COMPARATIVE FUNDAMENTAL CRYOBIOLOGY OF MOUSE EMBRYONIC STEM CELLS

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Abstract

Mouse embryonic stem cell (mESC) lines are central to projects such as the Knock-Out Mouse Project, which seek to create thousands of mutant mouse strains using mESCs for the production of human disease models. The ability to efficiently cryopreserve these cell lines for banking and transport is crucial to the success of these programs. The post-thaw recovery of viable cells varies significantly by genetic background, therefore there is a need to improve the efficiency and reduce the variability of current mESC cryopreservation methods. We employed the principles of fundamental cryobiology to improve the cryopreservation protocol of five mESC lines from different genetic backgrounds (BALB/c, C57BL/6, CBA, FVB, and 129R1 mESCs). Using methods outlined in this dissertation, a protocol utilizing 1 M propylene glycol, a cooling rate of 1°C/minute, and plunge into liquid nitrogen at -41°C, combined with subsequent warming in a 22°C water bath significantly improved post-thaw recovery for most mESC lines. Additionally, the effects of Latrunculin A (LATA), 1.5 M dimethyl sulfoxide (Me_2SO), and temperature were examined on C57BL/6 mESC osmotic response and permeability parameters. Temperature, Me_2SO, and LATA significantly influenced isosmotic cell volume, and LATA significantly affected adjusted osmotically inactive cell volume as well as permeability parameters for the C57BL/6 mESC line.