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APPLICATION NOTE

Megaruptor® genomic DNA shearing for large insert sequencing library preparation

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Introduction

Wageningen University has a strong background in plant genome sequencing including contributions to the Arabidopsis, potato and tomato genome research as partner of various international consortia. The sequencing facility of Wageningen University operates several 2nd and 3rd generation sequencing platforms including a Pacbio RS-II.

The rapid developments of this third generation sequencing resulting in continuously increased read length requires a reliable DNA fragmentation into large molecules. The benefit of these large DNA molecules is their ability to span repetitive sequences which appeared to be crucial for de-novo reconstruction of large and complex genomes.

To stay ahead of new developments intended for sequencing library preparations we tested the Megaruptor® from Diagenode for optimizing our library preparation procedure targeting very large DNA molecules used for Pacbio sequencing.For this purpose we used the Megaruptor® using large hydropores, evaluating target fragmentation size, fragment range, reproducibility and the ease in usage.

Shearing results

To test the reproducibility of Megaruptor® DNA fragmentation, gDNA from three plant from the same family (Solanaceae) i.e. tomato, potato and pepper was used. Using large hydropores two size settings per sample were applied, aiming fragmentation in 30 kb and 50 Kb large molecules.

Since DNA shearing is highly dependent on the quality of the input material, unprocessed genomic DNA for each plant was assessed in parallel. As each DNA isolation was done using different protocols, the molecules sizes of the independent gDNA isolates used in this test showed size ranges typically obtained from different plant gDNA isolations (40 to →100 Kb) and thus reflecting a realistic sample assortment.

The input material of tomato consisted mainly of HMW DNA with a small fraction of degradation. The input DNA of potato however appeared to be already degraded with peak fragment size around 50 Kb. Finally the pepper DNA could be regarded as an intermediate quality sample containing both HMW and partial fragmented DNA .

Megaruptor® sheared DNA clearly showed that the obtained fragment sizes are highly comparable between the three species and thus is independent on the initial input DNA. All Megaruptor® DNA fragmentations performed clearly resulted in a small range of DNA fragment sizes with the aimed peak fragment size of 30 or 50 Kb (Figure 1) except for the slightly

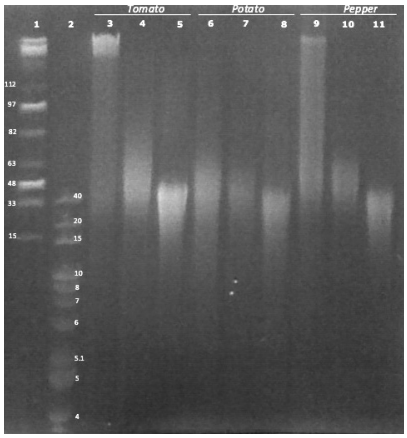

Figure 1: Field-inversion gel electrophoresis (FIGE) of unprocessed and Megaruptor® fragmented DNA.

Samples were loaded on 1% Megabase Agarose gel (Bio Rad) and analysed using a PippinPulse device (Sage Science). Two different DNA ladders (kilobase size numbers next to individual bands) were used for size comparison (lane 1; NEB Midrange PFG Marker, lane2; Invitrogen 1kb DNA Extension Ladder). Unprocessed gDNA (lane 3,6,9), Megaruptor 50kb (lane4,7,10)and 30 kb (lane 5,8,11) fragmentation of tomato potato and pepper respectively.

smaller potato DNA fragmentation using the 50 kb settings due to the insufficient input molecule size of this sample.

All test described above have been performed with non-disposable large hydropores. Meanwhile disposable large hydropores were released. Therefor we tested additional fragmentation of tomato gDNA using disposable hydropores. For this, the aimed peak fragmentation size was again set at 30 and 50 kb. Resulting fragmentation was compared with the previously sheared tomato gDNA. The tomato gDNA sheared with both types of large hydropores clearly show a comparable fragmentation (Figure 2).

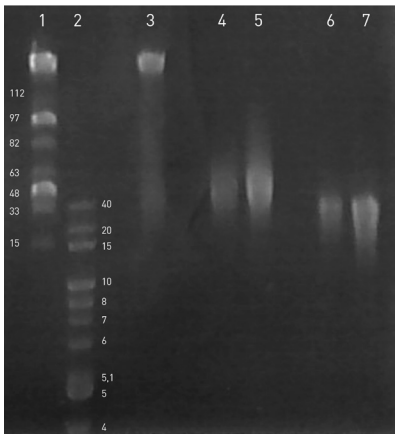

Figure 2: Comparison of fragmentation using different Megaruptor® Large assembly-types. Field-inversion gel electrophoresis (FIGE) of unprocessed and fragmented tomato DNA. Samples were loaded on 1% Megabase Agarose gel (Bio Rad) and analysed using a PippinPulse device (Sage Science). Two different DNA ladders (kilobase size numbers next to individual bands) were used for size comparison (lane 1; NEB Midrange PFG Marker, lane2; Invitrogen 1kb DNA Extension Ladder). Unprocessed gDNA (lane 3), Megaruptor 50kb from non-disposable hydropore (lane4), 50kb from disposable hydropore (lane5), 30 kb from non-disposable hydropore (lane 6) and 30kb from disposable hydropore (lane7).

To assess the versatility of Megaruptor® shearing prospects to get smaller DNA fragments we applied disposable small hydropores to process tomato gDNA aiming 6 and 8 kb fragmentation sizes.

For this medium size fragmentation we compared the Megaruptor® results with results obtained from Covaris gTubes very popular and frequently used for this purpose. Fragmentation results were visualized on a BioAnalyzer 7500 chip (Agilent Technologies, Figure 3).

The size distribution of both methods used are very comparable. Moreover, fragment sizes corresponded well to the aimed peak size and showed a small size range.

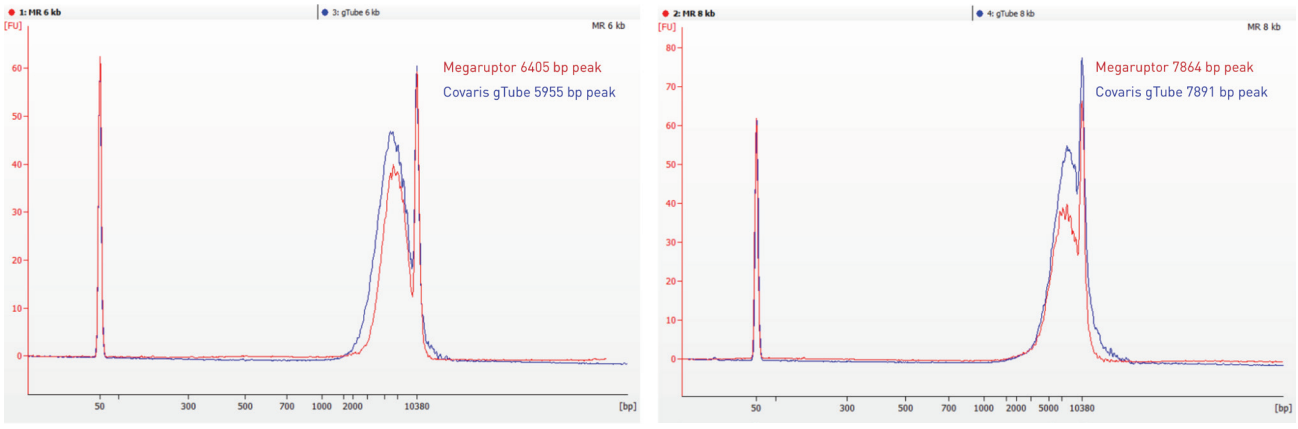

Figure 3: Fragmentation size analysis.

Electropherogram of DNA fragments analysed on a BioAnalyzer 7500 DNA chip showing Megaruptor® sheared gDNA (red) and Covaris gTube sheared DNA aiming 6 kb and 8 kb fragmentation (upper panel and lower panel respectively).

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Conclusions

Our assessment clearly showed that the Megaruptor® has a broad range of DNA fragmentation applications resulting in tight ranges of desired fragment sizes. Our findings obviously show that the Megaruptor® is able to shear gDNA samples from different sources in a highly reproducible way.

The Megaruptor® instrument is very easy to use with a fully automated operation and intuitive software making it possible to shear one or two samples with very little actual hands-on time.

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