£39,501.52

Total project cost	£44,191.50
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Earlham Institute

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vizgen

Earlham Institute
Norwich Research Park
Colney Lane
Norwich
Norfolk
NR4 7UZ

earlham.ac.uk
+44 (0) 1603 450 001

single-cell.enquiries@earlham.ac.uk



YouTube