Supplementary File 1

Marc Niere, April 2020

1. **Cultivation of adherent human cells**

**(Exemplified for parental 293 cells as well as stably transfected 293 cells encoding geneticin (G418)**

Medium: most commonly used:

DMEM (high glucose, **without** glutamine, e.g., Sigma/Merck D6546) or

DMEM (high glucose, **without** glutamine and **without** sodium pyruvate,

e.g., Sigma/Merck D5671)

supplement medium with

* 10 % (v/v) fetal calf serum, heat-treated for 30 min at 56 °C
* Penicillin/Streptomycin

(Final concentrations: Penicillin: 100 U/ml, Streptomycin: 100 µg/ml)

* 2 mM L-glutamine (final concentration)
* if needed (see above): 1 mM sodium pyruvate (final concentration)

additionally, supplement medium for cell lines stably transfected with plasmids encoding geneticin, puromycin etc. resistance with the corresponding antibiotic.

**For stably transfected 293 cells:**

* supplement medium for cell lines stably transfected with plasmids encoding geneticin resistance with G418-sulfate as outlined below.
* supplement medium for cell lines stably transfected with plasmids encoding puromycin resistance with puromycin as outlined below.

Concentration of selection antibiotics

It is recommended to cultivate cells in presence of 10-20 % of the antibiotic concentration that was used for selection to maintain the selective pressure. Recommended antibiotic concentrations for some cell lines are listed in the SOP “Generation of stably transfected cells”.

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**Update 2019**:

**G418**:

G418-resistant stably transfected 293 cell lines are by default maintained in medium supplemented with 550 µg/ml G418, which is the concentration that is used for selecting them when they are generated.

**Puromycin**:

Puromycin-resistant stably transfected **293 cells** are by default maintained in medium supplemented with 2 µg/ml puromycin, which is the concentration that is used for selecting them when they are generated.

Puromycin-resistant stably transfected **HeLa S3 cells** are by default maintained in medium supplemented with 2 µg/ml puromycin, which is the concentration that is used for selecting them when they are generated.

**Cultivation:**

* pipet 9 ml complete medium (at least prewarmed to room temperature) into a sterile 15 ml tube
* quickly thaw a vial of cells
* spray the cryotube with 70 % (v/v) EtOH (watch out, the label may disappear)
* pour the cells into the medium
* spin down cells (300 x g, 5 min)
* remove supernatant
* resuspend cells in complete medium
* transfer the cell suspension into a 10 cm cell culture dish
* exchange medium between 8 – 16 h after seeding (i.e., when cells are attached)

1. **Maintenance/ subculturing cells**

* Split cells every 7-10 days at 370C.
* Wipe down all interior surfaces of the laminar flow hood with 70% EtOH. Also disinfect all items introduced into the hood with 70% EtOH e.g. media, containers and pipettors.
* Load work area with appropriate pipettes, bottles, 10 cmculture dish etc.
* Make certain culture medium over cells is optically clear and free of evidence of microbial or fungal contamination.
* Examine cell sheet with an inverted light microscope. Culture should be 70-80% confluent.
* Aseptically aspirate out medium.
* Wash cells with sterile 1x PBS and add 1.5 ml 0.05% trypsin/EDTA solution to cells. Spread evenly and incubate at 37 0C. **NB:** Cells will appear rounded when examined under inverted light microscope.
* Watch cells under microscope and inactivate trypsin by adding 8.5 ml FBSsupplemented medium. Knock carefully on the culture dish until the cells are loosened.
* Split cells at 1:10 ratio.

**CELL QUANTIFICATION USING A NEUBAUER CHAMBER, 0.100 mm DEPTH, 0.0025 mm2/HAEMOCYTOMETER**

* Cell quantification shall precede transfection, cryopreservation, and subculture routines.
* Trypsinate cells and resuspend in 10 ml (i.e., for 10 cm culture dish). Be sure to dilute cells (with known dilution factor) for sufficient segregation.
* Clean the haemocytometer and glass cover with 70% EtOH.
* Moisten the coverslip with water or exhaled breath. Slide the coverslip over the chamber back and forth using slight pressure until Newton’s refraction rings appear (rainbow-like rings).
* Fill both sides of the chamber with cell suspension, approximately 10 μl on the loading groove. Allow sample to diffuse by capillarity.

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* View under an inverted phase contrast microscope using 10x objective; identify the nine main squares of counting chamber delimited by three lines (Fig. 1).
* **NB:** Cells that touch the upper and left border are counted (black dots, Fig.2) whilst cells that touch the right and lower border are not counted (red dots).
* Perform the procedure in four main squares. If the number is too high consider making a dilution.
* The count should proceed from the top left-hand corner and follow the direction in Fig.

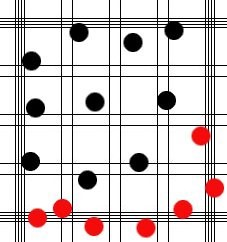
3.

* **Calculating number of cells:**

Multiply the dilution factor by the total number of cells, divide by the # of corner squares counted, and multiply by 104 to obtain cell concentration (cells/ml).

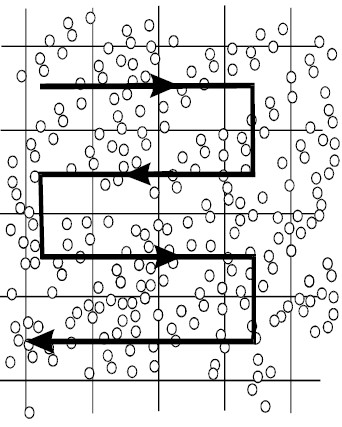
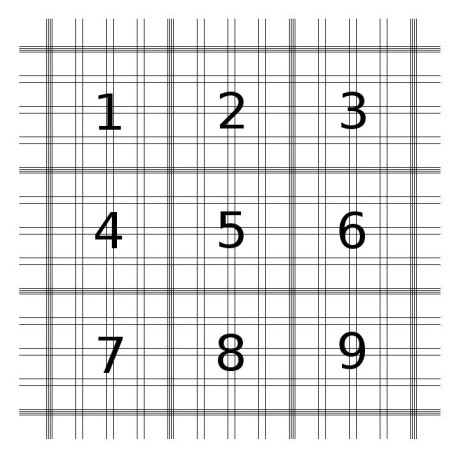
cell concentration equation

Clean haemocytometer and glass cover slip with 70% EtOH.

**Fig. 1****Fig. 2**

**Fig.**

**3**



1. **Transfection of cells with peroxisomal and mitochondrial PARP1cd plasmids using XtremeGene9 reagent**

**Day 1:**

1. Seed 800,000 293 cells in a 6 well plate the day before transfection. Grow the cells in a total volume of 2-2.5 ml medium. => Make sure to prepare enough wells for all controls.

**NB:** *Transfection is not required for 293 SLC25A51 knockout cells (SLC25A51KO) stably expressing mitochondrial PARP1cd (mitoPARP) constructs. The 293 NUDT12 cells do not stably express peroxisomal PARP1cd constructs (PexPARP) and so must be transfected*. Find below an example of plasmid map for PexPARP:

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Automatisk generert beskrivelse

1. Find below a suggested experimental setup for a 6-well plate:

# PLATE 1

|  |  |  |
| --- | --- | --- |
| 293-Parental (i.e., wildtype control) | 293-Parental + PexPARP |  |
| 293+NUDT12 | 293+NUDT12 + PexPARP |  |
| **PLATE 2** |  |  |
| 293-Parental | 293-Parental + mitoPARP |  |
| 293-SLC25A51KO | 293-SLC25A51KO + mitoPARP |  |

**Day 2:**

1. Prepare the plasmid DNA solution using sterile water at a concentration of 0.5 μg/μl (Provided, the concentration of the plasmid stock solution is high enough. If not, dilute the plasmid to a lower concentration and use accordingly more in step 5.)
2. Remove the medium and carefully add 2 ml fresh medium to the cells.
3. Briefly vortex the X-tremeGENE 9 DNA Transfection Reagent vial.
4. In a sterile tube, dilute 3 µl X-tremeGENE 9 DNA Transfection Reagent in 100 µl Opti-MEM and mix gently by vortexing. Prepare a total volume of diluted X-tremeGENE 9/Opti-MEM that is suffcient for the number of transfections planned.
5. Add 1 μg of plasmid DNA (i.e., 2 µl from a 0.5 µg/µl solution, see above) to 100 μl of diluted X-tremeGENE 9 DNA Transfection Reagent. Vortex gently (i.e., avoid any droplets getting stuck in the lid or at the inner wall of the tube). Adjust the total volume of samples with the number of transfections planned with each plasmid.
6. Incubate the transfection reagent:DNA complex for 20 minutes at room temperature.
7. Add the transfection complex to the cells in a dropwise manner.
8. Distribute the transfection complexes by gently shaking the 6 well plate.

Day 3:

* 1. Check cells under a fluorescent microscope to confirm successful transfection.
  2. To make protein lysate, add 150 µL of lysis buffer supplemented with 1 mM 3aminobenzamide, an inhibitor of endogenous poly ADP ribose polymerase (PARP).
  3. Pass the lysates through a 23-gauge needle to reduce viscosity.
  4. Determine protein concentration using the BCA assay.
  5. Dilute each sample to 50 µg protein for SDS-PAGE.

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1. **Immunocytochemistry of transiently transfected cells**

**Day 1:**

* Place a cover slip into the cavity of a 24 well plate

(use precision cover slips no. 1.5H for confocal laser scanning microscopy)

* If needed, coat the cover slip with collagen (or poly-L-lysine) => see respective SOP
* Seed cells (e.g., 75,000 HeLa S3 or 150,000 293 cells) in the cavity of a 24 well plate
* Add medium to a final volume of 1 ml

**Day 2 (optional):**

* Transfect cells with the transfection reagent of choice
* Incubate the cells for at least 24 h

**Day 3: Immunocytochemistry**

Perform all steps in the cavity of a 24-well plate

Wash the cells with 1 ml PBS

30 min 4 °C Fix the cells with 500 µl 4 % (v/v) ice-cold formaldehyde or paraformaldehyde solution prepared in 1x PBS

1 x Wash with 1 ml PBS

15 min RT Permeabilize cells with 500 µl 0.5 % (v/v) Triton X-100 (v/v) in PBS

1 x Wash with 1 ml PBS

1 h RT Block with 1 ml complete medium

[i.e., supplemented with 10 % (v/v) FCS]

**Immunodetection (part 1):**

at least 2 h at RT or Dilute primary antibody in complete medium overnight at 4 °C-10 °C [i.e., supplemented with 10 % (v/v) FCS]

Add at least 250 µl diluted antibody solution to the cells

1 x Wash with 1 ml PBS (3 min)

1 x Wash with 1 ml 0.1 % (v/v) Triton X-100 (v/v) in PBS (3 min)

1 x Wash with 1 ml PBS (3 min)

**Immunodetection (part 2):**

1 h RT Dilute secondary antibody in complete medium

[i.e., supplemented with 10 % (v/v) FCS]

Add at least 250 µl diluted antibody solution to the cells

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[optional, but recommended: stain nuclei with DAPI diluted 1:20,000 in PBS (500 µl), 5-10 min RT]

1 x Wash with 1 ml PBS (3 min)

1 x Wash with 1 ml 0.1 % (v/v) Triton X-100 (v/v) in PBS (3 min) 1x Wash with 1 ml PBS (3 min)

Mount the cover slips onto glass slides using a commercial mounting solution or a homemade mounting solution (see below for protocol)

**DAPI:**

* stock solution: 5 mg/ml in DMF
* dilute to a final concentration of 0.5 µg/ml (1:20,000) in PBS

**Mounting solution:**

* add 2.4 g Mowiol (G488) to 4.8 ml 100 % glycerol
* add 6 ml H2O → stir at RT for several hours
* add 12 ml 0.2 M Tris-HCl pH 8.5 → 50° C → stir until Mowiol is dissolved
* add 0.45 g DABCO [final conc. = 2.5 % (w/v)] → 4° C → stir until DABCO is dissolved • store aliquots at -20° C

1. **MitoTracker staining**

* One day post transfection / cell seeding, replace the culture medium with prewarmed medium supplemented with 0.2 µM MitoTracker

The stock solution is often prepared as a 200 µM solution (i.e., the stock solution is usually diluted 1:1000 with the medium).

* Incubate the cells at 37 °C in the cell incubator for 30 min
* Carefully wash the cells once with prewarmed medium
* Fix the cells with 4% paraformaldehyde (or 4% formaldehyde) in PBS
* Continue with the ICC protocol

Try to protect the cells as good as possible from light after MitoTracker has been added.