Creative Bioarray

# Metaphase Slide Preparation Protocol

## Introduction

This protocol describes the method approved by Creative Bioarray scientists to prepare slides for assays requiring metaphase cells. These include G-banding, metaphase fluorescence in situ hybridization (FISH) and others.

## Supplies and Reagents

- De-ionized water
- Positively charged glass microscope slides, 75 x 25 mm (SciGene CytoDrop® Slides Cat# 2060-01-01 or equivalent)
- Glacial acetic acid
- Molecular grade methanol
- Lint-free paper towels
- 100% Ethanol, molecular grade

## Equipment

- Micropipette and tips (20–200µL range) or Pasteur pipettes and bulb
- Chemical fume hood
- 50 mL Glass bottle with screw cap
- 50 mL Glass Coplin jars
- Benchtop slide warmer set to 40°C
- Inverted phase contrast light microscope with 40X objective
- 4°C refrigerator or ice bucket
- Laboratory centrifuge with buckets compatible with 15 mL conical tubes
- Electronic timer
- Benchtop oven set to 90°C
- Slide folder or plastic slide box
- Hygrometer for ambient relative humidity measurement
- Thermometer for ambient temperature measurement
- Optional: Cytogenetic slide prep chamber (Thermotron CDS-5 or equivalent)

## Procedure

### Cleaning the microscope slides (Optional)

1. Fill one clean Coplin jar with 100% ethanol, another with DI $H_2O$ and place a paper towel on the benchtop.

2. Dip each microscope slide in and out of the ethanol jar three times.
3. Tap the excess ethanol off the slide onto the paper towel and wipe both sides with a fresh paper towel.
4. Once dry, place each slide in a Coplin jar with DI $H_2O$ and store the jar in a 4°C refrigerator or an ice bucket for at least 30 minutes to chill.

## Preparing the cell pellets and checking cell density

*Note: Perform the remainder of this protocol in a fume hood to reduce exposure to fixative fumes.*

5. Prepare fresh 3:1 methanol to acetic acid fixative (Carnoy's fixative) in a 50 mL glass bottle and loosely cap it.
   a. The amount of Carnoy's fixative needed will vary depending on the number of samples and whether the samples require a fixative change.
6. Samples stored more than 24 hours after harvest must undergo a fixative change following the steps below.
   a. Centrifuge the samples at 1000 rpm / 232 g for 10 minutes at room temperature.
   b. Carefully aspirate supernatant, leaving about 0.5 mL of media behind with the cell pellet.
   c. Resuspend the cell pellet by gently flicking the bottom of the conical tube.
   d. Add 5 mL of fresh Carnoy's fixative, cap the tube and gently invert several times.
7. Centrifuge the samples at 1000 rpm / 232 g for 10 minutes at room temperature.
8. Carefully aspirate the supernatant and re-suspend the cell pellet by gently flicking the bottom of the conical tube.
   a. The amount of supernatant removed should result in a slightly turbid appearance once the pellet is resuspended. The appropriate turbidity will vary by sample.
   b. If in doubt as to sample quality or concentration and there is sufficient sample available, a single test drop can be applied to a fresh slide to check the cellular density using an inverted phase contrast light microscope with 40X objective.

## Metaphase Dropping Quality Control and Corrective Actions

9. Do a test drop. Figure 1 shows an example of good cell density
10. If there are too many cells packed together, chromosomes will not spread freely. There may appear to be a haze or film over the sample overall, and there may be dark, residual cytoplasm around the chromosomes.
    a. Add a small amount of fresh Carnoy's fixative to the sample tube
    b. Cap it, mix by gentle flicking and attempt another small test drop
11. If there are too few cells, more slides will be needed overall to complete the analysis.
    a. Re-centrifuge at 1000 rpm / 232 g for 10 minutes.
    b. Carefully aspirate a little more supernatant than in the initial preparation
    c. Resuspend by gentle flicking and attempt another small test drop.

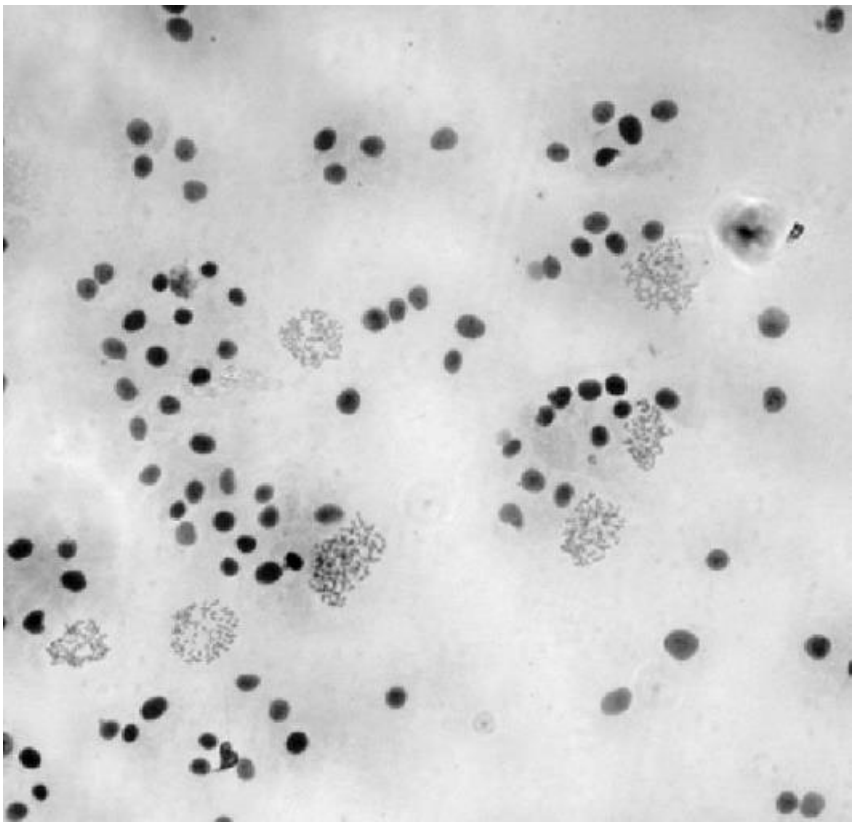

Figure 1: Example of good cell density after dropping sample on a slide. (Rondón Lagos, S. M., & Rangel Jiménez, N. E. (2012). Cytogenetic Analysis of Primary Cultures and Cell Lines: Generalities, Applications and Protocols. InTech. doi: 10.5772/34200 )

## Dropping sample onto slides

Note: Ambient conditions have a substantial impact on the quality of metaphase slides. A temperature range of 23°C to 24°C and relative humidity of 45% to 50% will give the best results. Under optimal conditions, the sample should dry completely on the slide after 30 to 45 seconds. A laboratory timer, a hygrometer and a thermometer are strongly advised to measure these variables. Alternatively, a cytogenetic slide prep chamber (e.g., Thermotron®) can be used to create a controlled environment in which to prepare slides. Users may apply a few drops of an expendable sample on a single slide to assess metaphase quality before risking more important samples.

12. Place an empty Coplin jar on top of a paper towel so its weight holds the paper towel in place.
13. Using a Pasteur pipette and bulb or a 200µL micropipette with fresh tip, draw up approximately 35µL of cell suspension.
14. Take a fresh slide from the chilled Coplin jar. While it is still wet, hold it at a 45° angle and release three to four drops of cell sample across the top of the unfrosted part of the slide, letting the suspension drip downwards away from the label as in Figure 2.

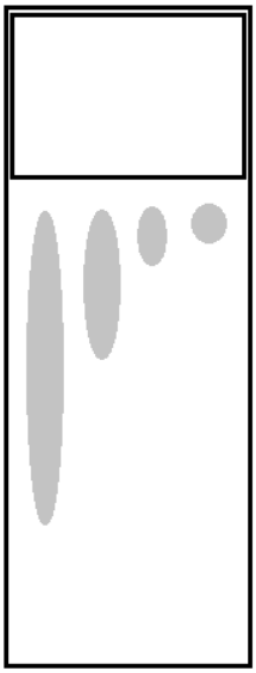

Figure 2. Example of cell sample drops streaming down slide surface.

15. Prop the slide up against the empty Coplin jar as in Figure 3.

Figure 3. Slide propped on Coplin jar.

16. The sample on the slide's surface will look grainy as it dries. At that point, and before it has dried completely, draw up about 50 µL of Carnoy's fixative with a clean micropipette tip or

Pasteur pipette and rinse the cells by dropping the fixative over the slide liberally, letting the liquid run down the slide as before.

17. Allow the slide to air dry, then examine it under a phase contrast microscope.

## Assessing sample quality

18. Low ambient humidity (e.g., sample dries in about 30 seconds) will produce metaphase spreads with a small diameter. Multiple chromosomes may overlap, making analysis challenging. Either one, or both, of the following steps can help mitigate low relative humidity.
    a. Place a thin stack of damp paper towels on a benchtop slide warmer set to 40°C. When dropping, lay the slide on the paper towels. The steam will lengthen the drying time, helping chromosomes spread before they fix onto the glass.
    b. Chill the sample tube before dropping. Lowering the temperature of the liquid medium will lengthen its evaporation time.
19. High ambient humidity (e.g., samples take about 45 seconds to dry) will allow chromosomes to drift too far and outside of the field of view. These are termed “broken metaphases.” Either one, or both, of the following steps can help mitigate high relative humidity.
    a. When dropping, lay the slide directly on a benchtop slide warmer set to 40°C. The surrounding air will be drier and warmer, shortening drying time.
    b. Drop sample onto dry slides. There will be less liquid, shortening drying time.
20. Figures 4 through 6 contain example images of G-banded metaphase cells showing a range of chromosome spreading outcomes.

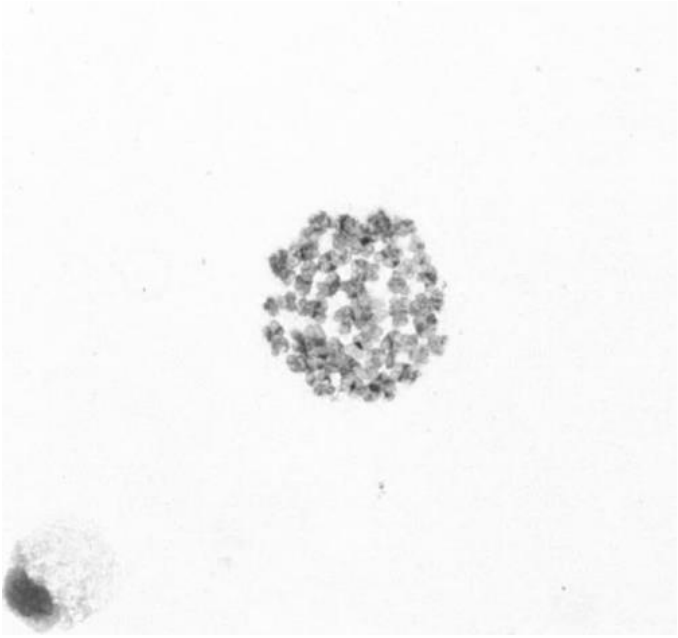

Figure 4. Metaphase cell with insufficient spreading; spread is too compact.

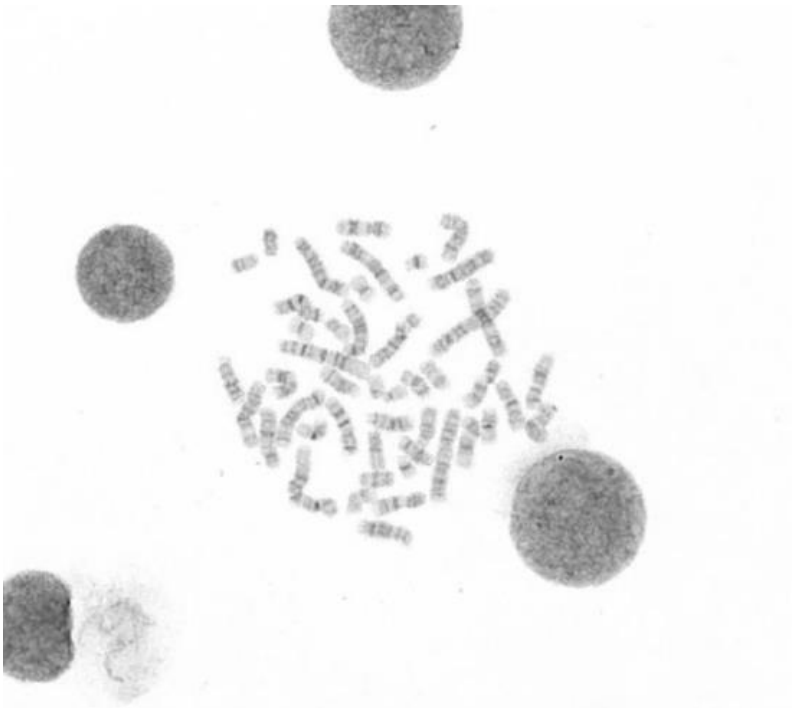

Figure 5. A metaphase cell with good spreading; whole, and few to no overlapping, chromosomes.

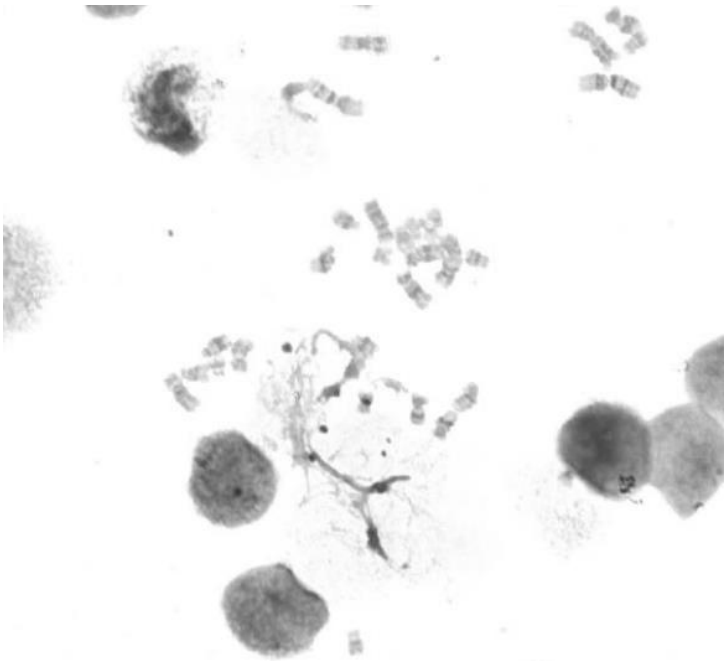

Figure 6. “Broken” metaphase cell. Part of the chromosome complement is outside the field of view.

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## Aging slides

21. Once dry, slides are ready to be aged. slides may either be left at room temperature for 24 hours or be placed in a 90°C oven for ten minutes.
22. Once slides have been aged, they should be stored at 4°C in a slide folder or plastic slide box to protect them from dust until hybridization.