

# Measurements for the ENCODE common cell types

The ENCODE Consortium has designated common cell types that will be used by all investigators to aid in the integration and comparison of data produced using different technologies and platforms. To ensure consistency in cell cultures prepared in different laboratories, investigators should take the measurements below for each cell harvest.

## Required Measurements

- **Growth time/passage number.** The growth time of cell lines should be determined by recording the date when cells were put into culture and when they were harvested. Investigators should go back to the original stock after growing a culture for one month. Passage number should be assessed for primary cells. The passage number for primary cells should not exceed 3-4 passages.
- **Cell density.** Cell density should be assessed for each cell culture.
  - The density of GM12878 cells should be maintained between  $2.0 \times 10^5$  cells/ml and  $1.0 \times 10^6$  cells/ml.
  - K562 cells should be grown to a maximal density of  $7.5 \times 10^5$  cells/ml.
  - HepG2 cells should be grown to a maximum of 75% confluence.
  - HeLa-S3 should be grown to a maximal density of  $5 \times 10^5$  cells/ml.
- **Cell cycle state.** Cell cycle state should be measured by FACS analysis within 2 weeks of the time of harvest. Samples can be prepared at the time of harvest for later analysis. Staining should be done immediately before FACS analysis. A protocol for performing FACS analysis is available below. Sample FACS profiles for K562 and HeLa-S3 are available at the end of this document.

## Other Issues to Consider

**Presence of mycoplasma.** Cell cultures should be tested at monthly for the presence of mycoplasma. The mycoplasma testing protocol used by Bionique, which does mycoplasma testing for ATCC, is available below.

## Freezing Cell Aliquots

As Tier 1, 2, or 3 cells are grown, each ENCODE group should freeze an aliquot of cells for potential future phenotyping.

# Preparation of cells for FACS analysis:

Farnham lab protocol 2008, February 26

## Prepare cells (this can be done up to two weeks in advance)

1. Trypsinize adherent cells using standard protocol (non-adherent cells can be counted directly).
2. Determine cell number. Use  $10^5$ - $10^6$  cells/sample.
3. Add appropriate volume of cells to a conical tube and spin them down at 1000 rpm for 3 min. Aspirate off the media.
4. Vortex pellet at low speed, add 0.5 ml of cold PBS, vortex again for 2-3 seconds. ***It is very important to achieve a single cell suspension.*** If cell clumping is a problem, you can use calcium- and magnesium-free PBS.
5. Resuspend the pellet in 5 ml of cold PBS. Centrifuge cells for 6 min at 1000 rpm.
6. Aspirate off the PBS.
7. Add 0.5 ml cold PBS and pipette up and down to achieve a single cell suspension.
8. Prepare a tube with 4.5 ml ice-cold 100% ethanol. Begin vortexing the tube with ethanol as you slowly add 0.5 ml of cells in PBS, dropwise.
9. Incubate on ice or at 4°C for at least 30 minutes. At this point, samples can be stored in the refrigerator for up to two weeks.
10. Make an appointment with the Flow Cytometry Facility!

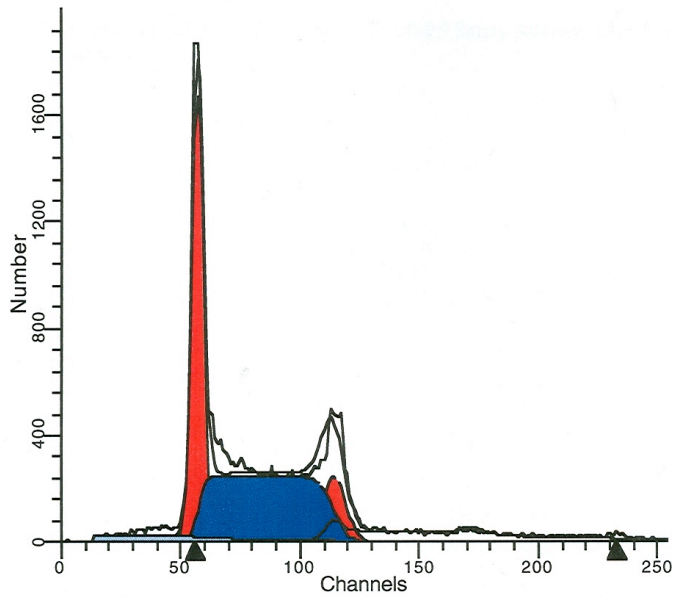
## Stain cells (this is done immediately before FACS analysis)

1. Prepare propidium iodide (PI)/Triton staining solution with RNase A.  

Recipe: 10 ml of 0.1% (v/v) Triton X-100 (Sigma) of PBS (Triton is a viscous liquid), 2 mg DNase-free RNase A (Sigma), 200 microliters of 1 mg/ml PI (Molecular Probes). Prepare fresh!
2. Warm tubes to 37°C for 5-10 minutes (otherwise ice crystals can tear apart your cells).
3. Centrifuge the ethanol-suspended cell for 5 min at 200 g (=1000 rpm). Decant ethanol thoroughly.

4. Suspend cell pellet in 5 ml of PBS, wait about 1 min, centrifuge 5 min at 200 g (=1000 rpm) for 5 min. Discard the supernatant.
5. Suspend cell pellet in 1 ml PI/Triton X-100 staining solution with RNase A. Keep in the dark at RT for 30 min or at 37°C for 15 min.
6. Immediately before analysis, vortex each sample and filter using a 5 ml polystyrene round-bottom tube with cell-strainer cap (Becton Dickinson, cat# 352235). Pour cell suspension onto the cap and centrifuge the tubes for a short period of time at 1000 rpm.
7. You are ready to do the FACS analysis.

K562 cell FACS, example 1



Sync Wizard Model

Date acquired: 04-Feb-08  
File: 02.04.08.005  
Source: SAMPLE ID  
Case: PATIENT ID  
Analysis type: Manual analysis  
Prep: Unknown

G0G1:  
37.74 % Mean: 57.07  
CV: 4.27 %

G2M:  
10.97 % Mean: 114.14  
G2/G1: 2.00

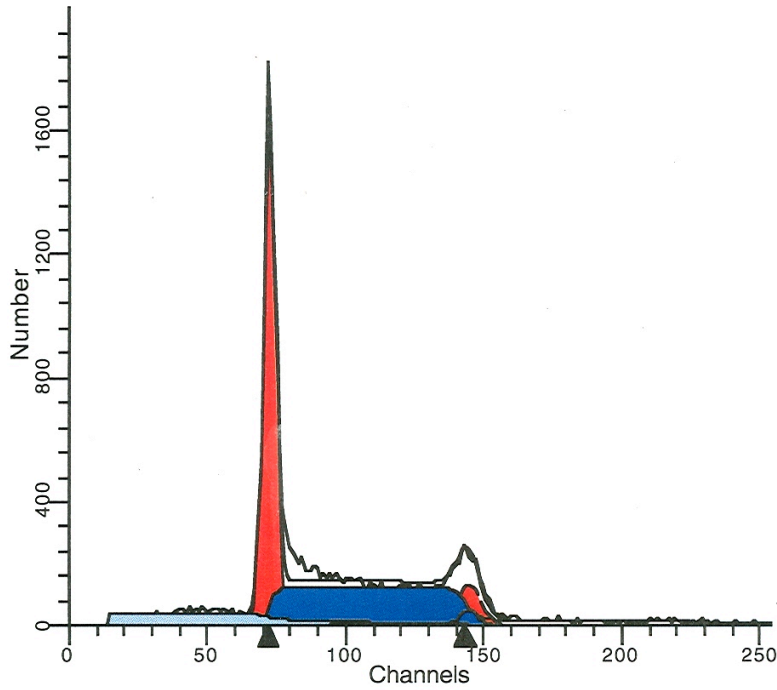
S-Phase:  
51.29 % Mean: 85.76

Compartment 1: 51.29 %

Debris: 5.16 %  
Aggregates: 13.77 %  
Modeled Events: 33632  
RCS: 6.710

S-Phase Assessment:  
Tissue Type: All  
Model type: Diploid  
Diploid S: High  
Calculated p-value:  $p < 0.01$   
S-phase Boundaries: 5.0 and 10.0

K562 cell FACS, example 2



Sync Wizard Model

Date acquired: 04-Feb-08  
File: 02.04.08.004  
Source: SAMPLE ID  
Case: PATIENT ID  
Analysis type: Manual analysis  
Prep: Unknown

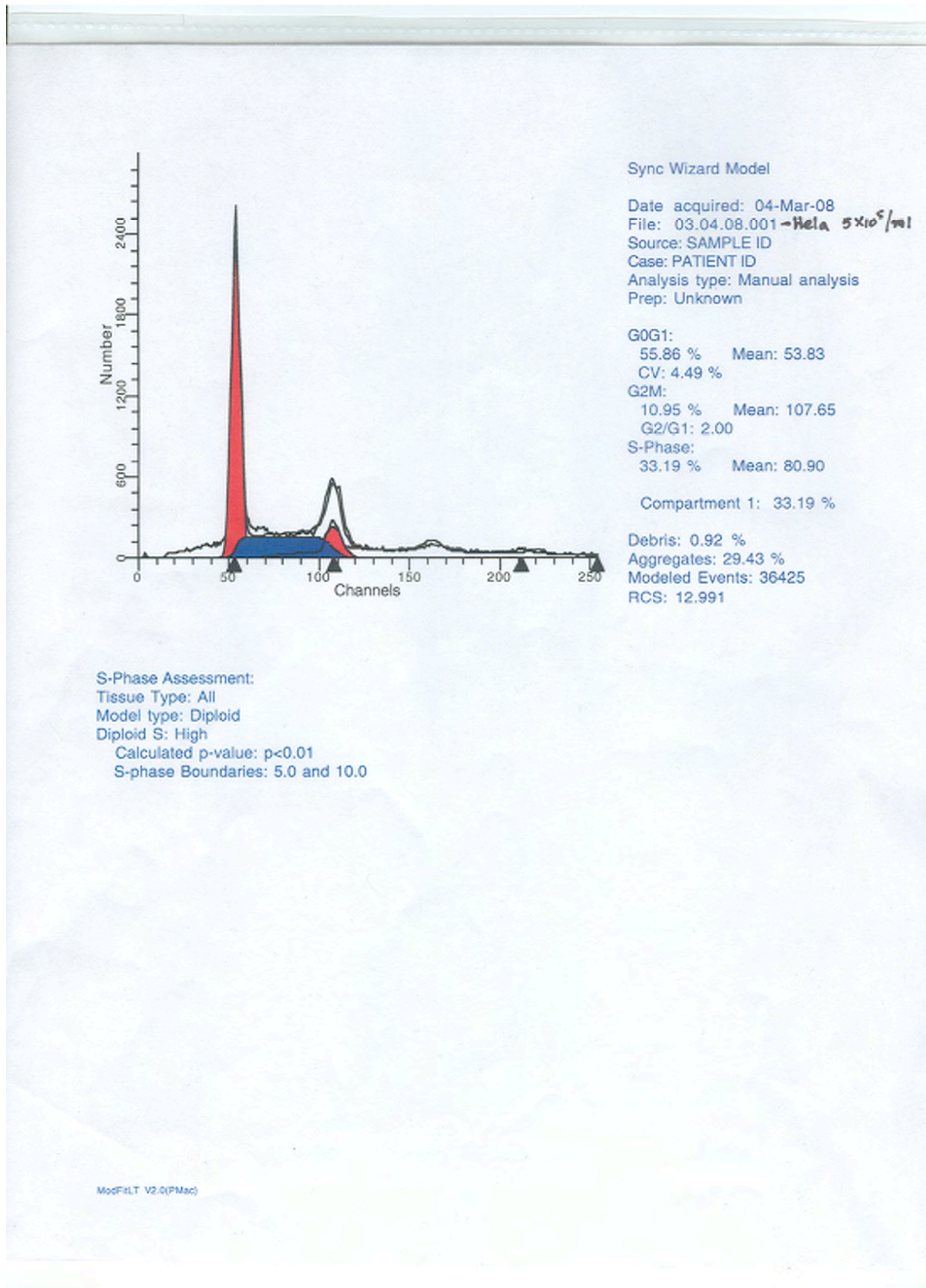
G0G1:  
45.10 % Mean: 72.35  
CV: 3.01 %  
G2M:  
7.51 % Mean: 144.69  
G2/G1: 2.00  
S-Phase:  
47.39 % Mean: 108.62

Compartment 1: 47.39 %

Debris: 13.25 %  
Aggregates: 8.55 %  
Modeled Events: 24567  
RCS: 3.863

S-Phase Assessment:  
Tissue Type: All  
Model type: Diploid  
Diploid S: High  
Calculated p-value:  $p < 0.01$   
S-phase Boundaries: 5.0 and 10.0

HeLaS3 FACS example



# Bionique Mycoplasma Testing Protocol

## **Bionique<sup>®</sup> Testing Laboratories, Inc.**

156 Fay Brook Drive  
Saranac Lake, NY 12983  
Phone: 518-891-2356  
Fax: 518-891-5753

### **Catalog ID: M-250 Multi-media Direct Culture and DNA Fluorochrome Test**

The purpose of this test is to determine whether or not mycoplasmal contaminants are present in a cell culture sample, be it a primary culture, hybridoma or continuous cell line.

Cells should be cultured at least once, preferably 3 passages without antibiotics &/or selective agents to increase sensitivity. This procedure combines an indirect DNA fluorochrome assay (see M-150) to detect non-cultivable mycoplasmas with a direct culture assay utilizing three mycoplasma media formulations. The DNA fluorochrome procedure is a non-selective indirect DNA fluorochrome assay which involves the inoculation of a 1.0 ml sample into a mycoplasma free indicator cell culture (VERO), incubation for 3 to 5 days, then performance of a DNA fluorochrome (Hoechst) staining assay on a slide prepared from the co-incubated sample/Vero cell culture (see M-100 CellShipper<sup>®</sup>). This assay is designed to enhance the level of sensitivity by reducing background from genetically unstable cell lines (e.g. hybridomas, etc.) and amplifying the titer of mycoplasma contaminants. Appropriate positive (<10<sup>3</sup> CFU/ml) and negative controls are processed with each sample.

The direct culture procedure utilizes three mycoplasma media formulations: Fortified Commercial (FC), Modified Hayflick (MH), and Heart infusion (HI) broth and agar formulations. A 0.5 ml sample is inoculated into 6 ml of each broth (FC, MH, & HI). A 0.1 ml sample is inoculated onto duplicate plates for each agar formulation. Each broth is subcultured onto like agar plates on Day 7 post setup. The agar plates are incubated aerobically and microaerophilically and examined microscopically at 7 day intervals. Appropriate positive (< 100 CFU) and negative controls are processed with each sample. The theoretical sensitivity is approximately 50 CFU. The accuracy of the combined test is approximately 99%. Total testing time is 28 days.