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

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1 Works for me dx.doi.org/10.17504/protocols.io.6knhcve

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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.

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

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