Metaphase Chromosome Spread Prep

This is a baseline protocol. Some steps may need to be optimized by the researcher.

All solutions should be made with molecular grade DNase/RNase free water unless otherwise specified.
All steps are at room temperature (RT) unless otherwise specified.
All slide rinses and incubations are done in coplin jars/slide dishes with gentle agitation/rocking.

This protocol is optimized for mammalian cells at ~70%-75% density in a T175 flask.

Preparation of Culture Cells:

Preheat to 37°C – all sterile;
Media
TrypLE
DPBS- Magnesium Chloride and Calcium Chloride free
Colcemid (10 µg/ml)
0.075M KCl (3 ml 1M + 37 ml Molecular Grade DNase/Rnase free water)

1. Add 10 µl Colcemid stock per ml of media for final concentration of 0.1 µg/ml.
    - Incubate at 37°C for 30 minutes to overnight. A good starting point is 1 hr.
2. Remove media and save in 50 ml conical tube (one for each 175T flask).
    - If cells are in suspension go directly to step 5.
3. Add 10 ml DPBS to the flask, gently rinse-add to the 50 ml conical with media.
4. Add 10 ml TrypLE to the flask and incubate at RT for 2 min.
    - Check under the scope to confirm cells are lifting.
    - When cells begin to lift off add 5 ml media, squirt directly onto adherent cells to remove, do not rap the flask, pipet up and down to break up clumps.
        - Add to 50 ml conical above.
5. Spin at 175 g for 5 min at RT.
6. Gently pour off supe, leave behind ~ 0.5 ml, resuspend by “flicking” tube.
7. Add 2-50 ml pre-warmed 0.075M KCl - amount is pellet size dependent. Use 2 ml if the cell pellet is just coating the bottom of the tube. If there is 0.5 ml – 1 ml cells add 10-50 ml to the tube.
    - Add slowly, flowing down the side of the tube. Continuously mix by flicking the tube. The solution should appear slightly cloudy, not clear.
8. Incubate the tube at 37°C for 30 min with gentle mixing.
    - Combine into one 50 ml tube if more than one tube is used.
9. Make fresh Modified Carnoy’s Fixative.
    - 3:1 MetOH:Glacial Acetic Acid.
    - Carefully add 10 ml Glacial Acetic Acid to 30 ml MetOH and set aside 12 ml on ice.
10. Use 1 ml pipettor, add 1 drop Modified Carnoy’s fixative/ml KCl with flicking-keep cells suspended.

- Gently invert to mix.

11. Spin cells at 250g for 5 min at RT.
    - Remove supernatant, leave ~ 0.5 ml behind, flick to resuspend.
12. Slowly add 5 ml Modified Carnoy's fixative with flicking to keep cells suspended.
    - Gently invert to mix.
13. Spin at 250g for 5 min at RT.
    - Remove supernatant, leave ~ 0.5 ml, flick to resuspend.
14. Add 5 ml Modified Carnoy's fixative as before, Spin at 250g for 5 min at RT.
    - Remove supernatant, leave ~0.5 ml and flick to resuspend cells.
15. Repeat step 15. Remove supernatant with caution, the pellet may be loose.
16. Add 8-15 ml fresh ice cold Modified Carnoy's fixative – final vol appropriate for 3:1 fix:cell suspension ratio.
    - The solution should appear cloudy yet translucent.
    - Cap and seal with parafilm. Store at -20°C for up to a year or more.

Adapted from:
Padilla-Nash et al., Spectral Karyotyping Analysis of Human and Mouse Chromosomes, Nat Proto 1, 3129-3142 (2006). https://doi.org/10.1038/nprot.2006.358

Metaphase Chromosome Spreads on Slides:

Preheat appropriate volume of 10 mM HCl to 37°C.

1. Place “Precleaned Fisherbrand Superfrost Plus” Microscope slides in Milli-Q water, store at 4°C overnight.
2. Remove metaphase chromosome cell preparation from -20°C freezer, gently invert to mix and place on ice. Remove slides from 4°C and place on ice.
3. Spin cells @250g for 5 min RT, carefully decant supernatant, add appropriate volume of fresh ice cold Modified Carnoy’s fixative and resuspend the pellet.
4. Remove a slide from water, shake off excess water, use 50 ul transfer pipettor drop one drop of cell suspension on cold slide.
    - Distribution of chromosomes may improve with tilting the slide ~11° longways and letting the drop slowly run down the slide.
5. Let the slides air dry minimum 1 hr RT, overnight is best. Cover to protect from dust.
6. Incubate slides in formaldehyde solution, 10 min (save solution for following incubation).
7. Rinse in 2X SSC, 5 minutes.
8. Just before use add 500 µl pepsin (10 mg/ml) to dry slide dish and add pre-warmed 10 mM HCl..
9. Incubate in freshly made pepsin solution at 37°C for 3-6 minutes (6 minutes is best).
10. Rinse in PBS/Mg $MgCl_2$ for 5 minutes
11. Incubate in formaldehyde solution for 10 minutes
12. Rinse in PBS/$MgCl_2$ for 5 minutes
13. Dehydrate, 70%, 90%, 96% EtOH, 5 minutes each. Air dry 20 minutes, cover to protect from dust.
14. Use immediately or store at -20°C.

REAGENTS

Modified Carnoy’s Fixative

3:1 Methanol:Glacial Acetic Acid – make fresh

- 30 ml of 100% Methanol
- 10 ml Glacial Acetic Acid
- Make appropriate volume needed for experiment.

PBS/$MgCl_2$

1X PBS, 50mM $MgCl_2$

- 20 ml 10X PBS
- 10 ml 1 M MgCl2
- Bring to 200 ml with molecular grade DNase/RNase free water

Formaldehyde Solution

1% Formaldehyde in PBS/$MgCl_2$

- 3 ml Molecular Biology Grade Formaldehyde (36.5-38% Sigma-Aldrich, #F8775)
- 97 ml PBS/$MgCl_2$

Pepsin solution

10 mg/ml in Molecular Grade DNase/RNase free water

- Dissolve 250 mg of Pepsin (Sigma Cat #P7012) in 25 ml molecular grade DNase/RNase free water.
- Store aliquots at -20°C.
- Use 500 µl/100 ml 10 mM HCl prewarmed to 37°C.

Adapted from:

Liehr et al., How to Obtain High-quality Metaphase Spreads for Molecular Cytogenetics, Current Protocols, 2, e392, https://doi.org/10.1002/cpz1.392