Beating Moore's law but now what?
Reduced sample prep cost for genetic analysis

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introduction

The use of better sequencing reagents and powerful analysis software has reduced the cost of next-gen sequencing dramatically and even beaten Moore's law in 2008. Moreover, in the past few years, the detection limits and throughput of analytical instruments, such as fragment analyzers and thermocyclers have significantly improved, allowing analysis of a lower amount of genetic material. However, one of the bottlenecks for high-throughput genomic applications has now significantly shifted towards the cost of sample preparation.

Miniaturizing the sample preparation volume provides the opportunity for significant cost savings and scaling down the required sample input to pg values.

This is especially essential for high-throughput applications, such as single-cell analysis or when the sample prep cost is significantly high.

In this poster we will present two case studies, in which the miniaturization of reaction volumes has reduced the cost of sample prep up to 5 times due to its accurate, true-positive displacement and cross-contamination free technology.

positive displacement, low-volume liquid handling

mosquito® HTS (25 nL – 1.2 µL) and **mosquito® HV** (0.5 – 5 µL) are automated 8- or 16-channel liquid handlers. Being based on true-positive displacement technology they enable fast, accurate, gentle and contamination-free liquid transfer, essential for genetic analysis applications where viscose gDNA and reagents are being handled (Fig1a).

mosquito® X1 (25 nL – 1.2 µL or 0.5 – 5 µL) is an automated single channel liquid handler. It's low dead volume (< 0.5 µL) is ideal for DNA/RNA normalization. mosquito's easy-to-use software calculates the required volumes of buffer and DNA/RNA in order to perform the normalization process seamlessly (Fig 1b).

Using low-volume liquid handling in normalization and sample prep of genomics applications is especially beneficial when working with precious and low abundance samples such as DNA/RNA derived from patient specimens or single-cell analysis.

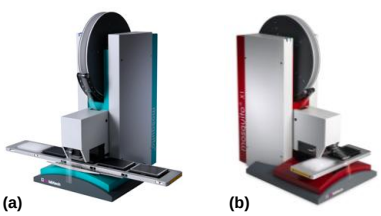

Fig 1. (a) mosquito HTS (8- or 16-channel) and, (b) mosquito X1 (single tip) liquid handler

Table 1. mosquito HTS and HV accuracy and precision, measured by Artel-MVS

volume (nL)	% inaccuracy	% CV
50	5.94	7.02
100	-0.57	5.43
600	-1.25	2.88
1200	-1.07	3.91

volume (nL)	% inaccuracy	% CV
500	1.93	3.17
1000	4.39	1.55
2500	5.18	0.93
5000	6.38	0.88

UC San Diego

1. characterization of exRNA isolated from urine

Extracellular RNAs (exRNA) have been discovered in various biological fluids, including, but not limited to serum, plasma and urine [1]. Urine is an ideal specimen as it can be collected in large quantities without invasive procedures or expensive equipment [2]. However, the low abundance of RNA and the high abundance of RNases in urine pose a challenge in exRNA isolation. Here, we investigate the efficiency of both commercial exRNA isolation kits and a "homebrewed" lysis buffer.

methods

Urine specimens were collected from 10 healthy individuals (18 – 40 years). exRNA was isolated from pooled and individual samples. The isolated RNA concentration was measured by NanoDrop™ (ThermoScientific, USA) and RiboGreen assays. exRNA profiles were studied using Agilent 2100 BioAnalyzer system (Agilent, USA). The isolation procedures were compared by quantifying the relative abundance of miRNA fractions using qRT-PCR TaqMan Gene Expression Assays. The reaction volume was reduced by 20% of the recommendation (4 *vs.* 20 µL) which was made possible using TTP Labtech's mosquito true-positive displacement liquid handling technology.

results

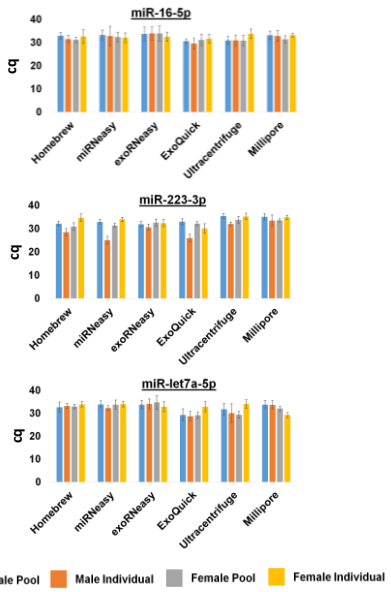

Fig 2. Quality control analyses of exRNA extraction using different kits from 500 µl of urine using qRT-PCR

discussion

qRT-PCR results (Fig 2) show a 2-fold greater yield, for the miRNeasy compared to the home brew technique (1 lower Cq cycle). We have demonstrated exRNA isolation from relatively small volumes of urine, with yields adequate for downstream analyses, eliminating the need to handle large volumes of lysis and extraction reagents, such as Qiazol and chloroform.

The use of mosquito low-volume liquid handler facilitated analyzing multiple miRNAs and mRNAs with relatively low input RNA and reaction volumes.

Future studies will include small RNA sequencing of the purified exRNA to determine whether the sequencing profile is influenced by the RNA isolation method.

references

[1] Anal Biochem, 2009. 387(2): p. 303-14.
[2] Front Immunol, 2012. 3: p. 45

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2. gDNA sequencing of environmental samples

At Stanford University (Stanford, CA, USA) gDNA from bacteria, found in environmental samples from Yellowstone National Park were studied to identify different bacterial species present, and to learn about their genetic composition. Isolated gDNAs from 49 different samples were normalized using TTP Labtech's mosquito X1 liquid handler (single tip), and Nextera XT (Illumina Inc.) libraries were prepared using mosquito HTS (16 channel) at 4 µL total volume (12.5% of the original volume).

methods

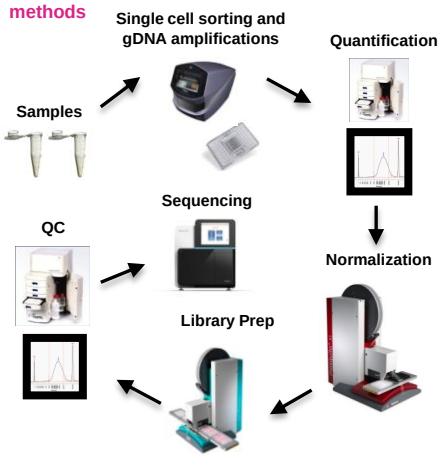

Fig 3. Single cell gDNA sequencing workflow

results

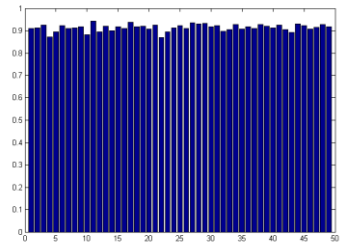

Fig 4. Percent of reads mapped to the contigs

discussion

The data shows that more than 90% of the reads mapped to the contigs for all 49 samples, confirming that miniaturized volumes did not affect the data quality, while saving on reagent cost and sample input.

conclusions

Using nL to µL true-positive displacement technology of mosquito liquid handlers, these systems provide:

- low cost sample prep via miniaturizing reagent volumes
- accurate and precise pipetting of all sample and reagent types, including gDNA, without the need for liquid classification
- very low sample input down to pg values
- fast, accurate and reliable low-volume liquid handling
- simplicity of use, small footprint, low cost of the instrument and being fully integrable
- accurate normalization of DNA at very low volumes, essential for variety of sample prep protocols

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