Aug 16, 2019 Version 1

Direct cDNA synthesis and pre-amplification of single embryos for RT-PCR V.1

DOI
dx.doi.org/10.17504/protocols.io.6knhcve

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.6knhcve

External link:
https://animal.ifas.ufl.edu/hansen/lab_protocol_docs/Yao%20and%20Hansen%20%20Direct%20cDNA%20synthesis%20and%20preamplification%20of%20single%20blastocysts%20for%20RT-PCR.pdf

Protocol Citation: Yao Xiao, Peter J Hansen 2019. Direct cDNA synthesis and pre-amplification of single embryos for RT-PCR. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6knhcve

Protocol status: Working
We use this protocol and it's working

Created: August 16, 2019

Last Modified: August 16, 2019

Protocol Integer ID: 26990

Keywords: RT-PCR, Fluidigm, cDNA synthesis, embryos, gene expression analysis within single embryo, cdna without rna extraction, target genes for analysis, direct cdna synthesis, cdna synthesis, extensive use of the cellsdirect kit, cellsdirect kit, rna extraction, target gene, gene expression analysis, rna, single embryo, additional gene, pcr this protocol, number of gene, dnase treatment, gene, intron in the target region, best approach to primer design, cdna, pcr, primer design, time pcr

Abstract

This protocol allows gene expression analysis within single embryos on multiple targets in either conventional real-time PCR or using the Fluidigm platform. The protocol makes extensive use of the CellsDirect Kit from ThermoFisher Scientific (catalog number 11753). References to the kit refer to this product. This protocol describes a method that converts RNA directly into cDNA without RNA extraction, which avoids the variation due to extraction process. The number of genes of interest can be up to more than 100. However, the target genes for analysis have to be determined prior to cDNA synthesis. Once the cDNA is made, there is no way to add additional gene to analyze. The best approach to primer design is to have primers span an intron in the target region. This is not a requirement, however, because of the DNase treatment included in the protocol.

Attachments

PDF

Yao and Hansen Dire...

295KB

Troubleshooting

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