

Materials List for:

# Using Fluorescence *In Situ* Hybridization (FISH) to Monitor the State of Arm Cohesion in Prometaphase and Metaphase I *Drosophila* Oocytes

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## Materials

Name	Company	Catalog Number	Comments
<b>Kits</b>			
Midi Prep kit	Qiagen	12143	Prep BAC clone DNA
GenomePlex Complete Whole Genome Amplification (WGA) Kit	Sigma	WGA2	Amplify BAC clone DNA
ARES Alexa Fluor 647 DNA labeling kit	Invitrogen	A21676	Label BAC clone DNA
PCR purification kit	Qiagen	28104	Remove non-conjugated dye following labeling of BAC clone DNA
<b>Name</b>			
<b>Company</b>			
<b>Catalog Number</b>			
<b>Comments</b>			
<b>Chemicals &amp; Solutions</b>			
<b>Note:</b> All solutions are prepared using sterile ultrapure water and should be sterilized either by autoclave or filter sterilization.			
Bovine serum albumin (BSA)	Fisher Scientific	BP1600-100	Prepare 10% stock Freeze aliquots
Calcium chloride	Fisher Scientific	C75-500	
DAPI (4', 6-Diamidino-2-Phenylindole, Dihydrochloride)	Invitrogen	D1306	<b>Toxic:</b> wear appropriate protection. Prepare 100µg/ml stock in 100% ethanol, store in aliquots at -20 °C. Prepare 1 µg/ml solution in 2X SSCT before use.
Dextran sulfate	Sigma	D-8906	
Drierite	Drierite Company	23001	
Dithiothreitol (DTT)	Invitrogen	15508-013	Prepare 10 mM stock
dTTP (10 µmol, 100 µl)	Boehringer Mannheim	1277049	Prepare 1 mM stock
EDTA (Disodium ethylenediamine tetraacetic acid)	Fisher Scientific	S311-500	Prepare 250 mM stock
100% ethanol (molecular grade, 200 proof)	Decon Laboratories	2716	
Tris (Ultra Pure)	Invitrogen	15504-020	
EGTA (Ethylenebis(oxyethylenenitrilo)tetraacetic acid)	Sigma	E-3889	
16% formaldehyde	Ted Pella, Inc.	18505	<b>Toxic:</b> wear appropriate protection
Formamide	Invitrogen	AM9342	<b>Toxic:</b> wear appropriate protection
Glucose	Fisher Scientific	D16-1	
Glycogen	Roche	901393	

HEPES	Boehringer Mannheim	737-151	
Heptane	Fisher Scientific	H350-4	<b>Toxic:</b> wear appropriate protection.
Hydrochloric acid	Millipore	HX0603-4	<b>Toxic:</b> wear appropriate protection.
Hydroxylamine	Sigma	438227	Prepare 3 M stock
4.9 M Magnesium chloride	Sigma	104-20	
Na <sub>2</sub> HPO <sub>4</sub> · 7H <sub>2</sub> O	Fisher Scientific	S373-500	
NaH <sub>2</sub> PO <sub>4</sub> · 2H <sub>2</sub> O	Fisher Scientific	S369-500	
Poly-L-lysine (0.1mg/ml)	Sigma	P8920-100	
Potassium acetate	Fisher Scientific	BP364-500	
Sodium acetate	Fisher Scientific	S209-500	Prepare 3M stock
Sodium cacodylate	Polysciences, Inc.	1131	<b>Toxic:</b> wear appropriate protection. Prepare 400mM stock
Sodium citrate	Fisher Scientific	BP327-1	
Sodium chloride	Fisher Scientific	S271-3	Sodium chloride
Sucrose	Fisher Scientific	S5-500	
10% Tween 20	Thermo Scientific	28320	Surfact-Amps
10% Triton X-100	Thermo Scientific	28314	Surfact-Amps
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>Solutions</b>			
<b>Note:</b> All solutions are prepared using sterile ultrapure water and should be sterilized either by autoclave or filter sterilization.			
TE buffer			10 mM Tris, 1 mM EDTA, pH = 8.0
20X SSC (Saline Sodium Citrate)			3 M NaCl, 300 mM sodium citrate
2X cacodylate fix solution			<b>Toxic:</b> wear appropriate protection. 200 mM sodium cacodylate, 200 mM sucrose, 80 mM sodium acetate, 20 mM EGTA
1.1X Hybridization buffer			3.3X SSC, 55% formamide, 11% dextran sulfate
Fix solution			<b>Toxic:</b> wear appropriate protection. 4% formaldehyde, 1X cacodylate fix solution
PBSBTx			1X PBS, 0.5% BSA, 0.1% Triton X-100
PBSTx			1X PBS, 1% Triton X-100
Extraction buffer (PBSTx + Rnase)			1X PBS, 1% Triton X-100, 100 µg/mL RNase
2X SSCT			2X SSC, 0.1% Tween 20
2X SSCT + 20% formamide			<b>Toxic:</b> wear appropriate protection. 2X SSC, 0.1% Tween 20, 20% formamide
2X SSCT + 40% formamide			<b>Toxic:</b> wear appropriate protection. 2X SSC, 0.1% Tween 20, 40% formamide
2X SSCT + 50% formamide			<b>Toxic:</b> wear appropriate protection. 2X SSC, 0.1% Tween 20, 50% formamide
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>Enzymes</b>			
AluI	New England Biolabs	R0137S	

HaeIII	New England Biolabs	R0108S	
MseI	New England Biolabs	R0525S	
MspI	New England Biolabs	R0106S	
RsaI	New England Biolabs	R0167S	
BfuCI	New England Biolabs	R0636S	
100X BSA	New England Biolabs		Comes with NEB enzymes
10X NEB buffer #2	New England Biolabs		Restriction enzyme digestion buffer. Comes with NEB enzymes
Terminal deoxynucleotidyl transferase (TdT) 400 U/μl	Roche/Sigma	3333566001	
TdT buffer	Roche/Sigma		Comes with TdT enzyme
Cobalt chloride	Roche/Sigma		<b>Toxic:</b> wear appropriate protection. Comes with TdT enzyme
RNase A (10 mg/mL)	Thermo-Scientific	EN0531	
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>Cytology Tools etc.</b>			
Forceps	Dumont		#5 INOX, Biologie
9" Disposable glass Pasteur pipettes	Fisher	13-678-20C	Autoclave to sterilize
Shallow glass dissecting dish			Custom made
Deep well dish (3 wells)	Pyrex	7223-34	
Fisherfinest Premium microscope slides	Fisher Scientific	22-038-104	Used to cover deep well dishes
Frosted glass slides, 25 x 75 mm	VWR Scientific	48312-002	
Glass slides, 3 x 1 in, 1 mm thick	Thermo-Scientific	3051	
Coverslips, 10 x 10 mm, No. 1.5	Thermo-Scientific	3405	
Tungsten needle			homemade
Prolong GOLD mounting media	Molecular Probes	P36930	
Compressed-air in can	Various		
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>Equipment</b>			
PCR machine	Various		
Nanodrop 2000, spectrophotometer	Thermo-Scientific		microvolume spectrophotometer
Vortexer	Various		
Table top microfuge at room temperature	Various		
Table top microfuge at 4 °C	Various		
Heat block	Various		
Hybridization oven or incubator with rotator	Various		
Nutator	Various		
A1RSi laser scanning confocal	Nikon		40X oil Plan Fluor DIC (NA 1.3)
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>Consumables etc.</b>			
50 mL conical tubes	Various		
15 mL conical tubes	Various		
1.5 mL microfuge tubes	Various		

500 µl microfuge tubes	Various		
200 µl PCR tubes	Various		
Plastic container with tight fitting lid	Various		To hold Drierite
Kimwipes	Various		disposable wipes
Parafilm	Various		paraffin film
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>Other</b>			
HPLC purified 5'-labeled oligonucleotides	Integrated DNA Technologies		Cy3-labeled probes that recognize the 359 bp satellite repeat of the X chromosome
Volocity 3D Image Analysis Software	PerkinElmer		Version 6.3