FACS-GAL isolation of β-galactosidase expressing cells from mid gestation mouse embryos

Kelly Lammerts van Bueren
University of California at San Francisco
Peter Scambler
University College London Institute of Child Health

Method Article

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Abstract

Introduction

Microarray analysis and RNA-Seq are powerful tools for investigating differential gene expression in animal models of human genetic disease. Often, these models are loss of function mutations introduced by gene targeting or trapping. One difficulty in such experiments, especially where work on congenital malformations is concerned, is that whole embryo or regional dissections will commonly contain cells that do not express the gene of interest. Thus where cell autonomous effects are of primary interest, for example in the case of transcriptional regulators, such experiments will be compromised by dilutional effects and perhaps secondary effects on surrounding, non expressing tissues. Isolation of the relevant expressing lineage can mitigate these effects. Flow cytometry offers one route to cell purification but requires a fluorescent marker, and relatively few fluorescent protein gene reporters are available. FACS-Gal \(1) offers the potential to capitalize upon the large existing repository of LacZ trapped ES lines now encompassing approximately 33% of all mouse genes "http://www.genetrap.org/index.html":http://www.genetrap.org/index.html. Moreover, the existence of Cre-reversible traps opens the way to within group comparisons of flow sorted cells, one pool having had expression restored using a tamoxifen activated Cre enzyme \(2).

Reagents

1. Tissue culture medium: Dulbeccos Modified Eagle's Medium \(DMEM) + 25 mM HEPES \(pH7.2) +/- 10% Fetal Calf Serum \(FCS) 2. Detectagene Green kit \(Molecular Probes D-2920) 5-chloromethylfluorescin di-β-D-galactopyranoside \(CMFDG) LacZ gene expression kit \(note, we obtained inferior results with the ImaGene Green C\(12)FDG kit) 3. 0.3mM chloroquine diluted in DMEM + 25 mM HEPES \(pH7.2) containing 5% FCS 6. 50 µM CMDFG, 200 µM verapamil in 5% FCS/DMEM + 25 mM HEPES \(pH7.2) 5. 1mM 2-Phenylethyl β-D-thiogalactoside Sigma, Cat. P4902 7. 1.5 µM propidium iodide Sigma, Cat. P4170 8. Phosphate buffered saline \(PBS) 9. 0.25% trypsin \(Gibco) in PBS

Equipment

Low speed, bench top microfuge Plastic, bulb topped pipettes Tissue culture incubator

Procedure

A. Isolation of embryos 1. Dissect out mouse embryos in DMEM + 25 mM HEPES 2. Embryos should be staged by somite counting to avoid noise due to normal intra and inter litter variation in development 3. If rapid genotyping \(or assignment of genotype by phenotype) is possible embryos may be pooled, otherwise process separately in microtitre well plates **B. CMFDG labelling of a single cell suspension** 1. In order to reduce background fluorescence block endogenous lysosomal β-galactosidase activity by incubating embryos in 0.3mM chloroquine diluted in DMEM + 25 mM HEPES containing 5% FCS and incubated at 37 °C, 5% CO_2 for 30 minutes 2. Rinse embryos three times in PBS, then incubate in 0.25% trypsin in PBS for approximately 30 minutes 3. During step 2 dissociate embryos into a single cell suspension by trituration every 5-10 minutes using plastic pipettes, starting with larger tips and moving down to smaller tips 4. Spin down the cell suspension at 300 x g for 3 minutes and resuspend in pre-warmed \(10 minutes at 37 °C) 50 µM CMDFG, 200 µM verapamil in 5% FCS/DMEM + 25 mM HEPES and incubate for 30 minutes at 37 °C, 5% CO2. \(Note: the verapamil is used to inhibit efflux of the fluorescent, CMFDG-glutathione adduct from the cells \(3)) 5. Place cells on ice and dilute to approximately 1-3 million cells/mL with 5% FCS/DMEM + 25 mM HEPES 6. Add 1mM PETG to stop the reaction 7. Add 1.5 µM propidium iodide to identify dead cells 8. Flow sort as soon as possible \(standard procedures) **C. Post FACS Treatment Notes** 1. For RNA isolation collect sorted cells immediately at 300 x g for 4 minutes and resuspended in 800 µl Trizol \(Gibco BRL). 250 µg/ml glycogen \(Roche) or 50 µg/mL Glycoblue \(Ambion) can be added as an RNA co-precipitant to improve yield 2. Homogenize cells by passing them twice through a 25-gauge needle \(shears genomic DNA) and store at -80 °C, or process immediately using standard techniques 3. For microarray, we found exponential amplification worked well \(4) and preserved transcript abundance

Timing

Approximately 6 hours \(including FACS).

Critical Steps

B2. This has to be as gentle but as complete as possible in order to maximize the yield of cells. It is worth practising on wild type embryos prior to the experiment and using a standard viability stain to assess operator competency. B8. Once again this helps with cell viability and improves RNA transcript integrity.

Troubleshooting

1. Poor signal:noise ratio due to low LacZ-induced fluorescence. This may be inherent due to the expression level of the gene concerned. Variations of the protocol include: a. alteration of the CMDFG incubation period b. try incubating at 4 °C rather than 37 °C 2. Poor cell viability a. decrease number of trituration steps, but use a cell strainer \(70 µm mesh size, BD Biosciences Cat. 352350) b. omit the chloroquine incubation step 3. Try FDG loading "http://www.natureprotocols.com/2008/08/06/detection_of_lacz_expression_b.php":http://www.natureprotocols.com/2008/08/06/detection_of_lacz_expressi

Anticipated Results

The loading technique typically results in 50% cell death in the fluorescent population. A population of highly fluorescing cells should be detectable.

References

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