Molecular beacon nanosensors for live cell detection and tracking differentiation and reprogramming - DTU Orbit (09/11/2017)

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High-sensitive and high-affinity methods to measure gene expression inside living cells have proven to be invaluable in regards to understanding fundamental processes such as cell differentiation, reprogramming, regeneration and cancer genesis. One tool for transcription visualization on single cell level is molecular beacons (MBs). They are stem-loop structured antisense oligonucleotide probes labelled with a reporter fluorophore at one end and with quencher at the other end. Upon hybridization with complementary target, hydrogen bonds between stem nucleotide bases brake, resulting in separation of fluorophore from quencher and thereby emission of a fluorescent signal that can be detected. In this project the usability and applicability of MBs for live cell detection and tracing of gene expression was demonstrated. MBs library targeting gene markers for pluripotent stem cells as well as markers for different stages of cell differentiation into the three germ lineages were designed. The focus was on stem cell differentiation into neuronal lineage as well as
reprogramming into a more immature state after plasmid transfection or under the influence of the environment and growth conditions. We have been able to detect gain of expression of neuronal markers during differentiation of embryonic mesencephalon derived cells (LUHMES) into dopamine neurons. Loss of expression of stem cell markers was also observed suggesting that MBs are able to dissociate from target mRNA and regenerate from open to closed state within living cells. Using MBs targeting pluripotent stem cell markers we demonstrated reverse into a more immature state of LUHMES induced by neurosphere-like growth conditions. Moreover, we have been able to trace localisation of this particular population during differentiation and thus demonstrate the usability of MBs for monitoring cell behaviour within 3D clusters. Finally, MBs detection of expression of human pluripotent markers after reprograming of adult somatic cells with plasmid codding for mouse transcription factors was demonstrated. In conclusion, the method of using transfected MBs is an easy and rapid way to detect gene expression. Target cells do not need to be genetically modified. Furthermore, MBs are able to detect both up- and down regulation of gene expression on the single cell level and dynamic tracing of specific cell populations within 3D clusters.

General information

State: Published
Organisations: Department of Micro- and Nanotechnology, Fluidic Array Systems and Technology, Bioanalytics
Authors: Ilieva, M. (Intern), Dufva, M. (Intern), Emnéus, J. (Intern)
Number of pages: 220
Publication date: 2013

Publication information

Original language: English
Main Research Area: Technical/natural sciences
Electronic versions:

Thesis.pdf
Source: dtu
Source-ID: u::8613
Publication: Research › Ph.D. thesis – Annual report year: 2013