

# APPLICATION NOTE

## Extraction of Nucleic Acids from Sugar Beet Leaves

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Within the framework of a TILLING project (Targeting Induced Local Lesions In Genomes) nucleic acids from leaf material are to be isolated from potential mutants. First a representative range of EMS (Ethyl methane sulfonate)-mutants is produced from 2000-5000 M2 plants and identified through TILLING. TILLING technology is a new and very effective method of reverse genetics for the production and identification of loss/gain of function-mutations of commercially valuable genes without genetic modification. To establish a TILLING platform with automated DNA extraction and PCR amplification of target genes, nucleic acid extraction of consistent yield and good quality is necessary. The effective detection of point mutation occurs via hetero duplex formation between DNA molecules of wild type and mutant.

### Experiment

During the first project year, DNA from 2688 individual plants was isolated by using a 96-well micro titer plate kit. (NucleoSpin Multi-96 Plant, Machery & Nagel, Düren). The plant tissue (0.5-1 g) was freeze dried in a 2ml Eppendorf vessel and then ground in the Geno/Grinder 2000 with two metal balls at a setting of 1000 (1000 strokes min<sup>-1</sup>) for 2 x 1 minute. For this, four mounts (plastic blocks) were produced in-house, each holding 24 Eppendorf vessels, and clamped into the Geno/Grinder. This type of sampling allows a flexible configuration of the individual samples. After grinding the tissue to a fine powder the samples were charged with

300 µl C1-buffer and homogenized in the Geno/Grinder for 30 seconds at a setting of 1000 (1000 strokes min<sup>-1</sup>).

Following lysis, the centrifuged samples are transferred into micro titer plates (round wells). After mixing, filtration, several rinse steps and drying the samples are finally eluted. DNA quantification is carried out by photometry and gel electrophoresis. Afterwards the samples can be stored long-term at -20°C.

### Yields

Approximately 40% of the samples yielded between 100 and 1500ng DNA. Only 2% of the samples did not yield an adequate amount of DNA. The maximum yields were around 16 µg. For a routine PCR 1ng of high-pure DNA is used. A further reduction of the DNA amount is possible. The selection of the mutant population is carried out with DNA pools. With a pool composition of 8 different samples the utilized DNA amount is reduced to 0.125ng/individual plant so that with 100ng DNA, the selection of DNA pools with 800 different target sequences becomes possible. The in vitro reproduction of linear DNA with GenomiPhi (GE Healthcare) allows further DNA analyses with small sample sizes.

Application Note SP011:  
**DNA Extraction**

Apparatus:  
**Geno/Grinder®  
2000**

Application:  
**Leaf Material**



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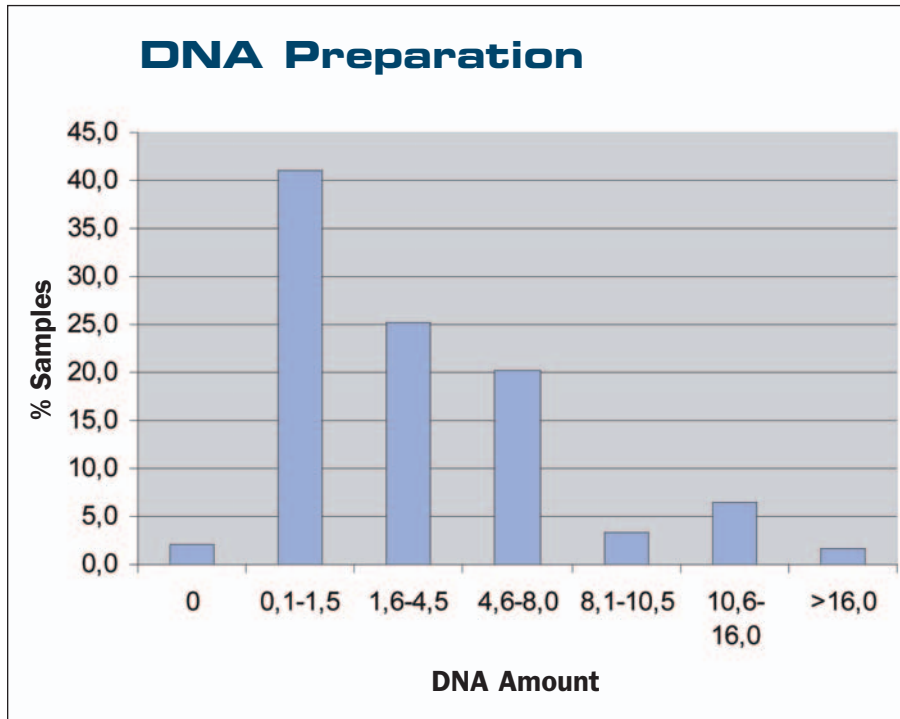


Figure 1. DNA yields from sugar beet leaf material using the Geno/Grinder 2000 and a 96 well micro titer plate kit (NucleoSpin Multi-96 Plant, Machery & Nagel). => **98% of the samples achieved good yields.**

### Conclusion

Homogenization of sugar beet leaves for the purpose of extracting and isolating DNA for subsequent PCR amplification requires a sufficient amount of good quality nucleic acid yield (>1ng). With a basic grinding process of 2 x 1 minute grinds of dry leaf material and subsequent wet homogenization of 30 seconds only 2% of more than 2600 did not yield suitable DNA material. This demonstrates the excellent suitability of the Geno/Grinder 2000 for high throughput processing of sugar beet leaf material.