FISH with EdU labelling

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Introduction

DNA Fluorescent In-Situ Hybridization (FISH) is a technique to visualize stretches of chromatin in fixed cells the technique has been originally developed in the early 80s[1] and extensively optimized by Cremer lab[2–4]. This protocol describes the combination of EdU labeling (as a marker for cell cycle stage) and 3D DNA-FISH in HCT116 cells, but with optimization can be used for all adherent cells.

Materials

All buffers should be filtered before use to reduce the noise from floating particles. This is specially important for the buffers containing

- DIG and/or biotin labelled probes
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Procedure

Cell Seeding

Cells seeded at 0.6e6/ml, 0.5 ml culture media well.

EdU Pulse

EdU pusled (A:C)*(1:4) by replacing with the media containing EdU(5mM). 6.5µl 10mM EdU in 13 ml media. Pulsed for 10 minutes.

Cell Fixation

1. Rinse cells 3 times, with warm(37°C).
2. Transfer coverslips to a new 24 well plate containing warm PBS.
   - This is to leave behind all the debris from the cells attached to plate.
3. Fix with 3% PFA in 1X PBS for 10 min at RT.
   - After this step you either need to gradually exchange PFA with PBS, or quench it with PBS-Glycine really quick.
4. Quench with 0.5M Glycine-PBS for 5 min at RT.

5. Permeabilize with 0.5% Tx-10 in PBS. 10 min at RT.
6. Rinse the cells with PBS, 3 times.
7. Incubate cells in 20% Gylcerol in PBS for 2 hr at RT.
8. Freeze the cells by dipping in liquid nitrogen and thaw on a paper towel or kimwipe.
    - Once the cells are thawed put back in PBS-Glycerol.
    - Repeat freeze-thaw cycle 4 times.
9. Wash cells in PBST 3 x 10 minutes at RT.
10. Rinse the cells with 0.1 N HCl in DW and incubate with fresh portion for 10 min at RT.
11. Wash cells with 2XSSC 3x 5 minutes at RT.
12. Incubate in 2XSSC/50% Formamide at 4$deg;C.
    - Incubated at least overnight.
    - Cells can be stored in 2XSSC/50% formamide for month.
    - The more cells stay in formamide, the more chromatin decondenses.

Hybridization

Probe washes

Probe staining

Click reaction

Counterstaining with DAPI

Mounting

References

1. Langer-Safer, P. R., Levine, M. & Ward, D. C. Immunological method for mapping genes on Drosophila polytene chromosomes. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 4381–5 (1982).

2. Solovei, I. *et al.* Spatial preservation of nuclear chromatin architecture during three-dimensional fluorescence in situ hybridization (3D-FISH). *Experimental Cell Research* (2002).doi:10.1006/excr.2002.5513

3. Solovei, I. & Cremer, M. 3D-FISH on Cultured Cells Combined with Immunostaining. 117–126 (2010).doi:10.1007/978-1-60761-789-1_8

4. Markaki, Y., Smeets, D., Cremer, M. & Schermelleh, L. Fluorescence In Situ Hybridization Applications for Super-Resolution 3D Structured Illumination Microscopy. In *Nanoimaging* **950**, 43–64 (Humana Press, Totowa, NJ, 2013).