

## Methods

# A protocol for preparation of chromosome spread and processing for transmission electron microscopy

Tatsuo Oguro<sup>1</sup>, Mohammad Ghazizadeh<sup>2</sup>

<sup>1</sup>Pathology Division, Chiba Hokusoh Hospital, Nippon Medical School, Chiba, Japan

<sup>2</sup>Central Institute for Electron Microscopic Researches, Nippon Medical School, Tokyo, Japan

**Abstract.** Transmission electron microscopic (TEM) observation of human chromosome ultrastructure may provide useful information on various abnormalities. Due to the difficulties in detaching metaphase chromosome spreads from the glass slide, this approach has been restricted. We introduce a simplified protocol in which the metaphase chromosome spreads are made on a flexible thermoplastic membrane (Aclar film) glued to the routine microscopic glass slides. The chromosome spreads are then impregnated with 1% osmium tetroxide, stained with freshly prepared 2% tannic acid, dehydrated, and flat-embedded in epoxy resin. After polymerization, the epoxy resin sheet is easily detached from the Aclar film and all chromosome spreads retain on the resin sheet. Each chromosome spread is identified under a light microscope, demarcated, trimmed then attached to a pre-polymerized blank epoxy resin block. Ultrathin sections are cut and observed under a transmission electron microscope. Application of this method to chromosome research is suggested to provide useful information on the chromosome morphology and ultrastructure in relation to various conditions and/or function.

**Keywords:** Method, flat embedding, transmission electron microscopy, human chromosome, ultrastructure

## Introduction

Transmission electron microscopy (TEM) is used to observe ultrastructural details of the human cells in health and disease. However application of this method to the human chromosomes has been faced with various problems mainly related to the success in separation of chromosome spreads from the glass slides for TEM processing [1]. Accordingly, the chromosomes could be sporadically and incompletely separated with no guarantee for separation of a whole chromosome spread. Wen et al. reported a repeated chilling and warming of the glass slide carrying chromosome spreads and epoxy resin block but this method was also associated with a partial success [2]. We previously reported a method to overcome the problems inherent in the process of chromosome spreads for TEM observation [3]. In this communication, we revisit this issue and provide the protocol used in that study in order to facilitate further use of the method. It is a simple and reproducible protocol which can be applied for preparation of chromosome spreads and processing for TEM observation.

## Materials and Methods

Normal human lymphocytes were obtained from a volunteer donor after obtaining informed consent. The blood (1 ml) was collected in a heparinized tube.

## Protocol

The protocol for preparation and TEM processing of chromosome spreads from human blood lymphocytes as adopted from the routine methods are summarized as below:

- Cut Aclar film (Nissin EM Co. Ltd., Tokyo, Japan) into the size of a coverslip (24 x 50 mm), mount on plain microscopic glass slides using a clear conventional mounting medium and dry overnight in an oven at 40°C.
- Clean in absolute ethanol and chill on dry-ice before use.
- In a culture flask, add 0.7 ml of heparinized blood to 10 ml of RPMI 1640 complete medium containing 12.5 µg/ml phytohemagglutinin (PHA-P, Sigma) and incubate in a CO<sub>2</sub> incubator for 72 hr at 37°C.
- Add 0.025 µg/ml Colcemid (Gibco, BRL), mix well and incubate in a CO<sub>2</sub> incubator for 72 hr at 37°C.
- Transfer the content into a 15 ml centrifuge tube and centrifuge at 1200 rpm for 10 min.
- Remove supernatant leaving 0.5 ml medium and re-suspend the cells in this volume.
- Add 2ml of pre-warmed (37°C) 0.075 M KCL in distilled water drop wise while gently agitating.
- Add an additional 8 ml of KCL for a total of 10 ml, mix well and incubate for 15 min at 37°C in a water bath.
- Add a few drops of freshly prepared fixative (methanol/glacial acetic acid, 3:1), close the tube's cap

\* Corresponding author: Mohammad Ghazizadeh, MD  
[ciem@nms.ac.jp](mailto:ciem@nms.ac.jp)



**Figure 1** A chromosome spread on Aclar film attached to a glass slide after flat-embedding in epoxy resin.



**Figure 2** Transmission electron microscopic observations of normal human chromosomes. Longitudinal section of chromosomes clearly show preserved morphology and internal ultrastructural details.

and invert to mix.

- Centrifuge the cells and remove the supernatant leaving 0.5 ml and re-suspend cells in this volume.
- Fix by adding 10 ml of the fixative with the first 2 ml being added drop wise with gentle agitation.
- After 10-15 min at room temperature, centrifuge the cells and remove the supernatant leaving 0.5 ml and re-suspend cells in this volume.
- Repeat the fixation procedure for two more times and suspend the cells in a small amount of the fixative then drop onto the surface of the pre-cleaned slides chilled on ice.
- Before drying, quickly re-hydrate in 90%, 70%, 50% ethanol each for 1 min, rinse in distilled water and fix with 2.5% glutaraldehyde for 30 min.
- Impregnate with 1% osmium tetroxide for 10 min followed by staining with a freshly prepared 2% tannic acid 3 times each for 10 min.
- Dehydrate in 50%, 70%, 90%, 100% ethanol, soaked in propylene oxide, and flat-embed by adding a few drops of epoxy resin mixture on the slide and placing a matched size pre-cut Aclar film on top as a covering weight to flatten the resin.
- Polymerize in oven as for the routine transmission

electron microscopic epoxy resin block.

- After polymerization, peel off the overlaid Aclar film and separate the epoxy resin sheet from the bottom layer Aclar film.
- Place the thin epoxy resin sheet on a plain glass slide and screen under a light microscope.
- Identify a desired chromosome spread, demarcate, cut out the area by scissors and mount on a blank epoxy resin block.
- Cut serial ultrathin sections on an ultramicrotome and further stain with or without uranyl acetate and lead citrate and observe under an electron microscope.

## Results

The results of observation of chromosome ultrastructure are shown in Fig. 1 and Fig. 2. The chromosome spreads were easily detached from the Aclar film with no residual chromosomes left. As it can be seen the chromosomes from human lymphocytes clearly show details of their internal ultrastructure. The preservation of chromosome morphology and structure was excellent.

## Discussion

The present protocol facilitates comprehensive analysis of internal ultrastructure of individual chromosomes by TEM. It allows a preliminary light microscopic screening and identification of the desired chromosomes. Since the whole chromosome spread is detached from the slides, essentially all 46 chromosomes per cell can be observed ultrastructurally. In addition, this allows studies on changes in chromosomal ultrastructure during the cell cycle or differences between normal and tumor chromosomes or gene mapping on chromosomes.

In conclusion, the protocol introduced here allows observation of chromosome spreads at the TEM level which makes it possible to analyze internal organization of DNA content in pre-identified individual chromosomes in various chromosomal regions. Application of this method to chromosome research is suggested to provide useful information on the ultrastructure of different chromosomes in relation to function.

## Conflict of Interest

The authors declare no conflicts of interest.

## References

1. Squarzoni S, Cinti C, Santi S, Valmori A, Maraldi NM. Preparation of chromosome spreads for electron (TEM, SEM, STEM), high and confocal microscopy. *Chromosoma* 103:381-392, 1994.
2. Wen GY, Jenkins EC, Goldberg EM, Genovese M, Brown WT, Wisniewski HM. Ultrastructure of the fragile X chromosome: New observations on the fragile site. *Am J Med Genet* 83:331-333, 1999.
3. Ghazizadeh M, Sasaki Y, Oguro T, Sato S, Egawa S, Inoue K, Shimizu H, Kawanami O. A novel technique for observing the internal ultrastructure of human chromosomes with known karyotype. *Microsc Microanal* 14:357-361, 2008.