

Providing Answers to Problems in Genomics Since 1996



- custom BAC library construction
- DNA library screening
- HMW DNA prep. for BioNano Irys® system
- NGS grade DNA prep.
- PacBio RSII >30kb library prep.
- BAC clone sequencing

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custom BAC library construction

Amplicon Express(AEX) has made over 1,800 custom BAC libraries since its inception in 1996. AEX's protocols are optimized for constructing high quality BAC libraries with fast turnaround. Average insert size of BAC library will be 115kb or higher. AEX specialize in delivering high quality libraries with limited of difficult-to-work-with starting materials.



BAC library screening

AEX has more than 20 years of library screening experience with all kinds of organisms, especially for very difficult regions of interests (duplicated regions, high GC regions, complex targets). AEX can provide below screening tools or screening service using below tools.

Pools & Superpools®

Amplicon Express PCR-based Pool or Superpool technology is used to screen your library up to 3000 times, and is a powerful tool to find your clone of interest. Amplicon Express had developed proprietary software to automate analysis of your Pool or Superpool results, increasing speed and accuracy in interpreting results.

nylon filter sets

Amplicon Express Nylon Filter sets have Screening filters using DNA/DNA hybridization .All clones printed in duplicate and filters can be customized to meet your needs.

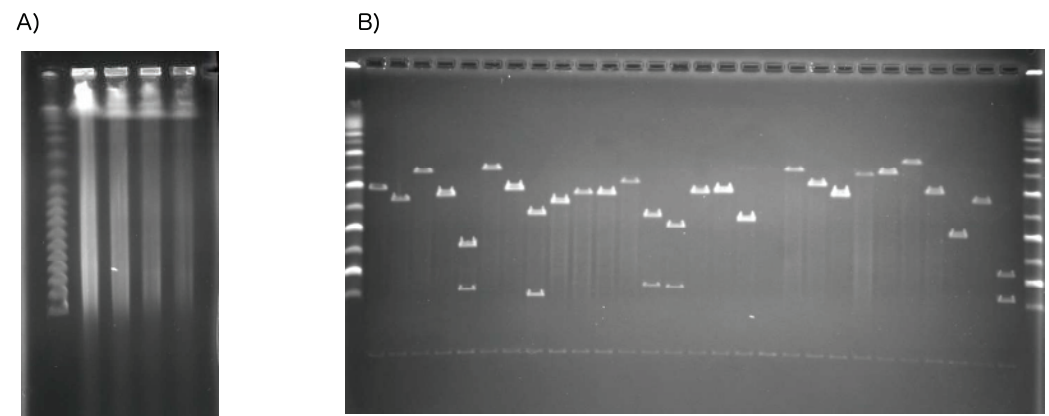


Figure A) PFGE data of extracted HMW DNA from tissue. B) randomly picked 28 BAC clones and digested by Not I. average insert size is 145kb



BAC clone sequencing

Many BAC libraries were made before and it takes lots of cost and time to determine BAC DNA insert (>100kb). Many researchers want detailed and accurate sequence of just a few BAC clones, from a few regions, not the entire genome. AEX is able to accomplish BAC clone sequencing inexpensively, because AEX makes a single PacBio library by combining 12 BAC clones provided by one or more clients. And also BAC clones are sequenced using illumina platform to identify individual clones. Sequencing data will be 50x-150x coverage for each BAC clone and consist of PacBio 20kb library reads and 500-700bp paired-end reads.

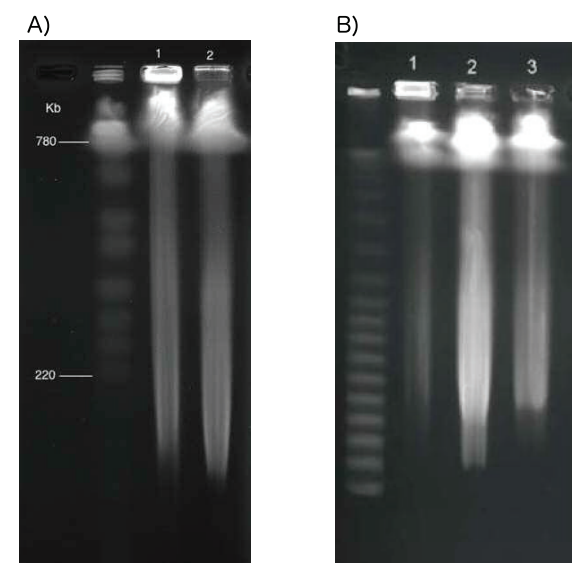


<BAC clone sequencing overview>

1. Make High quality GS grade BAC DNA (low E. coli genomic DNA)
2. Combine 12 pooled BAC clones in one NGS PacBio 20kb library.
3. Prepare individual Paired-End libraries for each BAC clone and run on a MiSeq instrument.

HMW DNA prep. for Irys®

Amplicon Express provides HMW DNA which is need for BioNano Genomics Irys optical mapping system. Deliverables are TWO ultra pure plug with 5µg, TWO regular pure plugs with 5µg and PFGE photo of the HMW DNA quality and quantity.



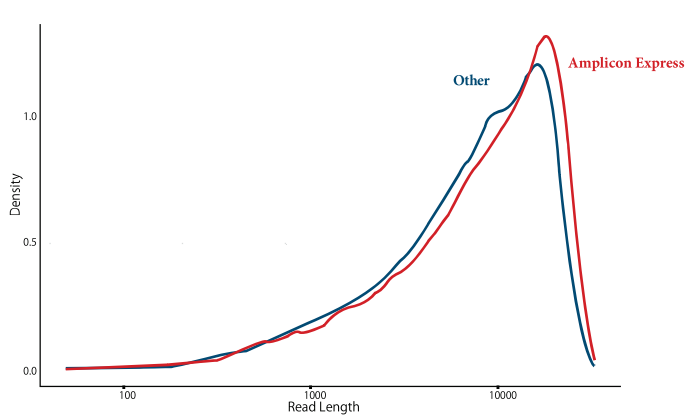
example of HMW DNA extraction
 (A) sample: Zea Mays (Corn)
 Lane 1: regular purity HMW DNA plug
 Lane 2: ultra pure HMW DNA plug

(B) sample: beetle
 Lane 1: regular purity HMW DNA plug
 Lane 2: ultra pure HMW DNA plug
 ※DNA maker: ladder has bands every 50kb

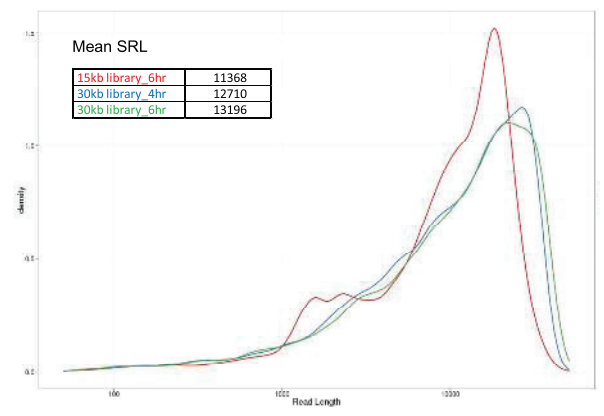
For the HMW DNA isolations Amplicon Express has done so far, the DNA quality for all samples was excellent. When HMW DNA concentration is desirable, we usually obtain fragment sizes N50 > 200 Kb. For some of the samples which the DNA concentration is low, a few extra steps are needed by our team to obtain reasonable concentration for BioNano IRYS Optical Mapping procedure, but it still generates good data."
 - Professor Mingcheng Luo, UC Davis

NGS grade DNA prep. for PacBio RSII & >30kb library prep. (coming soon)

Lead length of NGS platform is longer year by year in long-read sequencer as represented by PacBio RSII. The quality of starting material DNA becomes to be very important and essential to get high quality sequencing data. AEX provides NGS grade DNA prep. service optimized for PacBio RSII and PacBio library prep.

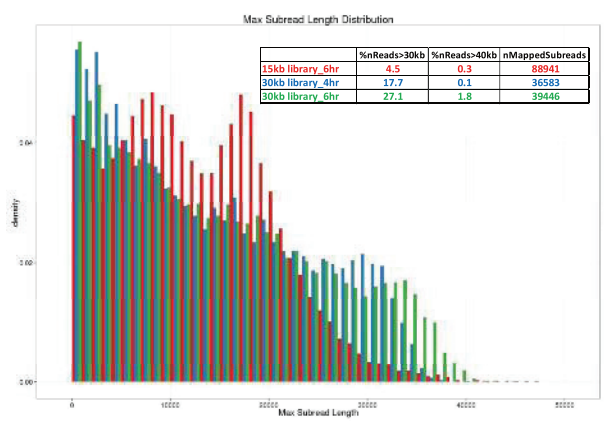
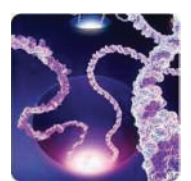


(A) Genomic DNA of E.Coli K12 strain MG1655 was extracted by AEX and another commercial supplier (other) and >15kb library were prepared and sequenced by PacBio RSII (P5_C3 chemistry, 3hrs movie). Mean read length of library prepared by Amplicon Express was 15% longer than that of a library from another commercial supplier (other).



Mean SRL	
15kb library_6hr	11368
30kb library_4hr	12710
30kb library_6hr	13196

(B), (C) Genomic DNA of E.Coli K12 strain MG1655 was extracted and >15kb and >30kb were prepared then performed PacBio sequencing using P6_C4 chemistry. 30kb library showed longer subread length than >15kb library.



	%nReads>30k	%nReads>40k	nMappedSubreads
15kb library_6hr	4.5	0.3	88941
30kb library_4hr	17.7	0.1	36583
30kb library_6hr	27.1	1.8	35446