PIXELBIOSCIENCES GMBH
NovaFISH WHOLEMOUNT USER GUIDE v0.4

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).

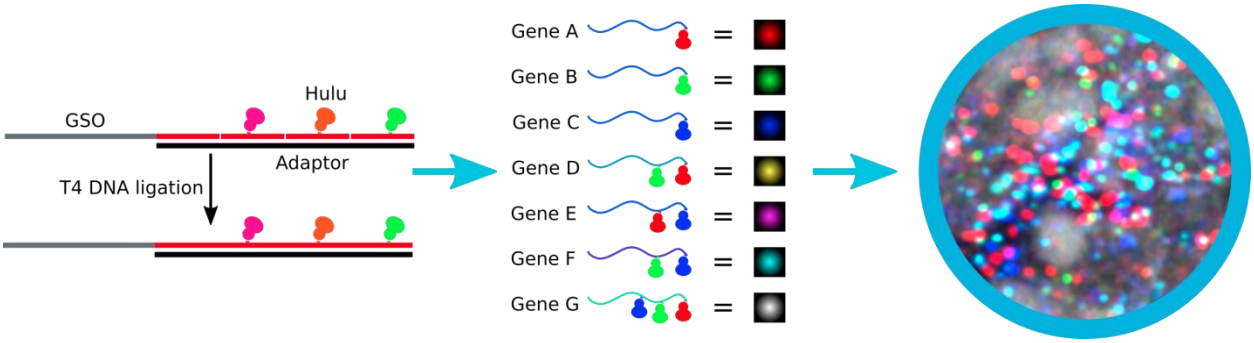

Figure 1. NovaFISH technology. NovaFISH probe is generated via our patented technology, the first enzymatic multiple fluorophore labeling for oligonucleotide pool (left). Currently, up to 7 genes can be barcoded with NovaFISH probe (middle). Each gene will have a distinct combination of 3 base color 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.

Step 1 Probe Preparation

Resuspend NovaFISH probe in 40 ul DNase/RNase Free water (i.e. DEPC treated water or commercial water aliquots for molecular biology use, for NovaFISH midi and maxi, use 200 ul and 500 ul water to resuspend).

Tips

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

repeated use by freezing-and-thawing the probe at room temperature. All water used in following steps to prepare the buffer should also be DEPC treated to minimize the RNase contamination (for a detailed protocol of how to make DEPC treated solution, check https://en.wikipedia.org/wiki/Diethyl pyrocarbonate)

Step 2 Sample Preparation

Fix with sample (whole embryo, tumorsphere, etc.) 4% formaldehyde in 1x PBSfor overnight at 4 degrees in a 1.5 ml epi tube. Remove formaldehyde bycentrifugation at 500-1000rpm 1min (alternatively allow the settle of the sampleby gravity) and then wash once with 135mM Glycine in 1x PBS to quenchres idual formaldehyde for 10 min.

Tips

Fixation could also be done with 4% paraformaldehyde in 1x PBS. Fixation time can be shortened into 10 min at the room temperature.

Step 3 Washing the residual formaldehyde

Wash once with 1x PBS to remove residual for 10 min. Remove the supernatant by spin off briefly (500-1000 rpm, 1min), then store the sample in 70% Ethanol at 4 degrees overnight. Then the embryo could store in 70% Ethanol at -20 degrees for several months.

Step 4 Washing the cells with NovaWash

Before hybridization, wash the coverslip with 2xSSC, 2M Urea (NovaWash) for 2 times, each time 10 min at room temperature.

Tips

Washing steps could be done on a shaker to have better removal of residual ethanol.

Step 5 Staining with NovaFISH probe

Dilute 0.5 ul of NovaFISH probe in step 1 into 50 ul 1x NovaHyb solution (2xSSC 2M Urea, 10% dextran sulfate, 5x Denhardt's solution). Take the NovaFISH

working solution into the sample tube. Hybridize at 37 degrees overnight. Optionally with shaking at 500 rpm.

Tips

Cover with an aluminum foil to protect from the light. NovaFISH probe is stableover ambient light. However, long time exposure to strong light is detrimental to the probe quality.

Step 6 Washing the unbound probe

Wash the coverslip with NovaWash for 4 times, each time 10 min at room temperature.

Tips

Washing can also be 2 times, 30 min each at 37 degrees. Or some PixelBiosciences users have tried 4 degrees overnight washing. And the signal is still well maintained. In the last 5 minutes of the last washing step, add DAPI solution to have final 1 ug/ml to stain nuclei.

Step 7 Mounting

Remove the residual buffer on the coverslip by dipping onto a clean tissue paper. Pipette 10 ul Prolong Gold/Glass mounting solution on a clean glass slide, then immediately cover the mounting solution with the 13mm coverslip having stained cells. Cell side should be again facing down. Allow the sample to cure for 24-48 hours at room temperature according to the instruction from Prolong Gold/Glass.

Tips

Imaging the sample on the coverslip by epifluorescence or confocal microscope with an appropriate laser (newer model of confocal microscope usually has better imaging outcome). NovaFISH probe is labeled 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 label. 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