

Automated single cell expression profiling in intact tissue by highly multiplexed in situ hybridization

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Technology description

PL-ISH is a technology for profiling the RNA molecules in isolated cells or in the single cells of an intact tissue specimen. It enables combinatorial, single molecule fluorescence in situ hybridization. It surpasses the capabilities of commercially available products such as RNAscope and ViewRNA because (i) the reagent costs are much lower, which enables high throughput experiments, (ii) many more RNA species can be queried simultaneously, and (iii) it offers an ultra high-throughput method for optimizing in situ hybridization probes.

A team of Stanford researchers has developed a highly sensitive, single molecule in situ hybridization system called Proximity Ligation- in situ hybridization (PLISH), which merges the specificity of proximity ligation, the sensitivity of probe tiling, and the high signal intensity of rolling circle amplification. PLISH enables efficient, high level multiplexing by iterative detect-image-erase cycles of four transcripts per round following a single hybridization step, with automated calculation of single cell expression profiles. Downstream data analysis can include clustering algorithms and t-SNE display, but unlike with tissue dissociative approaches for single cell profiling, spatial and morphological data is retained for each cell. Thus, PLISH enables spatial re-mapping of individual cells or cell classes back onto the tissue, allowing for inference of signaling centers, for instance, by localizing cells expressing ligands and cognate receptors. PLISH is highly versatile, performing well on archival FFPE (formalin fixed, paraffin embedded) or cryo-embedded tissue, and is compatible with conventional immunostaining for correlation of RNA and protein expression. PLISH is technically simple and inexpensive, employing conventional fluorescence microscopy and open source software, and can therefore be performed by any laboratory without requiring investment in new equipment. This technology can be used in combination with immunohistochemistry on primary tissue samples, including formalin-fixed and cryopreserved patient samples. Because PLISH is inexpensive and technically simple, it can be used by even non-expert biomedical researchers for in situ profiling to provide spatial context of newly-defined cell populations and reveal mechanistic aspects of human disease pathophysiology either alone or in conjunction with single-cell sequencing. In addition, it could potentially be adapted for diagnostics applications such as liquid biopsy or rare cell identification.

Multiplexed in situ analysis: Inexpensive and technically simple: Fast

Specific and sensitive detection: Compatible with

Application area

Low level combinatorial in situ hybridization (ISH) +/- immunostaining for:

research - co-stain for up to five transcripts and/or proteins in histological sections to identify and localize cell types of interest, correlate RNA (mRNA, non-coding RNA, etc.) and protein expression by cells, determine subcellular localization of RNAs of interest, integrate gene expression with morphologic and histologic features in tissue

In situ single cell expression profiling for:

research - validate single cell RNA-sequencing (sc-RNAseq) results, localize and map novel cell classes inferred from sc-RNAseq, empirically identify and map molecularly distinct cell sub-types, interrogate and map activity of entire signaling pathways in tissue by co-staining ligand and receptor families

diagnostics using primary patient samples with potential end user applications such as:

quality control and optimization of in vitro directed differentiation protocols of induced pluripotent

stem cells analysis of archived tissue - FFPE samples and legacy tissue bank samples of common or rare diseases identify and/or validate disease biomarkers based on specific tissue context

Advantages

unlimited capacity for multiplexing ISH with single hybridization step followed by detect-image-erase of 4 transcripts per cycle "on-demand" capacity to analyze any gene of interest, without requiring pre-existing antibodies or other reagents provides spatial context and localization of individual cells of interest in intact tissue

commodity oligonucleotides and enzymes (reagents cost ~\$1/gene vs. >\$1000 for conventional in situ kits) no specialized equipment - signal detection with conventional fluorescent microscope optimized probes reduce both background signal and the number of probes required for imaging

- single-step barcoding with rapid label-image-erase cycles and data collection

specificity from coincidence detection targets multiple sites in RNA sequence to detect low-abundance transcripts high signal-to-noise from enzymatic amplification renders tissue autofluorescence

inconsequential single molecule resolution with low false positive rate (less than 0.05 RNA counts per cell)

enables deep profiling with increasingly complex mixtures of hybridization probes

immunohistochemistry - can be performed concurrently archival FFPE tissue samples

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