DNA-DNA FISH on Metaphase Preps

This is a baseline protocol. Some steps may need to be optimized by the researcher.

All solutions are made with molecular grade DNase/RNase free water unless otherwise specified.
All steps are at room temperature (RT) unless otherwise specified.
All slide washes are done in coplin jars/slide dishes with gentle agitation/rocking.

For reconstitution of shipped myTags *in situ* hybridization probes see:
myTags Product Reconstitution Protocol

Pretreatment of Stored Metaphase Prep Slides

1. Slides may be refixed and cleaned, especially if stored for more than 1 week.
   - Place in Modified Carnoy's fix for 10 min, wash 2X in 96% ethanol for 10 min each, air dry.
2. Add 200 µl RNase solution to the slide, cover with parafilm coverslip, incubate in a humid chamber for 1 hr at 37°C.
3. Carefully remove the coverslip and wash slides in 2X SSC twice for 5 min.
4. Dehydrate slides through EtOH series, 70%, 90% and 96%, 2 min ea.
   - Air dry inverted with label side down and covered with paper towel.
5. Proceed to denaturation and hybridization.
   - Slides can be kept overnight at 4°C.

Denaturation and Hybridization

1. Add 60 µl Hybridization Mixture with myTag probe/s to each coverslip/hybridization slip, invert the slide onto the slip to avoid trapping bubbles
2. Seal the coverslips with rubber cement or appropriate sealant (Cytobond, ScieGene cat#202-00-1) and let cure for 5 minutes.
3. Incubate the slides at 75°C in a humid chamber for 5 min to denature the target DNA.
4. Move slides directly to 37°C in humid incubator and hybridize for 16-20 h (overnight)
   - Time depends on amount and length of probe and amount of target.
     - For highly repetitive sequences 2-4 hrs will suffice.
     - Can be advantageous to leave single-copy sequences to hybridize for 72 hrs or more.

Post-Hybridization Washes

Preheat appropriate volume of 2X SSC and 6X SSC to 42°C.
Heated rinses are done in 42°C waterbath with gentle shaking.
Do not let slides dry out between rinses.

Remove slides from hybridization incubator and observe for anything unusual, drying, drops of water, bubbles etc.

1. Wash slides in 6X SSC at RT, for 30 min and repeat.
2. Wash slides in 6X SSC at 42°C for 2 min.

3. Wash slides in 2X SSC at 42°C, for 5 min and repeat.
4. Wash slides with 2X SSC at 42°C for 3 min and repeat.
5. Take coplin jar/slide washing dish out of heated waterbath and let cool to RT.
6. Proceed to nuclear staining.
    - Recommend Hoechst 33342 (Invitrogen Cat #H3570).
        - Dilute stock (10 mg/ml) 1:2000 in PBS for final concentration of 5 µg/ml
        - Incubate slide 30 sec and rinse in PBS.
7. Coverslip with anti-fade media.
    - Recommend Prolong Diamond (Invitrogen Cat #P36961).
    - Cure at RT overnight before imaging.
8. Store slide at -20°C for long term storage.

REAGENTS

Modified Carnoy's Fixative
3:1 Methanol:Glacial Acetic Acid – make fresh

- 30 ml of 100% Methanol
- 10 ml Glacial Acetic Acid
- Make appropriate volume for number of slides in experiment.

RNase solution
DNase free Ribonuclease A from Bovine Pancreas (Sigma cat #R4642, 50% solution)

- Dilute to 10 mg/ml stock in 10 mM Tris-HCl, pH 8.0
- Aliquot stock and store at -20°C
- Dilute stock to 500 µg/ml in 10 mM Tris-HCl, pH 8.0
    - Dilute 24 µl 10 mg/ml stock in 01 mM Tris-HCl, pH 8.0 to final volume of 1.2 ml.

2X SSC and 6X SSC

- Dilute 20X SSC to appropriate concentration with molecular grade DNase/RNase free water.
- pH to 7.4 and autoclave.

Hybridization Mixture for Oligonucleotides
50% Formamide, 2X SSC, 1 mM Tris-HCl, 1mM EDTA, 10% Dextran Sulfate

- 250 µl 100% DI Formamide (final concentration 50%)
- 50 µl 20X SSC (final concentration 2X SSC)
- 1 µl 500 mM Tris-HCl, pH 8.0 (final concentration 1 mM)
- 1 µl 50 mM EDTA (final concentration 1 mM)
- 100 µl 50% Dextran Sulfate (final concentration 10%)
- 15 µl molecular grade DNase/RNase free water

Can be upscaled to larger vol of Hybridization Mix, aliquot and store at -20°C

Hybridization Mixture with myTag Probe
1X Hybridization Mixture for Oligonucleoties, myTags probe/s at appropriate concentration

- Dilute aliquot of reconstituted myTag probe/s to appropriate concentration (start with a concentration of 10 pmol/rxn) in DNase/RNase free H_2O to final volume of 10 µl.

- Add 50 µl Hybridization Mixture for Oligonucleotides, vortex to mix well and spin down, place on ice and minimize exposure to light.
- Final volume including diluted probe for 22 X 50 mm Coverslip/HybriSlip (Thermofisher HybriSlip Hybridization Cover) is 60 µl.
    - Final volume can be adjusted for smaller Coverslip/HybriSlip but keep the amount of myTags probe/rxn the same.

Adapted from:
Schwarzacher and Heslop-Harrison, “Practical *in situ* Hybridization”,
BIOS Scientific Publishers Limited, Oxford UK, Springer-Verlag, New York Inc., 2000.