# Direct reprogramming of mouse embryonic fibroblasts into renal tubular epithelial cells (iRECs)

## CURRENT STATUS: POSTED

Sebastian Arnold
Sebastian Arnold Lab (Freiburg)
sebastian.arnold@uniklinik-freiburg.de*Corresponding Author*

Soeren Lienkamp
Soeren Lienkamp Lab (Freiburg)
soeren.lienkamp@uniklinik-freiburg.de*Corresponding Author*

Michael Kaminski
Soeren Lienkamp Lab (Freiburg)

Jelena Tosic
Sebastian Arnold Lab (Freiburg)

Cataena Kresbach
Soeren Lienkamp Lab (Freiburg)

Hannes Engel
Sebastian Arnold Lab (Freiburg)

Jonas Klockenbusch
Soeren Lienkamp Lab (Freiburg)

Anna-Lena Müller
Soeren Lienkamp Lab (Freiburg)

Roman Pichler
Soeren Lienkamp Lab (Freiburg)

Florian Grahammer

Oliver Kretz

Tobias Huber

Gerd Walz

**DOI:**
10.1038/protex.2016.070
**SUBJECT AREAS**
*Cell biology* *Biological techniques*
**KEYWORDS**
*iREC, direct reprogramming, renal tubule cells, fibroblasts*

## Abstract

Here, we describe how to reprogram mouse embryonic fibroblasts into renal tubular epithelial cells (iRECs) by lentiviral transduction of four transcription factors. First, MEFs are isolated from limbs of E12.5 reporter mice. Second, lentivirus is generated to overexpress transcription factors. Finally, reprogrammed iRECs are selected by flow cytometry.

## Introduction

Direct reprogramming of one cell type into another can be achieved by overexpression of transcription factors. This process, also referred to as transdifferentiation or direct conversion, is fast and efficient as it overcomes the need for pluripotent stem cells. Here, we present a protocol for conversion of mouse embryonic fibroblasts to induced renal epithelial cells (iRECs). Overexpression of 4 transcription factors in vitro (i.e. Emx2, Hnf1b, Hnf4a and Pax8) generates cells that exhibit a global gene expression profile and functional properties resembling primary renal tubule cells. Therefore, iRECs may represent a useful platform for toxicity testing and disease modelling.

## Reagents

- Distilled water (B.Braun Melsungen AG, cat. no. 0082479E)
- DMEM (Dulbecco’s modified Eagle’s medium, Lonza Group AG, cat. no. BE12-604F)
- DMSO (Sigma-Aldrich, cat. no. D2650)
- EDTA (0.5M, pH 8,0) (Thermo Fisher Scientific Inc., cat. no. AM9260G)
- FBS (foetal bovine serum) (Biochrom GmbH, cat. no. S0115)
- Gelatine (Sigma-Aldrich, cat. no. G1890)
- L-Glutamine (200mM) (Thermo Fisher Scientific Inc., cat. no. 25030123)
- Mice: Gt(Rosa)26Sortm4(ACTB-tdtomato,-EGFP)Luo/Jmice (The Jackson Laboratory, #007676 Strain of origin: B6.129(Cg)) are bred with KSP/Cre/+ (#012237 B6.Cg)
- Nitrogen (Messer Industrigase GmbH, cat. no. 0092)
- PBS (Biochrom GmbH, cat. no. L182-10)
- Penicillin/Streptomycin (Sigma-Aldrich, cat. no. P0781)
- Polybrene (Santa Cruz Biotechnology Inc, cat. no. sc134220)
- Trypsin-EDTA (0,25%) (Thermo Fisher Scientific, cat. no. 25200056)

## Equipment

• 15ml tube

• Cell culture plate, 100mm

• Cell culture plate, 12-wells

• Cell culture plate, 24-wells

• Cell culture plate, 6-wells

• Cellstrainer (50µm, unsterile) (Sysmex Deutschland GmbH, cat. no. 04-0042-2317)

• Centrifuge

• 5% CO2 Incubator, 37°C

• Cryovials (Greiner Bio-one GmbH, cat. no. 123263)

• Disposable Scalpel No10 (PFM Köln, cat. no. 200130010)

• FACS-Tubes (Corning, cat. no.352052)

• Stereo microscope

• Cell sorter, detecting green (GFP) and red (TOM) fluorescence

(e.g. BD FACS AriaFusion)

## Procedure

Cell culture medium preparation

• Gelatine solution: stock solution: 2% gelatine in H2O

working solution: 0,1% gelatine in H2O

• MEF-Medium (MEFM): 10% foetal bovine serum (FBS), 2mM L-glutamine, 1% penicillin/streptomycin in Dulbecco’s modified Eagle’s medium (DMEM)

• Freezing Medium: 50% MEFM, 40% FBS, 10% DMSO

• FACS Buffer: FBS (3%), EDTA (5mM), Penicillin (40units/ml), Streptomycin (40µg/ml), Glutamine (800µM) in PBS

• Collection medium: 20% FBS, Penicillin (100units/ml), Streptomycin (0,1mg/ml), L-Glutamine (2mM) in DMEM

Extraction of mouse embryonic fibroblasts

• Sacrifice a pregnant mouse by cervical dislocation 12.5 days after positive plug check.

• Disinfect the mouse with 70% ethanol and open the peritoneum.

• Dissect the uterus containing the embryos.

• Isolate the embryos in ice-cold PBS.

• Isolate the limbs of the embryos to obtain murine embryonic fibroblasts (avoid contamination with renal tissue). Collect limb tissue in a well of a 24 well plate (filled with 1ml cold PBS). Besides, collect some tissue for genotyping, e.g. tail of the embryo.

• Mince tissue with a scalpel. Then, transfer the embryonic tissue into a 15ml tube containing 8ml 0.25% Trypsin-EDTA and digest the tissue for 30min at 37°C, vortexing the tube every 10min. Centrifuge the tube with the minced tissue for 5min at 1200rpm

• Coat 10cm dishes with 0.1% gelatine solution for at least 10 min and remove excess gelatine solution directly before plating the cells.

• Remove supernatant and resuspend the cell pellet in 5ml MEFM to stop trypsinisation. Seed onto a 10cm dish and culture in a 37°C 5% $CO_2$ incubator. After two days change medium. When cells are firmly attached, change medium every day carefully without detaching loosely attached cells. When cells are firmly attached, change the medium daily.

• Proceed to next step when cells are 80% confluent, approx. after 2 to 4 days.

Freeze MEFs

Note: For optimal efficiency do not freeze or split MEFs. Infect at P0.

• Aspirate the medium and wash cells with 8ml PBS. Add 2,5ml trypsin und put cells back into the incubator for 2-5 minutes.

• Gently tap the plate after 2-5 minutes so that the cells dissociate from the plate. Add 8ml cold MEFM. Resuspend and gently rinse the plate with the mixture. Then transfer the suspension into a 15ml tube. Centrifuge the cells for 5min at 1200rpm

• Remove supernatant and resuspend the pellet in 1ml freezing medium. Transfer the suspension into a Cryovial and store the vial first on -80°C for 3 days, then transfer the Cryovial into liquid nitrogen for long term storage.

Thaw Cells

In the morning:

• Coat four 6 well dishes with 0,1% gelatine solution.

• Thaw frozen vial of MEFs at 37°C. As soon as freezing solution is free of ice crystals add 1ml pre-warmed MEFM and transfer MEF solution into 15ml tube already containing 5ml of pre-warmed MEFM. Rinse vial again with MEFM, then centrifuge cells for 5 minutes at 1200rpm

• Remove supernatant and resuspend the pellet in MEFM. Seed one vial (or 1.2x106 cells) onto four 6-well plates (3x105 cells/ 9cm2 in each well) and culture in a 37°C 5% CO2 incubator for 7-8 hours.

Lentiviral Transduction of MEFs

In the evening:

Note: For optimal transduction efficiency do not freeze/thaw virus. Each freeze/thaw cycle will decrease virus titre by 20%.

• For generation of induced renal epithelial cells 4 different lentiviruses should be generated, carrying each of the four transcription factors (i.e. Emx2, Hnf1b, Hnf4a and Pax8). To create lentivirus pWPXLd (backbone), psPax2 (packaging) and pMD2.G (envelope) can be used, all available from Didier Trono via Addgene (#12258; #12260; #12259).

• Produce and concentrate virus (~100X) using polyethylene glycol precipitation as described before1. We produce virus by transfecting 12 15cm cell culture plates (293T cells), harvesting 204ml of virus-containing supernatant and concentrating supernatant to 2.4ml before freezing. Virus concentrate can be frozen at -80°C.

• Thaw virus directly before transduction of cells.

• Mix equal amounts of the 4 viruses.

• Dilute the mixture of viruses 1:100 to 1:1000 in MEFM containing 10µg/ml Polybrene.

• Aspirate cell culture medium covering MEFs, which should be at 20% confluency.

• Add 750µl virus containing MEFM per well of a 6-well plate. Incubate infected MEFs in a 37°C 5% CO2 incubator for 12h.

• After 12h (next morning) remove virus solution. Wash cells once with MEFM and add 2ml fresh

MEFM per well.

• Repeat infection cycles on five to seven consecutive days.

Isolation of iRECs

• Cultivate transduced MEFs in MEFM for 14 days without splitting.

• Change medium every second day.

• Wash cells with PBS, add 0,25% Trypsin-EDTA and incubate for 2min at 37°C until the cells detach.

• Resuspend cells in FACS Buffer. Centrifuge for 5min at 1200rpm.

• Remove the supernatant.

• Resuspend in 1ml FACS Buffer or PBS and filter suspension through a 50µm cell strainer in FACS buffer. Use sterile cell strainers or autoclave prior to use if continued culture of sorted cells is planned.

• Isolate GFP+ cells using a sorter detecting green and red fluorescence (e.g. BD FACS AriaFusion) into collection medium.

• Seed sorted cells on gelatin coated 12-well plates.

• Change medium every day and split cells onto a 6-well plate after they reach confluency.

## Timing

Day 0: Preparation of murine embryos

Day 4: Freeze MEFs when they have grown to confluency or split directly into 6-well plates for infection in the morning. When using frozen MEFs thaw them in the morning.

Day 4-10: Start lentiviral transduction in the evening. Repeat transduction daily as described above 5-7 times.

Day 11-24: Change medium daily.

Day 25: Isolation of GFP+ cells by FACS. Expand iRECs for further experiments.

## Troubleshooting

Problem/Solution:

Massive cell death during infection: Decrease virus titres; Titrate virus for optimal multiplicity of infection (MOI)

Notice: small amount of apoptotic cells is to be expected

Cells do not transdifferentiate: Before starting lentiviral transduction, a low initial cell density is crucial for transduction; Check for correct genotype of reporter MEFs; Check for expression of the four transcription factors by qRT-PCR or western blot.

Low efficiency: For highest efficiency do not freeze or passage MEFs before virus infection.

High virus titre increase efficiency.

Freshly prepared virus concentrates increase efficiency, do not freeze/thaw lentivirus.

Increase incubation time after transduction.

Avoid splitting MEFs after lentiviral transduction.

## Anticipated Results

First GFP+ cells can be observed 3 days after viral transduction. To enhance efficiency SV40 can be introduced in MEFs prior to expression of the 4 factors. If sorting is performed 14 days after transduction as described above, 6% of GFP+ cells can be expected.

## References

1 Kutner, R. H., Zhang, X. Y. & Reiser, J. Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors. Nature protocols 4, 495-505, doi:10.1038/nprot.2009.22 (2009).

# Direct reprogramming of fibroblasts into renal tubular epithelial cells by defined transcription factors

by Michael M. Kaminski, Jelena Tosic, Catena Kresbach, +10

Nature Cell Biology (11 January, 2017)