Materials List for:

Robust 3D DNA FISH Using Directly Labeled Probes

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Materials

Name	Company	Catalog Number	Comments
Reagent/Material			
NTB buffer			Step 1.1.1 (See 'FISH buffers and reaction mixes' list below)
DTT	Invitrogen	D-1532	Step 1.1.1
dNTPs	Bioline	BIO-39025	Step 1.1.1 (See 'FISH buffers and reaction mixes' list below)
Aminoallyl-dUTP	Ambion	AM8439	Step 1.1.1
DNA Polymerase I	New England Biolabs	M02095	Step 1.1.1
DNase I recombinant RNase free	Roche	4716728001	Step 1.1.1
QIAquick PCR purification kit	Qiagen	28104	Step 1.1.4, 1.2.3
NaOAc	VWR	27653	Step 1.1.5, Step 2.1.1.2
NaHCO3	Sigma	S5761	Step 1.2.1
Alexa Fluor Reactive Dye Decapacks for Microarray Applications	Invitrogen (Molecular Probes)	A32750, A32756, A32757	Step 1.2.2
DMSO (anhydrous)	Sigma Aldrich	276855	Step 1.2.2
SYBR Safe DNA gel stain	Life Technologies	S33102	Step 1.2.4 (alternative to ethidium bromide)
Cot-1 DNA	Invitrogen	18440-016	Step 2.1.1.1 (Cot-1 DNA can be home-made in large quantities and works just as well)
Single stranded DNA from salmon testes	Sigma	D7656	Step 2.1.1.1
Deionised formamide	Sigma	F-9037	Step 2.1.1.3 (Health hazard)
Dextran sulphate	Sigma Life Sciences	D8906	Step 2.1.1.4 (See 'FISH buffers and reaction mixes' list below)
XMP painting probes	MetaSystems	000000-0528-837	Step 2.1.1.4
Poly-L-lysine coated slides	Sigma Aldrich	P0425	Step 2.1.2
Hydrophobic pen (ImmEdge)	Vector Laboratories	H-4000	Step 2.1.2
70 µm sieve (Mouse f–tal liver cells only)	BD Falcon	352350	Step 2.1.2.1
PFA	Sigma Aldrich	P6148	Step 2.1.3 (Flammable, corrosive, acute toxicity, health hazard) (See 'FISH buffers and reaction mixes' list below)
Tris-Cl			Step 2.1.3 (See 'FISH buffers and reaction mixes' list below)

Saponin from Quillaja bark	Sigma Aldrich	47036	Step 2.1.4, 2.1.11 (Acute toxicity), (See 'FISH buffers and reaction mixes' list below)
Triton X-100	Sigma	T9284	Step 2.1.4, 2.1.11 (Acute toxicity, hazardous to the environment)
10 x PBS	Life Technologies (GIBCO)	70011-036	Step 2.1.4, 2.1.5, 2.1.6, 2.1.8, 2.1.10, 2.1.11, 2.1.12, 2.2.7, 2.2.9,
Glycerol	Fisher Scientific	G/0650	Step 2.1.6
HCl	VWR	20252	Step 2.1.9 (Acute toxicity, corrosive)
Formamide	Sigma Aldrich	47670	Step 2.1.14, 2.2.3 (Health hazard)
SSC			Step 2.1.14, 2.2.2-2.2.6, 2.2.8 (See 'FISH buffers and reaction mixes' list below)
Coverslips 22 x 22	Menzel-Glaser	BB022022A1	Step 2.1.15
Coverslips 22 x 50	Menzel-Glaser	BB022050A1	Step 2.1.15
Rubber cement	Marabu	2901 (10 000)	Step 2.1.15 (Flammable, health hazard, danger to the environment)
DAPI	Invitrogen Molecular Probes	D3571	Step 2.2.8 (Health hazard)
SlowFade Gold	Invitrogen	S36936	Step 2.2.10 (mounting medium)
Clear nail polish	Any supplier		Step 2.2.10
Equipment			
Nanodrop 2000	Thermo Scientific		Step 1.1.6, 1.2.4 (microvolume spectroscopy)
Typhoon FLA 7000 phosphoimager	GE Life Sciences		Step 1.2.4
Coplin jars	Sigma-Aldrich	S6016 (6EA)/S5516 (6EA)	Step 2.1.3 onwards
Liquid nitrogen dewar	Any supplier		Step 2.1.7
Base with Black Lid (light-tight chamber)	Simport	M920-2	Step 2.1.17
Thermomixer comfort	Eppendorf	5355 000.011	Step 2.2.3 (or use shaker in a 37 °C room)
Imaging and Image processing			
Metafer - Imaging Automation Platform	MetaSystems		automated imaging software
MetaCyte - Automated Interphase FISH analysis	MetaSystems		automated imaging software
Axio Imager Z2 upright	Zeiss		epifluorescence microscope used with automated imaging
IX81 confocal microscope (FV1000)	Olympus		confocal microscope
Bitplane, version 7.3	Imaris		image analysis and 3D modelling software
Scientific volume Imaging, version 4.1	Huygens		image analysis and deconvolution software

FISH buffers and reaction mixes

Name	Composition	Comments
10 x NTB buffer	0.5 M Tris-HCl, pH 7.5	
	0.05 M MgCl2	
	0.5 mg/ml BSA	
dNTP Mix	0.5 mM dGTP	Store 50 µl aliquots at -20 °C
	0.5 mM dATP	

	0.5 mM dCTP	
	0.125 mM dTTP	
4 % PFA	Weigh 4 g/100 ml PFA in PBS and heat to 62 °C. Adjust pH with NaOH to 7.0 to dissolve remaining PFA.	Store 50 ml aliquots at -20 °C, do not re-freeze
2 % saponin solution	Dissolve 2 g saponin per 100 ml PBS by extended stirring. Dissolve and adjust pH to 7.0 with NaOH. Bring to 1 l with ddH20 and autoclave.	Store 5 ml aliquots at -20 °C
20 x SSC	Weigh 175.3 g NaCl and 88.2 g sodium citrate and add to 80 ml ddH20 . Dissolve and adjust pH to 7.0 with NaOH. Bring to 1 l with ddH20 and autoclave.	
Dextran sulphate mix	20 % dextran sulphate in 2 x SSC buffer; Vortex solution and mix on a rocker overnight.	Store 500 µl aliquots at -20 °C