

Standard operation procedure: Importation of biological material into DU-041

SOP nbr: 7-07

Created: 05.05.2021

Revised: 22.05.2023

Valid to: 27.09.2024

Created by: Ingunn Jermstad, Shaista Khan,

Katarzyna Joanna Zelewska

Revised by: Katarzyna Joanna Zelewska

Approved by: Espen Engh

IMPORTATION OF BIOLOGICAL MATERIAL INTO DU-041

1.0 PURPOSE

- 1.1 To describe the special protocols to be followed for room DU-041 (animal housing room) and DU-039 (lab) and related to the operations of the Norwegian Transgenic Center (NTS).
- 1.2 To provide an overview of important measures to prevent contamination from NTS operations spreading into the Barrier.

2.0 DIVISION OF RESPONSIBILITY

- 2.1 After consultation with the responsible veterinary surgeon, the PMSK is responsible for approving the importation of animals ordered by NTS for housing in DU-041.
- 2.2 NTS and the Section for Comparative Medicine (KPM) are responsible for making sure that these procedures are followed at all times.

3.0 PROCEDURE

- 3.1 This procedure is adapted to the current situation, where room DU-041 has SPF status, while room DU-036 has SOPF status.

Routines governing access to DU-041

- 3.2 KPM is divided into three units, each with a different health status and where DU-044 and the Barrier have the highest health level. You must not enter the Barrier from another KPM unit, from units where animals are handled or from other animal departments on one and the same day. A full body wash and a complete change of clothes are mandatory requirements before entering the Barrier.
- 3.3 Place your shoes outside the cloakroom used for access to the Barrier and follow KPM's clothing procedures (ref. SOP 2-02 "Clothing and Hygiene in the Barrier").
- 3.4 NTS personnel are not permitted to use the zones belonging to DU-036.

Importation of biological material

- 3.5 Imported biological material is limited to the following two categories: cryopreserved sperm (stored in liquid nitrogen) and embryos (stored in culture media).
- 3.6 This material is used for *in vitro* fertilisation (IVF), resuscitation of cryopreserved embryos (embryo implantation), rederivation from live mice (embryo implantation) or the generation of unique transgenic lines (implantation of injected embryos).
- 3.7 All biological material that is brought into DU-041 (see above) and DU-039 is either intended to generate fertilised embryos for implantation in foster mothers, or consists of ready fertilised embryos to be implanted in foster mothers.
- 3.8 The outer surface of the container to be imported into the Barrier must be disinfected with Virkon (or other suitable disinfectant). Use protective glasses and chemical resistant gloves. If there is insufficient ventilation or when spraying a large amount of equipment, respiratory protection equipment must be used with a combined A1/P1 filter for moisture/particles. 70% ethanol should only be used if the container is brand new/clean and if the articles to be disinfected do not tolerate Virkon.

Rederivation of cryopreserved sperm (IVF)

- 3.9 PPE is recommended for the handling of liquid nitrogen: protective glasses/visor and cold-tolerant gloves.
- 3.10 Cryopreserved sperm (straw) stored in liquid nitrogen is imported into the clean MDU zone via the material sluice adjacent to the reception.
- 3.11 The container must be sprayed with Virkon or other disinfectant, in compliance with KPM's routines.
- 3.12 The container is then brought into the cloakroom leading to the Barrier, where liquid nitrogen and straw (with the sperm) is transferred to a new, clean nitrogen container, while avoiding contact between the two containers.
- 3.13 The "clean" container is usually stored in DU-039, DU-041 or in the cloakroom.
- 3.14 The clean nitrogen container with sperm straw is then taken directly into DU-039.
- 3.15 Before start-up of the procedure, all surfaces and microscopes must be disinfected with 70% ethanol.
- 3.16 All work must be carried out on an open bench top.
- 3.17 The microscopes and incubator should be placed immediately adjacent to one another so that the dish (containing the sperm and the eggs) is subject to the minimum of movement and the dish is kept at room temperature for as short a time as possible.
- 3.18 The procedure is as follows: sperm are thawed and placed in the dish together with eggs from donor animals. The fertilised eggs are then washed 5 times and incubated in clean dishes overnight in a cell incubator (at 37°C, 5 % CO₂) before embryo implantation is carried out the following day (see separate section on embryo implantation).

Embryo transfer (from cryopreserved embryos)

- 3.19 Thawing of cryopreserved embryos (in both internal and external laboratories, including KPMe or conventional unit) takes place in NTS' laboratories (L-143).

- 3.20 The microscopes and incubator are placed on the bench normally reserved for work on embryos. All surfaces and the microscopes must be disinfected with 70% ethanol before and after use.
- 3.21 Cryopreserved embryos are thawed and washed 5 times (in addition to the 5 washes carried out before cryopreservation) and are then transferred to a clean dish, which is put in the incubator for 1-3 hours.
- 3.22 The dish with the embryos is protected with additional packing (a larger petri dish or box) and is then taken to KPM via the cloakroom, where its surface is sprayed with Virkon.
- 3.23 NTS personnel change their clothing before entering the Barrier, in accordance with KPM's routines, and the embryos are brought in via the cloakroom.
- 3.24 The dish is then taken directly into DU-039 and immediately placed in an incubator. Embryo implantation is carried out afterwards (see separate section on embryo implantation).

Rederivation from live mice

- 3.25 The fallopian tube is dissected from superovulated females inseminated overnight and housed at KPME or conventional unit.
- 3.26 The fallopian tubes are taken to our own laboratories, L-143, while observing the disinfection procedure described above, under the point relating to embryo transfer from cryopreserved embryos.
- 3.27 Fertilised embryos are extracted from the fallopian tubes, washed 5 times and then transferred to a new medium/dish for incubation overnight.
- 3.28 The following day, the embryos are taken to DU-039, while observing the routines described for embryo transfers (from cryopreserved embryos).
- 3.29 Sometimes, embryos may be implanted on the same day. In this case, one person carries out the embryo transfer in KPME/MDU/conventional unit and washes the embryos, while another person carries out embryo transfer in DU-041. This is to avoid staff bringing agents/pathogens into DU-041 from KPME/MDU/conventional unit.

Embryo implantation of injected embryos (generation of new lines)

- 3.30 Donor embryos are isolated from females housed in DU-041, rack 503b. (As a rule, C57BL/6NRj donor females are purchased during the preceding week for this purpose, then superovulated and mated with males (studs) which are permanently housed in the same rack).
- 3.31 Oviducts are taken from DU-039 and brought to NTS (L-143). The embryos are isolated and washed 5 times before they are manipulated in our own laboratories.
- 3.32 After manipulation, they are transferred to a new medium/dish and incubated for 1-3 hours before being moved to DU-039 for embryo implantation.
- 3.33 These embryos are moved to DU-041, following the same procedure as described above under the section on embryo transfer from cryopreserved embryos.

Embryo implantation

- 3.34 The embryos are implanted in RjOrl:SWISS pseudo pregnant females after copulation with a vasectomised CD1 male.
- 3.35 The females are housed in DU-041, rack 503a. The vaginal plug is checked and the female(s) selected for implantation are taken to DU-039 for surgery.

- 3.36 All work surfaces and microscopes must be cleaned with 70% ethanol before starting the procedure. The incubator where the embryos are kept must also be regularly cleaned with 70% ethanol.
- 3.37 The females are sedated and the embryos are implanted. After implantation, all work surfaces must again be cleaned with 70 % ethanol.
- 3.38 The females are put in cages which are placed on a heated plate overnight - the filter lid is loosened so that the animals have access to air. The following day, the cages are moved to DU-041, rack 903.

Routines for the handling of animals and cages

- 3.39 All RjOrl:SWISS females that have undergone embryo implantation are placed in rack 903 and their cages are given red cage cards.
- 3.40 KPM and NTS follow their own routines regarding these cages. This involves changing gloves between each cage and handling these cages last when working in this room.
- 3.41 When offspring are separated from the mother, they are housed in cages in the same rack as the mother and handled according to the same routines until the results from health monitoring are received.


Health monitoring


- 3.42 In addition to normal health monitoring of sentinels, separate health monitoring (Idexx Oslo annual) is carried out on animals that have undergone rederivation from live animals, cryopreserved embryos or sperm.
- 3.43 Biopsies are taken from the ear of the offspring when they are two weeks old.
- 3.44 As soon as the genotyping results are available and we/the customer can verify that the offspring are of the correct genotype, samples are taken for health monitoring purposes. At this point, the offspring are ca. 3-4 weeks old. The aim is to obtain the results from the health monitoring tests before the offspring are separated from their foster mother.
- 3.45 Serology (opti spot) is always taken from the foster mother, while the remaining tests (fur swab, oral swab and faeces sample) are taken from both the foster mother and the offspring.
- 3.46 When generating new transgenic lines, all biological material originates from animals housed in DU-041 (see the point above about generating unique transgenic lines). For foster mothers/offspring of this type, NTS limits its health monitoring to sentinels and index animals. NTS does not carry out additional health monitoring of offspring generated from external embryos or sperm, unless particular projects are being conducted where the embryos injected originate from a room other than DU-041.
- 3.47 If Murine parvo virus (MPV - all variants), Lymphocytic choriomeningitis virus (LCMV) or Sendai virus are discovered on oocytes and embryos, biological material cannot be brought into rooms DU-041 and DU-039.

4.0 HEALTH, SAFETY AND ENVIRONMENT (HSE)


- 4.1 Everyone must have undergone sufficient training in order to ensure that they have the proper clothing and know how to use protective equipment correctly.

- 4.2 Everyone must have undergone sufficient training in order to ensure that these procedures are followed and the animals are handled correctly.
- 4.3 Work must be carried out on a ventilated bench in order to minimise exposure to allergens and microorganisms.
- 4.4 All those handling chemicals must have undergone sufficient training and have access to the proper protective equipment in order to ensure that the chemicals are used safely.
- 4.5 NTS must register all chemicals stored in DU-039 in a KPM location in Chemical Manager.

Rely+On Virkon Tablets	CAS no.	Pictogram	Hazard statements	Precautionary statements
Pentakalium-bis(peroksymonosulphate)-bis(sulphate)	70693-62-8		H315: Skin irritant.	P102: Keep out of the reach of children. P273: Avoid escape into the environment. P280: Use protective gloves/clothing/eye and face protection. P305+P351+P338: IF IN CONTACT WITH EYES: Rinse carefully with water for several minutes. Remove contact lenses if used and easy to do. Continue rinsing. P310: Contact a POISON CENTRE immediately or a doctor. P501: Dispose of contents/container in accordance with local, regional and national regulations.
Malic acid	6915-15-7		H318: Causes serious eye damage.	
Sulphamidic acid	5329-14-6		H335: May cause respiratory irritation.	
Sodium dodecylbenzenesulphonate	25155-30-0		H412: Harmful to aquatic life with long-lasting effects.	
Dipotassium peroxodisulphate	7727-21-1		EUH 208: Contains Dipotassium peroxodisulphate, which may produce an allergic reaction.	

Ethanol	CAS no.	Pictogram	Hazard statements	Precautionary statements
Ethanol	64-17-5		H225: Highly flammable liquid and vapour. H319: Causes severe eye irritation.	P210: Keep away from heat sources, hot surfaces, sparks, open flames and other sources of ignition. No smoking. P280: Use protective gloves/clothing/eye and face protection. P305+P351+P338: IF IN CONTACT WITH EYES: Rinse carefully with water for several minutes. Remove contact lenses if used and easy to do. Continue rinsing.

				<p>P370 + P378 In case of fire: Extinguish with carbon dioxide (CO₂), foam, powder or water.</p> <p>P403+P235: Store in a cool, well-ventilated place.</p> <p>P501: Dispose of contents/container in accordance with local, regional and national regulations.</p>
--	--	--	--	---

Nitrogen (refrigerated liquid)	CAS no.	Pictogram	Hazard statements	Precautionary statements
Nitrogen	007727-37-9		H281: Contains refrigerated gas that can cause severe frostbite	<p>P282: Wear cold-insulating gloves and a visor/eye protection.</p> <p>P336+P315: Warm the frostbitten parts of the body with lukewarm water. Do not rub the injured areas. Seek medical help immediately.</p> <p>P403: Store in a well-ventilated place.</p>

5.0 EQUIPMENT AND MAINTENANCE

- 5.1. Gloves: ordinary Nitril gloves and chemical-resistant gloves
- 5.2. Cold-insulating gloves
- 5.3. Protective glasses/visor
- 5.4. Microscopes
- 5.5. Soapy water
- 5.6. Paper towels
- 5.7. Virkon
- 5.8. 70% ethanol

6.0 HISTORY AND EDITING

- 6.1 Revised 22.05.2023 (Katarzyna Zelewska)

7.0 REFERENCES

- 7.1 SOP Norsk Transgensenter "Prosedyre BAR-041".