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BENCHMARK

Optimization of small-scale sample preparation for high-throughput OpenArray analysis

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Abbreviations used: CV%, coefficient of variance; C_{rt}, relative threshold cycle; qPCR, quantitative PCR

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ABSTRACT

OpenArray is one of the most high-throughput qPCR platforms available but its efficiency can be limited by sample preparation methods that are slow and costly. To optimize the sample workflow for high-throughput qPCR processing by OpenArray, small-scale sample preparation methods were compared for compatibility with this system to build confidence in a method that maintains quality and accuracy while using less starting material and saving time and money. This study is the first to show that the Cells-to-CT kit can be used to prepare samples within the dynamic range of OpenArray directly from cultured cells in a single well of a 96-well plate when used together with a cDNA preamplification PCR step. Use of Cells-to-CT produced results of similar quality and accuracy to that of a preparation method using purified RNA in less than half the sample preparation time. While Cells-to-CT samples also exhibited slightly increased variance, which affects the ability of OpenArray to distinguish small differences in gene expression, overall gene expression mean results correlated well between small-scale methods. This work demonstrates that Cells-to-CT with preamplification can be used to reliably prepare samples for OpenArray analysis while saving time, money, and starting material.

Keywords: Cells-to-CT, OpenArray, qPCR, RNeasy

INTRODUCTION

RNA expression analysis by quantitative PCR (qPCR) is a fundamental technique used widely across academia and industry to understand cellular responses to biological and external stimuli, characterize disease states, and advance therapeutic development. These efforts usually assess many genes at once, in order to understand changes in signaling pathways and to test for downstream or off-target effects. There are several qPCR methods available to assess RNA expression, some of which are amenable to automation, but most require high-quality sample preparation by column-based RNA extraction coupled with a reverse-transcription cDNA synthesis reaction, which can create a workflow bottleneck in instances where there are large sample numbers, as is the case in most drug screening efforts. This prompts a need for a high-throughput qPCR method that can combine qPCR automation with a sample preparation that maintains the quality and purity of traditional methods while saving on time, cost, and starting material.

The QuantStudio 12K Flex OpenArray (Thermo Fisher Scientific) is a commercially-available high-throughput qPCR platform that utilizes a small microscope slide-sized plate containing an array of through-holes coated with TaqMan assays to allow the user to simultaneously run 3072 reactions [1-2]. Despite its ability to assay a large number of genes at once, OpenArray has a more limited dynamic range than traditional qPCR due to its use of smaller volume reactions and thus is best suited for moderate to highly expressing genes [1,3]. However, adding a cDNA preamplification PCR step before running the qPCR has been shown to lower relative threshold cycle (C_{rt}) values into the dynamic range of OpenArray without altering the results, expanding the range of the assay [4-6]. The current manufacturer's recommendation for OpenArray RNA input is 2 µg in a 20 µl reverse-transcription reaction [5,7], which is quite high and not obtainable from a 96-well microplate cell culture that is typically used for drug screening or titration experiments, or when the number of cells are limited. While it has since been shown that 10–50 ng/µl of cDNA is compatible with OpenArray [2], this yield can still be difficult to achieve from a single 96-well sample. In addition, sample preparation for OpenArray has been validated only

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with column- [3] or TRIzol-based [5,8] RNA extraction methods that are expensive and time-consuming for high sample counts.

Cells-to-CT is a commercially available RNA extraction method in which cDNA is generated from crude cell lysates rather than purified RNA [9,10]. It is optimized for collecting cells from 96- or 384-well formats, providing enough RNA to proceed directly to a qPCR reaction from one well, and can be combined with a preamplification protocol to enhance mRNA analysis [9,10]. Although this method can introduce variability due to the use of unpurified RNA that does not allow for RNA normalization prior to the qPCR, it maintains good accuracy and demonstrates increased sensitivity in comparison to the traditional workflow of column-based RNA extraction and cDNA generation [11]. It also allows for scaling large experiments down to a smaller format, ultimately saving time, money, and material. While Cells-to-CT has been tested against other microfluidic platforms [12], it has not been reported on for use in conjunction with the OpenArray system.

In this study, we evaluated two methods of small-scale sample preparation from 96-well plates for OpenArray qPCR. We directly compared a traditional column-based RNA extraction and cDNA synthesis method to the Cells-to-CT workflow, with and without an additional cDNA preamplification step, in order to build confidence in a higher-throughput method that produces high quality data while using less starting material and saving time and money. We demonstrate that Cells-to-CT is compatible with the OpenArray workflow when combined with a cDNA preamplification step, which increases the accuracy and sensitivity of OpenArray for quantifying low abundant genes and enables gene expression analysis from very small amounts of sample.

MATERIALS AND METHODS

Generation of *in vitro* samples

HeLa cells (ATCC, Manassas, VA) were cultured at 37°C in high glucose DMEM (Gibco, Grand Island, NY #31053036) supplemented with 2 mM GlutaMAX (Gibco # 35050061) and 10% FBS (Sigma-Aldrich, St. Louis, MO #12107C-500ML) under a humidified atmosphere of 5% CO_2. 15000 cells per single 96-well or 300000 cells per single 6-well were seeded 24 h prior to treatment. Cells were treated with DMSO (Sigma-Aldrich) or YM201636 (Selleck Chemicals, Houston, TX) for 24 h before collection. Collection and RNA extraction of column-based extraction samples was completed with the RNeasy Plus Mini kit (Qiagen, Germantown, MD, #74134). SuperScript™ IV VILO™ Master Mix (Thermo Fisher Scientific, Waltham, MA #11756050) was used to generate cDNA from purified mRNA in a 20 µl reaction, using 1200 ng input RNA for 6-well samples and 150 ng for 96-well samples. Collection and RNA extraction of Cells-to-CT samples was completed using the TaqMan™ Gene Expression Cells-to-CT™ Kit (Thermo Fisher #AM1728), taking 22 µl lysate into the reverse-transcription reaction. Biological replicates consist of distinct samples seeded on separate days.

Live-cell imaging

Cells were seeded as above into black-walled CellCarrier-96 Ultra plates (PerkinElmer, Hopkinton, MA, #6055302) and treated for 24 h with compound. Cells were coincubated with 5uM Incucyte Caspase-3/7 green dye (Essen BioScience, Ann Arbor, MI, #4440) for 24 h or 10 µg/ml DQ-Red BSA (Thermo Fisher, #D12051) for 6 h. NucBlue Hoechst 33342 (Thermo Fisher, # R37605, 1:200) was added 6 h before imaging at 37°C on a PerkinElmer Opera Phenix high-content confocal imager. Images were captured and analyzed by batch processing using PerkinElmer Harmony and Columbus software, respectively. Per condition, 8–9 fields at 10X (caspase-3/7) or 19 fields at 20X (DQ-Red BSA) were imaged in 7–8 wells for each biological replicate.

cDNA preamplification and qPCR analysis

Preamplification of 96-well samples was completed in a 5 µl reaction volume, scaled down from the manufacturer's protocol, containing 2.5 µl Taqman Preamp Master Mix (Thermo Fisher #4488593), 1.25 µl of undiluted cDNA, and 1.25 µl of a custom pool of 0.2X Taqman assays ordered alongside the OpenArray plates to ensure assays were from the same batch. The thermal profile of the reaction was: 95°C for 10 min and 14 cycles of 95°C for 15 s followed by 60°C for 4 min. Preamplified samples were diluted to 100 µl with water and kept on ice until needed. To perform OpenArray analysis, 2 µl of either original cDNA or diluted preamplified sample was combined with 3.15 µl TaqMan OpenArray Real-Time Master Mix (Thermo Fisher #4462159) and 3.15 µl water. OpenArrays were then loaded using a QuantStudio 12K Flex Accufill System (Thermo Fisher #4471021) and run using a thermal profile linked to an OpenArray barcode by a .tpf file from the Sample Tracker Software as described previously [2,5]. Manual 384-well qPCR of 6-well samples was completed in a 10 µl reaction volume containing 5 µl Taqman Gene Expression Master Mix (Thermo Fisher #4369016), 2 µl cDNA (diluted 1:5), and 0.2 µM gene-specific TaqMan assay. For both qPCR methods, quantification of gene expression was performed in triplicate using an Applied Biosystems QuantStudio 12K Flex qPCR machine and associated SDS software (Thermo Fisher). The thermal profile of the manual reaction was: 95°C for 10 min and 40 cycles of 95°C for 15 s followed by 60°C for 30 s and 72°C for 30 s. Amplification of the sequence of interest was normalized to one or more reference endogenous genes: the geometric mean of ACTB, HPRT1, and XPNPEP1 for manual qPCR, and the geometric mean of ACTB, GAPDH, HPRT1, RPL13A, and XPNPEP1 for OpenArray qPCR. Fold-change values were calculated using the ΔΔCt method and the ratio of drug- to control-treated cells expressed. Data were analyzed with the Thermo Fisher ConnectTM cloud web tool and Microsoft Excel.

TaqMan gene expression assays

20X Taqman™ assays were purchased from Thermo Fisher (TFEB: Hs01065086_m1; ASAH1: Hs00602774_m1; ATP6V1B2: Hs00156037_m1; CD68: Hs02836816_g1; CTSA: Hs00264902_m1; CTSB: Hs00947439_m1; GALNS: Hs00975732_m1; GBA: Hs00986836_g1; MCOLN1: Hs01100653_m1; PPARGC1A: Hs00173304_m1; VPS35: Hs01084421_m1; UPP1: Hs01066247_m1; SQSTM1: Hs01061917_g1; XPNPEP1: Hs00958021_m1; ACTB: Hs01060665_g1; HPRT1: Hs02800695_m1; RPL13A: Hs04194366_g1; GAPDH: Hs99999905_m1). The same Taqman™ Assays were used to custom order OpenArray plates (Thermo Fisher, #4471124) and perform preamplification.

Statistical analysis

One-way analysis of variance test followed by a Tukey's post-hoc comparison was performed using Graphpad Prism v8.3.0 (GraphPad Software). Coefficient of variance (CV%) calculations were performed in Microsoft Excel. Both were calculated using fold-change values. Representative average CV% range per method was determined by calculating the CV% for each gene to establish a range for each treatment

group and averaging the lows of each treatment group and the highs of each treatment group. Error bars represent SD of either technical or biological replicates, as indicated in the legend. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$. Only statistically significant comparisons are indicated in graphs.

RESULTS

Comparison of OpenArray and manual qPCR methods

To evaluate different cDNA preparation workflows by OpenArray, samples from 96- and 6-well plates were first generated by treating HeLa cells for 24 h with DMSO or YM201636, a PIKfyve inhibitor known to activate the transcriptional activity of transcription factor EB (TFEB) [13], prior to collection *via* RNeasy Plus Mini kit or Cells-to-CT Gene Expression kit protocols. RNA extracted using RNeasy was reverse-transcribed using SuperScript IV VILO Master Mix and then the dose-dependent effects of YM201636 on TFEB and 12 literature-reported TFEB target genes were assessed and compared between methods.

The highest normalized input RNA yield that could be achieved for the 6-well RNeasy cDNA reactions was 1200 ng (60 ng/µl) which is lower than the manufacturer recommendation (2000 ng) [5,7], but assuming a rough cDNA output equivalent to half the RNA input, 60 ng/µl is still above previously reported input concentration limits [2]. Therefore, these samples were used to validate the accuracy of OpenArray since sufficient RNA input concentrations could be obtained for qPCR relative to the 96-well samples (150 ng, 7.5 ng/µl). In five of six experimental groups, the technical replicates for the majority of genes assayed exhibited higher coefficient of variance percentages (CV%) in OpenArray compared to manual qPCR (**Table S1**). Additionally, the overall average CV% range was higher with OpenArray (1.44%–27.43%) than with manual qPCR (1.42%–11.25%). This higher variation between technical replicates is likely due to the fact that less cDNA is used in the 33 nl reaction volume of OpenArray compared to the 10 µl reaction volume used for manual qPCR. Despite this difference in variation, the gene expression means showed good correlation between the two qPCR methods (**Fig. S1**), demonstrating the accuracy of OpenArray and confirming it as a worthwhile time-saving alternative to manual qPCR.

Comparison of OpenArray technical replicate variation between 96-well sample preparation methods

Samples generated from 96-well plates for a single biological replicate were then analyzed by OpenArray. While the raw C_{rt} values from the RNeasy preparation were within the dynamic range of OpenArray for all genes tested, use of Cells-to-CT resulted in the C_{rt} values of several genes falling above the upper limit of the dynamic range (**Fig. 1A** and **1B**). Due to the 33 nl volume of each OpenArray reaction, a C_{rt} of 28 is equivalent to one copy in the reaction and any data points above this limit should be dismissed [14]. The addition of a 14-cycle cDNA preamplification PCR step using a custom pool of TaqMan assays corresponding to the custom OpenArray card lowered the raw C_{rt} values of both RNeasy and Cells-to-CT samples while keeping them within the dynamic range. Furthermore, this preamplification step reduced variability between technical replicates for those Cells-to-CT samples that had previously approached or exceeded the upper limit of the OpenArray C_{rt} dynamic range (**Fig. 1C** and **1D**). Therefore, preamplification is necessary for Cells-to-CT to be compatible with the sensitivity of OpenArray.

The gene expression results between the 96-well RNeasy and Cells-to-CT preamplification methods showed good correlation across technical replicates (**Fig. 2**). The Cells-to-CT samples had larger variability between technical replicates for the majority of genes assayed in four of six experimental groups (**Table 1**). Similarly, the average CV% range for technical replicates is slightly higher for Cells-to-CT (2.04%–23.61%) than for RNeasy (1.41%–17.19%). This is likely attributable to the use of crude cell lysate instead of purified RNA in the Cells-to-CT reverse transcription reaction. While gene expression trends were similar between the two methods, this increased variation could explain why only 30 of the 37 groups (81.1%) across all genes assayed that had statistically significant changes in expression upon YM201636 treatment compared to DMSO using RNeasy were also significant when using Cells-to-CT (**Fig. 2**). Therefore, the ability of OpenArray to call out small significant differences between groups appears slightly reduced when combined with Cells-to-CT and this combination may be best suited in instances where large expression changes are expected or more replicates can be analyzed. However, the average CV% ranges for both 96-well preparation methods with preamplification were lower than that of 6-well samples assessed *via* OpenArray (1.44%–27.43%), indicating that the small-scale preparation methods did not introduce additional variability.

Comparison of OpenArray biological replicate variation between 96-well sample preparation methods

Gene expression and variation were next assessed across biological replicates for small-scale sample preparations from 96-well plates using RNeasy without preamplification, RNeasy with preamplification, and Cells-to-CT with preamplification. The majority (89%) of mean C_{rt} values across biological replicates for all three methods were within the dynamic range of OpenArray (**Fig. S2**). For those C_{rt} values outside of the range, all but one were associated with the RNeasy with no preamplification method, suggesting that 150 ng input RNA may be approaching the limit of RNA needed for OpenArray analysis without preamplification.

Table 1. Percentage of genes assayed that showed higher CV% with Cells-to-CT with preamplification compared to RNeasy with preamplification.

	Treatment group					
	DMSO	0.123 µM	0.370 µM	1.111 µM	3.333 µM	10 µM
Percentage of genes assayed	53.85	61.54	61.54	15.38	53.85	0.00

All three methods produced highly correlated results (**Fig. 3**), although of the 15 groups across all genes assayed that had statistically signif-

icant changes in expression upon YM201636 treatment compared to DMSO using RNeasy alone, only 6 groups are also significant when using RNeasy with preamplification and only 5 groups are also significant when using Cells-to-CT with preamplification. Furthermore, preamplification resulted in fewer statistically significant changes in expression overall for both RNeasy (7) and Cells-to-CT (12) methods compared to RNeasy alone (15), suggesting that preamplification itself introduces some level of variation.

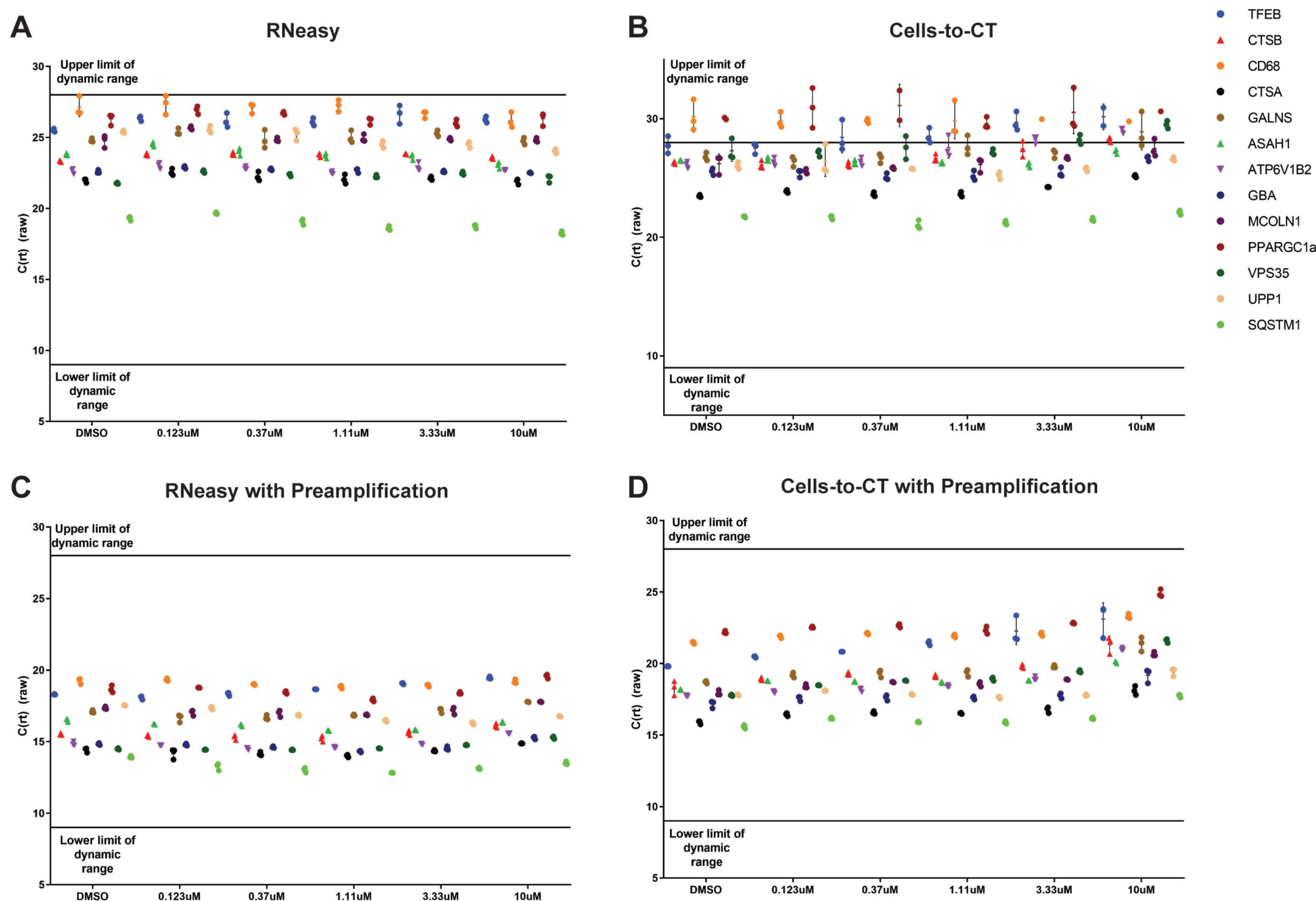

Figure 1. Preamplification is necessary for use of Cells-to-Ct with OpenArray. Raw C(rt) values of technical replicates resulting from use of RNeasy (**A**), Cells-to-CT (**B**), RNeasy with preamplification (**C**), Cells-to-CT with preamplification (**D**). Data are means ± SD (*n* = 3).

When comparing variation across all three preparation methods, Cells-to-CT with preamplification resulted in lower CV% in the majority of genes assayed in the DMSO group and the first two doses of YM201636 but increased CV% in about half or more of the genes assayed in the highest doses of YM201636 (**Table S2**). This is likely a reflection of cellular dysfunction or death caused by the compound at higher concentrations resulting in less RNA starting material for any subsequent reverse transcription reaction. Indeed, incubating cells with DQ-Red BSA, which gets internalized by the endolysosomal pathway and fluoresces upon hydrolysis in the lysosome, confirmed that YM201636 reduced the total DQ-Red BSA spot intensity per cell, indicating a reduction in endolysosomal function that further corresponded to an accumulation of enlarged vesicles (**Fig. S2A** and **S2B**). YM201636 also caused a dose-dependent increase in caspase-3/7 staining of apoptotic cells resulting in a significant decrease in the live cell nuclei count (**Fig. S2C** and **S2D**). Since the Cells-to-CT protocol does not include normalization of input RNA like the RNeasy protocol, this cytotoxicity would be expected to have a greater effect on the Cells-to-CT qPCR data, and this is reflected in the progressive increase in C_{rt} values and higher variability of C_{rt} replicates at higher doses of YM201636 that is, notably, not observed using the RNeasy methods (**Fig. S3**). However, even with this increased variance, the overall average CV% range for Cells-to-CT with preamplification (4.38%–47.08%) is only slightly higher than RNeasy with preamplification (8.13%–44.23%) across biological replicates, and both are lower than RNeasy alone (5.65%–57.01%). Therefore, while there are caveats to Cells-to-CT usage with the OpenArray platform, the similarity between preparation methods in both variance and gene expression means across biological replicates suggests that these data represent a true biological response. Cells-to-CT with preamplification is thus a reliable way to prepare small-scale samples that are compatible with the high-throughput OpenArray automated platform without compromising accuracy, provided an appropriate number of biological replicates are performed.

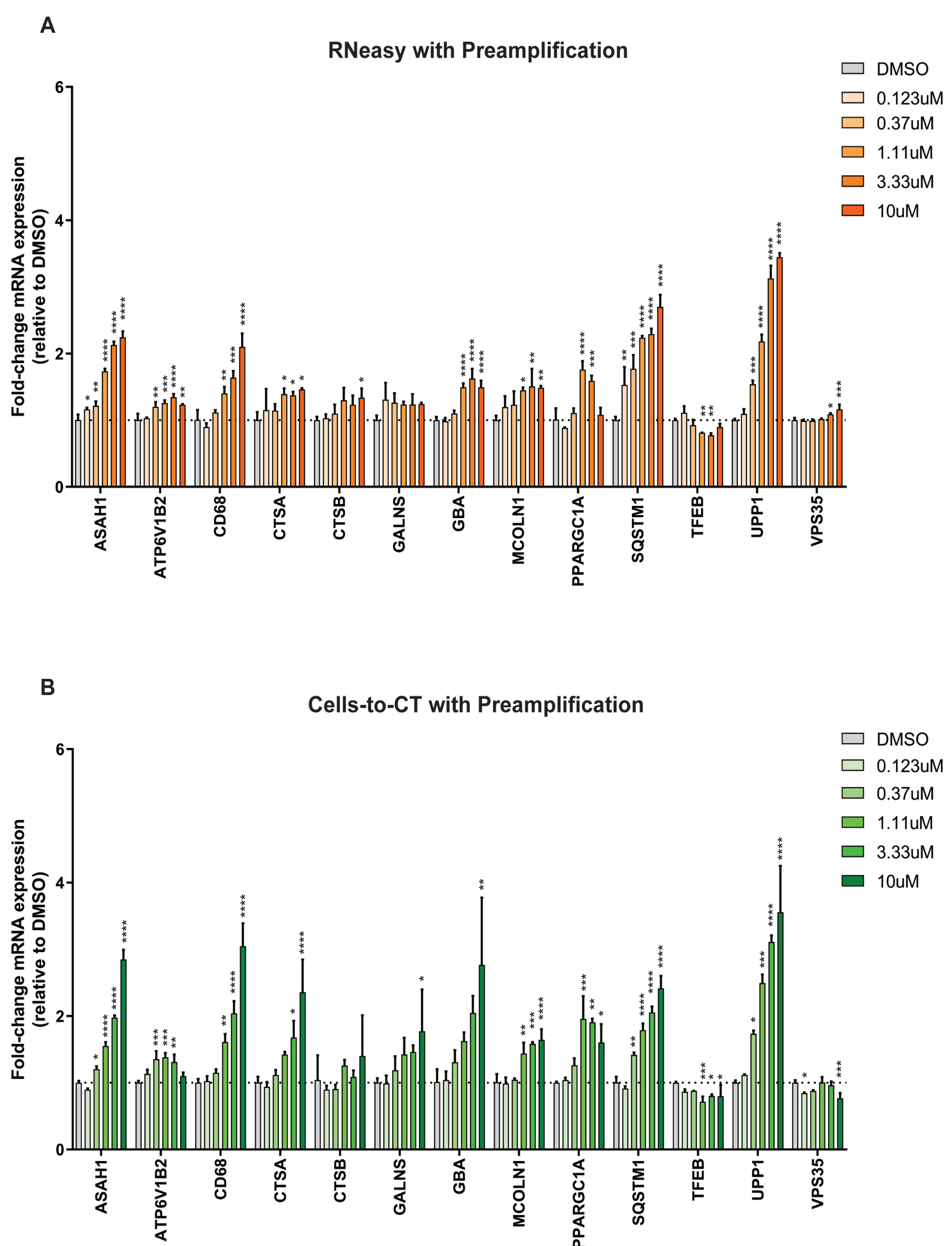

Figure 2. Comparison of small-scale preparation methods across technical replicates. Samples prepared using RNeasy with preamplification (**A**) or Cells-to-CT with preamplification (**B**) were assessed *via* OpenArray. Data are means ± SD (n = 3 technical replicates). $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$ compared to DMSO by one-way ANOVA.

DISCUSSION

In this study, we compared RNeasy Plus Mini kit column-based RNA extraction with subsequent cDNA generation to the Cells-to-CT kit to determine a reliable workflow for small-scale sample preparation for OpenArray analysis. Our results showed for the first time that Cells-to-CT combined with a preamplification PCR step is compatible with the OpenArray platform and can be used to prepare small-scale samples, such as single 96-well cell cultures, without markedly introducing variance compared to methods that use purified RNA. C_{rt} values within the dynamic range of OpenArray were achieved for all genes tested when starting with 150 ng input RNA from RNeasy 96-well samples in a 20 µl cDNA reaction, which is much lower than the manufacturer's recommended amount of 2000 ng [5,7], with few exceptions across biological replicates. Notably, the higher raw C_{rt} values of the Cells-to-CT 96-well samples obtained prior to preamplification indicate that there is less than 150 ng input RNA for these samples, but when combined with preamplification these samples can produce data of similar quality and accuracy. There are a few factors to keep in mind when choosing the Cells-to-CT workflow for OpenArray analysis. First, the use of crude cell lysate in the reverse transcription reaction can introduce some variability. While this variability appears to be within the same range as that resulting from a larger scale sample preparation using purified RNA, the data presented here suggests that this factor may still decrease

the ability of OpenArray to call out small, significant differences in gene expression. Second, lack of input RNA normalization in the Cells-to-CT protocol may increase sensitivity to instances of cell death thereby increasing data variability. Third, it is possible that culture heterogeneity may introduce some variance when using very small numbers of cells. These issues can be mitigated by use of a higher number of replicates per group, both technical and biological. To this point, the data from three biological replicates (**Fig. 3**) showed greater similarity across methods than the data from one (**Fig. 2**), emphasizing the importance of maximizing the number of biological replicates when using OpenArray to ensure accurate results. This can easily be accomplished by using Cells-to-CT in combination with the OpenArray platform since this workflow has the high-throughput capability needed to increase sample size without significantly adding time. Importantly, using this high-throughput method should not supersede further validation with methods that exhibit less variance and the user should determine the appropriateness of this platform before setting cut-off values to avoid discarding potentially important data.

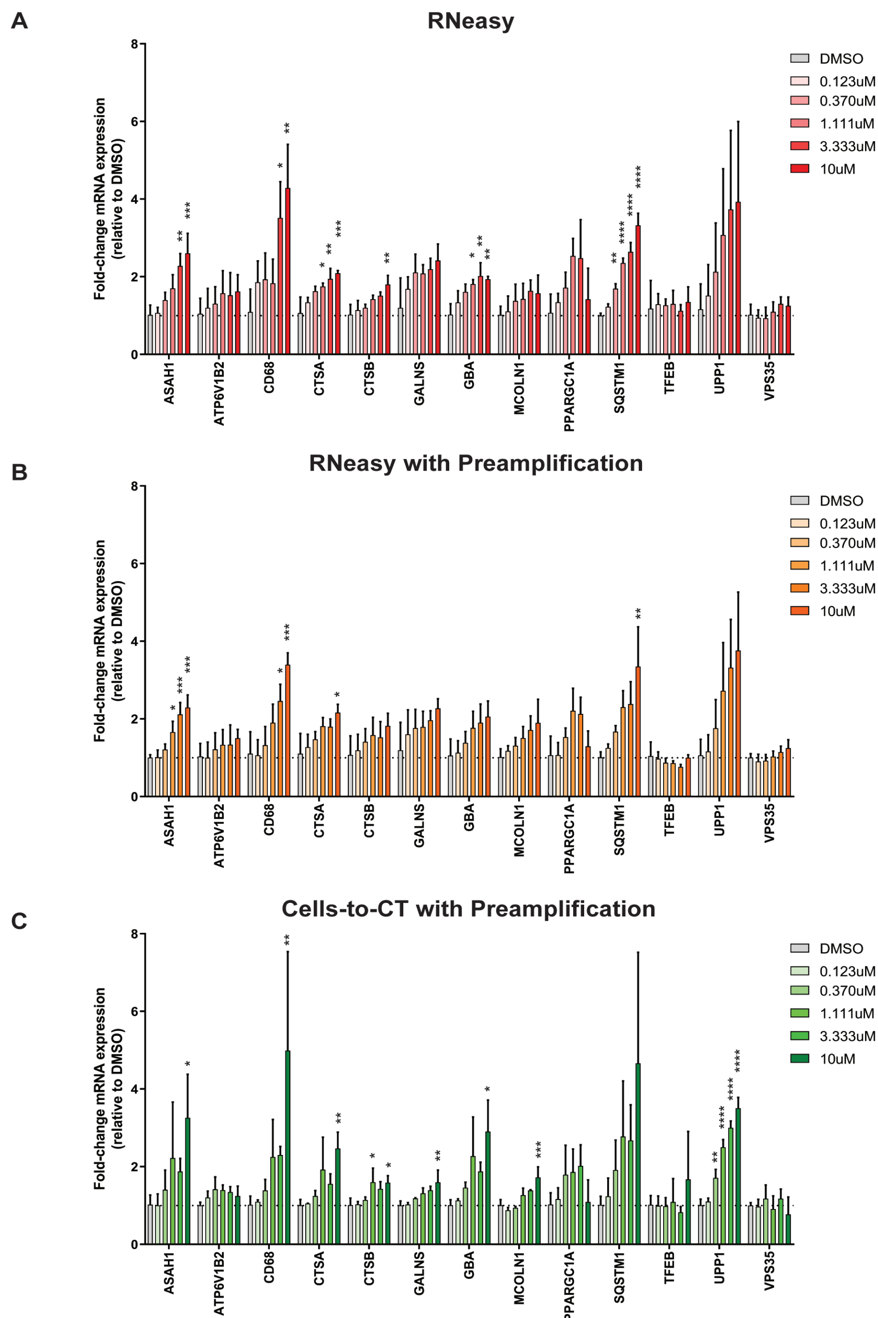

Figure 3. Comparison of small-scale preparation methods across biological replicates. Samples prepared using RNeasy (**A**), RNeasy with Preamplification (**B**), or Cells-to-CT with Preamplification (**C**) were assessed *via* OpenArray. Data are means ± SD (n = 3 biological replicates). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$ compared to DMSO by one-way ANOVA.

In a cost comparison analysis across methods using current prices listed on the manufacturers' websites, Cells-to-CT provides a minimum 23% cost savings per reaction compared to RNeasy with cDNA generation (**File S1**). Also, although this can vary user to user, Cells-to-CT can be completed in roughly 33% of the time needed for the RNeasy extraction kit and cDNA generation for the same number of samples. There are other options for RNA extraction not assessed in this study, such as the 96-well format RNeasy kit or less expensive column-based kits such as Thermo Fisher PureLink RNA Mini kit, that can reduce time and cost of extraction, but Cells-to-CT remains the option with the greatest time and cost savings.

While OpenArray itself is designed to be high-throughput with regards to the qPCR itself, sample preparation can be the rate-limiting factor in how much the platform can accelerate workflow. Use of Cells-to-CT with preamplification with the OpenArray platform can increase user throughput capability while only slightly increasing variance compared to a small-scale method using purified RNA, allowing users to further expand their qPCR processing.

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Author contributions

ACC and CLN designed the study. NAA, ACC, and CLN performed experiments and analyzed the data. NAA and CLN interpreted the results and wrote the manuscript. WDH contributed to editing of the manuscript and directs the research group within which this work was performed. All authors approved the final revised manuscript.

References

1. Morrison T, Hurley J, Garcia J, Yoder K, Katz A, et al. (2006) Nanoliter high throughput quantitative PCR. Nucleic Acids Res 34: doi: 10.1093/nar/gkl639. PMID: 17000636
2. Broccanello C, Gerace L, Stevanato P (2020) QuantStudio™ 12K Flex OpenArray® System as a tool for high-throughput genotyping and gene expression analysis. Methods Mol Biol 2065: 199-208. doi: 10.1007/978-1-4939-9833-3_15. PMID: 31578697
3. Prokopec SD, Watson JD, Waggott DM, Smith AB, Wu AH, et al. (2012) Systematic evaluation of medium-throughput mRNA abundance platforms. RNA 19: 51-62. doi: 10.1261/rna.034710.112. PMID: 23169800
4. Li P, Grigorenko E, Funari V, Enright E, Zhang H, et al. (2013) Evaluation of a high-throughput, microfluidics platform for performing TaqMan™ qPCR using formalin-fixed paraffin-embedded tumors. Bioanalysis 5: 1623-1633. doi: 10.4155/bio.13.125. PMID: 23822126
5. Patel SN, Wu Y, Bao Y, Mancebo R, Au-Young J, et al. (2013) TaqMan® OpenArray® high-throughput transcriptional analysis of human embryonic and induced pluripotent stem cells. Methods Mol Biol 997: 191-201. doi: 10.1007/978-1-62703-348-0_15. PMID: 23546757
6. Dalgard CL, Polston KF, Sukumar G, Mallon CTM, Wilkerson MD, et al. (2016) MicroRNA expression profiling of the armed forces health surveillance branch cohort for identification of "en viro-miRs" associated with deployment-based environmental exposure. J Occup Environ Med 58: doi: 10.1097/JOM.0000000000000764. PMID: 27501110
7. Life Technologies (2012) Applied Biosystems™ QuantStudio™ 12K flex real-time PCR system OpenArray® experiments: user guide. Available from https://www.thermofisher.com/content/dam/LifeTech/migration/files/pcr/pdfs.par.36605.file.dat/4470935b.pdf
8. Forreryd A, Johansson H, Albrekt A, Lindstedt M (2014) Evaluation of high throughput gene expression platforms using a genomic biomarker signature for prediction of skin sensitization. BMC Genomics 15: 379. doi: 10.1186/1471-2164-15-379. PMID: 24886304
9. Life Technologies (2012) TaqMan® gene expression Cells-to-CT™ kit: user guide. Available from https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_056225.pdf
10. Abruzzese R, Fekete R, Kephart D (2010) Cells-to-CT kits: next generation gene expression analysis workflows that eliminate sample purification. JALA 15: 362-368. doi: 10.1016/j.jala.2010.03.004.
11. Van Peer G, Mestdagh P, Vandesompele J (2012) Accurate RT-qPCR gene expression analysis on cell culture lysates. Sci Rep 2: 222. doi: 10.1038/srep00222. PMID: 22355736
12. Fox BC, Devonshire AS, Baradez M, Marshall D, Foy CA (2012) Comparison of reverse transcription-quantitative polymerase chain reaction methods and platforms for single cell gene expression analysis. Anal Biochem 427: 178-186. doi: 10.1016/j.ab.2012.05.010. PMID: 22617801
13. Wang W, Gao Q, Yang M, Zhang X, Yu L, et al. (2015) Up-regulation of lysosomal TRPML1 channels is essential for lysosomal adaptation to nutrient starvation. Proc Natl Acad Sci U S A 112: doi: 10.1073/pnas.1419669112. PMID: 25733853
14. Thermo Fisher Scientific (2016) Crt, a relative threshold method for qPCR data analysis on the QuantStudio 12K Flex system with OpenArray technology: application Note. Available from http://tools.thermofisher.com/content/sfs/brochures/CO28730-Crt-Tech-note_FLR.pdf

Supplementary information

Table S1. CV% comparison of OpenArray and manual qPCR.

Table S2. CV% comparison of small-scale sample preparation methods.

Figure S1. OpenArray and manual qPCR results from 6-well samples.

Figure S2. Lysosomal dysfunction and live/dead cell analysis.

Figure S3. Biological replicate C_{rt} values of small-scale sample preparation methods.

File S1. Statistical analyses and time and cost estimates.

Supplementary information of this article can be found online at http://www.jbmethods.org/jbm/rt/suppFiles/339.

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