REBUS BIOSYSTEMS™

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ESPER™ HIGH FIDELITY ASSAY

Multiplexed, quantitative gene expression analysis with subcellular resolution

Every transcript, every cell

The first assay developed for the REBUS ESPER™ spatial omics platform is the ESPER™ High Fidelity assay. It is based on cyclic single-molecule FISH chemistry and optimized for the Rebus Esper fluidics and imaging system.

A high-level of multiplexing is achieved without the use of barcoding or enzymatic amplification. A probe tiling strategy ensures signal is seen only from true targets.

- Up to 30 custom genes
- High sensitivity
- High specificity
- No barcoding
- No enzymatic amplification
- Access cytoplasm and nucleus

Whether the target is an mRNA molecule in the cytoplasm or a long non-coding RNA or nascent RNA within cell nucleus, the Esper High Fidelity assay shows consistent detection capability.

This assay is ideal for scientists who have done early, discovery-focused experiments who now need to validate their data and further refine their hypotheses.

High specificity and sensitivity, combined with the resolution and throughput of the Rebus Esper, uniquely position this assay for detection of critical biomarkers and gene signatures, especially in relatively rare cells.

Robust, reliable smFISH

The Esper High Fidelity assay is based on optimized cyclic single molecule fluorescent in situ hybridization (smFISH) chemistry. All steps are handled automatically by the Rebus Esper instrument.

An optimized on-instrument tissue pretreatment protocol allows high-quality data to be acquired from samples regardless of endogenous background.

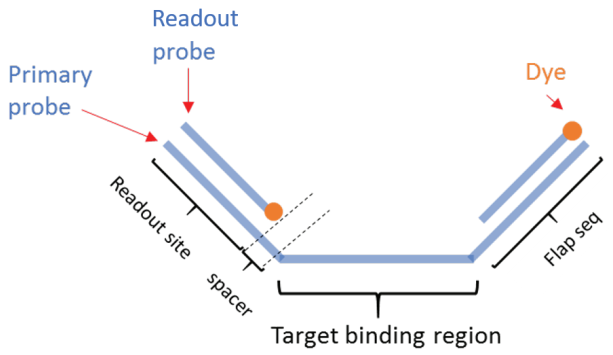
After pretreatment, specific primary probes are hybridized for each of up to 30 custom genes being analyzed. Multiple probes are used for each gene to ensure signal specificity.

During each subsequent cycle, fluorescently-labelled readout probes for three genes at a time are

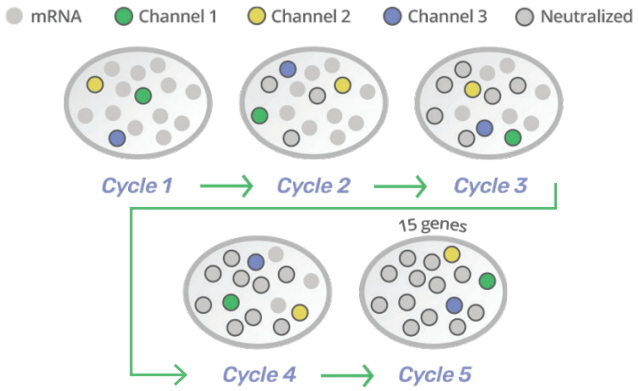
bound. The Esper High Fidelity assay primary probe architecture allows two readout probes to bind each primary probe.

Readout probes are imaged across the region of interest, and then inactivated. This process repeats until all genes in the experiment have been imaged.

Esper High Fidelity Probe Architecture



15 Gene Example (5 Cycles X 3 Genes)



Custom gene analysis

The Esper High Fidelity assay allows analysis of up to 30 custom genes. It can be used in any fresh frozen mouse or human tissue.

For each new experiment, you simply submit your gene list to Rebus Biosystems. The team will then design custom primary and readout probes for each gene and send you a ready-to-load Custom Probe Kit.

The total number of primary probes per gene will depend on the length of the common exon sequences for all isoforms of the target gene. The in-house developed algorithm ensures the number of probes and their sequences deliver optimal specificity.

The Custom Probe Kit along with the other Esper High Fidelity assay kits provide everything you need to use the Rebus Esper spatial omics platform.

Esper High Fidelity Assay Kits

- **Sample Preparation Reagent Kit**
  Fixation reagents, wash, and storage reagents
- **Flow Cell Assembly Kit**
  Pre-treated coverslips for tissue attachment, gasket, silicon top plate with laser drilled holes for reagent delivery
- **Custom Probe Kit**
  Primary and readout smFISH probes for analysis of up to 30 genes
- **Buffer Kit**
  Buffers for tissue pretreatment, hybridization, washes, DAPI staining, imaging, and signal removal

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Example: Precise spatial mapping with a small number of genes

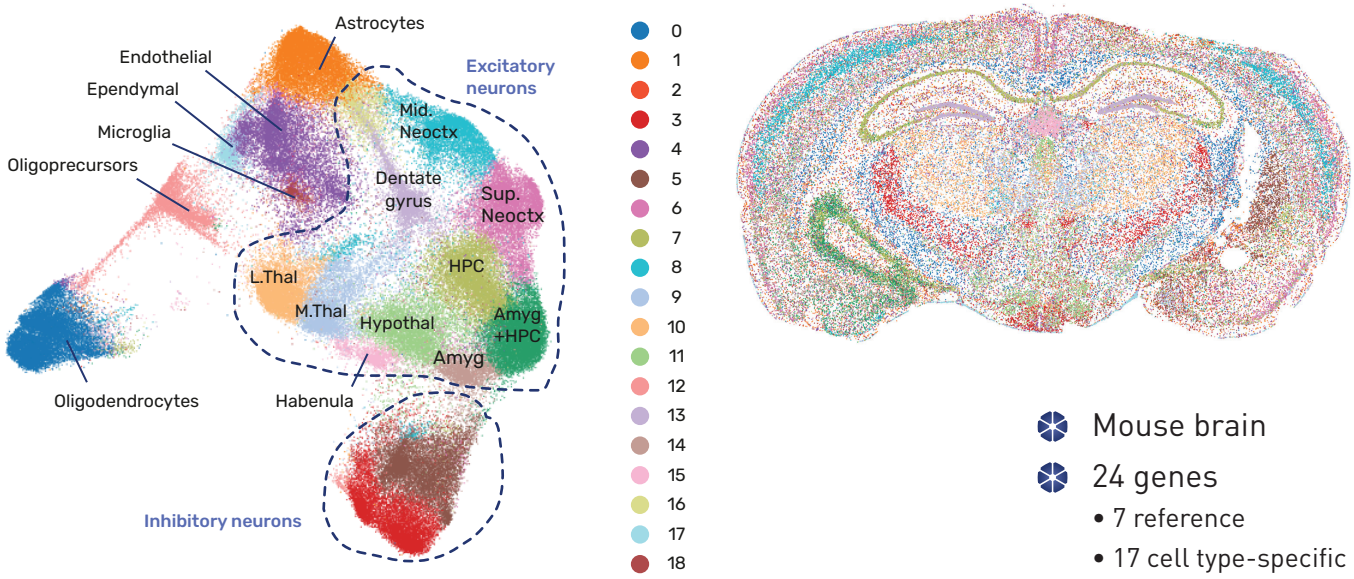
A coronal section of a 10-week-old mouse brain measuring about 50mm^2 was probed with a set of 24 genes - 7 reference and just 17 cell type-specific.

All major cell types of the mouse brain, both neuronal and non-neuronal, were identified. Unsupervised clustering using a Leiden algorithm and UMAP, produced the plot shown below on the left, where each color-coded cluster corresponds to a unique cell type.

When mapped back to X,Y space as shown on the right, these clusters clearly display the expected spatial organization and faithfully recapitulate the tissue architecture. Furthermore, the relative abundances of the different cell types precisely match previously published data.

Whereas other methods rely on a large number of genes detected with low sensitivity to type and map cells through imputation, the unmatched sensitivity of the Esper High Fidelity assay allows this level of precision with a small number of genes.

Cell Type Identification and Mapping



2255 Martin Avenue, Ste. F
Santa Clara, CA 95050 USA

www.rebusbio.com
info@rebusbio.com