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MEF Cell Culture (adapted from Qiu, et al. Bio Protocol 2016)

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We use this protocol and it's working

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Abstract

This protocol describes the process of isolating and culturing mouse embryonic fibroblasts from E14.5-15.5 mouse embryos. MEFs are a useful tool for examining cellular and molecular biology in transgenic mouse models.

Attachments

DOCX

MEF Cell Culture Pro...

12KB

Materials

Rinsing media

DMEM (high glucose, glutamine, and sodium pyruvate)
1% penicillin-streptomycin

Complete media

DMEM (high glucose, glutamine, and sodium pyruvate)
10% FBS
1% penicillin-streptomycin

Lab supplies

Sterile PBS
Sterile serologic pipettes
Sterile conical tubes
Sterile 6mm petri dishes
Sterile 10mm petri dishes
Dissection tools
Dissection microscope
Table-top centrifuge for conical tubes

Set up timed-matings between male and female mice

1 Place one male and one or two female mice into a single breeding cage.

1.1 Check females for vaginal plugs on the following morning.
The presence of a vaginal plug is considered embryonic day 0.5 (E0.5).

2 Record weight of female mice every 2–3 days.

3 Pregnant female mice of gestational days 14.5–15.5 are used.

Embryo removal and cell disaggregation

4 Euthanize a pregnant female mouse at E14.5 or 15.5.

4.1 Swab abdomen with 70% ethanol.
Cut open the abdomen and remove the uterus, placing it in a 10-cm petri dish containing 10 ml sterile PBS on ice.

5 Cut away the uterine decidua, separate and transfer each embryo along with its yolk sac to a fresh 60-mm petri dish with 5 ml sterile PBS on ice.

5.1 Carefully remove the yolk sac and placenta under a dissecting microscope, and examine the embryo to determine viability and/or morphological changes.
We routinely check for embryo size, color (pale, hemorrhage, etc.), and any obvious structural alterations including eye development and limb formation, as well as neurological abnormalities, such as spinal bifida and anencephaly. These are the two most common presentations in our experience, although both are rare in wild-type mice.

5.2 Cut the tail using clean scissors if DNA for genotyping is desired and collect in a 1.5mL Eppendorf tube. Keep tails on ice until tissue digestion.
Clean the instruments with 70% ethanol between embryos at this step to avoid carryover contamination.

6 Decapitate the head from the body with a pair of clean forceps.

7 Lay the embryo on its back, and remove the visible internal organs.

8 Put the body in a fresh sterile 60-mm petri dish containing 0.5 ml rinsing medium on ice.
Cover the dish and move to tissue culture hood.

9 Mince the bodies with fresh, sterile razor blades into small fragments as fine as possible.

9.1 Add 5 ml of cell culture-grade Trypsin-EDTA (0.25%).
Pipette up and down several times with a 5 ml pipette to disaggregate the tissue.
Transfer to a 50 ml conical tube.

10 Place the tube in a 37°C water bath with shaking for enzymatic digestion for 1–2 h.

11 Add 5 ml of rinsing medium, vortex and spin at 1,200 × g for 5 min at room temperature.

Culture the MEFs

11.1 Using a 5mL pipette, carefully remove the supernatant.
Leave 0.5-1mL supernatant in the tube.
Note: Don't try to aspirate the supernatant using a vacuum, since the DNA released from the cells makes it difficult to remove the supernatant without removing the cell pellets.

12 Use a P1000 to gently resuspend the pellet in the remaining media.

12.1 Transfer cells to a fresh 100-mm dish containing 10 ml complete culture media.
Cells from each embryo are processed separately and are plated in their own 100-mm dish.

13 Pipette cells up and down in the culture medium to disperse the cell clumps.

13.1 Allow cells to adhere and grow in a 37°C incubator supplied with 5% CO2.
This is passage 0.
The fibroblasts should start to attach to the bottom of dishes within 2 h of plating.

14 Change the medium the following day. Monitor cell growth. Once the cells reach confluence (~48 h), passage cells.

Protocol references

Qiu LQ, Lai WS, Stumpo DJ, Blackshear PJ. Mouse Embryonic Fibroblast Cell Culture and Stimulation. Bio Protoc. 2016 Jul 5;6(13):e1859. doi: 10.21769/BioProtoc.1859. PMID: 28573158; PMCID: PMC5448408.