



**PIXELBIOSCIENCES GMBH**  
**NovaFISH CELL USER GUIDE v0.5**

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NovaFISH is industry's first 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).

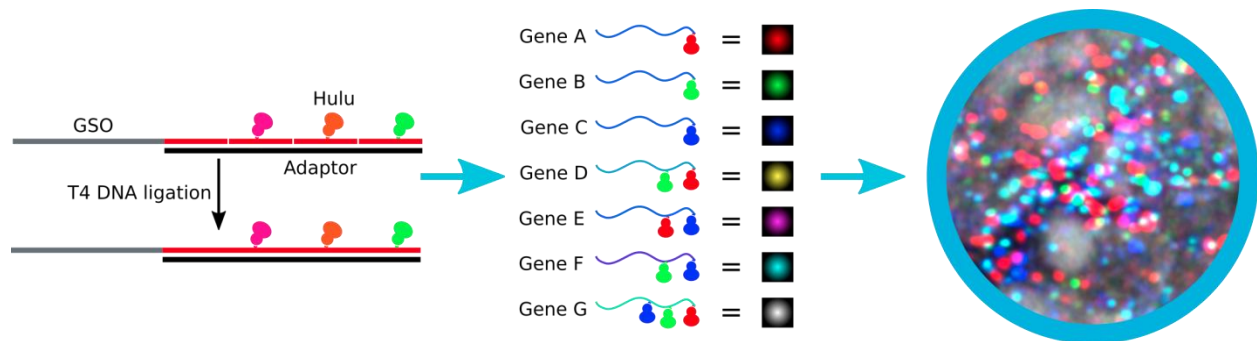


Figure 1. NovaFISH technology. NovaFISH probe is generated via our patented technology, the first enzymatic multiple fluorophore labeling for oligonucleotide pool (left). Currently, up to 7 genes can be barcoded with NovaFISH probe (middle). Each gene will have a distinct combination of 3 base color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



### **Step 2 Step 2 Cell Preparation**

Cultivate cells on 13 mm #1 coverslip. Fix with 4% formaldehyde in 1x PBS for 10 min at room temperature. Remove formaldehyde and then wash once with 135mM Glycine in 1x PBS to quench residual formaldehyde for 10 min.



#### **Tips**

*If the coating is required for your cell line, coat the coverslip with molecule required for your cell to attach and grow. Before fixing the cell with 4% formaldehyde, cells could grow at various confluence (10- 100%), but at least one overnight to allow enough stickiness of cells to the coverslip. Fixation could also be done with 4% paraformaldehyde in 1x PBS. Normally we use 12 or 24 well cell culture plate for cultivation, fixation, and the washing steps.*



### **Step 3 Washing the residual formaldehyde**

Wash once with 1x PBS to remove residual for 10 min. Then store the sample in 70% Ethanol at 4 degrees overnight. Then the cells could store in 70% Ethanol at 20 degree for several months.



#### **Tips**

*Seal the culture well plate containing the fixed cells on the coverslip with parafilm or tape to minimize the evaporation of ethanol. Or regularly check the ethanol level in the well to make sure there is no dry-out of the cells.*



### **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 on a clean piece of parafilm, then cover the solution with washed coverslip from step 4. Remember the side with cells should face down to the solution. Hybridize in a humidified chamber at 30 degrees overnight.*

**Tips**

*The cell side of coverslip can be easily distinguished if look the coverslip under the light. The cell side is less reflective than the non-cell side. Or experimenter could also check the coverslip on the light microscope and try to scrape some cells from the upside of the coverslip. If there is no cell can be scraped off, the upside is non-cell side.*

**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 room temperature. 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	<a href="#">P10144</a>
Prolong Glass	ThermoFisher Scientific	<a href="#">P36982</a>