

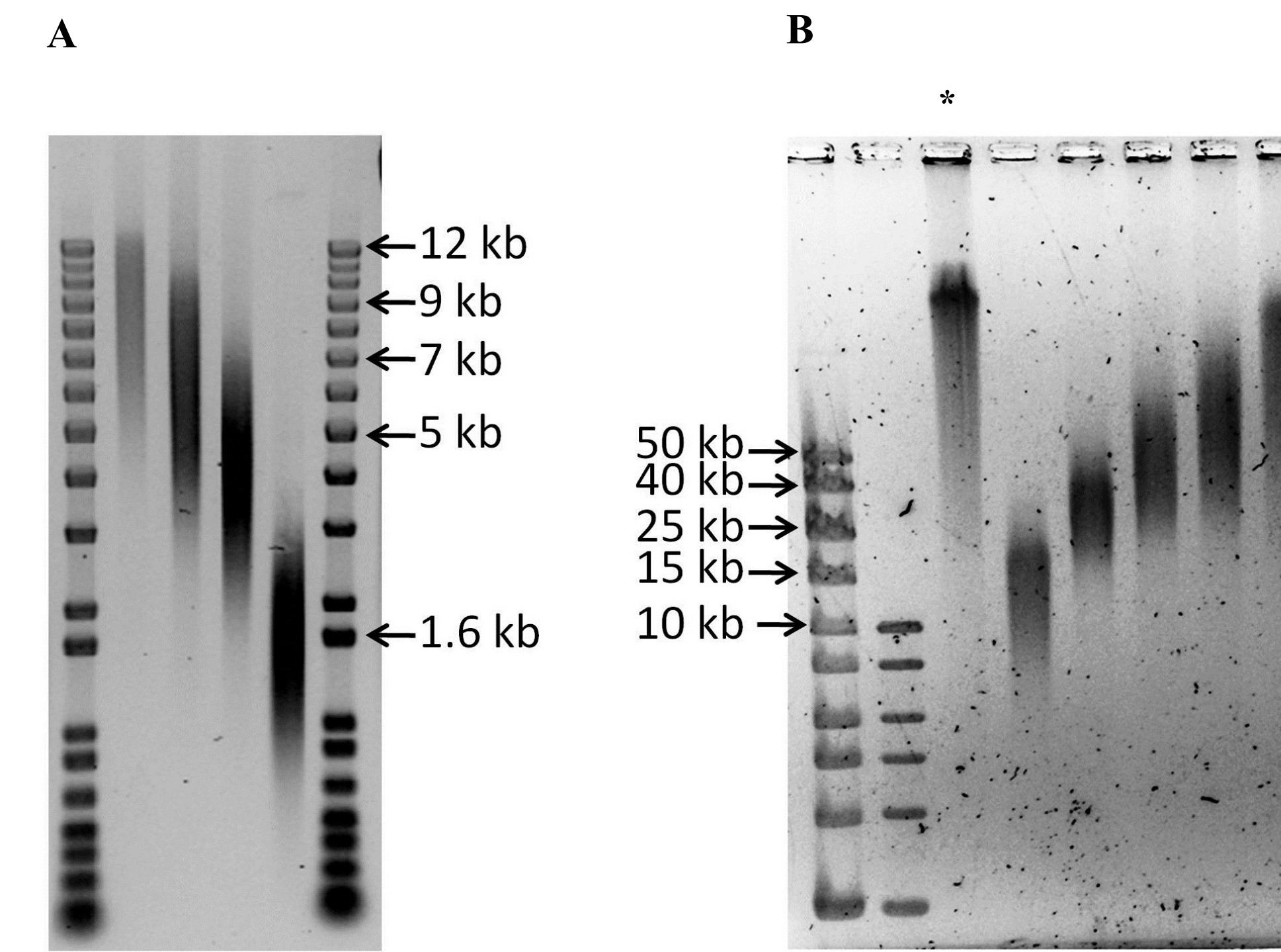
Improvements in Hydrodynamic Shearing Facilitates 20-50 kb True Mate Pair Library Construction

David Mead¹, Svetlana Jasinovica¹, Erin Ferguson¹, Amanda Krerowicz¹,
Brendan Keough¹, Michael J. Lodes¹, and Aric Joneja²

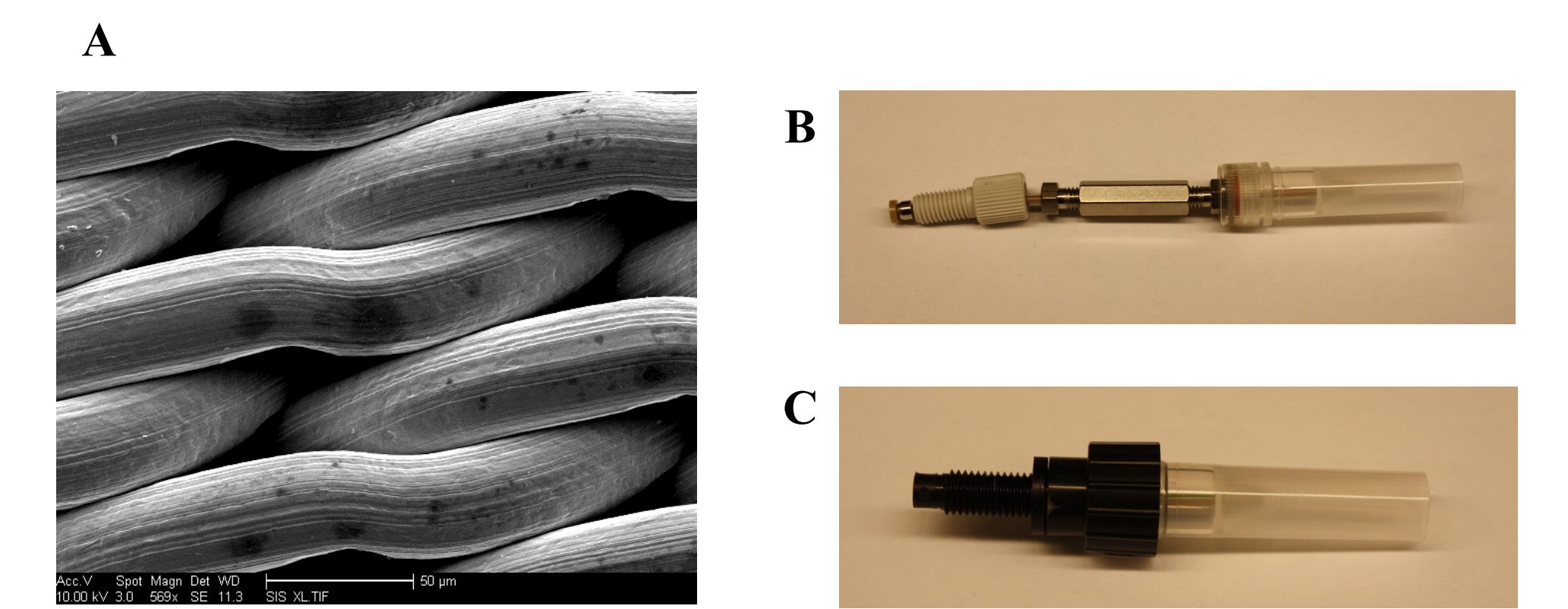
Introduction

Next generation sequencing technologies produce massive quantities of short reads covering entire Gb genomes. A major limitation is the proper assembly and phasing of genomes, as the current technology cannot resolve repeats, segmentally duplicated sequences, or differentiate between diploid chromosomes. We have developed a long span mate-pair technology that has demonstrated 70-90% efficiency for 20 kb libraries. It is predicted that 50 kb true mate-pair libraries should enable proper assembly of many genomes *de novo*. This application demands libraries of long DNA segments, but few methods are available for reliably generating fragments in excess of 2 kb. Diagenode's Megaruptor[®] utilizes hydrodynamic shearing to target libraries of between 2 kb and 90 kb, with the majority of fragments falling in a 2 to 3- fold size distribution. The Megaruptor consists of an automated syringe pump and ceramic valve with a unique disposable shearing assembly called a Hydropore. Significantly, each Hydropore contains an array of pores rather than a single shearing orifice. This eliminates the clogging issues that plague single orifice shearing devices. The disposable nature of the Hydropores reduces the possibility of sample cross-contamination. Sample processing is user-friendly due to the intuitive software interface, and two samples can be sheared sequentially. The Megaruptor has been used by several sequencing centers to produce high-quality small insert fragment libraries for Illumina and PacBio platforms. We have used the instrument to prepare 20-50 kb inserts for a new mate-pair library technology that is >70% efficient. Size range and the quality of sheared DNA with supporting sequence data are presented for 20 kb true mate-pair library construction.

Megaruptor: Hydrodynamic Shearing of DNA



(A) Agarose gel image of human genomic DNA sheared using the Hydropore for short fragments. As the flow rate increases, the fragment size decreases from a mean size of 9 kb to a mean size of 2 kb. (B) PFGE image of human genomic DNA sheared using the Hydropore for long fragments. Longer fragments are generated by very slow flow rates, * indicates unsheared DNA. The total range of sizes achievable with the Megaruptor is 2 kb to 90 kb.

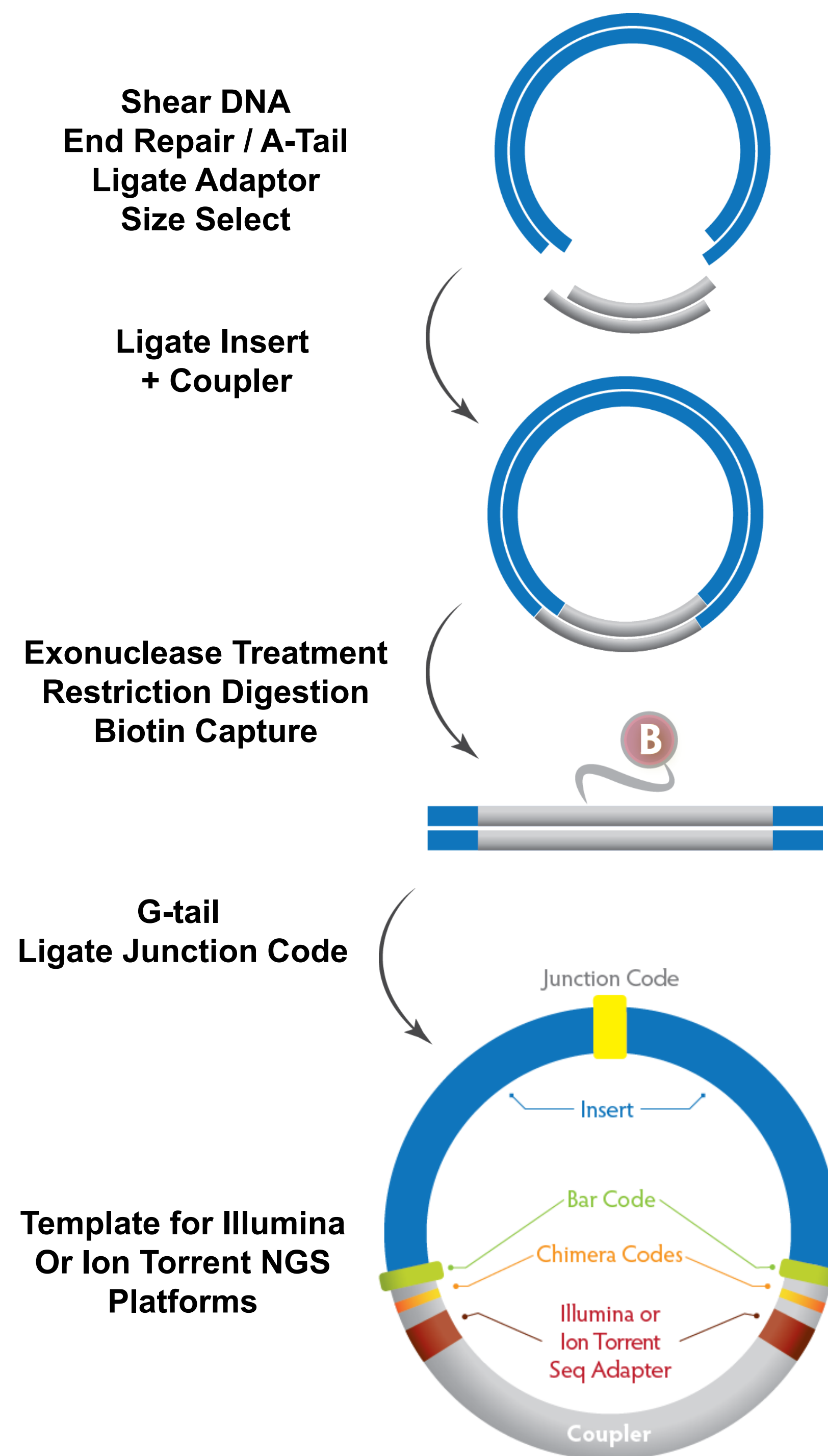


(A) SEM image of a pore array. An essential component of the Megaruptor Hydropores is the disposable pore array. These irregular but highly uniform pores shear DNA samples without clogging. (B) The metal Hydropore can contain pores of large or small size depending on the desired fragment length. The metal housing is washed and the pore array is disposable. (C) The plastic Hydropore (in production) will be fully disposable.

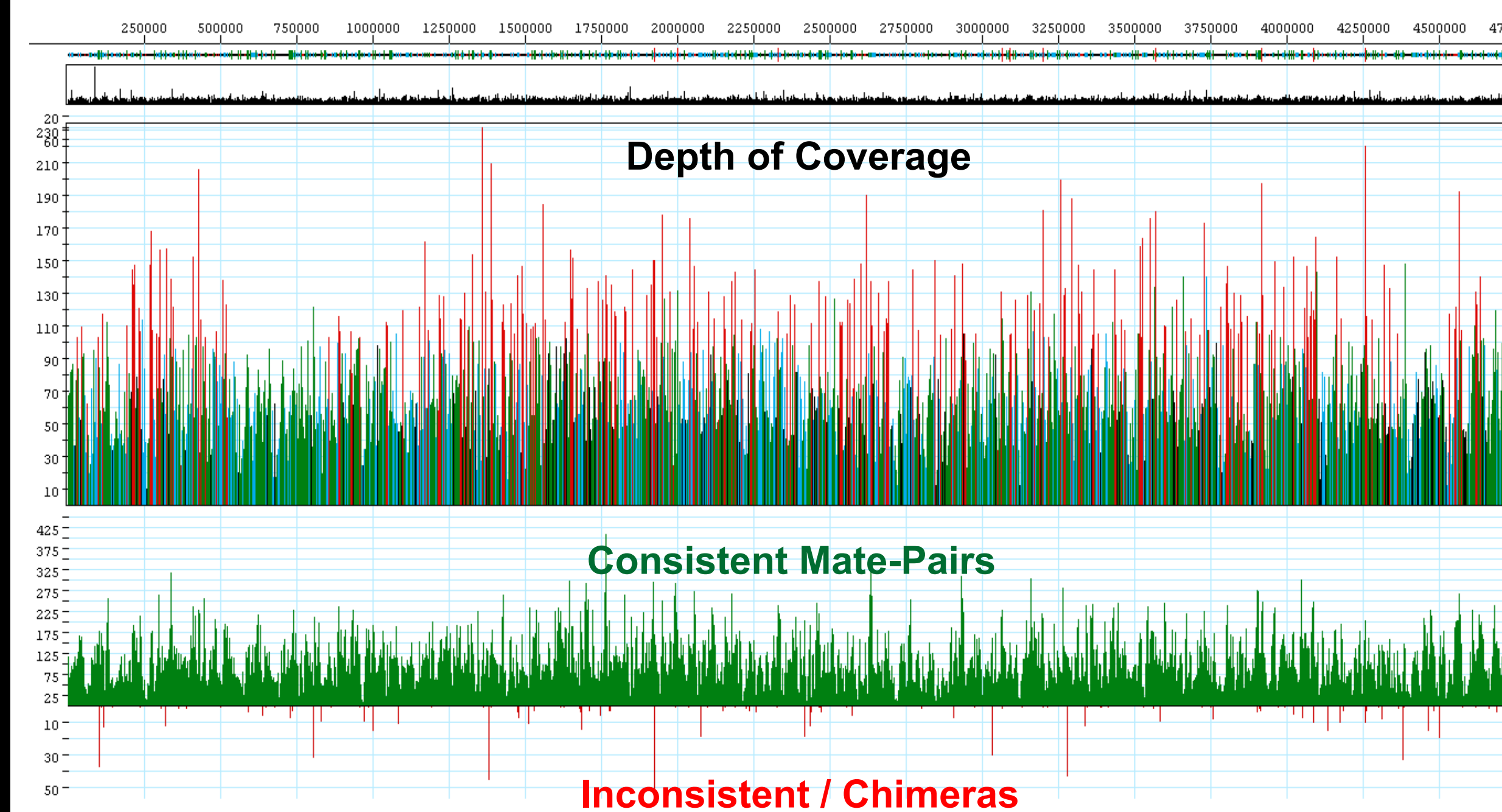


A Megaruptor system consists of a syringe pump, a 9-port ceramic valve, Hydropores for shearing DNA, a laptop running control software, and wash buffers.

NxMate NGS True Mate Pair Library Construction



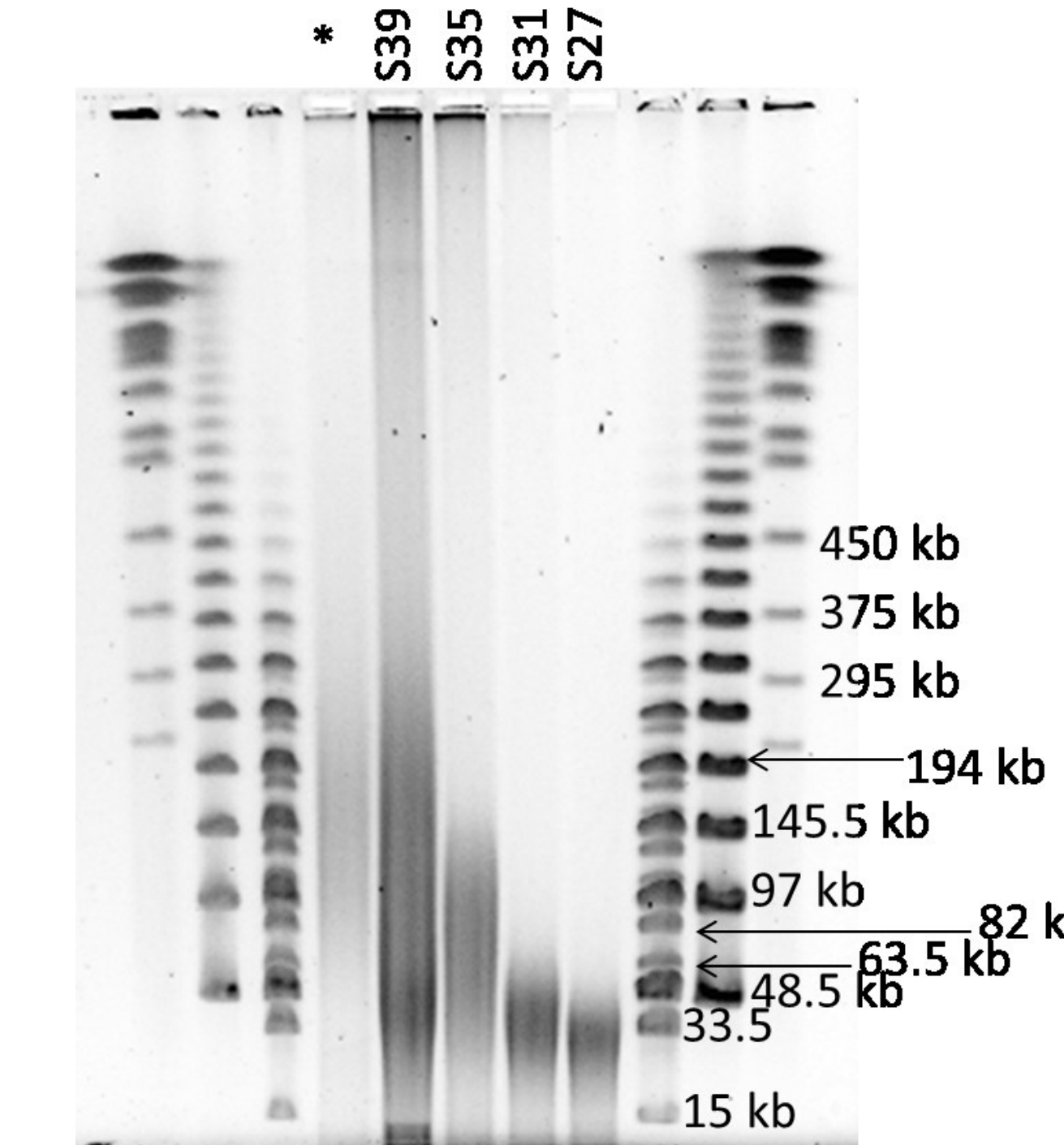
E. coli 20 Kb Genome Coverage Analysis With Ion Torrent Mate Pair Library



Ion Torrent Platform Large Scaffolds with Lucigen Mate Pair Data

Platform	Sequence Type	Pair Distance	Contig N50	No. Contigs	Max. Scaffold size	No. Scaffolds
Illumina	2X100bp	350bp	75kb	135	200kb	35
Ion Torrent	300bp	none	65kb	267	none	none
Ion Torrent	300bp + Lucigen Mate Pairs	20kb	55kb	337	2100kb	9

20-50 kb Shearing for Mate Pair Library Construction



PFGE image of *E. coli* DH10B HMW genomic DNA sheared with custom speed codes. S39, S35, S31, and S27 are custom speed codes developed by Aric Joneja (Diagenode, Inc.) to apply Hydropore technology to HMW shearing. As before, longer fragments are generated by very slow flow rates, and * indicates unsheared DNA. The total range of sizes achievable with the custom Megaruptor HMW speed codes is 30 kb to 90 kb.

The level of control afforded enables the construction of libraries whose mate pairs can span the long repetitive elements common in animal and plant genomes that confound genome assembly in a variety of projects from resequencing to BAC closure to *de novo* sequencing, particularly when HMW and LMW approaches are used in a complimentary fashion.

Randomly sheared DNA is end- repaired, size selected, gel eluted and processed for mate pair library construction.

Summary

Megaruptor[®] Device for Hydrodynamic Shearing

Advantages

- Can provide a tight distribution of fragment sizes from 2 kb to 90 kb.
- Two samples are processed in series
- Avoids the clogging issues commonly encountered with single-orifice devices
- Disposable element eliminates cross-contamination
- DNA source has minimal effect on shearing performance

NxMate[™] NGS Mate Pair Technology

Advantages

- High efficiency true mate pairs, few chimeras
- Low bias, low redundancy
- 2-20 kb mate pair libraries routinely
- Up to 50 kb libraries with expertise