Miniaturization technologies for cost effective library preparations for next-generation sequencing (NGS)

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overview

Importance of mosquito® HTS in long fragment read (LFR) sequencing technology and Nextera/Nextera XT library preparations due to its accurate, true positive displacement and cross-contamination free technology. These are two examples of miniaturization of the next-generation sequencing (NGS) sample preparation volumes and input DNA.

introduction

As the per base read cost of NGS has decreased, the library preparation cost, especially in high-throughput applications has become a larger portion of the total cost. Using the mosquito HTS liquid handler and miniaturizing the sample preparation volumes provide the opportunity for significant reagent cost savings, and lowering sample input.

mosquito HTS is a nanolitre to microlitre liquid handler (25 nL – 1.2 µL) enabling fast, gentle, cross-contamination free, and accurate true positive displacement technology (Figure 1).

Fig 1: mosquito HTS liquid handler

conclusions

We have proven that the mosquito HTS provides several advantages for the LFR technology and Nextera library preparation:

1) reduced cost through miniaturization of reagents
2) lowering DNA input from 50 ng to 1 ng for Nextera and from 1 ng to 60 pg for Nextera XT sample prep kits
3) the gentle pipetting is critical to avoid any shearing of gDNA (>50kb), essential for high LFR data quality
4) fast, accurate and reliable low volume dispensing. mosquito HTS takes 3 min to dispense into a 384-well plate

In addition, simplicity of use, small footprint, low cost of the instrument and being fully integrable into any automated process are also of great importance in choosing mosquito for these workflows.

1. LRF technology

Complete Genomics has developed a low-cost DNA sequencing and haplotyping process, called long fragment read (LFR) technology. LFR is similar to sequencing long single DNA molecules of fragments 10–1,000 kb [1] in length, without cloning or separation of metaphase chromosomes. LFR works by diluting DNA and spreading it across a 384- well plate such that each well contains approximately 10% of a haploid genome. The overall affect is that maternal and paternal overlapping fragments of the genome are rarely found in the same well. Since one DNA sample is spread across 384 wells it is imperative to keep reaction volumes very small to avoid excessive reagent costs downstream. The mosquito HTS plays an important role during the sample preparation by accurately and gently dispensing only 100 nL of long fragments of gDNA (>50kb) into each well.

methods

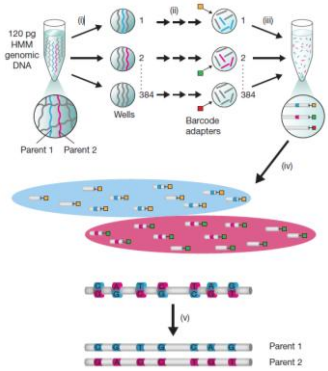

Fig 2: LFR technology and controlled random enzymatic fragmenting:

1) 100 nL (100–130 pg) of high molecular mass DNA is physically separated into 384 distinct wells using mosquito HTS
2) through several steps all within the same well and without intervening purifications, the gDNA is amplified, fragmented and ligated to unique barcode adapters
3) all 384 wells are combined, purified and introduced into the sequencing platform of Complete Genomics [2]
4) mate-paired reads are mapped to the genome using a custom alignment program and barcode sequences are used to group tags into haplotype contigs
5) the final result is a diploid genome sequence.

results

LFR can accurately phase up to 99% of all detected heterozygous SNPs, present in a genome, into long contiguous stretches of DNA (N50s 400–600 kb in length). See Figure 2 for a comparison of haplotyping performance across 114 anonymous donor samples. mosquito HTS has been essential in reducing the cost of the process and making it feasible for high throughput applications.

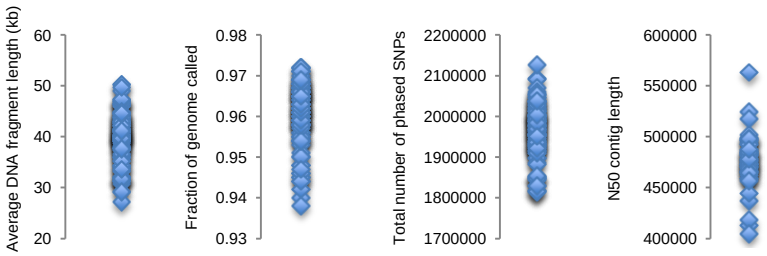

Fig 3: LFR performance metrics on 114 anonymous donor samples. Each blue dot represents a single sample. Accurate and gentle pipetting of mosquito HTS results in reproducible sequencing and haplotyping across different samples.

2. Nextera sample preparation

Nextera and Nextera XT (Illumina, Inc.) sample prep kits are used to prepare DNA libraries without mechanically shearing the DNA. However, having a precise and accurate ratio of tagmentation enzyme to DNA sample is essential in obtaining fragments of the correct size. Almost all library prep protocols recommend volumes that are within the range of manual pipettes, or that of larger volume liquid handlers. Here, for the first time, we present the use of miniaturized Nextera and Nextera XT sample prep volumes down to sub microliter, using a mosquito HTS liquid handler.

results

Nextera library prep

		Human	Unique
Sample 1	Average	0.986	0.963
	Stdev	0.001	0.002
Sample 2	Average	0.987	0.963
	Stdev	0.001	0.002
Sample 3	Average	0.988	0.958
	Stdev	0.001	0.001

Table 1: Average fraction of the reads of 3 different large MW (over 10 kb, Coriell Institute) gDNA libraries prepared in 1 µL in 8 replicates, mapping to the given characteristic: human and unique. Only 1 ng of gDNA was used per library. Very low standard deviations show the reproducibility of this miniaturized Nextera protocol.

Nextera XT library prep

example 1

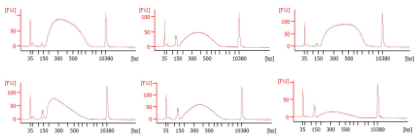

Fig 4: Bio-analyzer data of Nextera XT libraries from 6 different pancreatic progenitor cells in vitro differentiated from human embryonic stem cells. 4 µL libraries were prepared with only 400 pg of cDNA per library using mosquito HTS. cDNA was obtained through single cell isolation, RNA extraction and reverse transcription, using the C1 system (Fluidigm Corp.).

example 2

4 µL Nextera XT libraries were prepared from a mixture of 5 different bacteria species (6-20 kb), separated into single cells. Only 60 pg of input gDNA was used per library.

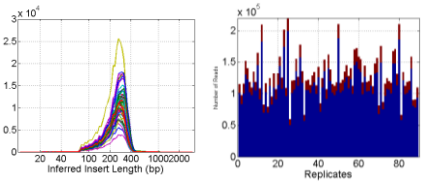

Fig 5: Inferred insert length graph (left) showing insert sizes of ~300 bp for 4 µL libraries, as expected in standard libraries. The percentages of trimmed reads (right) show well below 10% of the reads being rejected, confirming great data quality.

references:

1) Drmanac, R. Nucleic acid analysis by random mixtures of non-overlapping fragments. US patent 7,901 891 (2006).
2) Drmanac, R. et al. Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. Science 327, 78–81 (2010).

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