Chromatin immunoprecipitation from human embryonic stem cells.

Journal: J Vis Exp

Publication Year: 2008

Authors: Stephane Bertani, Alice Kan, Frank Sauer

PubMed link: 19066517

Funding Grants: Non-coding RNA as tool for the active control of stem cell differentiation

Public Summary:

Scientific Abstract:
The functional and structural complexity of the myriad of cells in metazoan organisms arises from a small number of stem cells. Stem cells are characterized by two fundamental properties: self-renewal and multipotency that allows a stem cell to differentiate into virtually any cell type. The progression stem cell to differentiated cell is characterized by loss of multipotency, structural and morphological changes and the hierarchic activity of transcription factors and signaling molecules, whose activities establish and maintain cell-type specific gene expression patterns. At the molecular level, cell differentiation involves dynamic changes of the structure and composition of chromatin and the detection of those dynamic changes can provide valuable insights into the functional features of stem cells and the cell differentiation process. Chromatin is a highly compacted DNA-protein complex that forms when cells package chromosomal DNA with proteins, mainly histones (4). Stemcellness and cell differentiation has been correlated with the presence of specific arrays of regulatory proteins such as epigenetic factors, histone variants, and transcription factors. Chromatin immunoprecipitation (ChIP) provides a valuable method to monitor the presence of RNA, proteins, and protein modifications in chromatin. The comparison of chromatin from different cell types can elucidate dynamic changes in protein-chromatin associations that occur during cell differentiation. Chromatin immunoprecipitation involves the purification of in vivo cross-linked chromatin. The isolated chromatin is reduced to smaller fragments by enzymatic digestion or mechanical force. Chromatin fragments are precipitated using specific antibodies to target proteins or protein and DNA modifications. The precipitated DNA or RNA is purified and used as a template for PCR or DNA microarray based assays. Prerequisites for a successful ChIP are high quality antibodies to the desired antigen and the availability of chromatin from control cells that do not express the target molecule. ChIP can correlate the presence of proteins, protein and RNA modifications, and RNA with specific target DNA, and depending on the choice of outread tool, detects the association of target molecules at specific target genes or in the context of an entire genome. The comparison of the distribution of proteins in the chromatin of differentiating cells can elucidate the dynamic changes of chromatin composition that coincide with the progression of cells along a cell lineage.