

Methylation assay and sequencing using the Advalytix AmpliCell platform

Simple, sensitive and affordable protocols on epigenetics.

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The Advalytix AmpliCell – cell culture & PCR system is based on the AmpliGrid 1 μL reaction slide. The 48 hydrophilic reaction sites of the AmpliCell are treated with fibronectin to create an optimal surface for attachment of adherent cells. The hydrophobic regions outside the reactions sites are covered with a special foil that is easily removed for downstream PCR analysis. Disposable chambers for cell culture medium are pre-mounted on the slide to allow optimal growth conditions for the cells of interest. This application report shows data on the methylation state of cells grown on the AmpliCell platform using a simple, sensitive and affordable workflow.

Methylation of CpG islands in the promoter region of different genes that are involved in development and cancer progression, is assumed to have an influence on the expression of these genes. Either a hypomethylation or a hypermethylation leads to an increase in gene expression or silencing effect. The methylation state of different cell types such as cancer cells, stem cells or primary cells such as neurons is often important to understanding the influence of the methylation of genes.

Introduction

HeLa cells (HPV18 positive cervical carcinoma epithelial cells) are grown on AmpliCell (figure 1). A methylation assay using the Qiagen EpiTect® Bisulfite Kit followed by sequencing is performed to analyze the methylation status of genes.

1 Figure 1: AmpliCell platform



In the current example, we deposit HeLa cells on the AmpliCell and incubate for 3 hours to allow attachment of the cells on the AmpliCell surface. Cells are washed, dried and a bisulfite reaction is performed using a conventional kit. A PCR with methylation specific primers followed by a sequencing reaction is done. On the basis of the sequencing data it is possible to analyze the percentage of methylated versus unmethylated events.

Protocol

Stain HeLa cells with Hoechst dye (Hoechst Bisbenzimid H 33342, Sigma) with a concentration of 1mg/mL (100x staining solution). Dilute the Hoechst 1:100 with the cell suspension and incubate for 5-10 min at room temperature. Deposit 4×10^3 HeLa cells on the AmpliCell chambers (each reaction site will contain approx. 1×10^3 cells) and incubate for 3 hours to allow attachment of the cells to the AmpliCell surface.

Afterwards, remove chamber and foil of the AmpliCell and wash the glass slide with 1x PBS (phosphate buffered saline, pH 7.4), 0.05x PBS and water. Prepare the Qiagen Bisulfite mix as described in table A:

A Table A: Bisulfite mix

Component	1 reaction
Nuclease free water	0.14 μL
Bisulfite Mix	0.61 μL
DNA Protect Buffer	0.25 μL
Total volume	1 μL

Pipette 1 μL Bisulfite mix on each reaction site of the AmpliCell and immediately cover with 5 μL of sealing solution. Transfer the AmpliCell slide to the AmpliSpeed slide cycler and run the program according to table B:

B Table B: Bisulfite program

Temperature	Time
99°C	5 min
60°C	25 min
99°C	5 min
60°C	85 min
99°C	5 min
60°C	175 min

Add 0.5 μL of 0.1M NaOH to each reaction site by pipetting on top of the sealing solution. Due to the physical conditions, the aqueous solutions will merge immediately. Incubate for 20 minutes at room temperature. Afterwards remove the sealing solution by dipping the slides into 100% hexane (please follow the safety precautions by handling with organic solvents). Wash the slide for 3 min in water and air dry it afterwards at 37°C using the incubation function of the AmpliSpeed slide cycler.

