BIOMEDICAL RESEARCH INSTITUTE

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Biomphalaria glabrata embryonic cell (Bge) culture

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1. **Shipped Bge cells:** high density cells are shipped in a 50mL cell culture flask filled with Bge medium to prevent excessive movement during shipping. Allow cells to settle overnight at 26°C.
    - Remove excess medium and proceed with cell culture. Decant all but 6mL of medium. The cells can then be split into 10-20 flasks.
    - Bge cells are not easily retrieved from frozen vials, therefore it is best to keep a culture growing at all times.

2. **Complete Bge Medium 1 liter**

Schneiders's Drosophila Medium	220mL
Lactalbumin hydrolysate	4.5g (27mL - 50x Lactalbumin hydrolysate soln 166.5g/L)
Galactose	1.3g
Gentamycin (10 mg/mL stock)	2.0 mL
Phenol Red (0.5% solution)	1.62mL
MilliQ H2O	up to 900mL
Adjust pH to 7.0	
Sterile filter	
7% Heat-inactivated FBS	

3. Bge cells are very sensitive to different lots of FBS therefore it is important to test several lots (BRI uses GIBCO #A38400-01)

4. **Grow cells at 26°C in complete Bge medium** (7% heat-inactivated FBS in Bge medium). Keep the cells growing at a high density. They like to form cell balls on top of a monolayer- this is normal. Split them when the cell balls get very dense, or about every 2 weeks.

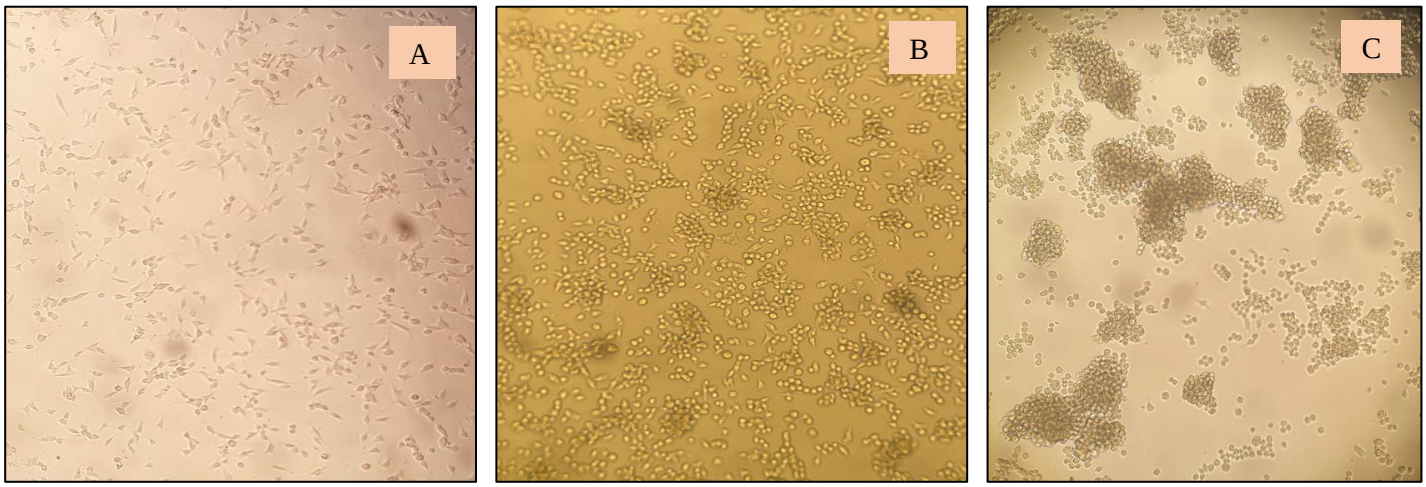
5. **Split the cells/passage:** dislodge the attached cells by hitting the flask with the palm of your hand and transfer the cell suspension into a 15mL tube. Resuspend cells in 1mL complete Bge medium and use 50-100 µL of cell suspension to re-seed into a new 50mL tissue culture flask with 6mL complete Bge medium.

6. **To freeze Bge cells**: remove the attached cells by using a cell scraper and transfer the cell suspension into a 15mL tube. Centrifuge at 700 rpm for 5 min. Resuspend cells in 0.5-1mL of complete Bge medium, then quantitate cells by cell counter. Dilute the cells to a final concentration at $1.0 \times 10^9 - 1.0 \times 10^{10}$ cells/mL in room temperature (RT) freezing medium [9 FBS: 1 DMSO (v/v)]. Aliquot 0.8-1.5 mL into cryovials then transfer into the freezing container

(pre-cooled in the refrigerator [4°C]), as soon as possible. Keep freezing container in -70°C for overnight. Thereafter, the vials are ready to store in liquid nitrogen.

7. **To revive frozen cells from liquid nitrogen**, thaw cells in 35-37°C water bath until ~80% liquid, then add 1-2mL of Bge medium at RT immediately. Transfer the cell suspension into a 15mL tube and centrifuge at 700 rpm for 5 min, RT. Resuspend the cell pellet with at least 7mL of Bge medium and centrifuge as above. Resuspend the cells with 5mL complete Bge medium, and then transfer the cells to 50mL tissue culture flask. Let the cells rest in culture at 26°C for a week (DO NOT CHANGE MEDIUM) to let the cells adapt and start dividing before changing medium.

8. Notes
    - BRI tests Bge cells for mycoplasma (Sigma Aldrich Lookout® Mycoplasma PCR Detection Kit) before shipment.
    - For more information contact André Miller at e-mail: amiller@afbr-bri.org



A) This image is the density that is needed to safely seed the cells. Bge cells require each other to survive and grow. Seeding at a lower density will guarantee failure.
B) This image shows the stage where the Bge cells are near confluency: the cells will form balls and detach from the cell culture flask.
C) This image shows the Bge cells at the "ball stage". The cells are not dead; most remain viable but are overgrown and ready to be split.