

Ultra Expansion microscopy protocol with improved setup for upright and inverted microscopes. v1

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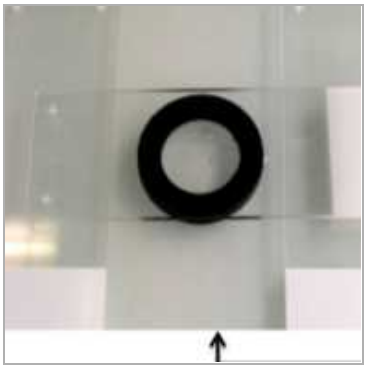
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Ultra Expansion microscopy protocol with improved setup for upright and inverted microscopes.

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Mélanie Bonhivers

ABSTRACT

Ultra

This protocol is based on the following papers:

CITATION

Gambarotto D, Hamel V, Guichard P (2021). Ultrastructure expansion microscopy (U-ExM).. Methods in cell biology.

LINK

https://doi.org/10.1016/bs.mcb.2020.05.006

CITATION

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.

LINK

https://doi.org/pii:jcs254300.10.1242/jcs.254300

CITATION

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.

LINK

https://doi.org/10.1371/journal.ppat.1009329

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

	Reagents	Company	Reference	Comment
	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

	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	

	Equipment	Company	Reference	Comments
	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.

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

	Stock solution	Stock solution	Volume to prepare per sample	Final concentration
	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.

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)

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

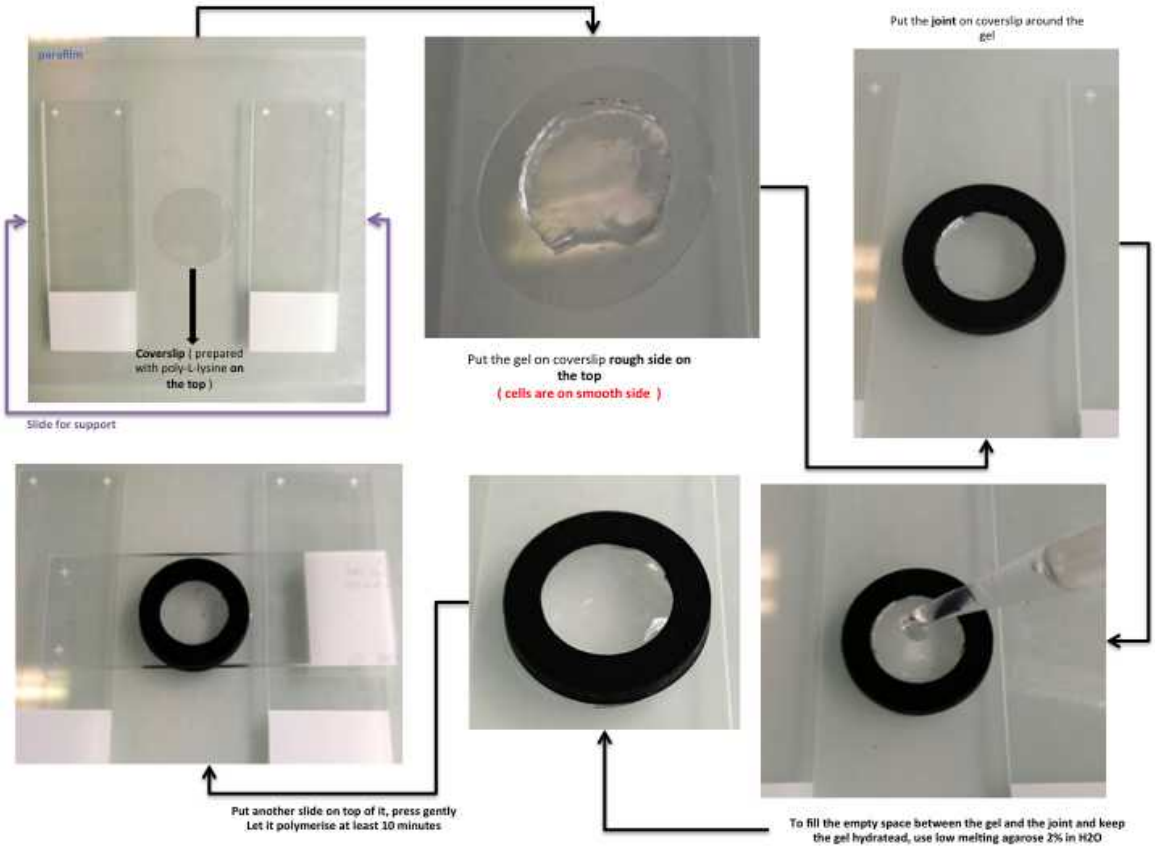
OR (- Change the water bath and incubate Overnight 37 °C 100 rpm)

- 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.



How to mount your gel.