mosquito® 应用：

基于Illumina测序平台的DNA文库构建微缩化

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简 介

近年来二代测序技术发展迅速，以其高通量、低成本的优势，广泛应用于生命科学研究中。

对于高通量测序核心平台而言，测序技术的发展使得文库构建流程面临更低交付成本及更快交付速度的挑战，而自动化液体处理工作站的出现缓解了这一瓶颈。本文阐述了 mosquito HV 如何成功完成基于Illumina测序平台的DNA文库构建微缩化，体积仅为手动标准流程的十分之一。

mosquito HV 是英国 SPT Labtech 公司研发生产的自动化液体工作站，采用独特的固相置换技术，一次性枪头内的不锈钢活塞直接与液体接触，通过控制活塞的移动来进行移液(图1)。因此，mosquito可以对低至25 nL的液体进行精准移液，无需进行枪头校准和液体定义。

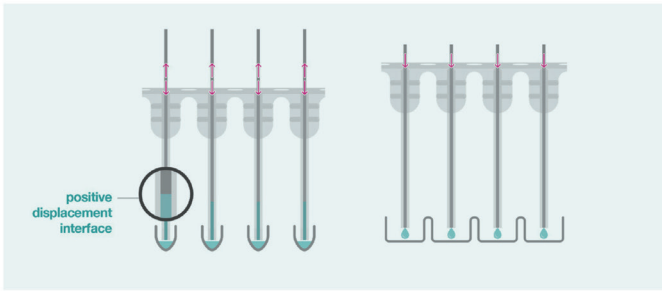
之所以选择 NEBNext Ultra II FS DNA 方法，是因为它使用酶切打断实现片段化，无需进行物理超声打断。使用 ZymoBIOMICS 微生物群落标准品作为基因组DNA，在使用不同起始量的 input DNA 情况下，将 mosquito HV 制备的微缩化文库与手动制备的标准体积文库进行比较。为了确定样本起始量较低时的结果，则使用单个菌株样品的微缩化 NEBNext Ultra II FS DNA 文库，和相同条件下手动制备的 Illumina TruSeq Nano文库进行比较。此外还对NEBNext Ultra II FS DNA 自动化建库与 TruSeq Nanoh、TruSeq PCR Free 手动或传统工作站建库进行比较。

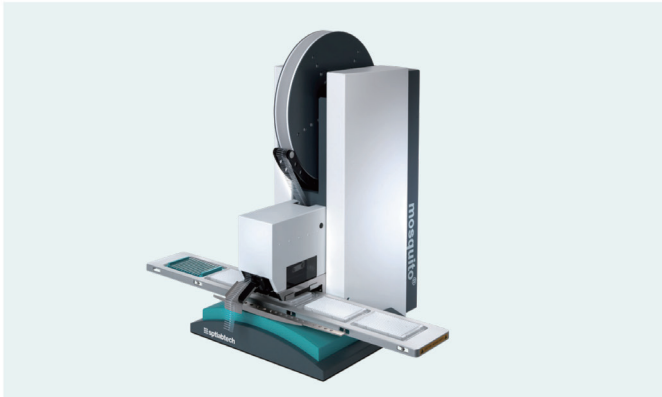
mosquito® HV

灵活开放的液体处理工作站

- DNA文库构建微缩化，大幅节省试剂成本
- 实现精准、稳定的纳升级加样，提高数据质量和可重复性
- 实现高效率、高通量的微生物组研究

Figure 1 (below). mosquito® positive displacement tip technology and mosquito® HV genomics





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手动 VS 自动建库

本次实验以ZymoBIOMICS微生物群落标准品(Cat. No. D6306)为样本，测试NEBNext Ultra II FS DNA文库试剂盒。该标准品包含10种微生物菌种的基因组DNA混合物，目前被广泛用于宏基因组学工作流程的验证和质量控制，它能反映实验过程中可能出现的偏差和错误，是测试文库构建试剂盒的理想工具。此外，它还可以直接与 CGR 先前测试过的建库试剂盒进行性能比较。测试起始量分别为50ng、1ng、0.1ng，一式三份。如图2所示。

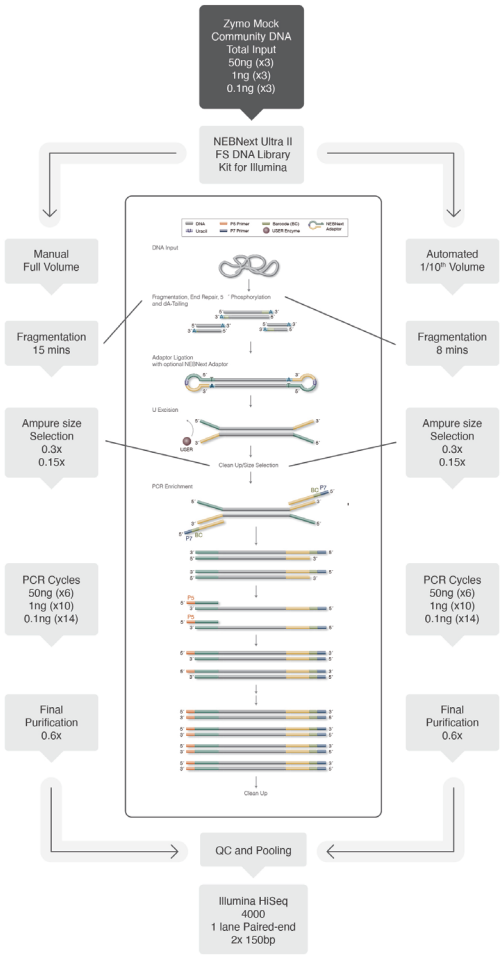


Figure 2. Schematic representation detailing how NEBNext libraries were generated using the ZymoBIOMICS Microbial Community DNA Standard as input DNA. Manual libraries were made using full volumes according to protocol, automated libraries were made using 1 in 10 volume on the SPT mosquito HV platform.

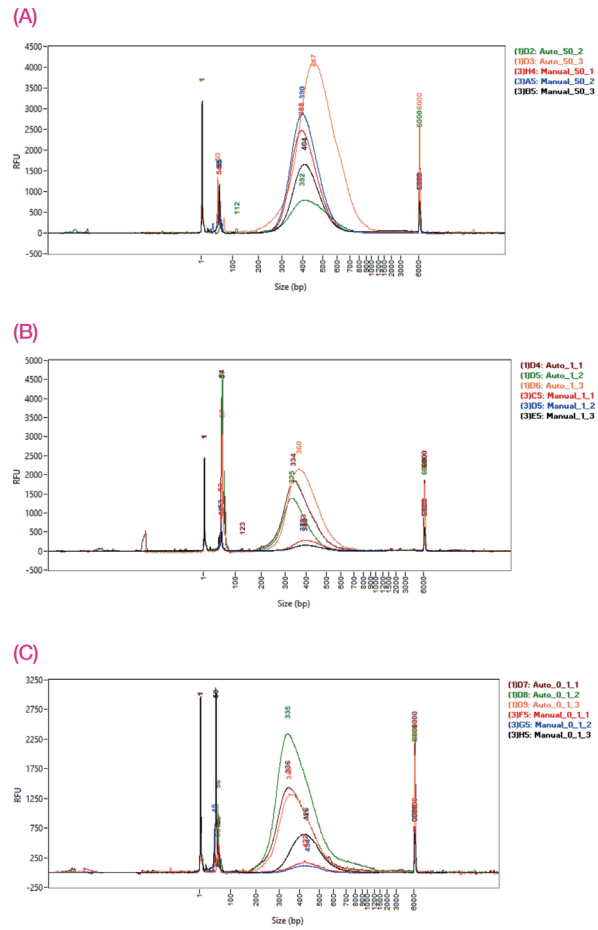


Figure 3. Fragment Analyzer traces comparing the size distribution of manual and 1/10 automated libraries for A) 50ng, B) 1 ng, and C) 0.1 ng of input DNA.

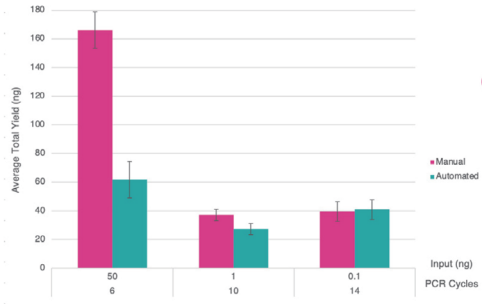


Figure 4. Bar chart showing average yields for the manual and 1/10 automated libraries with differing input amounts and PCR cycle numbers. Error bars indicate standard deviation.

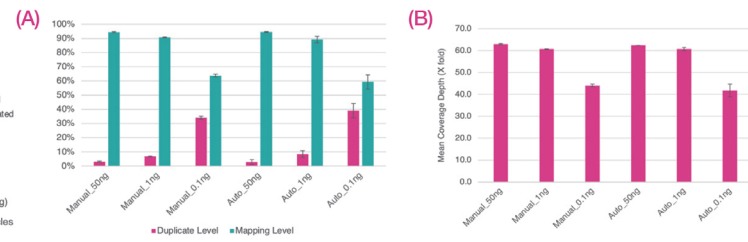


Figure 5. Sequence data were normalised to 13M reads per library, A) indicates average levels of duplicate and mapping percentages, B) shows average fold coverage. Error bars indicate standard deviation

临床与细菌分离物的性能比较

使用ZymoBIOMICS微生物群落DNA标准品得出了令人满意的分析结果，但这并不代表使用实际样本可以达到同样效果。此前曾手动制备TruSeq Nano文库，使用100 ng的input DNA，从临床分离的细菌样本中生成数据。现使用自动化平台，缩减样本量至1/10，在NEBNext Ultra II FS DNA加入10 ng DNA，并对结果进行比较。

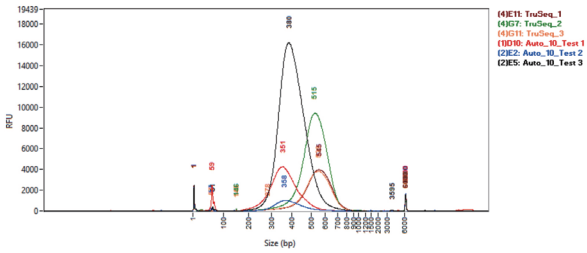


Figure 6. Fragment Analyzer traces comparing the size distribution of manual TruSeq Nano and 1/10 volume automated NEBNext Ultra II FS DNA libraries.

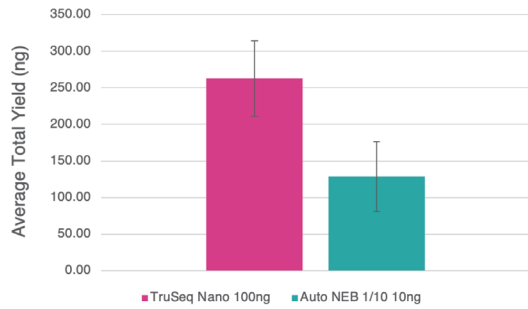


Figure 7. Bar chart detailing average yields for the manual TruSeq Nano and 1/10 automated NEB libraries generated from clinical bacterial isolates. 100 ng and 10 ng of input DNA were used for the TruSeq Nano and NEB 1/10 libraries, and 8 and 10 cycles of PCR, respectively. Error bars are standard deviation.

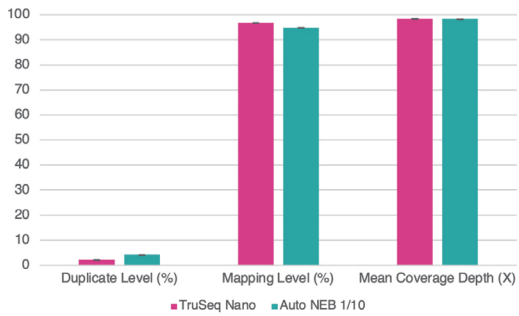


Figure 8. Sequence data were normalised to 1.3M reads per library. Bar chart shows averages of duplicate and mapping percentages, and fold coverage of the genome. Error bars are standard deviation.

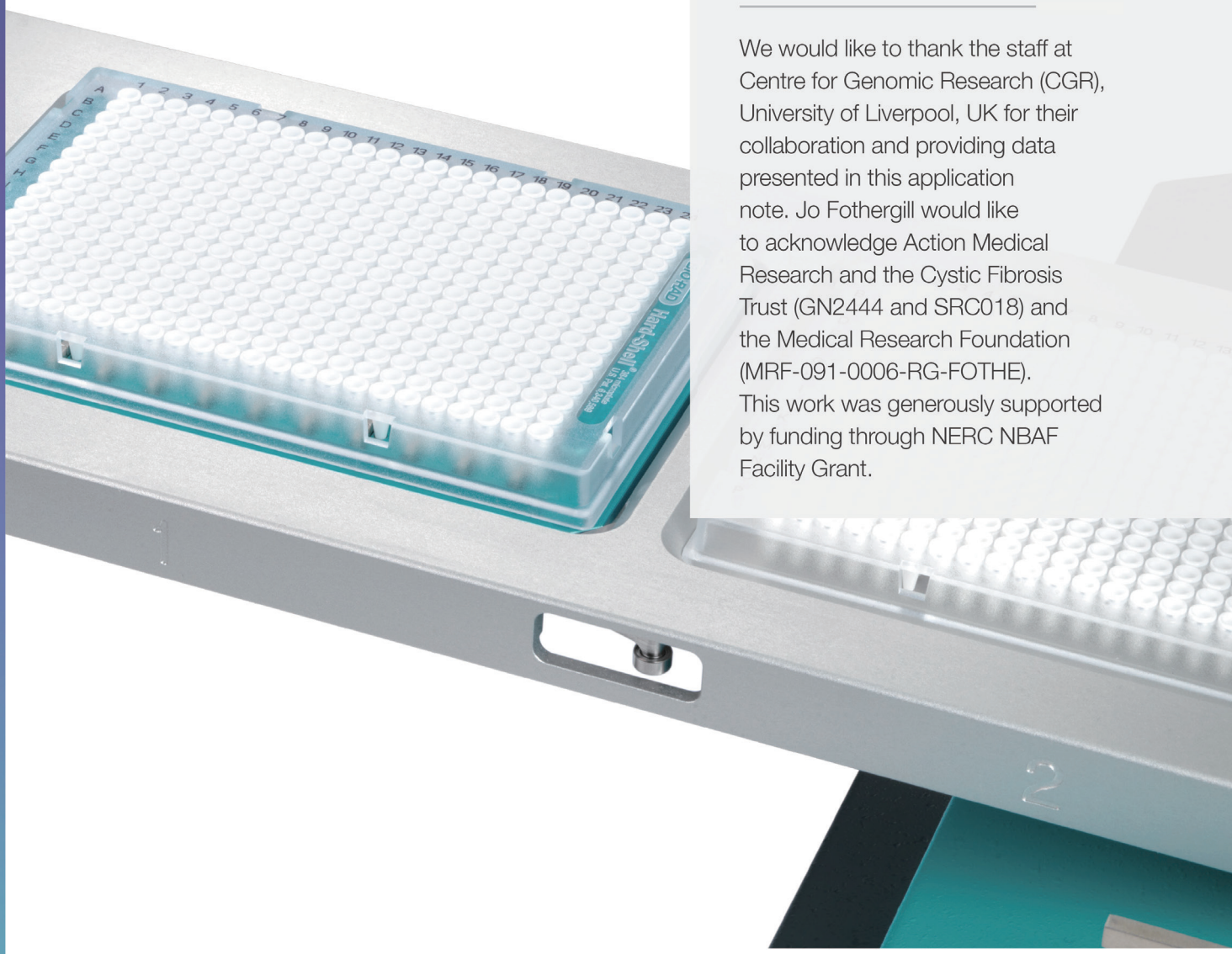
1/10 体积 NEB Ultra II FS 自动文库 VS 标准体积自动/手动 TruSeq Nano 和 TruSeq PCR free 文库

此前，CGR 的工作流程使用的是 Illumina TruSeq Nano 或 TruSeq PCR Free 建库。使用上述方法（原始体系的手动及贝克曼 FXP 自动化系统）获得数据。TruSeq Nano 或 TruSeq PCR Free 文库分别使用了 100 ng 和 1 µg 的 ZymoBIOMICS 微生物群落 DNA 标准品。当将 50 ng DNA 投入到 1/10 体积的 NEB NEBNext Ultra II FS 进行 DNA 文库构建时，三种方法的数据具有可比性。

Figure 9. Sequence data were normalised to 13M reads per library. Bar chart shows averages of A) percentage duplicates, B) percentage mapping, and C) fold coverage (X) of the mock community. Error bars are standard deviation.

结 论

本研究表明，NEBNext Ultra II FS DNA文库以1/10体系制备，其性能不亚于人工制备的原始体系文库，且通过反应体系的微缩化大幅节约了试剂成本。重复率、比对率、覆盖度和GC偏差 (未在本文呈现) 都与加入1ng DNA的情况下相当。整个工作流程可以在一天内完成，从而使样本量更多的大型项目得以运行，并有效减少技术误差。



致 谢

We would like to thank the staff at Centre for Genomic Research (CGR), University of Liverpool, UK for their collaboration and providing data presented in this application note. Jo Fothergill would like to acknowledge Action Medical Research and the Cystic Fibrosis Trust (GN2444 and SRC018) and the Medical Research Foundation (MRF-091-0006-RG-FOTHE). This work was generously supported by funding through NERC NBAF Facility Grant.

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