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Ultra Expansion microscopy protocol with improved setup for upright and inverted microscopes.

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1

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Ultra Expansion microscopy

This protocol is based on the following papers:

Gambarotto D, Hamel V, Guichard P (2021). Ultrastructure expansion microscopy (U-ExM).. Methods in cell biology. https://doi.org/10.1016/bs.mcb.2020.05.006

Amodeo S, Kalichava A, Fradera-Sola A, Bertiaux-Lequoy E, Guichard P, Butter F, Ochsenreiter T (2021). Characterization of the novel mitochondrial genome segregation factor TAP110 in Trypanosoma brucei.. Journal of cell science. https://doi.org/pii:jcs254300.10.1242/jcs.254300

Isch C, Majneri P, Landrein N, Pivovarova Y, Lesigang J, Lauruol F, Robinson DR, Dong G, Bonhivers M (2021). Structural and functional studies of the first tripartite protein complex at the Trypanosoma brucei flagellar pocket collar.. PLoS pathogens. https://doi.org/10.1371/journal.ppat.1009329

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Reagents	Company	Reference	Comment
Absolute ethanol			
Acrylamide 40%	Euromedex	EU0060-A	store at 4°C
Ammonium persulfate (APS)	Euromedex	EU0009	store as 10% in H2O at -20°C
Formaldehyde 37%	Sigma	252549	
HCl			1N solution
HOECHST (bisBenzimide H 33342 trihydrochloride)	Sigma	B2261	make 5 mg/mL in H2O. Store at -20°C as aliquotes
milliQ Water			0.2 um filtered
N,N'-Methylenbisacrylamide 2%	Euromedex	EU0560	store at 4°C
PBS			0.2 um filtered
SDS 20%			
Tris-Base			
NaCl			stock solution 5M
Sodium Acrylate 38%	AK Scientific	97–99%, R624	store the powder at -20°C
TEMED	Euromedex	50406	
Tween-20	Sigma	P-7949	store RT

Reagents

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Equipment	Company	Reference	Comments
12 mm round coverslips	VWR	6530021	Comments
Metal block			pre-cooled -20°C
Shaker			RT
Incubator 37°C			shaker or that can accomodate a shaker
homemade spatula			with a rigid plastic cover
1.5 ml tubes			
24 mm round coverslips			
24-well plates	NUNC		
6-well plates	NUNC		
Glass slides	Themoscientific	J1800 AMN7	
Laboratory wipes			
Petri dishes			
Plumbing joints		20x27 N14	
Thermoblock	Eppendorf		
Vortex			
Parafilm			

Equipment

compound	volume	Final concentration
Formaldehyde 37%	19 ul	0.7%
Acrylamide 40%	25 ul	1%
PBS 1x	956 ul	
Prepare just before use		

Activation solution (FA/AA)

Reagent	B	Final concentration	Comment
Sodium acrylate		38% w/v	Dissolve little by little in milliQH2O and on ice under the fume hood. 0.22 um filtered.This solution is critical. Use only solution that appears clear or very slightly yellow.

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Sodium acrylate stock solution (SA)

Stock solution	Stock solution	Volume to prepare per sample	Final concentration
Sodium Acrylate (SA)	38% (W/W)	500ul	23% (W/V)
Acrylamide (AA)	40%	250ul	10% (W/V)
Bis-acrylamide (BIS)	2%	50 ul	0.1% (W/V)
PBS	10x	100 ul	1x
Make 90 ul aliquotes and store at -20°C for up to 2-3 weeks Make 90 µL aliquots and store at -20°C for up to 2-3 weeks maximum. Note: the solution does not freeze.			

U-ExM Monomer solution (MS)

Reagent	Stock concentration	Volume / Weight	Final concentration
TRIS-Base	-	0.6 g	50 mM
HCl (fuming?)	-	pH to 9.0	
ddH2O	-	10 mL	
SDS	20% (694 mM)	28.82 mL	200 mM
NaCl	5 M	4 mL	200 mM
milliQ water		qsp 100 mL	

Denaturation solution (DS)

Day 0.

Preparation coverslips (24mm) coated with poly-L-lysine for mounting

- Place the coverslips on Parafilm and cover each of them with **400 µL** of poly-L-lysine **00:30:00**
- recover and store the poly-L-lysine solution
- wash the slides 2 times in a water bath
- Dry the coverslips up on adsorbing paper.

- Pre-cool overnight at -20°C a metallic tube holder

- If required, prepare your cells on 24 mm coverslips to grow.

Day 1- Sample preparatio, first expansion and primary antibody incubation

1- Prepare cells on 12 mm coverslips

In 24-well plates, prepare coverslips with your favourite cells. The cells density should not be too high to avoid non-isotropic expansion (non-confluent cells).

Wash the cells with PBS.

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2- Activation

- Place the coverslips in 24-well plate, cells up, and load **1 mL** of FA/AA solution
- Fill up the empty wells with water
- Incubate at **37 °C** **100 rpm** **04:00:00**

3- Gel polymerization

a- Prepare **On ice**

- a lid of a 24-well plate covered with a pad and parafilm.
- A -20°C-pre-cooled metallic tube holder with
  - **100 µL** of APS 10%
  - **100 µL** of TEMED 10%
  - a **90 µL** aliquot of MS solution

b- Prepare the MS + TEMED + APS solution and gel polymerization

- Add **5 µL** of TEMED 10% into the 90 ul MS aliquote, then add **5 µL** of APS 10%
- Vortex 2 sec and load on the parafilm 2 drops of **35 µL not more** . (Do 2 drops at once only as the polymerization is very rapid).
- Immediately, adsorb the excess FA/AA solution on a tissue and transfer the coverslips on the drop, cells side facing down.
- Incubate **On ice** **00:05:00** , then at **37 °C** **without shaking** **01:00:00** . During this step, prepare step 4.

c-Gel detachment

- After the polymerization and in a 6-well plate, add **1 mL** of denaturation solution.
- Transfer the coverslip+polymerized gel (gel facing up) to the 6-well plate
- Incubate **Room temperature** **00:15:00** **100 rpm** or until the gel detaches from the coverslip.

4- Denaturation

- Set the Thermoblock at 95°C
- Pre-heat at 95°C **1 mL** of DS per sample in an eppendorf tube.
- Collect the detached gel and load it into the DS.
- Fill up the tube with heated DS and incubate at 95°C **01:30:00** .

5- First expansion

- Load each gel in a petri dish with **40 mL** milliQ water
- Incubate **00:30:00** **Room temperature** **100 rpm**
- Repeat twice **01:00:00** **Room temperature** **100 rpm**

(-**OR** Change the water bath at least once and incubate **Overnight** **4 °C**)

- Measure the diameter of the gel (expect of a >4x expansion compared to the 12mm diameter of the coverslip)

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6- Primary antibody incubation

- Incubate the gel in 40 mL PBS **00:10:00** **Room temperature** **100 rpm**
- Repeat twice **00:20:00** **Room temperature** **100 rpm** a- In a 24-well plate, per sample, add **1 mL** of PBS, 2% BSA, 0.2% Tween-20.
- Fill up the empty wells with water
- Gel cutting
    - Use a 1 mL Tips to cut a circular piece of the gel (usually 4 pieces can easily be cut from one gel).
    - Place each piece of gel into the 24-well plate
    - Incubate in PBS, BSA, Tween-20 **00:10:00 or more** **37 °C** **100 rpm**
- Primary antibody(ies) incubation
    - Remove the PBS, BSA, Tween-20 solution and replace it with **350 µL** of primary antibody(ies) diluted in PBS, BSA, Tween-20
    - Incubate **37 °C** **Overnight** **100 rpm** , the lid sealed closed to avoid evaporation.

Day-2 Secondary incubation, second expansion and gel mount

1- Secondary antibody incubation

- Wash 3 times in **350 µL** PBS, BSA, Tween-20 **37 °C**
- Remove the PBS, BSA, Tween-20 solution and replace it with **350 µL** of secondary antibody(ies) and **Hoechst** (5µg/mL) diluted in PBS, BSA, Tween-20
- Incubate **37 °C** **03:00:00** **100 rpm** , the lid sealed closed to avoid evaporation, in the dark.
- Wash 3 times 10 min in **1 mL** PBS, BSA, Tween-20 **37 °C** **00:30:00** **100 rpm**

2- Second expansion

- Transfer the gel into a 6-well plate and wash with **5 mL** water **00:30:00**

OR (- Change the water bath and incubate **Overnight** **37 °C** **100 rpm**)

- Change twice the water bath and incubate for 30 min **01:00:00** **37 °C** **100 rpm**
- Measure the diameter of the gel, it should be consistent with the first expansion

(You can store the gels water in water at 4°C)

3- Mounting and imaging

This setup allows the imaging on upright and inverted microscopes. Also, the gels do not dehydrate during long microscopy sessions and can be further stored t 4°C.

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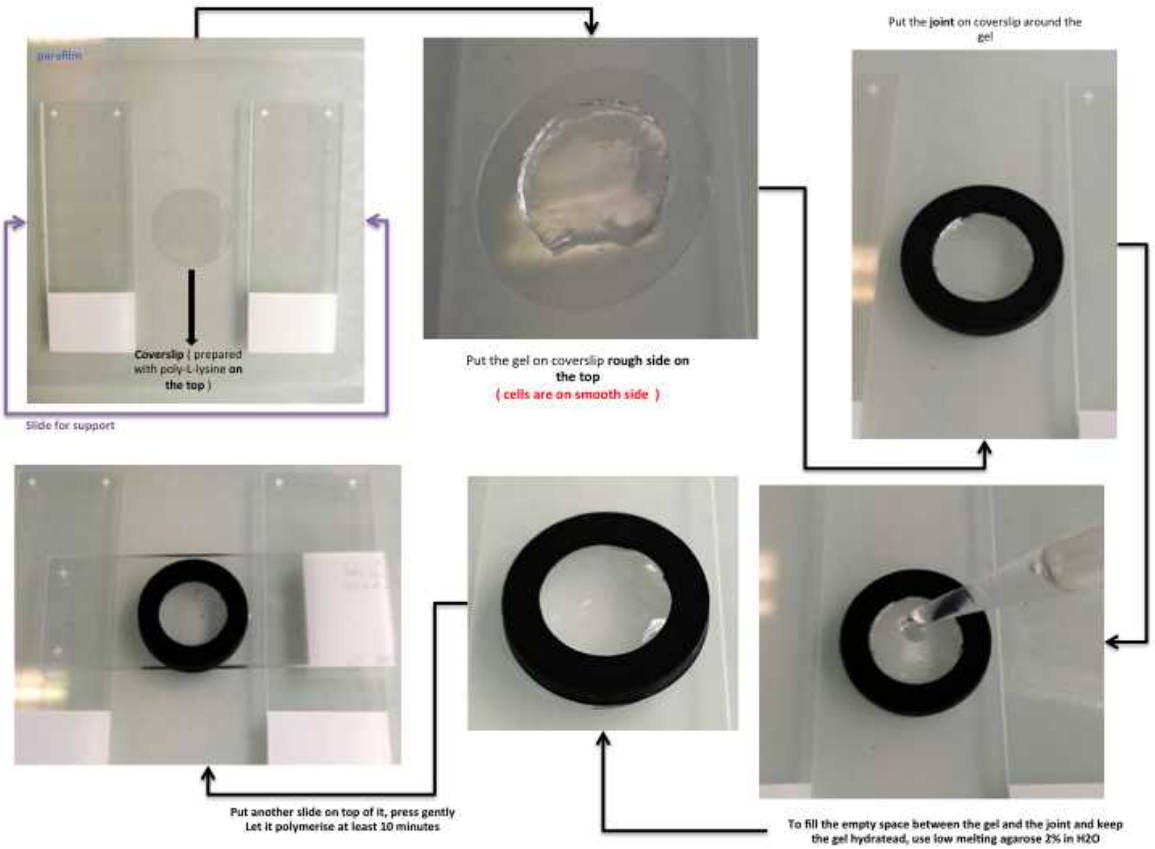

How to mount your gel.

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