In Vitro Culture and Live Imaging of Mouse Embryonic Sternums

Yaopan Mao (ymao@waksman.rutgers.edu)
Ken D. Irvine's Lab, Rutgers University
Ken Irvine (irvine@waksman.rutgers.edu)
Howard Hughes Medical Institute, Waksman Institute and Department of Molecular Biology and Biochemistry, Rutgers The State University of New Jersey
Philippa Francis-West (Philippa.francis-west@kcl.ac.uk)
Department of Craniofacial Development and Stem Cell Biology, King's College London
Ivan Crespo-Enriquez Crespo-Enriquez
Danielle Whiting
Tina Martin
Joanna Mulvaney

Method Article

Keywords: organ culture, live imaging, mouse, embryonic sternums

Posted Date: May 5th, 2016

DOI: https://doi.org/10.1038/protex.2016.034

Abstract

Methods describe how to set up in vitro culture of mouse embryonic sternums, and how to perform live imaging of sternum cell movement in time course with 20x lens resolution.

Introduction

Methods about mouse embryonic \(starting at E12.5-E13.0) sternum culture and subsequent live imaging at cell-level resolution are detailed. The methods may also helpful for in vitro culture and live imaging for other mouse embryonic bone organs.

Reagents

Mice strains: B6.129\(Cg)-Gt\(ROSA)26Sortm4\(ACTB-tdTomato,-EGFP)Luo/J and FVB.Cg-Tg\(CAG-cre/Esr1)5Amc/J \(both available from Jackson Lab). **CAUTION** Any experiments involving live mice must conform to relevant Institutional and National regulations. Tamoxifen. CO2-independent medium \(Gibico, Cat. # 18045). Costar Transwell inserts \(ref # 3450). ø50mm culture dishes \(FluoroDish, Cat # FD5040-100). Capillary-micropipets \(Drummond Scientific, Cat # 5-000-2050, ~1.5mm thick). DMEM/F12\(Gibco, Cat. # 11320) Fetal bovine serum. Antibiotics and antimycotics \(Gibico, Cat. # 15240).

Equipment

GFP Dissecting Stereo Microscope. Conventional cell culture incubator. PerkinElmer spinning disc confocal microscope with cell culture chamber. CO2 tank for cell culture. 20x objective lens with long working distance \(3.5mm, Leica Cat # 506147). Mouse embryo dissection forceps.

Procedure

1. Mice B6.129\(Cg)-Gt\(ROSA)26Sortm4\(ACTB-tdTomato,-EGFP)Luo/J are crossed to mice FVB.Cg-Tg\(CAG-cre/Esr1) 5Amc/J \(Jackson Lab). Record the plugging time at morning, count it as E0.5. This crossing will produce GFP-RFP fluorescent mosaic embryos after tamoxifen induction. 2. By E10.5, pregnant mice are intraperitoneally injected with tamoxifen at dosage of 1.2 mg per mouse. Tamoxifen is dissolved in corn oil at a concentration of 20mg/ml. To aid tamoxifen solving in corn oil, add 100 ul 100% ethanol to 20mg tamoxifen, prior to corn oil adding, and let soak for 5 minutes. 3.By E12.5 or E13.0, pregnant mouse moms are sacrificed, embryos are saved in PBS solution. Embryos are examined under ZEISS Stemi SV 11 Apo microscope with built-in GFP exciting illumination, and GFP positive embryos are saved in CO2-independent medium \(Gibico, Cat. # 18045). 4. GFP positive embryos are dissected in CO2-independent medium to recover sternums, under conventional micro-dissect scope. This is the tricky and the key step to secure sternums for successful culture. Practice is necessary. Cut the GFP positive embryo head off first along the neck. Under dissecting microscope with micro-dissecting forceps, cut muscles

together with the covering skin along the left side of backbone, and rip them off carefully from the backbone. Then peel muscles together with skin carefully along rib cage toward the sternum, which is hard to see, but can be discerned at the position where muscles disappear and only skin continues during the uni-directional peeling. Keep peeling until the skin is mostly peeled after passing the left side sternum. Repeat the same procedure for the right side. Make sure to peel left or right side muscles as a single unit. This makes peeling much easier, and less chance to damage the sternum. 5. Trim off lower trunk part from the muscle/skin peeled chest. With micro-dissecting forceps, cut all the left and right ribs near the sternum, and release the sternum with some rib parts still attached. Make sure the attached left and right side ribs are roughly symmetrical. Trim off extra parts if necessary. 6. Set up the in vitro culture device. Trim a Costar Transwell insert \(ref # 3450) wall, and put in a ø50mm culture dish \(FluoroDish, Cat # FD5040-100). The insert wall is too high, trimming is required to meet the height of the culture dish. Two length-trimmed capillary-micropipets \(Drummond Scientific, Cat # 5-000-2050, ~1.5mm thick) are put between the insert bottom and the culture dish glass cover to make space for accommodating culture medium. To prevent the two micropipets from rolling, it is recommended to make them L-shaped with some glass work technique. 7. Wet the Costar Transwell insert mesh with culture medium, DMEM/F12\ (Gibco, Cat. # 11320) with 10% fetal bovine serum plus 1x antibiotics and antimycotics \(Gibico, Cat. # 15240). Lay the sternum from step 5 on the insert so that the skin/ventral side touches the mesh. Suck off extra medium so that the sternum touches down the mesh firmly, and adjust the sternum with forceps to make sure it is fully stretched gently on the mesh surface. Fill the space beneath the mesh with culture medium, so that the sternum is only immersed by the medium, no more medium is needed. For successful culture, it is critical to have the sternums immersed only \(not submerged) in the culture medium, and to have the sternum skin/ventral side face insert mesh. 8.The set-up sternums are cultured first in conventional cell culture incubator for a few hours \(for E13.0) or late overnight \(for E12.5), with 5% CO2 and 37ºC settings. 9. Live imaging. Transfer the in vitro culture system from step 8 to the cell culture chamber of PerkinElmer spinning disc confocal microscope with culture conditions set to 37ºC and 5% CO2. Discern the location of sternum as well as its top and bottom under dim transmitted light. Live images are taken from 6 to 10 hours, at 1.5 or 2μm z-spacing and time interval of 5 minutes, under 20x objective lens with long working distance \(3.5mm) and water immersion. Both transmitted light and 488nm laser were used to visualize sternums contour and individual GFP-positive sternum cells respectively. After live imaging is finished, the in vitro culture system is returned to conventional cell culture incubator for further culture, with culture medium changed every two days. The culture can keep growing for up to one week. This step is also for examining if culture is successful. 10. Live images are analyzed with Volocity software.

Timing

Setting up in vitro culture system requires a few hours for each sternum for experience hands. Culture and live imaging takes about two days.

Anticipated Results

1. Dynamic intercalation movement of GFP-marked sternum cells is observed. 2. Successful culture will observe the process of left and right sternum halves merge together, and the growth of trimmed ribs.

References

N/A

Acknowledgements

Thanks to my colleagues Dr. Shuguo Sun for helping the usage of PerkinElmer spinning disc confocal microscope.

Figures

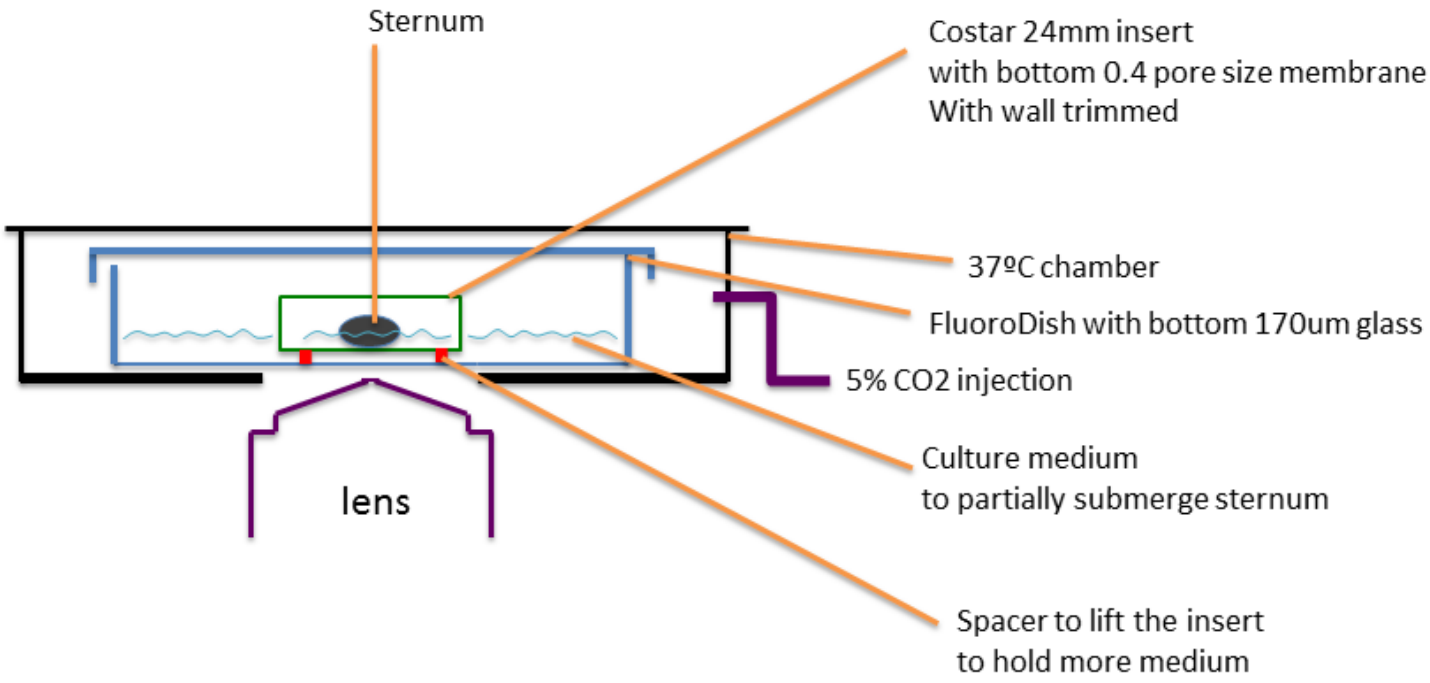

Figure 1

culture divice diagram of sternum in vitro culture device

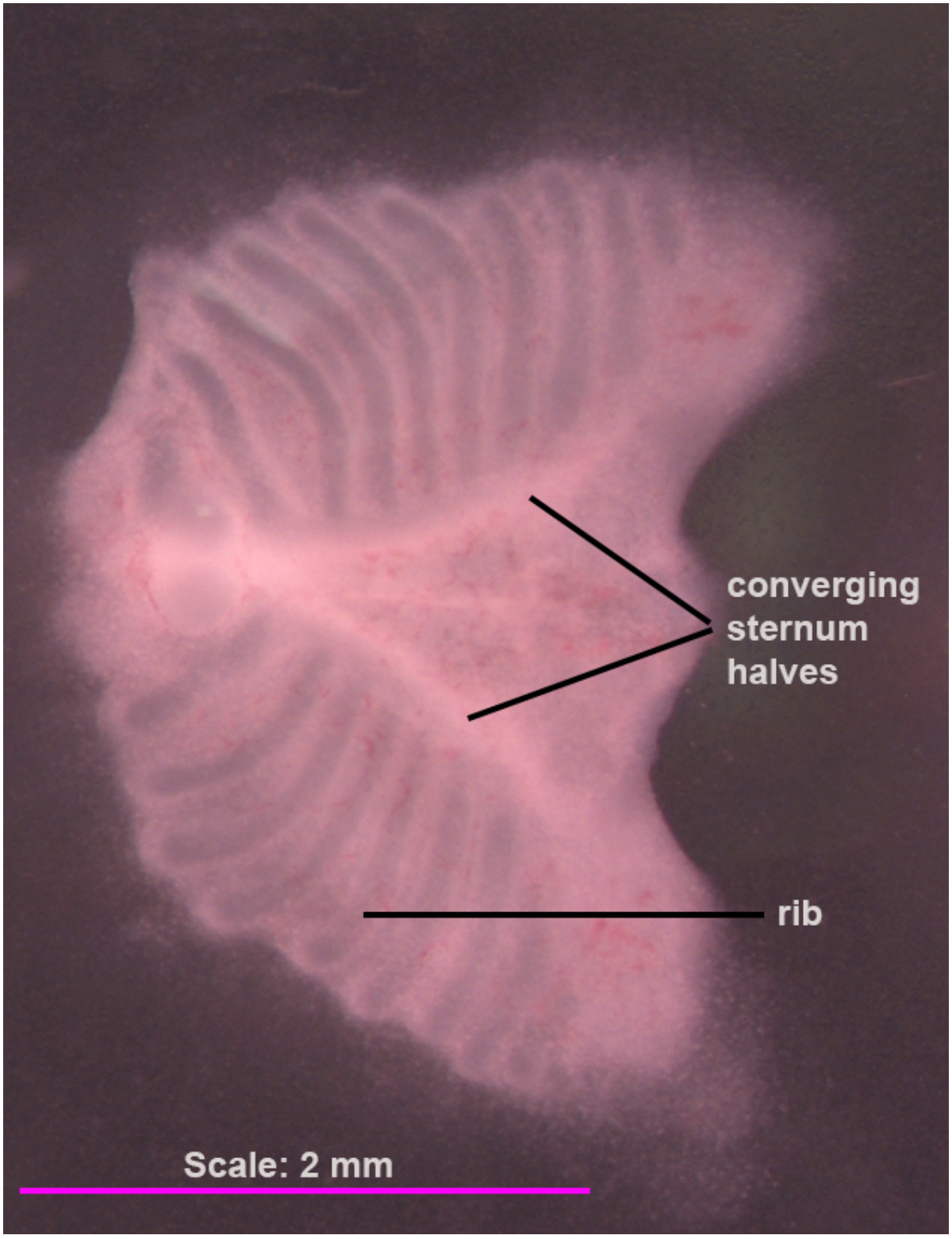

Figure 2

A photo of in vitro culture of mouse sternum A photo of in vitro culture of mouse sternum The sternum was from a E13.0 embryo, and cultured for about 24 hours before the image was taken under light microscopy, at 1.2x magnification.