

mutaFISH™

(mutation-specific Fluorescence *In Situ* Hybridization)

Single Cell, Single Molecule, DNA and RNA Mutation Detection at Single Nucleotide Resolution

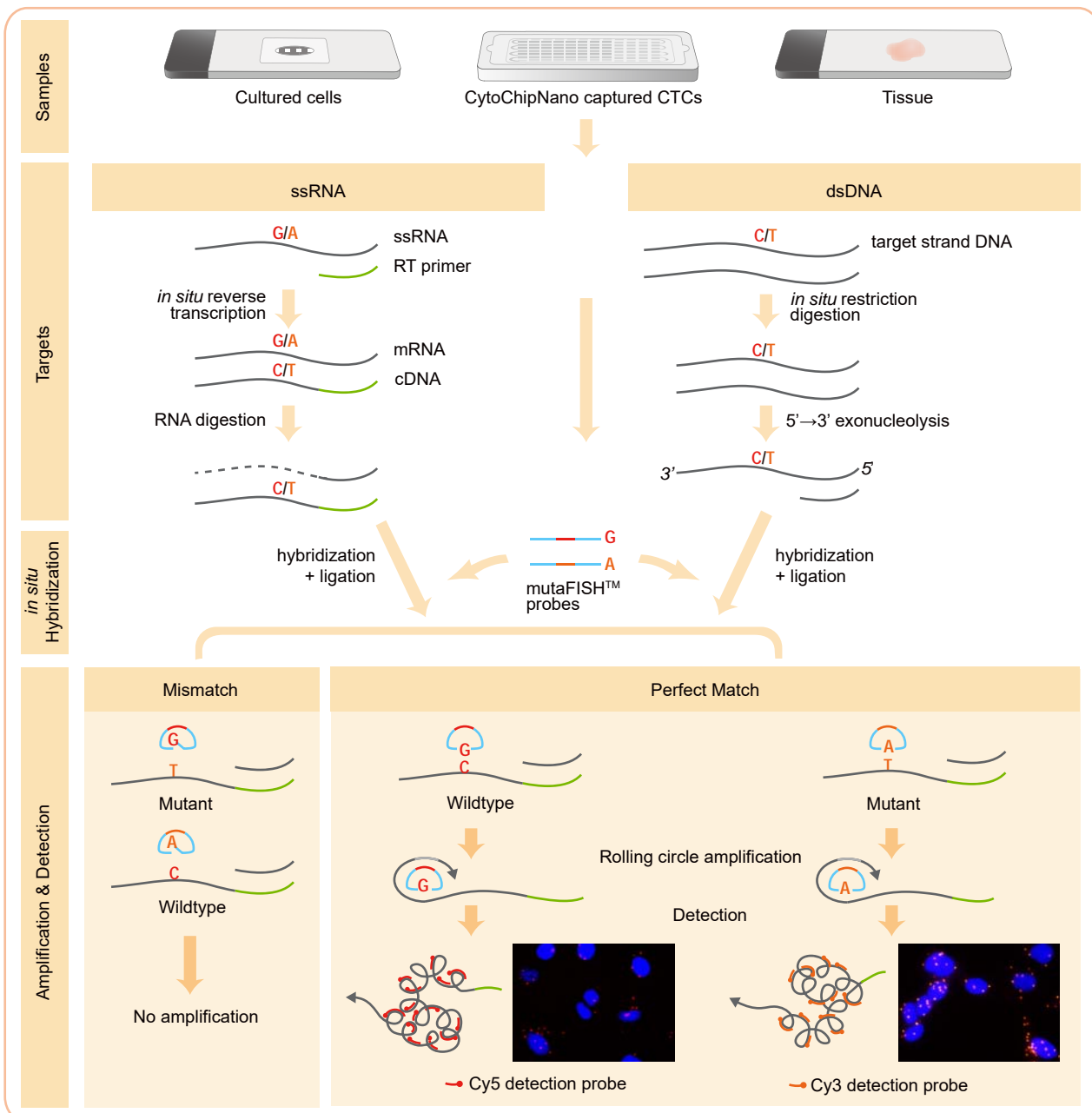


The ability to interrogate cellular heterogeneity at the single cell and single molecule levels has gained increased importance. Many bioreagents and techniques exist to address this issue separately. None has the unified approach to detect and quantify DNA and RNA changes across diverse spectrum particularly point mutations and single-nucleotide polymorphisms (SNP) while maintaining their spatial context. This challenge is even greater as different analytical platforms such as fluorescence, cyto/histochemistry, flow cytometry, and microfluidics are needed to study the complexity of biological states.

Abnova has integrated padlock probe and rolling circle amplification (RCA) for *in situ*, single cell, single molecule, DNA and RNA mutation detection at single nucleotide resolution, and obviated the technical challenges of PCR and hybridization optimization required for efficient and target-specific analysis. Abnova provides a growing portfolio of off-the-shelf, validated mutaFISH™ probes and accessory reagents to address the unmet needs in the research and clinical settings.

Technology

mutaFISH™ Workflow



Abnova mutaFISH™

Advantages

- Single Cell, Single Molecule Detection
- *In situ* Analysis of DNA & RNA
- Single Nucleotide Resolution
- Higher Sensitivity than dPCR and NGS
- Multiplexing Capability
- Cross Analytical Platforms
- No DNA or RNA Extraction

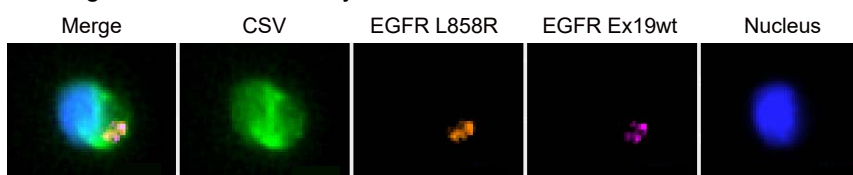
Applications

- Cell *In situ* Hybridization



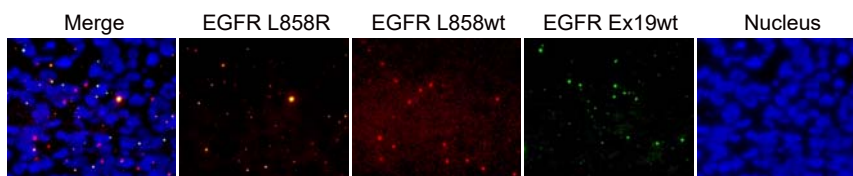
metaFISH™ staining was performed *in situ* in human CWR22Rv1 cells captured by CytoQuest™ CR. AR-V7 was detected via orange signal (Cy3), and ARwt was detected via red signal (Cy5).

- Circulating Tumor Cell *In situ* Hybridization



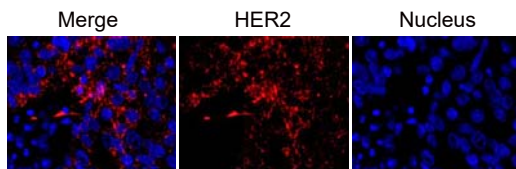
metaFISH™ staining was performed *in situ* in CTCs from NSCLC patient. CTCs were detected by using immunofluorescence staining for CSV (FITC, green). EGFR L858R point mutation was detected via orange signal (Cy3) and EGFR Ex19wt was detected purple signal (Texas Red).

- Fresh Frozen Tissue *In situ* Hybridization



metaFISH™ staining was performed *in situ* in human frozen lung adenocarcinoma tissue. EGFR L858R point mutation was detected via orange signal (Cy3), EGFR L858wt was detected via red signal (Cy5), and EGFR Ex19wt was detected via green signal (Texas Red).

- Formalin-Fixed Paraffin-Embedded Tissue *In situ* Hybridization



metaFISH™ staining was performed *in situ* in human breast cancer FFPE tissue. HER2 was detected via red signal (Cy5).

metaFISH™ Probes vs Conventional DNA and RNA FISH Probes

Items	metaFISH™ Probe	Conventional DNA FISH Probe	Conventional RNA FISH Probe
Point Mutation	Yes	No	No
Gene Amplification	Yes	Yes	No
Gene Deletion	Yes	Yes	No
Gene Translocation	Yes	Yes	No
Gene Expression	Yes	No	Yes

Publication Reference

1. *In situ* mutation detection and visualization of intratumor heterogeneity for cancer research and diagnostics. Grundberg I, Kiflemariam S, Mignardi M, et al. *Oncotarget*. 2013;4(12):2407-18.
2. *In situ* detection and genotyping of individual mRNA molecules. Larsson C, Grundberg I, Söderberg O, Nilsson M. *Nat Methods*. 2010;7(5):395-7.
3. Lock and roll: single-molecule genotyping *in situ* using padlock probes and rolling-circle amplification. Nilsson M. *Histochem Cell Biol*. 2006;126(2):159-64.
4. *In situ* genotyping individual DNA molecules by target-primed rolling-circle amplification of padlock probes. Larsson C, Koch J, Nygren A, et al. *Nat Methods*. 2004;1(3):227-32.