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IN VITRO CULTURE AND CHARACTERISATION OF EMBRYONIC STEM (ES) CELLS FROM THE MAROON CLOWN FISH *PREMNAS BIACULEATUS* (BLOCH, 1790)

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Introduction

Embryonic stem (ES) cells provide unique tools for cell-mediated gene transfer and targeted gene mutations due to the possibility of *in vitro* selection of desired genotypes. They have the intrinsic ability to self-renew and can be applied to biodiversity rescuing, gene targeting and germ-line transmission (Chen *et al.,* 2004). Establishment of ES gene targeting techniques in cultured fish provides a novel approach for genetic improvements; developmental biology, and analysis of gene function in fish. Successful ES cell lines have been developed from zebra fish and medaka (Hong *et al.,* 1996).

The present paper discusses *in vitro* culture of embryonic stem (ES) cells employing blastomeres isolated from mid-blastula stage embryos of the maroon clown fish *Premnas biaculeatus*

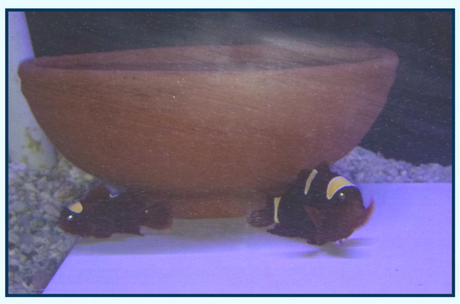
Materials and methods

Tissue culture medium

Tissue culture medium was formulated with Leibovitz-15, DMEM and Ham's F12 with various additives and supplements such as FBS , HEPES buffer, non-essential amino acids, antibiotics, fungizone and growth factors.

Isolation of blastomeres from embryos

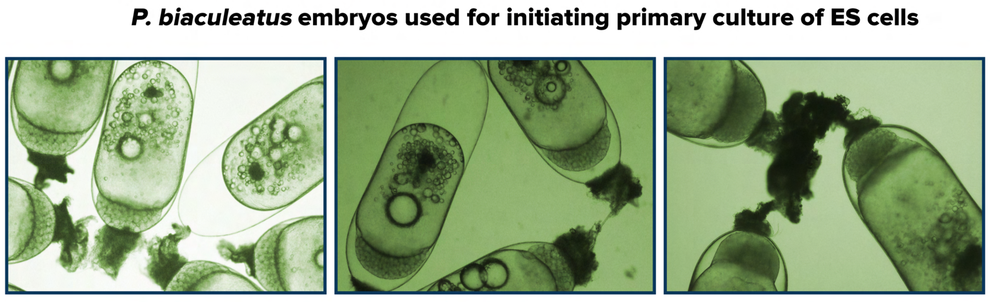
Embryos of *P. biaculeatus* 2 h post-fertilisation, were collected and the developmental stages were assessed. For isolating blastomeres, enzymatic (pronase treatnment) as well as manual method of dissecting out the blastomeres were employed. In order to standardize the optimum stage of the embryos for initiating primary stem cell culture, blastomeres were isolated at different stages viz., 64, 128 and 256 cell stage embryos.



Premnas biaculeatus brood pair

Premnas biaculeatus brood pair guarding the fertilized embryos

***P. biaculeatus* embryos used for initiating primary culture of ES cells**



64 cell stage | 128 cell stage | 256 cell stage

Substrate for blastomere attachment

Different substrates were compared for efficacy of blastomere attachment *viz.* Mitomycin treated feeder fibroblasts, polystyrene cell culture dishes (with and without gelatin / laminin coating) and glass dishes.

Initiation of primary ES cell culture

The isolated blastomeres were seeded onto tissue culture dishes with/without feeder fibroblasts. Within 24 h post-seeding, complete medium was replaced with media containing growth factors (bFGF and FGF-2). Subsequently, media was changed every four days and the cell morphology was examined under an inverted microscope.

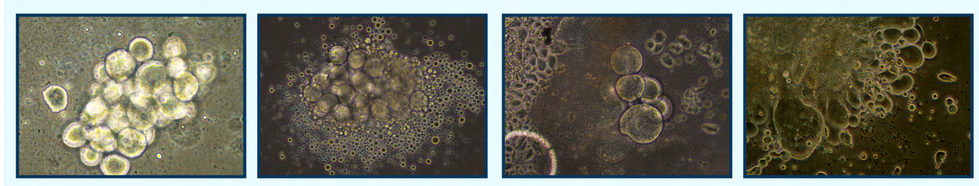
Optimisation of *In vitro* culture conditions and characterisation

Culture conditions of ES cells were optimized and the ES cell cultures were characterized for presence of stem cell specific markers *viz.* TRA 1-60 & SSEA 4 by immunoflourescence staining. To ascertain the efficiency of *in vitro* gene transfection, the ES cells were transfected with pMAX GFP.

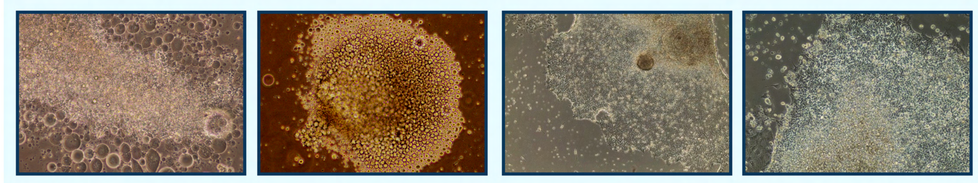
Results and discussion

Though enzymatic treatment and mechanical separation were attempted for isolating blastomeres, the latter method gave better results with minimum interference of yolk globules

Out of the different developmental stages attempted, the mid blastula stage with 256 cell stage gave better blastomere attachment and multiplication. Among the different substrates used, gelatin coated polystyrene dishes were found to have better blastomere attachment and multiplication.



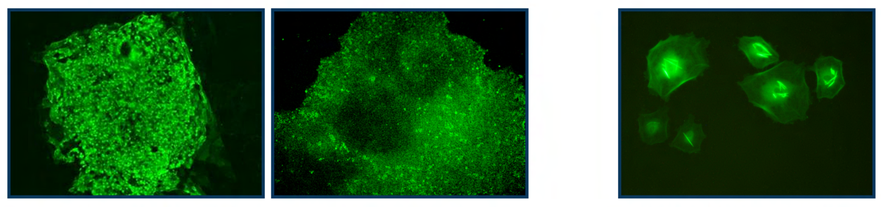
P. biaculeatus blastomeres seeded in tissue culture medium (X100) | Blastomere attachment soon after seeding (X200)



Blastomere attachment and formation of ES cell colonies | ES Cell colonies (PBES12)

Immunoluorescence staining: PBES12 cells were found to be positive for stem cell specific markers TRA1-60 and SSEA4

Transngene expression: PBES12 cells exhibited efficient transgene expression when transfected with pMAX GFP by nucleofection.



TRA1-60 positive cells (PBES12) | SSEA4 positive cells (PBES12) | Expression of GFP gene (PBES12)

Conclusion

Stable embryonic stem (ES) cell lines from *P. biaculeatus* will have potential use in transgenic studies and the methodology could be further utilized for deriving ES cells from farmed marine food fish species

Acknowledgements

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References

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