Protocol transcriptomics and DNA methylation assay

Transcriptomics is the study of the complete set of RNA transcripts produced by the genome under specific circumstances or in a specific cell. It provides insights into gene expression patterns, allowing researchers to understand how genes are regulated and how they contribute to various biological processes.

DNA methylation is a biochemical process involving the addition of a methyl group to the DNA molecule, typically at the cytosine base of a CpG dinucleotide. This modification can influence gene expression without altering the DNA sequence itself, often leading to transcriptional repression. Methylation patterns can change in response to environmental factors, developmental stages, and disease states.

Together, transcriptomics and DNA methylation studies can reveal how epigenetic modifications affect gene expression and contribute to cellular functions and disease mechanisms.

Chemicals & materials

Chemicals

Chemicals	Provider (article number)
DNA AWAY	Carl Roth (X996.1)
RNA AWAY	Carl Roth
DPBS without Ca and Mg	PAN Biotech (P04-361000)
RPMI 1640 medium	Gibco (11875093)
Trypsin inhibitor	Roth (2949.1)
Melanocyte Growth Medium M3	PromoCell (C-24310)
Keratinocyte Growth Medium M3	PromoCell (C-20011)

Materials

Material	Provider
T25 flasks	TPP
Serological pipette (5, 10 & 25 ml)	Sarstedt
PIPETBOY acu 2 pipette controller	Integra
Microliter pipette (10, 200, & 1000 µl)	Eppendorf
RNAse-free pipette tips	Sarstedt
RNAse-free SafeSeal reaction tubes	Sarstedt
Cell scraper	TPP
RNeasy Plus MiniKit	Qiagen
WT Plus Reagent Kit Clariom S Affymetrix	Thermo Fisher
NucleoSpin Tissue Kit	Macherey-Nagel

Cell preparation

- On the first day, seed the cells in T25 flasks with defined cell density in growth medium.
- Pre-culture the cells for 24 h.

Treatment

- On the second day, change the medium with 8 ml growth medium.
- Expose the cells to electromagnetic fields for defined exposure time.

- Incubate the cells in the incubator for further 1.5 cell cycle to allow micronuclei to form.
- If needed, change the medium after 48 h of seeding.

Cell harvest

- Wash the cells twice with 2 ml pre-warmed DPBS without Ca^+ and Mg^+.
- After removing the pre-warmed DPBS without Ca^+ and Mg^+, add 3 ml cold DPBS without Ca^+ and Mg^+.
- Detach the cells using cell scraper and move the cell suspension to a 15 ml centrifuge tube.
- Wash the cell culture flask using another 3 ml cold DPBS without Ca^+ and Mg^+ and move the solution in the 15 ml centrifuge tube.
- Resuspend the cell suspension.
- Take 100 µl aliquot of cell suspension for cell counting.
- Centrifuge cells at 4 °C, 1200 rpm for 10 minutes.
- Remove the supernatant.
- Snap freeze the cell pellets in liquid nitrogen.
- Store the cell pellets at -80°C until further use.

Transcriptomics using Affymetrix Microarray

- RNA is isolated from cell pellets using RNeasy Plus Mini Kit.
- Transcriptomics assay is performed with WT Plus Reagent Kit Clariom S Affymetrix.

DNA methylation assay

- DNA is isolated from cell pellets using NucleoSpin Tissue Kit.
- DNA methylation assay is performed by Diagenode using the Infinium Epic Array Service V2.