Immunostaining of spindle checkpoint proteins on Drosophila mitotic chromosomes from larval brains

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Method Article

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Abstract

Introduction

We describe a very simple method to immunostain Drosophila mitotic chromosomes for spindle checkpoint proteins and kinetochore components. By using appropriate primary antibodies, this procedure allowed us to detect Zw10, Zwilch, Cenp-C, Cenp-meta and BubR1 on kinetochores of larval brain chromosomes. However, the same method can be used for detecting also structural components of chromosomes \(our unpublished results).

Reagents

Saline \(0.7% NaCl), Hypotonic solution \(0.5% sodium citrate). Fixing solution \(1.8% Formaldehyde, 45% acetic acid in dH20) Liquid nitrogen Ethanol PBS-T \(PBS containing 0.1% TritonX). Appropriate primary and secondary antibodies Vectashield medium H-1200 with DAPI \(4,6 diamidino-2-phenylindole)

Equipment

Slides and coverslips Humid box Tweezers Dissecting Scope Epifluorescence Microscope

Procedure

1. Dissect Drosophila larval brains in saline \(0.7% NaCl), 2. Treat dissected brains for 10 min with a hypotonic solution of 0.5% sodium citrate. 3. Fix for 5 min in a drop of fixing solution \(1.8% Formaldehyde, 45% acetic acid) on a coverslip. 4. Gently lean a clean slide over the coverslip and squash the brain in the same fixing solution 5. Freeze the slide in liquid nitrogen 6. Remove the coverslip and immerse the slide in cold ethanol \(-20°C) for 10 minutes. 7. Wash the slide in PBS-T \(PBS containing 0.1% TritonX). 8. Incubate the slide overnight with appropriate primary antibody in a humid box at 4°C 9. The next day, wash the slide twice in PBS-T for 10 minutes 10. Incubate the slide with the secondary antibody for 2 h at room temperature, in a humid box. 11. Wash the slide twice in PBS-T for 10 minutes and let it air dry. 12. Mount the slide in Vectashield medium H-1200 with DAPI \(4,6 diamidino-2-phenylindole; Vector Laboratories, Burlingame, CA) to stain DNA and reduce fluorescence fading. 13. Analyze the immunostaining with a epifluorescence microscope