RASTRUM

Protocol

qPCR Gene Expression Analysis from RASTRUM™ 3D Cell Models

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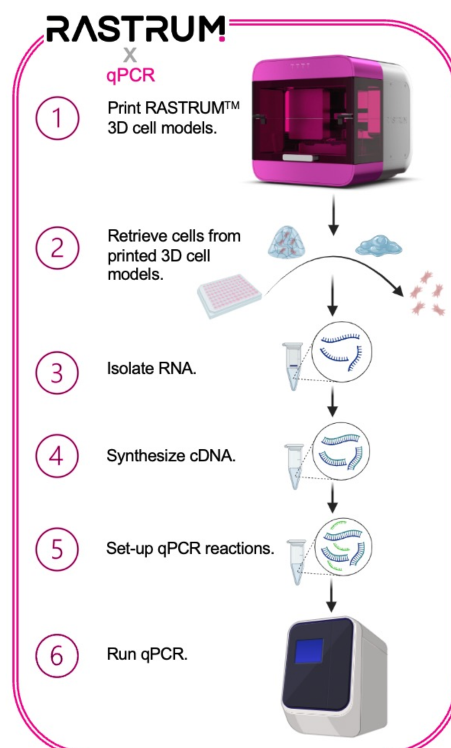
Introduction

qPCR is a popular method for characterising the differential gene expression profile of cells under various conditions. Herein, we have developed a simple protocol that allows you to implement standard 2D qPCR workflows with RASTRUM 3D cell models.

Equipment and reagents required, but not provided

- RASTRUM 3D cell models
- *Recommended:* ReliaPrep™ RNA Miniprep System (Promega, Z6010-12); RNeasy Plus Mini Kit (Qiagen, 74034; or similar)
- *Recommended:* Spectrophotometer e.g. NanoDrop™ 2000, Qubit™ or similar
- *Recommended:* M-MLV reverse transcriptase (Life Technologies, 28025013; or similar)
- RNaseOUT™ Recombinant Ribonuclease Inhibitor (ThermoFisher, 10777019)
- Random Primers (Promega C1181 or ThermoFisher, 48190011; or similar)
- Promega dNTP Mix (Promega, U1511)
- 7900HT Fast Real-Time PCR System (Life Technologies, 4351405; or similar)
- *Recommended:* Applied Biosystem Taqman Universal master mix (ThermoFisher, 4304437) or KAPA Probe Fast master mix (Roche, KK4705; or similar) or KAPA Probe Fast master mix (Roche, KK4705; or similar)
- *Recommended:* TaqMan™ Gene Expression Assay probes (Thermofisher; or similar)
- UltraPure™ DNase/RNase-Free Distilled Water (ThermoFisher, 10977015)

Graphical Protocol



Protocol

1. Retrieve cells from RASTRUM 3D cell models according to our Cell Retrieval protocol.

   Note: In some instances (e.g. printing of non-proliferative cells), the cellular material from multiple wells should be pooled to extract sufficient quantities of RNA for downstream cDNA synthesis. To estimate the approximate yield of RNA that can be expected from your starting material, we usually calculate that a typical mammalian cell contains 10–30 pg total RNA. The amount of RNA depends on experimental conditions.

2. Proceed to RNA extraction using the ReliaPrep™ RNA Miniprep System according to the manufacturer's instructions.

   Note: While we recommend using the ReliaPrep™ RNA Miniprep System to extract RNA from retrieved cells, the cellular material is compatible with similar silica membrane technologies and phenol/chloroform extraction. We also recommend performing gDNA removal to eliminate genomic DNA as a source of template in downstream qPCR.

3. Quantify the concentration of RNA extracted from the cellular material using a spectrophotometer such as the NanoDrop or Qubit.

4. Synthesise cDNA from RNA using a reverse transcriptase such as M-MLV according to the manufacturer's instructions.

   Note: Ensure that you work in an RNase-free environment and with DNase/RNase-free water to avoid RNA degradation.

5. Quantify the concentration of synthesised cDNA using a spectrophotometer such as the NanoDrop or Qubit.

6. Set up qPCR reactions with 20 ng cDNA, KAPA Probe Fast master mix and 10 µM of TaqMan™ gene expression assay probe according to the manufacturer's instructions.

   Note: While we recommend the use of Taqman™ probes for gene expression analysis, the cDNA samples are compatible with other nucleic acid detection technologies such as SYBR.

7. Run qPCR reactions on the 7900HT Fast Real-Time PCR System or similar.

8. Analyse data.

   Note: For each target gene, expression level is quantified in relation to the expression of a control gene using the ΔΔCt method to provide relative quantification. Gene expression values normalised to each control gene are calculated as the average of the expression value for each target gene.

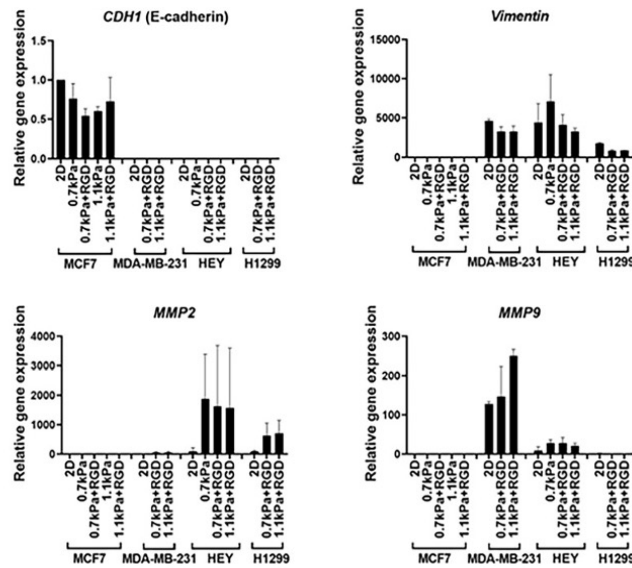


Figure 1: Gene expression analysis using cells recovered from RASTRUM matrices using Cell Retrieval protocol. Retrieved cells were analysed using qPCR to determine migration/invasion-relevant mRNA and protein expression levels, respectively. All experiments were repeated at least twice. Adapted from Jung et al., 2022.[1]

References

1. Jung, M., Skhinas, J.N., Du, E.Y., Tolentino, M.A.K., Utama, R.H., Engel, M., Volkerling, A., Sexton, A., O'Mahony, A.P., Ribeiro, J.C.C., et al. (2022). A high-throughput 3D bioprinted cancer cell migration and invasion model with versatile and broad biological applicability. Biomaterials Science, 10, 5876–5887.

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