IF/DNA FISH or co RNA/DNA FISH for Xist

Fixation:

1. apirate media, wash briefly w/PBS.
2. Fix for 10 minutes @ RT using 4% PFA in PBS.
3. Permeabilize on ice for 10 minutes with cold PBS/0.5% Triton X-100/1:200 NEB VR inhibitor complex
4. Store cells in 70% EtOH at -20 C.

IF/DNA

1. 2x 5 min PBS
2. block 30 min RT in PBS 0.2% Tween-20 0.2% IgG-free BSA (0.2 g /100ml)
3. 1 hr RT antibody in block
4. wash 3x 4 min in PBS 0.2% Tween-20
5. biotinylated Goat anti-rabbit (or whatever) secondary, 30 min
6. wash 3x 4 min in PBS 0.2% Tween-20
7. fix 5 min in 4% PFA RT
8. 2x 4 min PBS wash

DNA/RNA FISH start here:

1. Dehydrate in EtOH series, 3 min each: 75/85/95/100 % EtOH,
2. Air dry 5 min
3. Denature on 80 C heat block in 70% deionized formamide/2X SSC for 20 minutes (IF/DNA) or 9 minutes (RNA/DNA)
4. 2x SSC cool on ice block
5. DNA FISH with 7ul of probe pair overnight in 50% formamide/2x SSC humidified chamber, 37 C
6. Wash in 6-well well, 2 ml per wash, 5 minutes eah:
   1. @ 42 3x 50% form/2x SSC, @ 55 3x 1x SSC
7. IF/DNA: wash 1x 2 min in 4x SSC @ RT
8. Block 15 min w/2mg/ml BSA, 0.2% Tween-20 4x SSC
9. 1:100 avidin/alexa-488 30 min in 0.2% BSA, 0.2% Tween-20 4x SSC
10. 3x 4X SSC 4 min each
11. Fix to slide with Prolong Gold DAPI antifade, let dry O/N, sop up extra with carefully placed kim wipe