1. INTRODUCTION/PURPOSE

Xenopus oocytes are widely used as an expression system in order to functionally investigate membrane proteins alone or in combination with other proteins. Advantages of using oocytes over other heterologous expression systems include the simple handling of the giant cells, the high proportion of cells expressing foreign genetic information, the simple control of the environment of the oocyte by means of bath perfusion, and the control of the membrane potential. With this procedure, we aim to demonstrate the proper way of harvesting of oocytes from Xenopus lævis.

2. RESPONSIBILITIES AND SAFETY

See the general UiO procedure 'risk management policy in laboratories' for an overview of responsibilities at UiO. See also role descriptions for IMB's systematic Health, Safety and Environment (HSE) work in the laboratory.

General laboratory safety applies. For more information see IMB´s HSE webpages.

3. NECESSARY SAFETY EQUIPMENT



Nitrile


Safety mask

4. EQUIPMENT, MATERIALS AND SOLUTIONS

Materials for transport assay in oocyte

- ≈ 40 ml Low Barths medium (same as Modified Barths medium but without calcium)
- ≈ 25 ml Modified Barths medium (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HEPES (pH 7.5, NaOH)
- PenStrep (100 µg/mL penicillin, and 100 µg/mL streptomycin)
- 70 % Ethanol
- Scalpel, forceps, scissors, tongs
- Tissue paper
- Petri dish
- 2 types of surgical suture: for inner layer → violet (B/BRAUN, C1048220, 4/0, 45 cm); for outer layer → black (B/BRAUN, C0712060, 6/0, 45 cm, comes in green package)
- for anaesthesia: Sodium bicarbonate (S5761, Sigma)+ Ethyl 3-aminobenzoate methanesulfonate salt (A5040, sigma)
- plastic pipettes
- 2 boxes with ice: one plastic box for surgery, one styrofoam box for media
- Eppendorf tubes (2 and 1,5mL)

5. PROCEDURES: DESCRIPTION OF PROCEDURE

General:

- Make sure the working area and equipment is disinfected with 70% ethanol.
- Wear a labcoat
- Wear gloves
- Wear safety glasses
- Bench area should be covered with bench paper

Procedure A - Anesthetization

1. Mix 1.4 g of sodium bicarbonate and 2.0 g of Ethyl 3-aminobenyoate methanesulfonate salt in a 50 ml tube (red lid) (do not add water at this step!!!)
2. Fill a little bit of water from reservoir in 2 l Erlenmeyer flask
3. Add chemical mixture and dissolve it in water
4. Fill up with water (from reservoir) up to 2 l
5. Pour anaesthesia mixture in extra vessel
6. Choose one frog from the pool and transfer it quickly to the vessel with anaesthesia
7. Anesthetize frog until it is unconscious – takes between 15-40 min
8. Make sure that frog is unconscious: first, try to pull the frogs hind leg. When it is no longer responding to that, try to put it on its back. If it does not respond (move in any way) it is ready for surgery. (All this is done when the frog is still in the anaesthesia water solution.)

Procedure B – Surgery

1. Wipe all tools with ethanol and tissue paper
2. Place transparent film on top of the surgery box with ice and carefully drill some holes in the film
3. Open Modified Barth medium and place a plastic pipette in this tube
4. Place the frog ventral side up on crushed ice throughout the surgical procedure to maintain anesthesia
5. Gently wipe the abdominal skin where incision will be made with some tissue paper
6. Disinfect hands with alcohol but make sure that gloves are dry before touching the frog
   Note: It is extremely important to work fast so that frog is not dried out
7. Carefully lift the skin with the fingers and make a 1 cm incision in the lower abdominal wall near one leg (layer I of the skin) (when using the scalpel: “cut in the air” do not put the tip (sharp end) of the scalpel towards the frog)
8. Carefully lift the next layer with forceps and make a second incision of the same size (directly underneath the first incision – be careful not to shift the cuts)
9. Place transparent film over the whole frog so that the oocytes do not come into contact with the skin
10. Make an incision in the transparent film as well and remove eggs from ovaries using forceps and scissors
11. Pour some Low Barth medium in a petri dish
12. Place the oocytes in petri dish with Low Barth medium
13. Remove the transparent film and wash hands with ethanol (make sure that gloves are dry before handling the frog)
14. Regularly moisten the frog with Modified Barth medium
15. Stitch the wounds: use violet suture for inner layer, 3-5 stitches
16. Stitching: tongs in the right hand, forceps in the left hand; hold the needle (of the suture) in the tongs as stabbing it through the skin; pull the needle out with fingers or tongs; wrap the suture twice around the tongs; cut with small scissors; repeat to make a second knot. (You tube has nice videos for learning suturing techniques.)
17. Stitch the wounds: use black suture for outer layer, 3-5 stitches
18. Change the water in the vessel (temperate water)
19. Place the frog in the vessel
    Note: have some wet tissue paper in one corner so that the frog’s head is above water
20. Water the frog from time to time so that it does not dry out (or put some wet paper on top of its body)
21. Wait until frog is fully awake – takes 15-30 min

22. Transfer frog to an extra pool – single-housed for a few days (≈ 3 days) → make sure frog is starting to swim shortly after the surgery. After two days try to give food. It might not want to eat yet, but if it does it's a good sign. Make sure that all food is removed from the pool if the frog is not eating to prevent microbial growth and wound infection.
23. If frog is behaving normally it can go back to the common pool
24. Wash used instruments with water and soap
25. Disinfect work bench

6. RISK ASSESSMENT

The likelihood is assessed by assuming the user following the precautions stated in the step by step risk assessment (SJA) below.

6.1 List of chemicals and their H and P phrases

Chemical	Hazard symbol	H statements	P statements
Sodium bicarbonate		H1 Not a dangerous chemical	-
Ethyl 3-aminobenzoate methanesulfonate salt		H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation. H412 Harmful to aquatic life with long lasting effects.	P273 Avoid release to the environment. P302 + P352 IF ON SKIN: Wash with plenty of soap and water. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

6.2 Biological agent

If you are working with biological hazard you should use this table and information from the PSDS.

Animals used on this procedure are tested for the ff. pathogens once a year by the animal facility officer (see updated health report).

Classification	Pathogens	Risk group # 
Parasite	Cryptosporidium spp.	2
	Pseudocapillaroides xenopi	NC*
Fungus	Batrachochytrium dendrobatidis	NC*
Bacteria	Mycobacterium chelonae	2
	Mycobacterium gordonae	NC*
	Mycobacterium marinum	2
	Pseudomonas aeruginosa	2
	Salmonella spp.	2
Virus	Ranavirus spp.	NC*

*NC (not classified as risk group)

6.3 Risk assessment; step by step

Part of procedure		Unwanted scenarios	Precautions	Emergency planning	S*K
A1	Preparing anæsthesia (Ethyl 3-aminobenzoate methanesulfonate salt)	Inhalation	If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection. Wash hands before breaks and at the end of workday. Handle in accordance with good industrial hygiene and safety practice.	3*1
		Skin contact	Wash off with soap and plenty of water. Consult a physician.		
		Eye contact	Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.		
		Swallowing	Rinse mouth with water. Consult a physician.		
B10		Damage skin due to incision	Wash affected area of the skin with lots of water and disinfect with disinfectant.	Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.	3*1

6.4 Overall risk assessment for this SOP

Risk categories

- Red: S*K=10-25 the overall risk of making this solution is unacceptable risk. Access new precaution to reduce the risk should established.
- Yellow: S*K=4-9 the overall risk of making this solution is medium. Access new precaution to reduce the risk should considered.
- Green: S*K=1-4 the overall risk of making this solution is fully acceptable - minimal risk.

If S*K of the step by step risk assessment falls into different categories (as listed above), the overall risk is set to the highest S*K value.

When following this SOP, there is ACCEPTABLE risk associated with this procedure.

6.5 Substitution

According to Norwegian law, we have to assess the possibility of substitution of hazardous chemicals. This assessment needs to be documented.

The procedure has a minimal risk of accident and no substitution is required.

6.6 Special cautions necessary due to reproductive toxicity:

You will find this information in the SDS. Generally, it is not recommended to work with a chemical that has carcinogenic or reproductive effects if you are planning to be or are pregnant. If a chemical is proven to pass into breast milk it is not recommended to perform procedure if you are breast feeding.

If you are working with Class II biological agents that may cause infections, you should consider the risks using the relevant PSDS and other relevant documentation.

Planning pregnancy (women): Procedure is safe but precautionary measures must be taken.
Pregnant: Procedure is safe but precautionary measures must be taken.
Breast feeding: None

7. WASTE DISPOSAL

Waste	Volume	Disposal method	Enviromental risk
Pipet tips, gloves, eppendorf tubes, tissue paper, bench paper, other waste contaminated with blood (from frog surgery)	1 small yellow trash bin /semester	Yellow trash bins	None, since this is according to procedure and handled by trained staff and wastes are collected by approved personnel.
Ethyl 3-aminobenzoate methanesulfonate salt	2g in 2l	Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal. Diluted solution is safe for drain disposal (<40,9 mg/l).	Toxicity to fish LC50 - Oncorhynchus mykiss (rainbow trout) - 40,9 mg/l - 96 h

8. REFERENCES

(Chaudhry, Reimer et al. 1999), Wagner, Friedrich et al. (2000), (Hamdani el, Gudbrandsen et al. 2012)

1. Chaudhry, F. A., R. J. Reimer, D. Krizaj, D. Barber, J. Storm-Mathisen, D. R. Copenhagen and R. H. Edwards (1999). "Molecular analysis of system N suggests novel physiological roles in nitrogen metabolism and synaptic transmission." Cell **99**(7): 769-780.
2. Hamdani el, H., M. Gudbrandsen, M. Bjorkmo and F. A. Chaudhry (2012). "The system N transporter SN2 doubles as a transmitter precursor furnisher and a potential regulator of NMDA receptors." Glia **60**(11): 1671-1683.
3. Wagner, C. A., B. Friedrich, I. Setiawan, F. Lang and S. Bröer (2000). "The Use of Xenopus laevis Oocytes for the Functional Characterization of Heterologously Expressed Membrane Proteins." Cellular Physiology and Biochemistry **10**(1-2): 1-12.