

Materials List for:

Combined DNA-RNA Fluorescent *In situ* Hybridization (FISH) to Study X Chromosome Inactivation in Differentiated Female Mouse Embryonic Stem Cells

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Materials

Name	Company	Catalog Number	Comments
female mouse embryonic stem cells			will be made available upon request
mouse embryonic fibroblasts			can be derived from E13.5 embryos, or via commercial sources e.g. Millipore
ES cell culture medium			DMEM, 15% fetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin, non-essential amino acids, 0.1 mM γ -mercaptoethanol, and 1,000 U/ml LIF. Filter sterilize and store at 4 °C for maximum of 2 weeks.
LIF	Chemicon	ESG1107	
DMEM	Invitrogen	11960085	
Fetal Calf Serum	Invitrogen	10099141	
Penicillin–streptomycin (100x)	Invitrogen	15140-122	
Non-essential aminoacids	Invitrogen	11140-050	
γ -mercaptoethanol	Sigma-Aldrich	M7522	
ES cell differentiation medium			IMDM-Glutamax, 15% heat inactivated fetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin, non-essential amino acids, 37.8 μ L/L monothioglycerol and 50 μ g/ml ascorbic acid
IMDM-Glutamax	Invitrogen	31980-030	
Ascorbic acid (50 mg/ml)	Sigma-Aldrich	A4403	
monothioglycerol	Sigma-Aldrich	M6145	
T25 cell culture dishes	Greiner Bio-one	690160	
6-well cell culture dishes	Greiner Bio-one	662160	
cell culture grade PBS	Sigma-Aldrich	D8537	
Trypsin-EDTA 0.25% (v/v) trypsin/0.2% (w/v) EDTA in PBS	Gibco	25200-056	
Falcon tubes	Falcon	352196	
24 x 24 mm coverslips	Menzle	780882	
gelatin	Sigma-Aldrich	G1890-100G	prepare a 0.2% gelatin/cell culture PBS solution, and autoclave
Pepsin	Sigma-Aldrich	P7000	Dilute to 0.2% in 0.01 M HCl (pH 2.0)

absolute 100% ethanol	Sigma-Aldrich	32221-2.5L	use absolute 100% ethanol and RNase free water to make 70% and 90% ethanol solutions
RNase free water	Baxter	TKF7114	
sterile filter tips	Rainin Bio Clean	GP-L10F, GP-L200F, RT-1000F	
digoxigenin-labeling kit (DIG-Nick translation)	Roche	11745816910	
biotin-labeling kit (Biotin Nick translation)	Roche	11745824910	
Sephadex G50	Sigma-Aldrich	G5080	
2 ml syringe	BD Plastipak	300013	
sterilized cotton			
Eppendorf tubes	Eppendorf	30120086	
yeast tRNA 10 mg/ml	Invitrogen	15401-029	
Salmon Sperm DNA 10 mg/ml	Invitrogen	15632-011	
mouse cot-1 DNA 1 mg/ml	Invitrogen	18440-016	
Eppendorf bench top microcentrifuge	Eppendorf	Model 5417R	
Eppendorf refrigerated centrifuge	Eppendorf	Model 5810R	
2 M NaAc, pH 5.6	Sigma-Aldrich	S7670	
50+ hybridization solution			50% formamide, 2x SSC, 50 mM phosphate buffer pH 7, 10% dextran sulfate pH 7
formamide	Acros organics	3272350000	
dextran sulfate	Fluka	31403	
10 mM phosphate buffer, pH 7			add 57.7 ml of 1 M Na ₂ HPO ₄ and 42.3 ml 1 M NaH ₂ PO ₄ ; use DEPC treated water and autoclave
denaturation buffer			70% formamide/2x SSC/10 mM phosphate buffer pH 7
Tween-20	Sigma-Aldrich	P2287	
2 M Tris pH 7.5 solution			
Tris-saline-Tween washing solution (TST)			100 ml 10x saline, 50 ml 2M Tris, 500 µl Tween-20, add RNase free water till 1 L
10x saline solution			dissolve 85 g NaCl in 1 L RNase free H ₂ O, and autoclave
Tris-saline-BSA (TSBSA)			500 µl 10x saline, 250 µl 2 M Tris, 1 ml 100x BSA, 3.25 ml RNase free H ₂ O
BSA, purified, 100x	New England Biolabs	B9001S	
sheep-anti-dig antibody	Roche diagnostics		use 1:500 diluted in TSBSA
rabbit-anti-sheep antibody conjugated with FITC	Jackson labs		use 1:250 diluted in TSBSA
goat-anti-rabbit antibody conjugated with FITC	Jackson labs		use 1:250 diluted in TSBSA
mouse-anti-biotin antibody	Roche diagnostics		use 1:200 diluted in TSBSA
donkey-anti-mouse antibody conjugated with Rhodamine	Jackson labs		use 1:250 diluted in TSBSA
goat-anti-horse antibody conjugated with Rhodamine	Jackson labs		use 1:250 diluted in TSBSA

Tris-saline washing solution (TS)			10 ml 10x saline, 5 ml 2 M Tris, 85 ml RNase free H ₂ O
Vectashield mounting medium with DAPI	Vectorlabs	H-1200	
nail varnish			
fluorescent microscope			