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Evaluation of library preparation methods for Illumina next generation sequencing of small amounts of DNA from foodborne parasites

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ABSTRACT

Illumina library preparation methods for ultra-low input amounts were compared using genomic DNA from two foodborne parasites (*Angiostrongylus cantonensis* and *Cyclospora cayetanensis*) as examples. The Ovation Ultralow method resulted in libraries with the highest concentration and produced quality sequencing data, even when the input DNA was in the picogram range.

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Whole genome sequencing (WGS) is a promising technique to develop advanced diagnostic, molecular epidemiology, and source tracking tools for food-borne parasites of public health importance. A major bottleneck in genome sequencing is library construction, which refers to the preparation of the nucleic acid into a form that is compatible with the sequencing system to be used (Head et al. 2014). Most of the next generation sequencing platforms adhere to the same basic library production strategies, including DNA fragmentation, end repairs and adapter ligation. Standard library preparation methods require large quantities of nucleic acids, making it challenging to apply to microorganisms that cannot be propagated in the laboratory (Bhattacharya et al. 2002, Chandra et al. 2014, Lay et al. 2010). However, recent advances make it possible to produce libraries with much lower amounts of input material (Parkinson et al. 2012).

Previous comparisons of commercial Illumina library preparation kits revealed that methods used influence sequencing results (Lan et al. 2015; Rhodes et al. 2014). To identify method(s) suitable for WGS of eukaryotic parasites, we compared four library preparation kits intended for low input DNA amounts: NEBNext Ultra DNA Library Prep kit (New England Biolabs Inc.), Ovation Ultralow Library System (Nugen Technologies Inc.), ThruPlex FD Prep kit (Rubicon Genomics Inc.) and Nextera XT DNA Library Kit (Illumina). The comparison was made with genomic DNA from *Angiostrongylus cantonensis*, a nematode associated with eosinophilic meningitis worldwide (Wang et al. 2012) but whose genome is not fully characterized (Yong et al. 2015; Morassutti et al. 2013). DNA was extracted from an *A. cantonensis* adult worm using DNeasy® Blood and Tissue Kit (QIAGEN) and quantified using Qubit dsDNA HS Assay (Invitrogen). One nanogram of DNA was used as starting material for each kit: intact genomic DNA for Nextera XT (since it employs enzymatic fragmentation); and mechanically-fragmented DNA, using conditions for 300 base pairs in an M220 Focused-Ultrasonicator™ (Covaris Inc.), for the other three methods. The quality and quantity of the libraries were assessed in a 2200 TapeStation (Agilent Technologies).

The Ovation, ThruPlex and NEBNext libraries had similar size distribution, but the Ovation library was considerably higher in concentration (Fig. 1a). The NEBNext library produced adapter dimers, as evidenced by the presence of a smaller peak beside the main library. The Nextera

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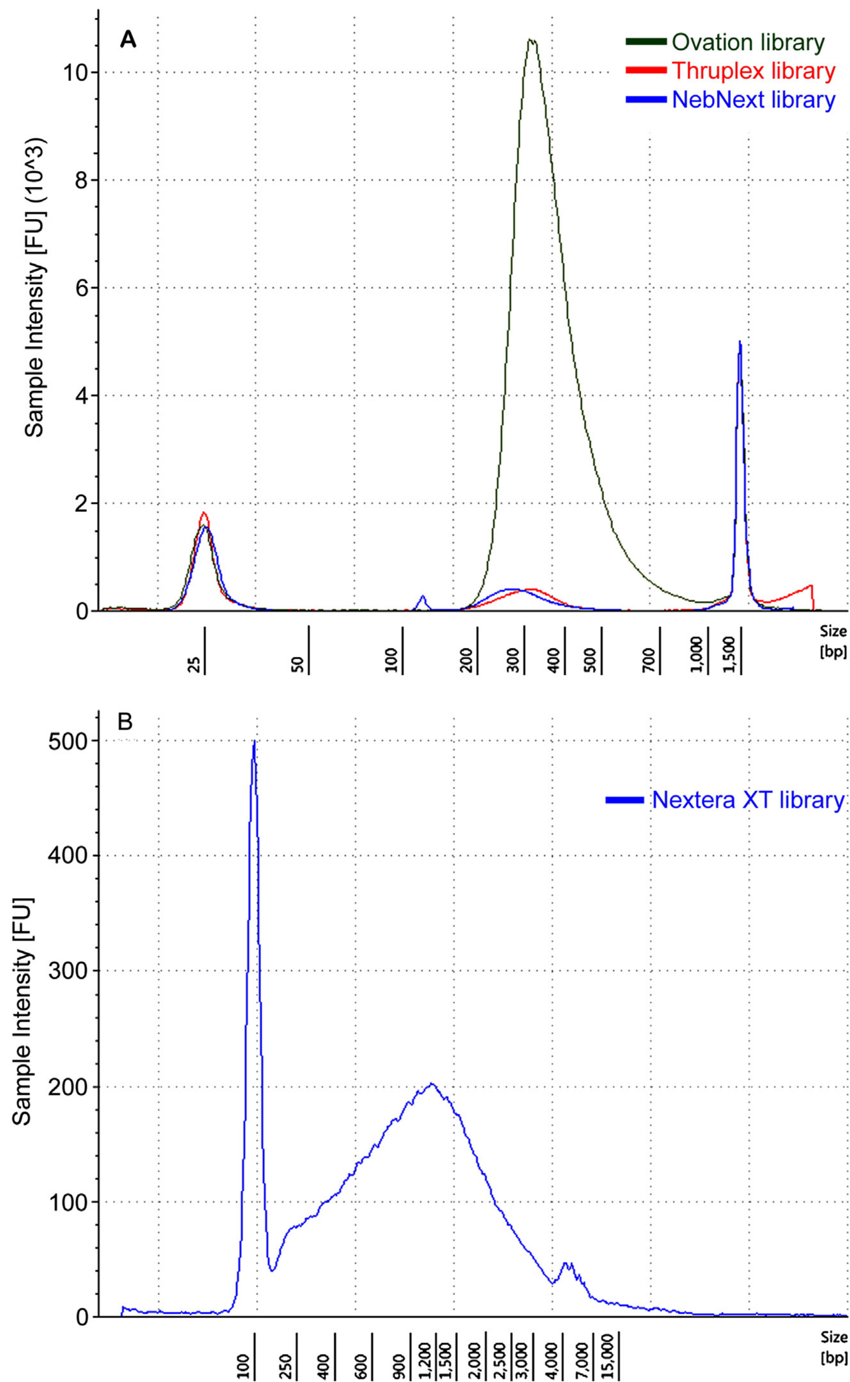

Fig. 1. Size distribution and concentration of Illumina libraries of *Angiostrongylus cantonesis* using different methods. A): Ovation (green), NEBNext (blue) and ThruPlex (red) libraries as measured on a D1000 Screen Tape. The 25 and 1500 base pair peaks are internal size markers included in each lane. B): Nextera XT library as measured on a Genomic Screen Tape. The prominent 100 base pair peak is a size marker included in each lane.

library could only be detected using a genomic DNA screen tape, revealing that most of the library consisted of very large fragments, indicating insufficient enzymatic fragmentation (Fig. 1b). Possible reasons for this are inadequate purity or composition of the parasite DNA. Inaccurate DNA quantification can reportedly lead to production of longer fragments due to an unfavorable ratio between the tagmentation enzyme and the number of DNA molecules accessible to the enzyme (Adey and Shendure 2012). Applying a size exclusion step can eliminate adapter dimers from the NEBNext library as well as the larger fragments from the Nextera library. However, size exclusion was not applied in order to maintain consistent standards for comparison of the different methods. Besides, size selection steps do not always remove long fragments in Nextera libraries (Kim et al. 2013; Lamble et al. 2013).

The libraries were sequenced using MiSeq Reagent Kit v2 (500 cycles) (Illumina). The quality of sequencing results depends both on the library quality (van Dijk et al. 2014) and the bioinformatics tools used to analyze the sequencing data, such as trimming and assembly algorithms (Ekblom and Wolf 2014). The quality of the sequence data obtained in this study was assessed using FastQC 0.11.4. The BBduk plugin in Geneious R9 (Biomatters Ltd.) was used for trimming. To ensure an unbiased comparison, the same number of trimmed reads was randomly extracted from each sequenced library and assembled using the

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