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 feeding and maintenance of Xenopus lævis frogs.

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 cleaning

- Nets
- Hose
- Brush
- Food: "Adult Xenopus Complete Diet" (340 g/package, product # 316518-18-2412, Zeigler Bros., Inc., USA)
- GH Salt (product # L 04840, NORAQ, Norway)
- pH/KH Plus (product # 581401 CE, Tetra Aqua, Germany)

5. PROCEDURES: DESCRIPTION OF PROCEDURE

A. Feeding Procedure:
Frequency of Feeding: 3 days a week; every morning at 10am (Monday, Wednesday and Friday)

1. Remove dirt from the pool with a net
2. Wash the net with tap water
3. Feed the frogs with 1 portion of food (measuring cup is provided inside the food bucket)
1. Note: Leave the frogs undisturbed while they are eating!

B. Normal Cleaning Procedure:
Frequency of Cleaning: 3 days a week; 2-3h after feeding (Monday, Wednesday and Friday)

1. Remove excess food with a net.
2. Wash the net everytime with tap water
3. Clean the buckets and artificial leaves (See Figure 1) from microbial growth using a brush.
4. Drain most of the water off the pool by turning the valves (See Figure 3) of the sewer pipe
   Note: Frogs must still be covered with water!!
   Note: Always close Valves 1, 2, 3 and Valve MAIN
5. Fill the pool with tempered water from the reservoir (See Figure 2) to half level of the black buckets (See Figure 1)
6. Add 2 spoons of "GH salt" and 2 spoons of "pH/KH Plus" in 500 ml of tempered water. Add ½ of this mixture to each pool.
2. Close the water tap for the reservoir (See Figure 2)

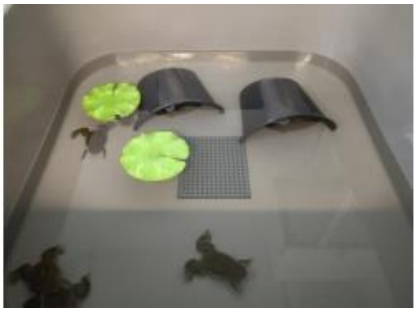

Figure 1: Pool with buckets and artificial leaves

Figure 2: Water reservoir

Figure 3: Water valve

C. General Cleaning Procedure:

Frequency of General Cleaning: once every 2 months

1. Fill a new clean pool that is available with tempered water from the reservoir
2. Transfer all the frogs to the newly water-filled pool
3. Drain completely the dirty pool by opening the valves (See Figure 3)
4. Connect an orange hose to the tap and use this to clean the pool.
   Note: Do not use water from the reservoir to clean the dirty pool
5. Clean the pool and drainage with a brush from microbial growth
6. Clean the buckets and artificial leaves (See Figure 1) from microbial growth using a brush.
7. Close the valves (See Figure 3) of the sewer pipe
   Note: Always close Valves 1, 2, 3 and Valve MAIN
8. Close the water tap for the reservoir (See Figure 2)

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
GH Salt		H1 Not a dangerous chemical	-
pH/KH Plus		H1 Not a dangerous chemical	-

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
B, C	Normal cleaning & General cleaning	Skin contact	Wash off with soap and plenty of water. Consult a physician.	Avoid contact with skin and eyes. Normal measures for preventive fire protection. Handle in accordance with good hygiene and safety practice. Wash hands before breaks and at the end of workday. Wear mask.	3*1
		Eye contact	Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.		
		Swallowing	Do not drink water from the pool or any water source from this room. Rinse mouth with water. Consult a physician.		
		Inhalation of dust from chemicals	If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.	Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.	
B, C	Normal cleaning & General cleaning	Slippage	Do not step on wet floor. Dry the floor with dry cloth or tissue paper before working.	Handle in accordance with good hygiene and safety practice. Consult a physician after a fall.	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
1	Glove and tissue paper	1 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.
2	Dirty water from the pool (including excess food and feces)	10L /cleaning day	Drain in the sewage	Health and environmental monitoring (including swab and water test) is performed yearly by trained staff from animal facility.

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.