PIXELBIOSCIENCES GMBH
NovaFISH ON-CHIP USER GUIDE v0.2

NovaFISH is the most powerful multiplexing smFISH (single molecule FISH) technology developed by Pixelbiosciences GmbH. Like conventional smFISH, NovaFISH detects DNA/RNA expression by staining individual molecule and later analyzing with our FREE intelligent cloud-based image analysis platform NovaREAD. The critical made we made with NovaFISH is the autonomous combinatorial color barcoding (Figure 1).

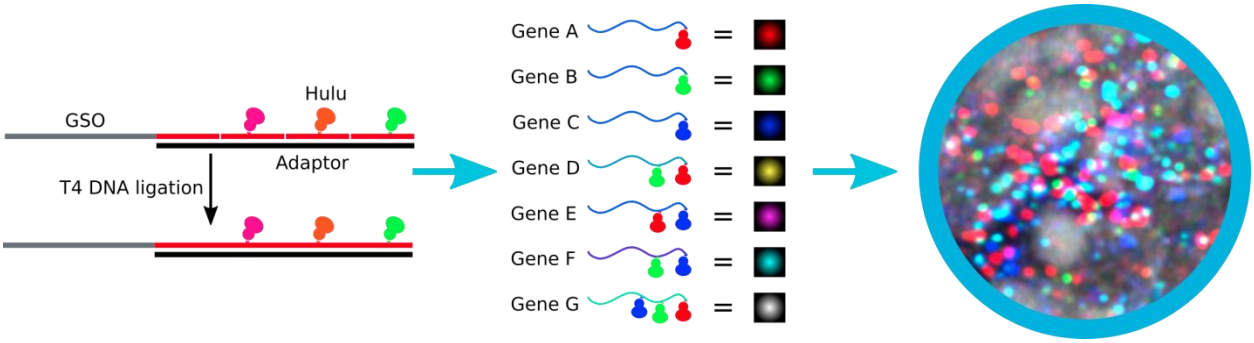

Figure 1. NovaFISH technology. NovaFISH probe is generated via our patented technology, the first enzymatic multiple fluorophore labeling for oligonucleotide pool (left). Currently, up to 7 genes can be barcoded with NovaFISH probe (middle). Each gene will have a distinct combination of 3 base color Nova (Red, Green, and Blue). The composite color dots will become the basis for our barcoding and decoding in multiplexing staining (right).

Each NovaFISH probe in the probe library for a certain gene will be labeled with the same stoichiometry of various fluorophores. The barcoding capacity of NovaFISH is exponentially growing with the number of lasers in the microscope for the imaging. With state-of-art microscope with 7 lasers, one can potentially detect 127 genes in one round of hybridization.

NovaFISH probe can be used to detect DNA/RNA expression in digital quantification. The sample can be in different format, including isolated DNA/RNA, fixed cell, fixed tissue sections, fixed whole mount embryo. This manual is for the detection of RNA/DNA in fixed tissue by NovaFISH.

The following procedures will be divided into 2 major parts: tissue section preparation, and NovaFISH staining.

Step 1 Probe Preparation

Resuspend NovaFISH probe in 33 ul DNase/RNase Free water (i.e. DEPC treated water or commercial water aliquots for molecular biology use).

Tips

Facilitate the dissolution of NovaFISH probe in water by tapping the tube for several times. Alternatively, leave the tube on bench at room temperature for 20min. The NovaFISH probe should be stored at -20 degree or lower. It is ok for repeated use by freezing-and-thawing the probe at room temperature.

Step 2 DNA/RNA on-chip capture

Take 1 ul of isolated RNA/DNA target in solution and hybridize with the glass slide immobilized with capture probe for the target. Hybridize in humidified chamber at 37 degree for 1 hour.

Tips

Design a capture probe for the target. The capture probe should contain a 5' end $T_{10}C_{10}$ tag for immobilization on regular glass slide, then followed by a sequence complementary to the 5' end sequence of the target (20 bp should be enough). Immobilizing the capture probe on clean glass slide by uv crosslinking according to published reference (Biotechniques. 2008 Sep45(3):261-71 doi: 10.2144/000112905).

Step 3 Washing the unbound probe

Wash the glass slide with 2xSSC, 10% formamide, 0.1 % Tween-20 (WashT) for 2 times, each time 10 min at room temperature.

Tips

Wash T should be prepared with DEPC treated water to eliminate any RNase/DNase contamination.

Step 4 Staining with NovaFISH probe

Dilute 0.5 ul of NovaFISH probe in step 1 into 50 ul 1xHyb solution (2xSSC, 10% formamide, 10% dextran sulfate, 1mg/ml tRNA E. coli, 2mM RVC complex, 0.2mg/ml BSA). Take 10 ul NovaFISH working solution on the spot of captured target on glass slide. Hybridize in humidifed chamber at 37 degree for 2 hours.

Tips

In order to minimize solvent evaporation during hybridization, one can reduce the hybridization temperature to 30 degree, or cover the solution with one 13 mm coverslip.

Step 5 Washing the unbound probe

Wash the glass slide with 2xSSC, 10% formamide, 0.1 % Tween-20 (WashT) for 2 times, each time 10 min at room temperature.

Tips

See tips in step 3

Step 6 Mounting

Remove the residual buffer on the spot of target. Pipette 10 ul Prolong Gold/Glass mounting solution on the spot and immediately cover with one 13mm coverslip (#1.5 or #1.0). Allow the sample to cure for 24-48 hours according to the instruction from Prolong Gold/Glass.

Tips

Imaging the target on chip by epifluorescence or confocal microscope with appropriate laser. NovaFISH probe is labelled with the combination of Atto488.Atto565 and Atto647N. All fluorophores are barcoded as G (Atto488), Y(Atto565), and R (Atto647N). Check your probe barcoding scheme on the tube labe. For example, Gapdh-1G1Y1R is standing for mouse Gapdh NovaFISH probe with one Atto488, one Atto565 and one Atto647N. GAPDH-2G1R is standing for Human GAPDH gene with 2 Atto488 and one Atto647N.

Appendix 1 Recommended Reagents from other vendors

Name	Vendor	Cat. No.
Prolong Gold	ThermoFisher Scientific	P10144
Prolong Glass	ThermoFisher Scientific	P36982
RVC complex	NEB	S1402S
BSA	Ambion	AM2616
tRNA E. coli	Sigma	000000010109541001