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Transfection of Atlantic salmon primary hepatocytes

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We use this protocol and it's working

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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.



Materials

- 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)
- Peristaltic pump
- Erlenmeyer flask with stir bar
- 70 μ M cell strainer
- Neon electroporator [**ThermoFisher, MPK5000**](#)
- Neon electroporation tip, 10 μ l [**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  7.4 , increase volume to 500 mL using dH₂O, filter sterilize and store at  4 °C .

 400 mL dH₂O

 50 mL 10x HBSS without Ca/Mg

 1 mL 0.5 M EDTA

 5 mL 1 M HEPES

Collagenase buffer: 1x HBSS **with** Ca/Mg, 10 mM HEPES, pH 7.5.

Mix reagents, adjust to  7.5 , increase volume to 200 mL using dH₂O, 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  8.4 , increase volume to 400 mL using dH₂O, 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 μ L 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.

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

1h

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 μ L 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.

- 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.

5m

Note

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 gall 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.

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

5m

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

Note

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

Digestion and washing

- 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.

1h



10 Filter the digested cells through a 70 μ m 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.**

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

5m

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

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

5m

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.

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

5m

STEP CASE

Electroporation 7 steps

If cells will be used for electroporation proceed here. This protocol is for 10 μ l 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 1×10^7 cells/mL to 4×10^7 cells/mL . (i.e. 1×10^5 cells to 4×10^5 cells per 10 μ l 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.

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

- 20 Add 1-5 μ g of plasmid DNA or 1 μ l 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 μ l 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.