2010

Geno/Grinder®

Tissue
Homogenization
Cell Lysis

APPLICATION NOTE

Effect of Lysis Time and Other Variables on DNA Extraction from Fresh Basil Lysed in 2 ml Tubes With the **Geno/Grinder®**

With kind permission of Sunrise Science, San Diego, CA

ABSTRACT

Mechanical cell lysers are used for effective extraction of genomic DNA from samples containing plant or animal tissue. However, all mechanical lysers do not provide the same outcome. The 2000 Geno/Grinder® was compared with a competitive cell lyser (Competitor A) for extraction of DNA from fresh basil. In addition, homogenization time and operating rate were varied for the Geno?Grinder, while buffers, grinding media and tube size remained constant. Results indicated that use of the Geno/Grinder provided DNA with higher molecular weight than the competitive instrument. In addition, optimal homogenization conditions using the Geno/Grinder were found to be 90 sec. at a rate of 2000 cyc/min.

SAMPLE SETUP

:: TUBES:
2 ml conical-bottom screw-cap tubes on a standard 48 well rack.

:: GRINDING MEDIA:
Garnet and single 1/4 inch ceramic bead ("Lysing Matrix A").

:: SAMPLE:
100 mg fresh basil leaf, harvested from live plant immediately prior to lysis.
>1 gram of leaves were chopped into small pieces and mixed. 100 mg of chopped leaf mixture was placed into each of 10 tubes.

:: LYSIS REAGENTS:
400 ml of Buffer Ap1 and 4 ml of RNaseA from commercially available genomic DNA isolation kit were added to each tube.

:: HOMOGENIZATION:

Tube #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Homogenizer*	CA	CA	GG	GG	GG	GG	GG	GG	GG	GG
Time (sec)	40	40	40	40	60	60	90	90	120	120
Speed (cyc/min)	6000	6000	2000	2000	2000	2000	2000	2000	2000	2000

*CA = Competitor A Instrument
*GG = Geno/Grinder® Instrument (SPEX SamplePrep LLC)

DNA PURIFICATION

SPEX SamplePrep®

:: APPLICATION NOTE SP017:
Lysis Time and Other Variables on DNA Extraction

:: APPARATUS:
Geno/Grinder® 2010

:: APPLICATION:
DNA Extraction from Fresh Basil Lysed

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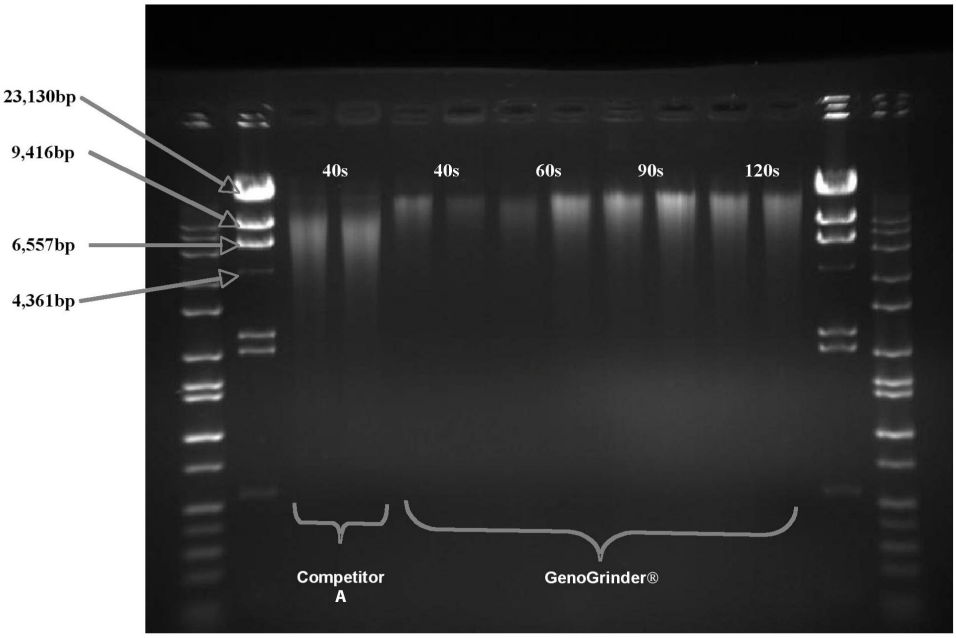
Post-lysis purification was performed using a commercially available plant kit according to the kit manual.

DATA



Outside Marker (lanes 1 and 14): Kb ladder
Inside Marker (lanes 2 and 13): Lambda Hind III

INTERPRETATION

After homogenization for 40 sec. using the competitive cell lyser, DNA of lower molecular weight (lower quality) was obtained than for samples processed at any of the four run times using the Geno/Grinder.

At both 90 and 120 sec., nearly all the DNA isolated using the Geno/Grinder was higher than 6,557 bp, indicating that significant shearing or degradation had not occurred, even at the longer processing time. Little to no difference in the results was observed at these two processing times. While run times for 40 and 60 sec. on the Geno/Grinder yielded high molecular weight DNA, insufficient quantities were obtained.

CONCLUSIONS

The best conditions for processing fresh basil were found to be 90 sec. at 2000 cyc/min with the 2000 Geno/Grinder. Increasing the processing time to 120 sec. did not appear to offer any benefit. In addition, use of the Geno/Grinder gave higher quality DNA than was obtained using the competitive lyser.

Further optimization of the processing time may be possible by varying the type and size of grinding media. However, that was beyond the scope of this project and was not attempted.

Optimal lysing conditions for the re-designed 2010 Geno/Grinder may differ somewhat from those reported here.

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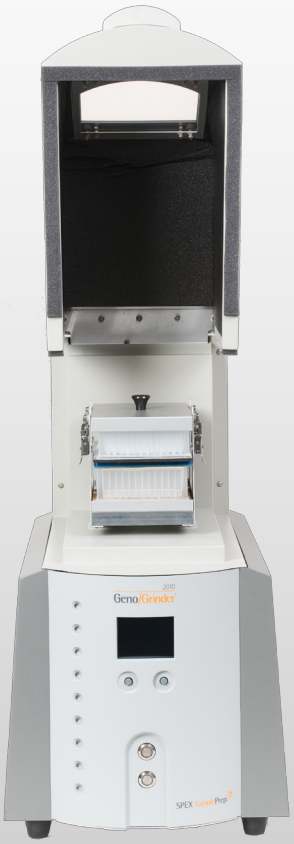

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