automated, low-cost, miniaturized RNA-Seq and DNAseq library preps

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introduction

For next-generation sequencing (NGS), the demand for high-throughput, low-cost, miniaturized sample preparation has increased both in academic and clinical settings.

Low-volume liquid handlers, such as mosquito® LV offer low dead volumes and the ability to work reliably with small amounts of, difficult and viscous, solutions at sub-microliter volumes which result in significant cost savings.

Low-volume liquid handlers offer reduced costs, higher throughput, improved reproducibility and less hands-on time. These advantages can benefit the NGS sample prep workflow, including library construction, quantification and normalization and pooling.

automated, low-volume liquid handling

mosquito LV (25 nL – 1.2 µL) and mosquito HV (0.5 – 5 µL) are automated 8- or 16-channel liquid handlers. mosquito X1 (25 nL – 1.2 µL or 0.5 – 5 µL) is an automated single channel liquid handler. Its low dead volume (< 0.5 µL) is ideal for DNA normalization. mosquito's easy-to-use software runs the required volumes of buffer and DNA in order to perform the normalization process seamlessly (Fig 1).

Based on true-positive displacement technology mosquito liquid handlers enable fast, accurate, gentle and contamination-free liquid transfer, essential for different genomics sample preparation methods.

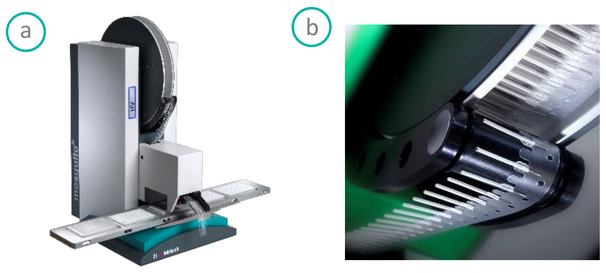

Fig 1. (a) a mosquito liquid handler, (b) mosquito tips based on true-positive displacement technology

1. low-cost NEBNext® Ultra™ DNA Library Prep

Automated sample preparation using mosquito liquid handlers was compared to manual set-up with the same or larger volumes.

methods

Single cell whole genome amplification (WGA) was performed the using Sigma Aldrich WGA4 kit. Using the NEBNext® Ultra™ DNA Library Prep kit (E7645S) input sample volumes were reduced up to 10-fold. In a 6.5 µL total volume the amount of input sample was 5-, 37- and 60- ng compared to 185 ng in a total volume of 65 µL for the manual set up.

results

Sequencing data showed that the results are not affected by lowering the sample input or volumes (Fig 2 and 3, Table 1). The most important factor is an accurate and precise liquid handler to avoid sample to sample variation when miniaturizing the volumes and sample inputs.

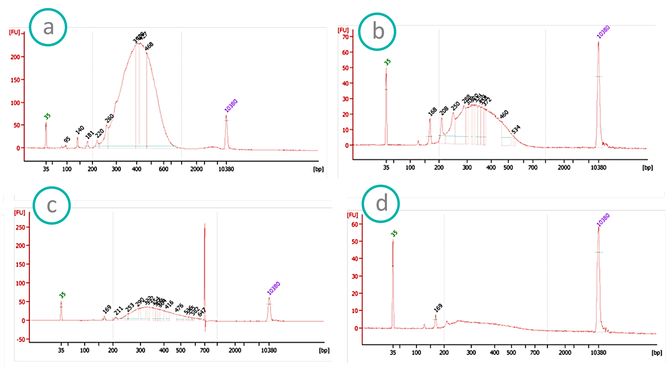

Fig 2. Sequencing results using NEBNext® Ultra™ DNA Library Prep kit. a) manual (65 µL, 185 ng), b) mosquito (6.5 µL, 60 ng), c) mosquito (6.5 µL, 37 ng), d) mosquito (6.5µL, 5 ng)

sample 1: WGA single cell DNA

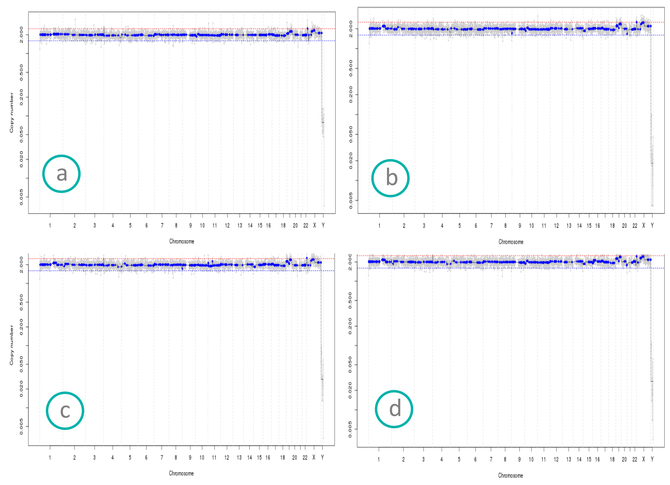

Fig 3. CNV profiling data a) manual (65 µL, 185 ng), b) mosquito (6.5 µL, 60 ng),c) mosquito (6.5 µL, 37 ng), d) mosquito (6.5 µL, 5 ng)

Table 1. Comparison of mappability between different sample prep methods on mosquito liquid handler and manually

Sample prep method	% Mappability
mosquito (5 ng, 6.5 µL):	59.66%
mosquito (37 ng, 6.5 µL)	65.20%,
mosquito (60 ng, 6.5 µL)	63.12%
manual (185ng, 65 µL)	59.84%

2. low-cost NEBNext® Small RNA Library Prep

Extracellular RNA (exRNA) can be used as a biomarker for early diagnosis and monitoring of various diseases. Small RNA, such as microRNA (miRNA) and piwiRNA (piRNA), found within exRNA populations, can be detected, classified and quantified by next generation sequencing (NGS) techniques. However, exRNAs are found in very low concentrations in most biofluids, therefore the cost of the library generation is significant.

Increasing the sensitivity of commercially available small RNA sequencing kits is necessary to maintain the reliability and robustness of these assays in detecting the low amounts of exRNAs. The high costs of library preparation can be addressed through reaction miniaturization and automation processes.

methods

Library preparation: exRNA was isolated from plasma samples using the miRNeasy kit (Qiagen #217004). Illumina based miRNASeq libraries were prepared using the NEBNext Small RNA Library Preparation Kit (NEB #E7580). The manufacturer's protocol was modified to facilitate automation using the mosquito LV liquid handler.

Modifications included:

- reduced reaction volume by 5- to 10-fold
- adaptors diluted 1:6
- <10 ng input RNA

Library characterizations were performed on a Bioanalyzer DNA High Sensitivity microfluidic chip and content analysis was performed through NGS (Illumina HiSeq 4000) (Fig 4).

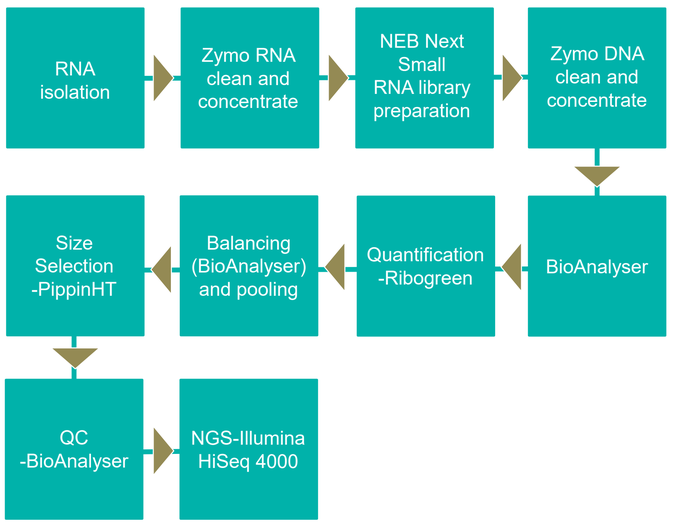

Fig 4. NEBNext Small RNA Library Prep workflow

results

We have demonstrated that 5-fold volume reactions are reliable. The primary roadblock to further volume reduction is evaporative sample loss.

Very low input RNA (up to 10-fold reduction compared to recommended volumes) routinely produced high value small RNA libraries (Fig 5).

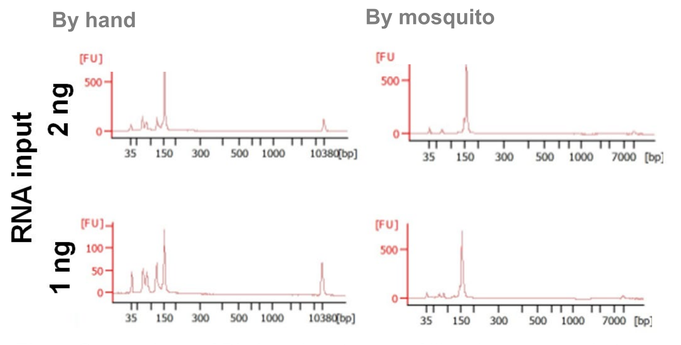

Fig 5. Comparison of BioAnaylzer traces of libraries prepared at recommended scale manually compared to a 5-fold reduction in scale using mosquito LV. A 5- to 10-fold reduction in recommended RNA input was used.

3. low-volume magnetic bead clean up

mosquito HV can be used for purification of DNA samples or libraries in as low as 4 µL total volume of sample and beads.

methods

A 384 well magnetic stand (LV Labtech) was used for the bead clean up steps on mosquito HV liquid handler (Fig 6). The same samples were cleaned up manually and the results were compared.

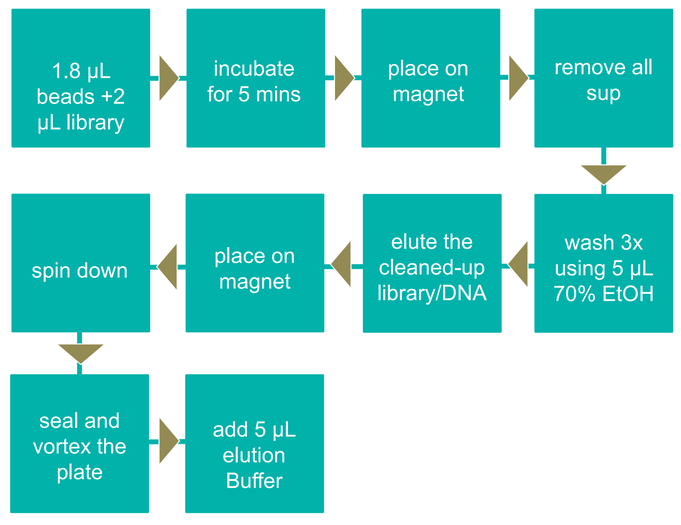

Fig 6. mosquito liquid handler bead clean up workflow.

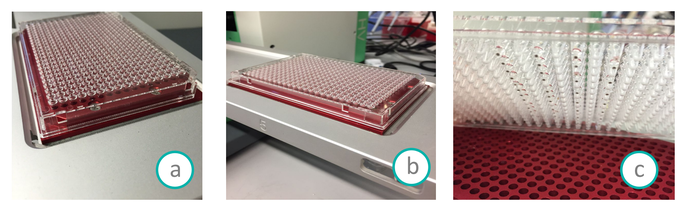

Fig 7. a) and b) magnetic bead plate on mosquito deck, c) beads pulled down using magnetic bead plate.

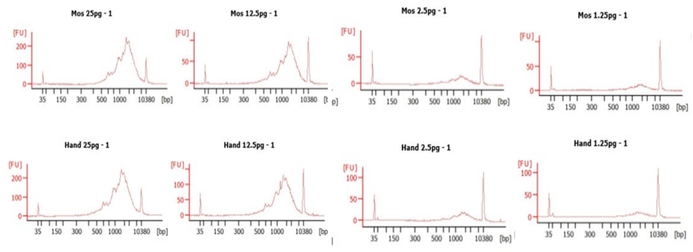

Fig 8. cDNA cleaned up manually and on mosquito HV liquid handler, showing similar results.

conclusions

mosquito liquid handlers (SPT Labtech, UK) have been essential in setting up reduced reaction volumes in genetic analysis applications. The system provides:

- a fully open platform liquid handler that is not specific to a specific kit or application
- fast, accurate and reliable low volume liquid handling without any liquid classification from 200-300 n/µL gDNA to ethanol
- low cost library prep through minimizing reagent volumes
- reduced sample input (pg to low ng values)
- reduced cost and reduced volume 384 well bead clean up

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