This protocol is provided as a guideline and may need to be optimized by the researcher

DNA Fluorescent *in situ* Hybridization Labeling of Metaphase Chromosomes

Baseline protocol

For chromosome spread preparation in human cells see:
Arbor Biosciences/myTags Protocols-"Metaphase Chromosome Spreads-Human Cells"

Avoid drying of slides between steps.
Prepared chromosome spread slides can be stored in PBS for one week at 4°C prior to use.
All rinses are done with gentle agitation/rocking.

For reconstitution of shipped myTags ISH probes see:
Arbor Biosciences/myTags Protocols-"Reconstitution of myTags ISH Probes"
Aliquot probes into working stock volumes to avoided repeated freeze-thaw cycles.

Day 1-Hybridization

1. Wash slides with 1X PBS at RT for 5 mins
2. Incubate slides in 2X SSCT at 65° C for 15 mins in humid chamber to prevent drying.
3. Wash slides in 2X SSCT at RT for 2 mins
4. Dehydrate the slides 70% EtOH, 90% EtOH for 5 mins each. Air dry
5. Denature the chromosomes with 0.07N NaOH for 3 mins at RT
6. Wash slides in 2X SSCT for 5 mins
7. Dehydrate the slides 70% EtOH, 90% EtOH for 5 mins each. Air dry
8. Prepare probe hybridization mix at the desired concentration (starting at 10 pmol/hybridization reaction).
9. Heat the probe hybridization mix at 70^{o} C for 5 mins and chill on ice until use.
10. Add hybridization mix to slides. Cover with HybriSlip™ (Grace Bio-Labs) or parafilm "coverslip" to prevent drying and to retain hybridization solution on the sample.
11. Incubate the samples overnight at 37°C in humid chamber to prevent drying.

Day2-Post Hybridization

1. 2X SSCT post hybridization wash buffer needs to be pre-heated at 37°C.
2. Remove probe hybridization mix by washing the slides at 37°C, 1 x 30 mins with pre-heated 2X SSCT.
3. Wash slides 2 x 5 mins with 2x SSCT at room temperature
4. Wash with PBS for 5 minutes at RT
    a. Add more SSCT and PBS washes if nonspecific binding background is detected
5. Stain with appropriate nuclear stain per SOP (recommend-Hoechst 33342 Solution, Thermo Fisher #62249), rinse and coverslip with antifade media (recommend-ProLong Diamond, Thermo Fisher # P36965 or # P36961)

REAGENTS

2X SSCT (SSC with 0.1 %Tween 20):

5 ml 20 x SSC
500 µl 10% Tween 20
Bring to volume of 50 ml with molecular grade dH_20
Stored at 4°C. Expiration: 1 month.

10X PBS (use molecular grade dH_20):

1.3 M NaCl
0.07 M Na2HPO4
0.03 M NaH2PO4

Probe Hybridization buffer:

In 2X SSCT:
50% formamide
10% dextran sulfate
40 ng/ µl RNaseA

Adapted from:
Carthew lab in Northwestern University, under section “Chromosome in situ hybridization using biotin labeled probes.”
The link: http://groups.molbiosci.northwestern.edu/carthew/manual/Manual.html