3D- (XIST)-RNA-hybridization

preparation of XIST hyb-mix:
For human/mouse XIST full length cDNA was nick-translated by DIG or Bio or direct labelled at a final concentration of 20 ng/µl.

Set up- no cot for cDNA probes
50 ng/µl labeled XIST cDNA
1 µl Salmon sperm (10mg/ml)
0.3 µl tRNA
2.5 x vol ETOH absolute

Spin 30' max 4deg
Dry the pellet
Dissolve dried pellet in (6 µl) of FA

30' shaking 42 deg

Mix probes in same volume of a 2x conventional hybmix (i.e. per 100 µl: 25 µl 20X SSC, 25 µl 50% Dextran sulfate, 25 µl BSA,12,5 µl VRC, 12,5 µl H20)

Den 5' 75 deg/5 min ice

perform XIST hybridization the same day as fixation!

Fixation and pretreatment
Wash cells 2x with PBS (cell culture)
Fix cells in 2% PFA (Formaldehyde) for 10min,

Permeabilize cells 10 min in 0.5% TritonX100/PBS+ VRC (500 µl in 50ml)
Wash 2x in PBST

Wash in 2 x SSC

Incubate in 50% FA/2xSSC for 2 h

Hybridization and detection

Put denatured (12 µl) probe on cover slip with cells, seal with fixogum

Let hybridize at least O/N at 37^{o} C

Washing
3X 3min 50%FA/2x SSC at 42 deg
3X 3 min in 2 X SSC at 42deg

PBS/ DAPI mounting for direct labeled probes

Detection as usual for bio/dig probes and **DAPI staining**