APPLICATION NOTE No. 420

Easy Automation of Metagenomic Library Preparation with the ep*Motion*® 5073m NGS Solution

Abstract

Metagenomic studies can determine the genetic composition of microbiological samples. These studies usually rely on the use of marker genes, such as 16S rDNA, for the phylogenic classification. Which specific marker gene and region will be examined and how deeply sequenced will depend on the aim of the study. Nevertheless, the basic approach of library generation is common to these experimental designs. It is important to have a robust workflow to correctly analyze the samples. In this application note we demonstrate such a workflow, which may be customized and scaled to specific customer needs.

Introduction

Scientific questions around the microbiome such as case – control and longitudinal studies require best practices and standardized plus reproducible laboratory workflows to process hundreds, sometimes thousands of samples for the identification and comparison of microbial community structure, composition, and genetics, as well as functional variation [1]. Automation of library preparation not only minimizes the loss of samples, wasted reagents and sequencing delays, but also reduces inter-operator variability as well as errors in sample tracking. This application note describes the automated processing of microbial DNA samples on the Eppendorf ep*Motion* into sequencing ready libraries (Figure 1).

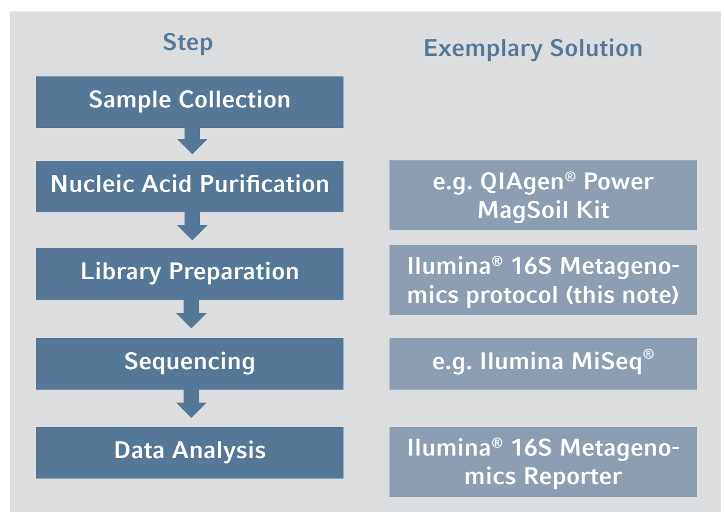

Figure 1: Sample workflow for metagenomics and relevance of this protocol. Here we do show the preparation of targeted 16S Amplicons on the ep*Motion* 5073m NGS Solution for up to 24 samples.

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Materials and Methods

ep*Motion* setup

The Eppendorf ep*Motion* family comprises of a series of multi-purpose liquid handling workstations different in size and functionality, hence providing flexibility and scalability to every laboratory. Here we present the ep*Motion* 5073m NGS Solution. The ep*Motion* 5073m NGS Solution comes with dispensing tools, covering a 1-300 µL volume range, and on deck Thermomixer, for incubation and bead resuspension. Additionally, a gripper can transport plates around the worktable with ease for magnetic bead cleanups, and allows working with tip holders to increase deck capacity on a small footprint. The ep*Motion* 5073m and other models in the family are ideal walk-away companions for labs that demand high efficiency, accuracy and automated workflow at an attractive price point. ep*Motion*'s user-friendly interface and modular programing capabilities, gives the users flexibility, and guides the operator through the run setup, including placement of the labware and required reagent volumes. Here we demonstrate how these features of the ep*Motion* 5073m can be used to automate a complex method, such as Illumina's 16S Metagenomic Sequencing Library Preparation protocol.
For automated liquid handling an ep*Motion* 5073m NGS Solution was used. Magnetic bead separation was performed using Eppendorf Magnum FLX Magnet Adapter. The automated protocol is split into 2 sub-methods (Figure 2), each ending at a safe stopping point. All amplifications were performed off-deck on the Mastercycler® X50.

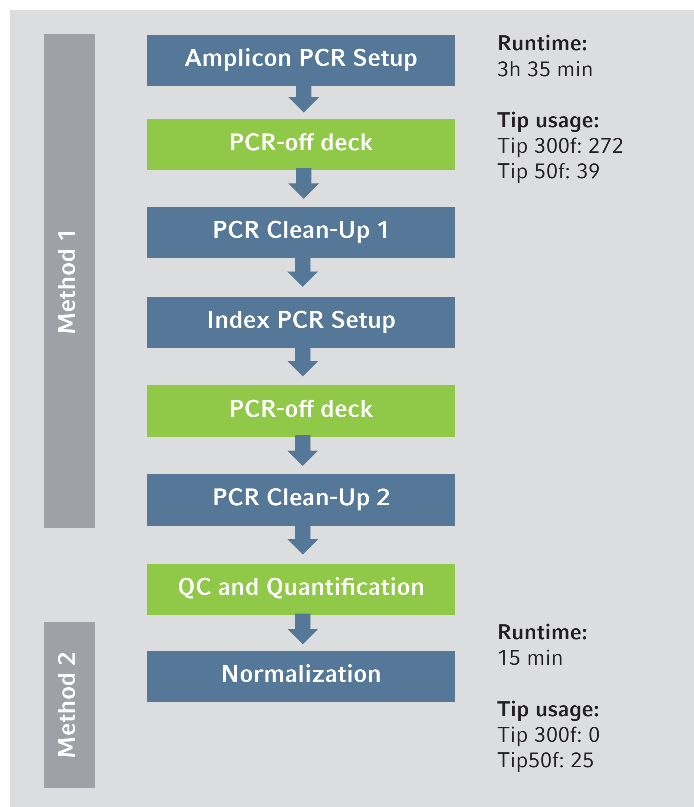

Figure 2: Demonstrated workflow for Illumina's 16S rDNA protocol on the ep*Motion*. The grey boxes show submethods processed on the ep*Motion*. Steps highlighted in green are performed off-deck. PCR amplifications were performed using the Mastercycler X50. In alignment with the protocol, workflows are compartmented into logical units ending at safe-stopping points. Detailed run times and consumable usage are shown next to each submethod.

Experimental design

Libraries were generated using an automated version of an Illumina protocol described above [2]. For the initial experimental setup an input of 12.5 ng genomic *Escherichia coli* MG1655 genomic DNA (ATCC® 700926D5™) was used. 24 samples were prepared per run. For each library, the quality control (library size distribution and quantification) was performed using an Agilent® 2100 Bioanalyzer® DNA 1000 kit.
For the second part of the experiment a mock community of 20 Strain Staggered Mix Genomic Material (ATCC MSA1003™) was used to compare 16 libraries prepared on the ep*Motion* vs. 8 manually prepared libraries.

To complete 24 samples for the automated run we included a control set of 4 *E. coli* samples and 4 non-template controls. Since the controls showed good results, sequencing of the pooled libraries was performed on an Illumina MiSeq System using paired-end mode (2x150 bp). Data was analyzed using Illumina's BaseSpace® 16S Metagenomics app.

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Results & Discussion

Establishing 16S rDNA method using *E.coli* genomic DNA
Initially we sought to demonstrate the functionality of our approach by amplifying the V3-V4 region of 16S rDNA from *E. coli*. We investigated the variability and yield across different 24 replicates of the same input DNA (Figure 3). The samples clustered tightly with a median amplicon size of 657 bp (CV 0.5%) and median yield of 218 nM (CV 10.9%), without primer dimers, suggesting, efficient PCR setups and bead purifications.

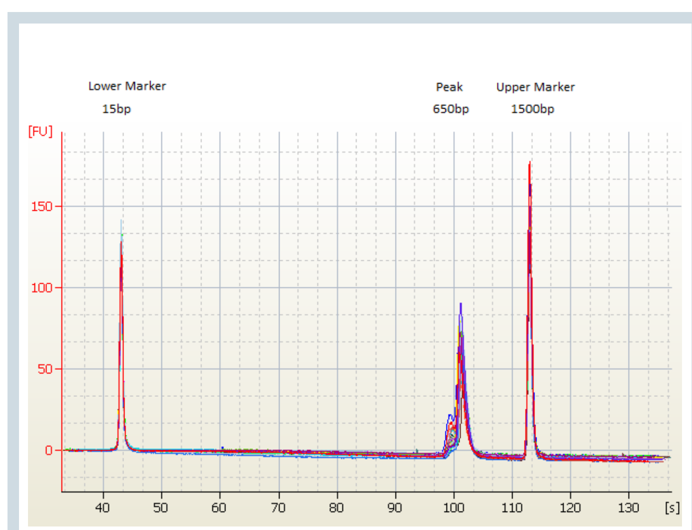

Figure 3: Overlapping Bioanalyzer Electropherogram of 24 replicates shows tight clustering and high yields of amplicons suggesting specific amplification of libraries. Peaks for the upper marker, PCR product and lower marker are indicated with their respective sizes.

A factor of potentially high negative relevance to metagenomic studies is cross contamination. To test this, we designed and executed a library preparation run with an alternating set of *E. coli* DNA template and non-template controls (NTC). The resulting gel electrophoresis did not reveal any amplicons in the NTC, but only some primer dimers (Figure 4).

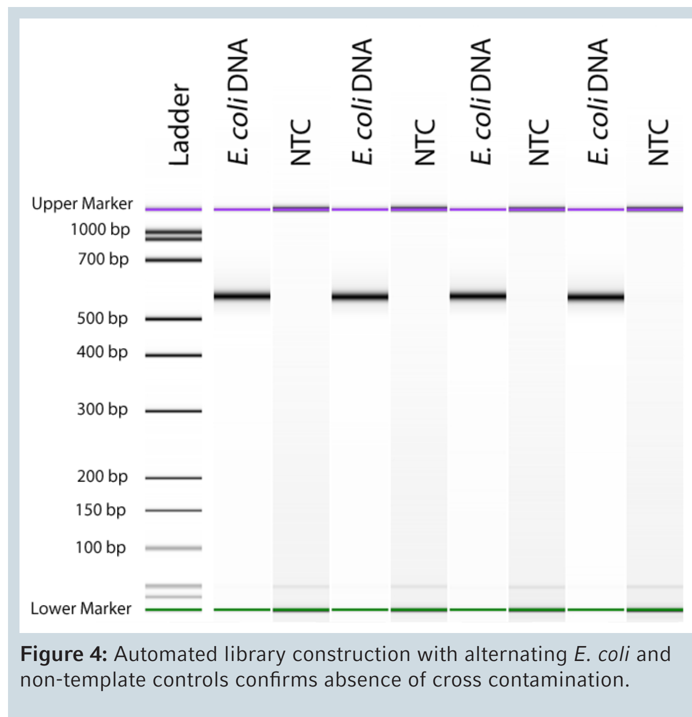

Figure 4: Automated library construction with alternating *E. coli* and non-template controls confirms absence of cross contamination.

Investigating strain to strain reproducibility using a 20-strain mock community

We thus sought to apply the method established above to test the reproducibility of detecting metagenomic diversity. To this end, we prepared 16 replicates of a 20-strain mock community on the ep*Motion*. For comparison we performed the same experiment on 8- replicates manually. The resulting 24 samples were pooled and sequenced on a 2x150 bp MiSeq run. An operational taxonomic unit (OTU) classification was performed on the resulting files using Illumina's 16S BaseSpace app to check whether the constituent genii of the mock community could be reidentified. As shown in Figure 5, the number of reads obtained from this experiment was high enough for all samples not to affect the % of reads assigned to genus, which was stable averaging at 87.1%. To assess potential differences in the community calls for manually and automatically prepared samples a principal coordinate analysis (PCoA) was performed on genus level (Figure 6). The PCoA analysis showed a very tight clustering of the samples suggesting little to no variation between the manual library preparation and the automated procedure for the assignment of genii. The results shown above qualify the automated method as delivering sequencing libraries similar or better than manually prepared ones.

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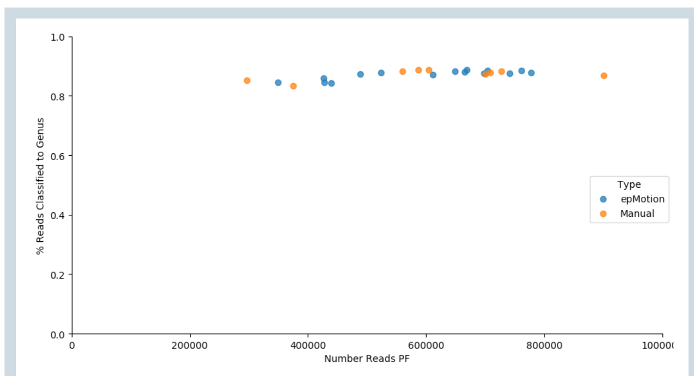

Figure 5: Number of reads passing filter (PF) vs the % reads classified to genus. The same percentage of reads classified to genus remained constant for all samples. This suggests, that the coverage obtained in the sequencing run was high enough to obtain consistent classification of all samples.

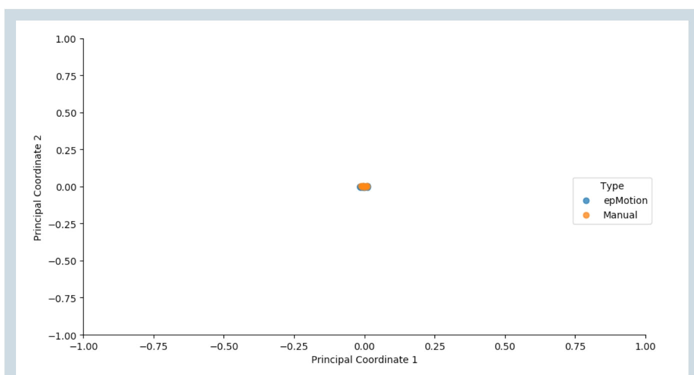

Figure 6: PCoA on the OTU classification of 24 replicates of the mock community. The principal coordinates are color coded per sample type for the 8 samples prepared manually (orange) and the 16 samples prepared on the ep*Motion* 5073m NGS solution. This scatterplot shows a Principal Coordinate Analysis (PCoA) of the normalized relative abundance of all samples. The PCoA measures differences in the distribution of taxonomic classifications between samples at the genus level. The genus level shows tight taxonomic clustering of results.

Conclusion

The increased demand for high-quality NGS libraries in laboratories has necessitated the development of robust, automated methods for library preparation. This application note demonstrates the successful and reproducible automation of 16S rDNA amplicon libraries on the ep*Motion* 5073m NGS Solution and equal performance as the manual protocol. Further upscaling and automation of the workflow can be performed using the ep*Motion* 5075, to process up to 96 samples per run to support a wide range of sequencing depths and throughputs.

References

[1] Knight, R. et al. Best practices for analyzing microbiomes. Nature Microbiology Review 2018; 16:410-422.
[2] Illumina. 16S Metagenomic Sequencing Library Preparation Guide. Part # 15044223 Rev. B

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Ordering information

Description	Order no. international
ep*Motion*® 5073m NGS Solution (w/o CleanCap and with EasyCon)	5073000930
ep*Motion*® 5073m NGS Solution (w/o CleanCap and with Multicon)	5073000949
ep*Motion*® 5073mc NGS Solution (w CleanCap and with EasyCon)	5073000957
epMotion® 5073mc NGS Solution (w CleanCap and with Multicon)	5073000965
Mastercycler® X50s	6311000010
Agencourt® AMPure XP 60 ml kit	Beckman Coulter® Genomics, part # A63881
Absolute Ethanol	General lab supplier
KAPA® HiFi HotStart Ready Mix (2x)	KAPA Biosystems, part#KK2601
Nextera® XT Index Kit Illumina	catalog # FC- 131- 1001 or Illumina®, catalog # FC- 131- 1002

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