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Pogona vitticeps (Agamidae: Squamata) autopod explant culture method

This protocol describes how to manipulate and investigate *ex vivo* autopod chondrogenic growth of bearded dragon embryos.

Protocol:

1. Incubate *Pogona vitticeps* embryo to days 14-17.
2. Make sure CO2 tissue incubator is clean and functioning properly. Wipe clean with alcohol between experiments only when there are no culture in the incubator. Incubator should be a humid, 32°C with atmosphere of 5% CO2.
3. Prepare culture media (see recipe below) to the amount needed for the week. Prepared media can be used for one week if kept in the refrigerator.
4. Prepare the organ culture dish with the media and PBS, and put it in the incubator to warm up. The outer rim of the culture dish is filled with ~4 ml sterile PBS (optional: add a drop of gentamycin to the PBS). The inner well is filled with ~0.7 ml chondrogenic culture media.
5. While the culture dishes are warming up, soak a piece of 0.8um nitrocellulose filter paper in sterile PBS, and transfer twice to fresh PBS. Cut the filter paper to small squares that are at least twice as big as the autopod. Leave papers in PBS in petri dish for later. They can be used for about a week. Keep in frig.
6. Open egg into a dish. Remove membranes using a sterile scissors – in sterile 1X PBS in clean petri dish.
7. Transfer autopods to fresh petri dish with 1X PBS. Keep right and left correctly labeled and if possible keep them palm-side down for consistency.
8. Measure the autopod length under the scope quickly, and label the organ culture dish.
9. Place the autopod onto the filter paper by gently prodding. Then cover the dish, and put into the tissue incubator.

10. Replace the media with fresh, pre-warmed culture media (at low level antibiotic) every day. Slightly nudge the limb once a day so the growing cells don't stick to the filter but be careful not to tear any tissue.

11. Image the growing digits every day during the media change. Use transmitted light in order to see the condensations and the developing joints.

Recipes for solutions:

Chondrogenic Culture Media (based on Kanczler et al., 2012)

Alpha-MEM (or DMEM) 15ml

Ascorbic acid 2-phospate 100uM (use 15ul of stock 100mM)

Gentamycin Low concentration = 5ug/mL (use 7.5ul of stock)

ITS-X Insulin-transferrin-selenium, ethanolamine, 10 nM (use 150 ul of ITS-X stock)

Transforming growth factor β3 (10 ng/mL) (use 0.6 ul of stock TGF-β3)

See: Kanczler, J.M.; Smith, E.L.; Roberts, C.A.; Oreffo, R.O.C. 2012. A novel approach for studying the temporal modulation of embryonic skeletal development using organotypic bone cultures and microcomputed tomography. Tissue Engeneering: Part C. 18(10): 747-760.

Ascorbic acid stock solution of 10mM (1000X) and working sol'n (1ul/ml)

176.12 MW

To make 1mL of 100 milimolar, use 17.612 mg of ascorbic acid in 1mL sterile RO water.

= 1000x concentration (i.e. stock is 100mM, we need 100uM)

To make 50 ml of culture media at 100uM, use 1ul per ml, therefore use a 50ul of stock ascorbic acid

To make 15 ml of culture media at 100uM use 15 ul of stock ascorbic acid (100uM0 in 15 ml media

Using gentamycin stock solution (10mg/ml)

For 5ug/mL, use 25 ul for 50 ml culture media; or use 7.5ul for 15 ml culture media volume

For 10ug/mL, use 1ul/ml; use 50ul for 50ml culture media

For 20ug/mL, use 100ul for 50mL culture media

Using 100xITS-X solution

For 50ml culture media, use 0.5ml of ITS-X stock

For 15ml culture media, use 150ul of ITS-X stock

Using TGF-β3

You need 10ng/mL

Therefore in 15ml culture media, you need 15x10ng = 150ng in 15ml media

Making stock = 5ug diluted into 20ul water

Use 0.6ul of stock of TGF-β3 in 15ml culture media