Adaptation of a commonly used, chemically defined medium for human embryonic stem cells to stable isotope labeling with amino acids in cell culture (SILAC)

Table of Contents

1 Protein Quantitation using the BCA method

The protein was estimated using the BCA method with the BCA protein assay kit (Pierce, Rockford, IL, USA). This method combines the reduction of cupric ions to cuprous ions by the protein and subsequent reaction of the cuprous ions with two molecules of BCA to give an intense purple color read at λ = 562 nm. The BSA standard with a working concentrations; 25, 125, 250, 500, 740, 1000, 1500, 2000 µg/ml were used in triplicate (25 µl/ well of 96-well plate). SDS (5 µl, 4%, v/v) was added to wells containing blank and standards to normalize for its presence in the SDS-extracted samples. BCA working reagent (obtained by mixing reagent A:B in proportion 50:1 as per kit manufacturer instruction) was added to each well of 96-well plate (200 µl/well), in a final volume of 230 µl. The samples were prepared by combining 5 µl of protein solution with 25 µl of water. The 96-well plate was incubated at 37°C temperature for 30 min and read at λ = 562 nm using EnVision Plate Readers (PerkinElmer, Waltham, MA, USA). Proteins were determined in three independent measurements.

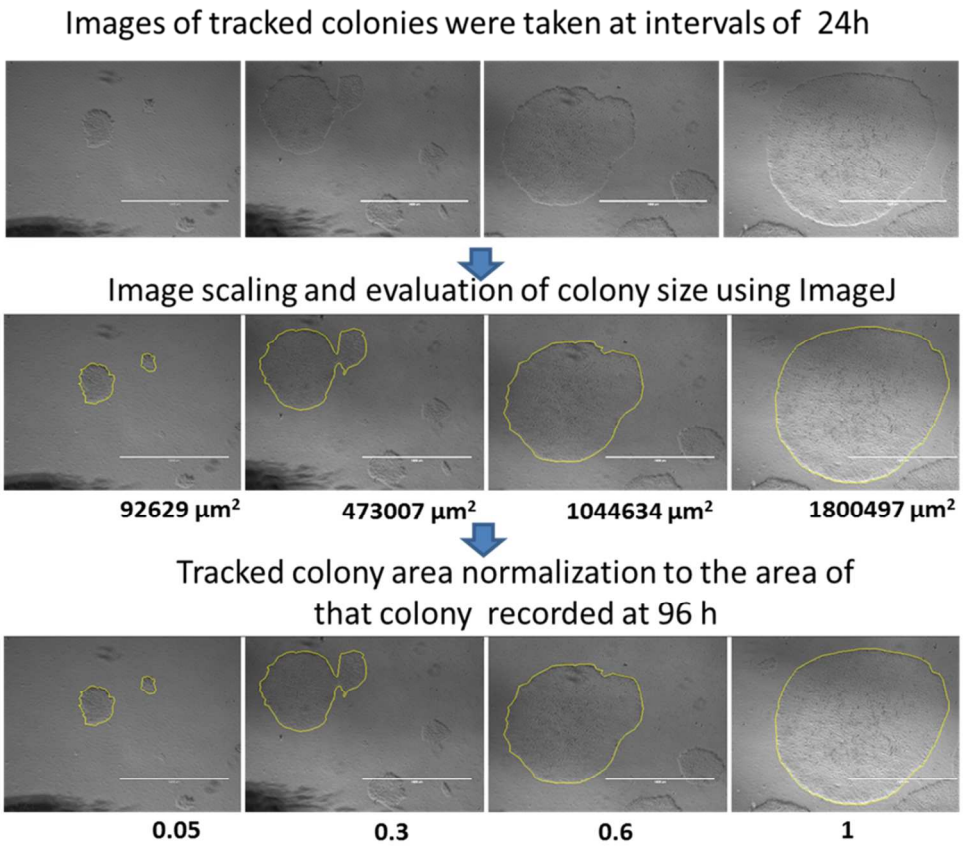
2 Protein Quantitation using Bradford protein assay

The protein was estimated using the Bradford reagent (Sigma-Aldrich, St. Louis, MO, USA). The procedure is based on the formation of a complex between proteins in solution and the dye, Brilliant Blue G. The protein-dye complex causes a shift in the absorption maximum read at λ = 592 nm. Bradford reagent was aliquoted in to polystyrene cuvettes (800 ul/each) The BSA standards with working concentrations; 250, 500, 740, 1000, 1500, 2000 µg/ml were used in triplicate (5µl/cuvette). To normalize for urea/ thiourea presence in the extracted samples, 1 µl of 6 M urea, 2 M thiourea in 10 mM

Hepes was added into cuvettes containing blank and standards. Typically, 1 µl of sample were used for measurements. Water was added up to the final volume of 1 mL, cuvettes were sealed with parafilm, contend was mixed and incubated at room temperature for 15 min and read at λ = 592 nm using spectrophotometer, NanoDrop2000C (Thermo Scientific, Rockford, IL USA). Proteins were determined in three independent measurements.

3 Details of growth rate evaluation using ImageJ

In total 182 colonies were tracked and 728 micrographs were obtained, for each tracked colony growth rate was evaluated as presented below.



4 hES cell differentiation protocol:

Undifferentiated hES cells are grown to reach sub-passage confluence. At that stage mTeSR1 is replaced with DMEM media (DMEM low glucose with 20% fetal bovine serum (FBS), and 1% penicillin/streptomycin). Differentiation was induced for 6 days in the FBS containing media. Morphologically changed cells were then passaged using trypsin (1.5ml/well) and plated on new matrigel in six well pates for a further six days culture. Media was changed every 2-3 days. After six days cells were passaged using trypsin and spread on plastic flasks. All cultures were performed at 37°C, 5% CO_2. Dead and non-adherent cells were removed the day after initial plating by washing with PBS. Media was then changed every three days. Cells were harvested when 80% confluent growth was achieved.

5 Quantitative PCR (qPCR) analysis:

Total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany) and followed by cDNA synthesis via the GoScript™ reverse transcription system (Promega, Madison, WI, USA) according to the manufacturer's protocols. Quantitation was performed by monitoring SYBER Green fluorescence signal

generated during amplification. PCR reactions were performed using 1 µl cDNA, PCR master mix 2X (Promega, Madison, WI, USA) and 1 µM of each primer pair (IDT, San Jose, CA, USA) per 20 µl reaction volume. Triplicate reactions in 96-well optical reaction plates were amplified under the following thermal cycling conditions: 95°C for 10 min with 40 cycles and 95°C for 15 min and 60°C for 1h in a 7500 Real Time PCR machine (Applied Biosystems Inc., CA, USA). Ct calculations based on the results were determined using the manufacturer's 7500 software (Version 2.0.5).