FIB-SEM / SEM Array Tomography Sample Preparation of Cell Monolyaer

Note: This protocol is designed to enhance signal for backscatter electon (BSE) imaging of epoxy embedded cell monolyar at low accelerating volatages (1-5 keV).

Day 1				
Steps		**Time**	**Temp**	**Check**
Fixation (I)	2.5% GA in 0.1M CB	5 m x 1	36.5 °C	☐
	EMS, Cat #15960	25 m x 1	RT	☐
Washing	0.1M CB	3 m x 3	RT	☐☐☐
Fixation (II)	1% $K4Fe(CN)_6$ + 1% OsO_4 in 0.1M CB	15 m x 1	ICE	☐
	0.2ml 10% postassium ferrocyanide K4Fe(CN)6, 1%			
	0.5ml 4% OsO4, 1%			
	0.5ml 0.4M CB, 0.1M			
	0.8ml MQW			
	2 ml Total			
Washing	Mili-Q/Distilled Water	3 m x 3	RT	☐☐☐
TCH	1% Thiocarbohydrazide (TCH) in Mili-Q/Distilled Water	5 m x 1	RT	☐
	✓ Make fresh and filter right before use! ✓ Dissove 0.02g TCH with 2ml Mili-Q/Distilled Water in glass vial at 60 °C! ✓ A toxic compound made by the reaction of carbon disulfide with hydrazine (hydrazinolysis). ✓ It is used in the silver proteinate specific staining of carbohydrates.			
Washing	Mili-Q/Distilled Water	3 m x 3	RT	☐☐☐
OsO4	1% OsO4 in Mili-Q/Distilled Water	15 m x 1	RT	☐
Washing	Mili-Q/Distilled Water	3 m x 3	RT	☐☐☐
en bloc Stain	1% UA in Mili-Q/Distilled Water	Overnight	4 °C	☐

Day 2				
Washing	Mili-Q/Distilled Water	3 m x 3	RT	☐☐☐
Walton's Lead Asparate Staining		10 m x 1	60 °C	☐
	✓ Prepare an aspartic acid stock solution by dissolving 0.998 g of L-aspartic acid in 250 ml of MQW. ✓ Dissolve 0.05g L-aspartic acid in 12.5ml boiled distilled water. ✓ Note: the aspartic acid will dissolve more quickly if the pH raised to 3.8. ✓ This stock solution is stable for 1-2 months if refrigerated. ✓ To make a lead asparate solution dissolve 0.066 g of lead nitrate in 10ml of asparatic acid stock and pH adjusted to 5.5 with 1N KOH/NaOH. ✓ 12 drops of 1N KOH/NaOH using 1 ml measurer disposible pipette. ✓ Note: the lead asparate solution is placed in a 60 °C oven for 30 minutes.			
Washing	MQW	3 m x 3	RT	☐☐☐

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Dehydration	10% EtOH	5 m x 1	ICE	☐
	30% EtOH	5 m x 1	ICE	☐
	50% EtOH	5 m x 1	ICE	☐
	70% EtOH	5 m x 1	ICE	☐
	80% EtOH	5 m x 1	ICE	☐
	90% EtOH	5 m x 1	ICE	☐
	95% EtOH	5 m x 1	ICE	☐
	100% EtOH (I)	5 m x 1	RT	☐
	100% EtOH (II)	5 m x 1	RT	☐
Infiltration	Durcupan : 100% EtOH = 1 : 2	30 m x 1	RT	☐
	Durcupan : 100% EtOH = 1 : 1	1 h x 1	RT	☐
	Durcupan : 100% EtOH = 2 : 1	4 h x 1	RT	☐
	Absolute Durcupan	Overnight	RT	☐

Day 3				
Polymerization	Absolute Durcupan	48 h	70 °C	☐

Day 4				
Re-sizing	✓ ~ 1.2 mm^3 of resin blocks ✓ Attach on resin cylinder for SEM Array Tomography ✓ Attach on SEM pin stub for FIB-SEM imaging			☐
Sectioning	✓ 750 x 750 x 70 nm for TEM imaging			☐
	✓ 800 x 800 x 50 nm for SEM Array Tomography			☐
Carbon Evaporation	✓ Sections on a Si wafer	Carbon Thread	7 nm	☐
Sputtering	✓ ~ 1.2 mm^3 of resin block on SEM pin stub	Au	10 nm	☐

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