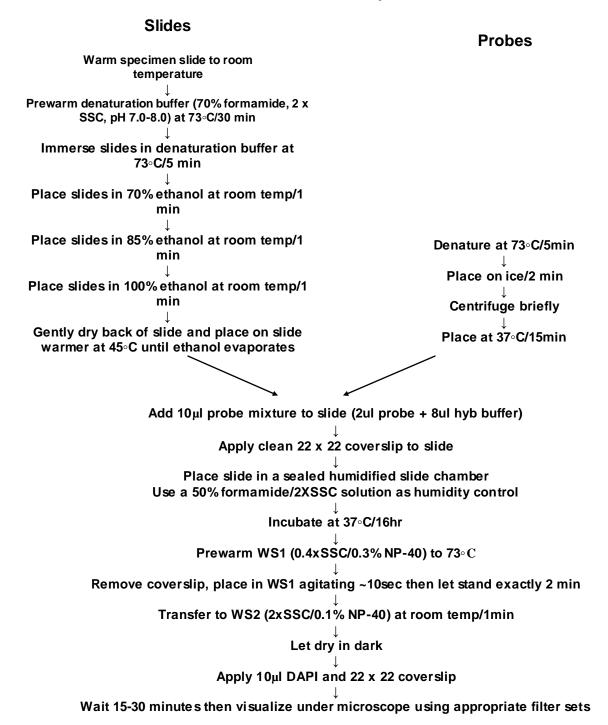


## **BAC DNA Fluorescent Probes**

## **Quick Reference – Hybridization Protocol**

# **Manual Denaturation/Hybridization**



### \*Optional Automated Hybrite/Thermobrite Method

Turn on Hybrite/Thermobrite

Set program (see guide below)

Presoak absorbant material in dH2O and position in Hybrite/Thermobrite

Add 10 μl probe mixture to slide (2ul probe + 8ul hyb buffer)

Apply clean 22 x 22 coverslip to slide

Apply rubber cement on edges of coverslip to seal

Place in Hybrite or Thermobrite & close lid

Start program

After at least 16 hrs

Prewarm WS1 (0.4xSSC/0.3% NP-40) to 73° C

Remove coverslip, place in WS1 agitating ~10 sec then let stand exactly 2 min

Transfer to WS2 (2xSSC/0.1% NP-40) at room temp/1min

Let dry in dark

Apply 10 μl DAPI and 22 x 22 coverslip

Wait 15-30 minutes then visualize under microscope using appropriate filter sets

#### Hybrite/Thermobrite Program Guide

For Peripheral Blood Preparations:

Denature at 72-73°C for 2 minutes/Hybridize at 37°C for at least 16 hours

For Paraffin Embedded tissue sections after pretreatment:

\*Denature at 83°C for 3 minutes/Hybridize at 37°C for at least 16 hours - May require trouble shooting

**Optional FISH Pretreatment:** 

We recommend the Abbott Molecular FISH Pretreatment Kit: http://www.abbottmolecular.com/FISHPretreatmentKit\_5792.aspx

**Paraffin Pretreatment:** 

We recommend the Abbott Molecular Paraffin Pretreatment Kits: I, II or III: http://www.abbottmolecular.com/PretreatmentKits\_5777.aspx