Naoji YUBUKI 2010

Transmission Electron Microscope (TEM) sample preparation

There is no perfect method for TEM preparation due to combinations of factors (fixative, buffer, concentration, temperature and processing time). A basic procedure to start TEM work is shown below. However, this should be regarded as a starting point, and methodologies may vary by sample in order to achieve better results. The most important thing to remember is the health of cell(s). It is my experience even that conditions such as time of day and position in cell cycle are important when considering TEM preparation when using synchronized cultures.

Dysnectes brevis

The followings are the factors one can change:

Buffer: sodium cacodylate buffer, phosphate buffer, collidine buffer
Fixation method: double fixation, simultanious fixation
Concentration of fixative: 0.5-5% for glutaraldehyde, 0.1-2% for osmium tetraoxide
Duration for fixing: 30 min to 24 hours for glutaraldehyde and osmium tetraoxide
Osmic control: with or without sucrose or sorbitol
Concentration of osmotic control: 0.1-0.2M for sucrose and 0.2-0.4M for sorbitol.
Temperature: on ice or room temperature for fixing process

Rictus lutensis

Method: Glutaraldehyde and Osmium Tetraoxide (OsO_4) double fixation

- Cool down cell suspention and a centrifuge beforehand.
- Prepare the fixative solution right before processing.
- Use a glass or polypropylene container. Solvents (aceton and propylene oxyde) dissolve polystyrene products.

Wobblia lunata

Cell suspension (4˚C)

Fixative solution (4 ˚C)
- ☐ Fixative: 5% Glutaraldehyde (EM grade)
- ☐ Osmotic control: 0.2M sucrose
- ☐ Buffer: 0.2M sodium cacodylate buffer

First fix: Mix equal volumes of the cell suspension and the fixative solution.
- ☐ Shake the mixture gently.
- ☐ Keep cool for two hours.

Caecitellus parvulus

Centrifugation: at 4 ˚C for 5 min to form a pellet.
- ☐ ☐ *Do not rotate very high speed. Preferably 1000g or lower.*

Wash: with 0.2M sodium cacodylate buffer (pH7.2) three times, 5min on ice for each wash.

Post fix
Remove the buffer and add the second fixative solution (below).
Keep it on ice for 1 hour.
- ☐ ☐ Fixative: 1% OsO_4
- ☐ ☐ Buffer: 0.2M sodium cacodylate buffer

Calkinsia aureus

Cafeteria roenbergensis

Naoji YUBUKI 2010

Transmission Electron Microscope (TEM) sample preparation

Wash: with 0.2M sodium cacodylate buffer (pH7.2) once, 5min on ice.

Room temperature hereafter.

Dehydration: with graded ethanol series.
- ☐ 10 to 20 min. for 30, 50, 75, 90, 95% Et-OH for each steps
- ☐ 4 times, 15 min for 100% Et-OH for each steps.

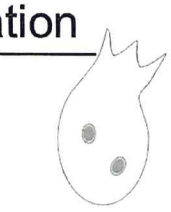
Substitution 1:
1. Ethanol and acetone (or propylene oxide) mixture (1:1) twice, 10 min. for each steps.
2. 100% acetone (or propylene oxide) twice, 10 min. for each steps.
3. Acetone (or propylene oxide) and resine mixture (1:1) for 6 to 12 hours in room temperature.

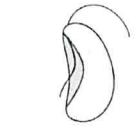
Cover the sample container loosely with aluminium foil. Concentration of the resin will increase as acetone (or propylene oxide) evaporates.

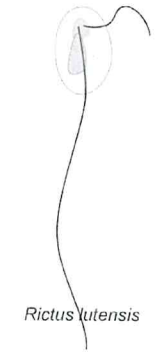
Substitution 2: Change with 100% resin twice, 5 hours for each steps.

Embedding:
- ☐ Change with 100% resin and put a label with the sample information written with pencil.
- ☐ Polymerize the resin in 70 ˚C oven for 10 to 12 hours.

Spurr's resin (Spurr 1969 J. Ultrastr. Res. 26:31) is made from four kinds of monomers and has to be mixed completely. The unpolymerized resine can be kept in a freezer. It needs to be stored separately from water. When removing resin from freezer storage, keep bottle at room temperature without opening the lid. Otherwise condensation forms on the bottle interior. This could be detrimental to the resin.


Stepahnopogon minuta

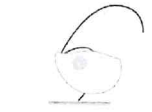

Dysnectes brevis


Rictus lutensis


Wobblia lunata


Caecitellus parvulus


Calkinsia aureus


Cafeteria roenbergensis