NOV 30, 2022

Transfection of Atlantic salmon primary hepatocytes

DOI
dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

Alex K. Datsomor[1], Ragnhild Wilberg[1], Jacob S. Torgersen[2], Simen R. Sandve[1], Thomas N Harvey[1]

[1]Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Centre for Integrative Genetics (CIGENE), Norwegian University of Life Sciences, Ås, Norway;
[2]AquaGen AS, P. O. 1240, 7462, Trondheim, Norway

Thomas N Harvey: Corresponding author;

Thomas N Harvey

ABSTRACT

This protocol is for isolation and transfection of primary hepatocytes from Atlantic salmon. We have confirmed plasmid transfection efficiency of up to 46% and RNP cutting efficiency up to 60%. Primary cells can survive in culture for at least three weeks, with transgene expression 48 hours post transfection. We find that coating of culture plates with polyethylenimine (PEI) is essential to cell adhesion.

DOI

dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

PROTOCOL CITATION

Alex K. Datsomor, Ragnhild Wilberg, Jacob S. Torgersen, Simen R. Sandve, Thomas N Harvey 2022. Transfection of Atlantic salmon primary hepatocytes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

FUNDERS ACKNOWLEDGEMENT

The role of whole genome duplication in vertebrate adaptation
Grant ID: 274669

KEYWORDS

Transfection, Electroporation, Primary cell, Hepatocyte, Atlantic salmon, CRISPR

CREATED

Oct 13, 2022

LAST MODIFIED

Nov 30, 2022

PROTOCOL INTEGER ID

71297

MATERIALS TEXT

- 10x Hanks balance salt solution (HBSS) -Mg/-Ca ThermoFisher, 14175095
- 10x Hanks balance salt solution (HBSS) +Mg/+Ca ThermoFisher, 14025092
- 1 M HEPES Sigma, H3537
- Boric acid Sigma, B6768
- L-15 medium ThermoFisher, 11415064
- Fetal bovine serum (FBS) Sigma, F7524
- Penicillin-Streptomycin ThermoFisher, 15140122
- Polyethyleneimine (PEI, branched) Sigma, 408719
- Collagenase ThermoFisher, 17018029
- Trypan blue Bio-rad, 1450021
- Cell culture plates (6, 12, 24 well, or 35 mm)
- Paristaltic pump
- Erlenmeyer flask with stir bar
- 70 µM cell strainer
- Neon electroporator ThermoFisher, MPK5000
- Neon electroporation tip, 10 ul ThermoFisher, MPK1025
- Neon electroporation buffer R ThermoFisher, MPK1025
- Neon electroporation buffer E ThermoFisher, MPK1025

SAFETY WARNINGS

See MSDS for safety information.

Prepare buffers and plates

1 Prepare the following:

Note

This can be done beforehand to save time.

Wash buffer: 1x HBSS **without** Ca/Mg, 1 mM EDTA, 10 mM HEPES, pH 7.4.
Mix reagents, adjust to pH 7.4, increase volume to 500 mL using dH_2O, filter sterilize and store at 4 °C.

400 mL dH2O

50 mL 10x HBSS without Ca/Mg

1 mL 0.5 M EDTA

Citation: Alex K. Datsomor, Ragnhild Wilberg, Jacob S. Torgersen, Simen R. Sandve, Thomas N Harvey Transfection of Atlantic salmon primary hepatocytes https://dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

5 mL 1 M HEPES

Collagenase buffer: 1x HBSS **with** Ca/Mg, 10 mM HEPES, pH 7.5.
Mix reagents, adjust to pH 7.5, increase volume to 200 mL using dH_2O, filter sterilize and store at 4 °C.

100 mL dH2O
20 mL 10x HBSS with Ca/Mg
2 mL 1 M HEPES

Borate buffer: 0.1 M boric acid
Mix reagents, adjust to pH 8.4, increase volume to 400 mL using dH_2O, filter sterilize and store at

Room temperature
350 mL dH2O
2.47 g Boric acid

100x Polyethylenimine (PEI) stock: 1 mg/mL polyethylenimine, branched
Mix and filter sterilize. Polyethylenimine is very viscous, take care when pipetting. Store at 4 °C for up to 3 months. Heat at 37 °C if a precipitate forms.

20 mL 0.1 M borate buffer
19 µL polyethylenimine, branched

Cell culture media: L15 media, 5% fetal bovine serum, 1x penicillin-streptomycin

47 mL L15 media
2.5 mL Fetal bovine serum
0.5 mL 100x penicillin-streptomycin

Collagenase: Resuspend collagenase powder in dH2O to a final concentration of [M] 1*10^5 U/mL.
Prepare 50 µL aliquots and store at -20 °C

2 Coat wells of the culture plate with 1x PEI.

Note

This can be done the day before to save time. Coated plates can safely be stored in the incubator overnight.

2.1 Dilute **PEI stock** 1:100 in **0.1 M borate buffer**.

2.2 Add sufficient volume to empty wells. Enough to cover the bottom of the well.

Citation: Alex K. Datsomor, Ragnhild Wilberg, Jacob S. Torgersen, Simen R. Sandve, Thomas N Harvey Transfection of Atlantic salmon primary hepatocytes https://dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

1h

2.3 Incubate for 01:00:00 at room temperature.

2.4 Wash wells 2x with **HBSS with Mg/Ca**

Dissection and perfusion

3 Dilute collagenase to a final concentration of [M] 150 U/mL in collagenase buffer.

45 µL collagenase

30 mL collagenase buffer

Note

This should be done the day of the experiment

4 Kill the fish with a sharp blow to the head. Immediately record length and weight.

Note

We have tested the protocol on 100-400g fish (freshwater stage, parr and freshwater smolt). Larger fish and saltwater stage should be possible

5 Open the fish from the side with a scalpal by cutting in an arc from the gill to the anus. Try to cut just below the kidney to open the body cavity.

5m

6 Locate the portal vein and insert the needle. Perfuse liver with **wash buffer** for 00:05:00. The liver should turn from red to yellow/white.

Note

Citation: Alex K. Datsomor, Ragnhild Wilberg, Jacob S. Torgersen, Simen R. Sandve, Thomas N Harvey Transfection of Atlantic salmon primary hepatocytes https://dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

It is important to insert the needle into the portal vein rather than the bile duct which is very close. If the needle is in the bile duct the gull bladder will begin to enlarge. If the needle is inserted into the portal vein then the liver will immediately begin to change color. If it is too difficult to find the portal vein then the needle can be inserted further into the liver in the vicinity of the portal vein for a less efficient perfusion.

5m

7 Without removing the needle switch the wash buffer tube to collagenase buffer from step 3. Perfuse with collagenase for 00:05:00 .

8 Excise the liver and move to an ice cold petri dish. Move to a LAF bench and gently rip the liver apart with tweesers until few large chunks remain. Shake the pieces to loosen the cells.

Note

Ripping rather than cutting produces jagged edges, increasing the suface area exposed to collagenase.

Digestion and washing

1h

9 Pour the entire liver solution into a sterile Erlenmeyer flask containing a sterile magnetic stir bar. Incubate at 15 °C for 01:00:00 with gentle stirring.

10 Filter the digested cells through a 70 µm cell strainer and collect in 50 mL conical tube. Rinse the cells with L-15 media until the tube is nearly full. **From this point on keep cells on ice at all times.**

5m

11 Centrifuge the cells at 100 x g, 4°C, 00:05:00

12 Remove the supernatant and resuspend in 5 mL HBSS without Mg/Ca

5m

13 Centrifuge again at 100 x g, 4°C, 00:05:00

Citation: Alex K. Datsomor, Ragnhild Wilberg, Jacob S. Torgersen, Simen R. Sandve, Thomas N Harvey Transfection of Atlantic salmon primary hepatocytes https://dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

14 Remove the supernatant and resuspend in 5 mL HBSS without Mg/Ca

15 Mix cells 1:1 with trypan blue and count using a hemocytometer.

Note

Cells may need to be diluted before counting. Typically a 1:10 dilution is sufficient. Try to only count hepatocytes which are large and round. Blood cells are oval shaped and should not be counted, but can indicate the efficiency of the perfusion.

5m

Electroporation and plating

5m

16 Divide appropriate number of cells into 1.5 mL eppendorf tubes (electroporation) or conial tubes (direct plating) for the number of conditions in the experiment. Centrifuge at 100 x g, 4°C, 00:05:00

Step 16 includes a Step case.
Electroporation
Direct plating

step case

Electroporation

If cells will be used for electroporation proceed here. This protocol is for 10 µl Neon electroporation tips.

17 Prepare culture plate by adding the appropriate amount of culture media **without** antibiotic to each well. Keep in the incubator at 15 °C until needed.

18 Remove supernatant completely and resuspend cells in buffer R at [M] 1*10^7 cells/mL to [M] 4*10^7 cells/mL. (i.e. 1*10^5 cells to 4*10^5 cells per 10 µl electroporation)

Note

Storing the cell suspension in buffer R for more than 15-30 minutes can reduce cell viability and transfection efficiency. Keep on ice.

Citation: Alex K. Datsomor, Ragnhild Wilberg, Jacob S. Torgersen, Simen R. Sandve, Thomas N Harvey Transfection of Atlantic salmon primary hepatocytes https://dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1

19 Insert the electroporation chamber into the Neon electroporator and add 3 mL buffer E.

20 Add 1-5 ug of plasmid DNA or 1 µl RNP to cells.

Note

Do not add more than 10% of the cell volume. Amount of plasmid depends on the number of cells. See Neon electroporation manual for guidelines.

Note

If using RNPs, prepare according to the IDT protocol "Delivery of ribonucleoprotein complexes into Jurkat T cells using the Neon™ Transfection System".

21 Aspirate cell suspension into a 10 µl Neon tip, insert into the Neon electroporator, and electroporate at **1400 V, 20 ms, 2 pulses**

22 Immediately add electroporated cells to media in prepared culture plate. Incubate cells at 15 °C overnight.

23 The next day, change antibiotic free media with culture media containing antibiotics. Cells can be kept at 15 °C for several weeks, changing media every 3-4 days. Transgene expression (GFP, luciferase, etc...) can be detected after 48 hours.

Citation: Alex K. Datsomor, Ragnhild Wilberg, Jacob S. Torgersen, Simen R. Sandve, Thomas N Harvey Transfection of Atlantic salmon primary hepatocytes https://dx.doi.org/10.17504/protocols.io.j8nlkw4p1l5r/v1