# Rapid Method for Soy DNA Extraction and Genotyping

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## ABSTRACT

Plant geneticists and seed companies need rapid and cost effective methods to accurately genotype large numbers of samples that are compatible with automated, high-throughput workflows.[1]

Crude plant extraction samples are often high in potent PCR inhibitors, such as polyphenolics and polysaccharides, and light absorbing pigments that, together, lead to a loss of sensitivity and specificity, and poor signal in fluorescence-based quantitative PCR applications.[2] This is particularly true for the current generation of probe-based and high-resolution melt (HRM) SNP genotyping PCR mixes, where both high-quality and high-purity DNA are critical.[3]

Here we present a rapid and inexpensive DNA extraction method developed by an international seed company. DNA extracted from soybean leaf and seed using this method was used directly in genotyping assays. The extracted DNA was tested in both TaqMan® SNP genotyping and HRM assays using reagents from Applied Biosystems, Bioline, KAPA Biosystems and Roche.

Only the SensiFAST™ Genotyping Kit and SensiFAST™ HRM Kit from Bioline were able to both amplify and correctly type the plant samples. The advanced buffer means that minimal optimization is required to achieve fast, reproducible and accurate results, thus providing a cost-effective and fully automated method to genotype large numbers of soybean and other plant samples.

## METHODS

DNA was extracted from soybean samples and subsequently amplified using a probe-based SNP genotyping PCR mix: lyophilized soybean leaves or ground seeds were incubated at 96°C for 10 minutes in 0.5 M NaOH; the alkaline extract was neutralized in Tris-HCl (pH 7.5) solution; 1µl of each neutralized extract was amplified in a 4µl TaqMan-based SNP genotyping PCR (see Fig. 1) using KAPA PROBE FAST qPCR Mix (KAPA Biosystems), LightCycler® 480 Probes Master Mix (Roche), SensiFAST Genotyping Kit (Bioline) and TaqMan® GTXpress™ Master Mix (Life Technologies).

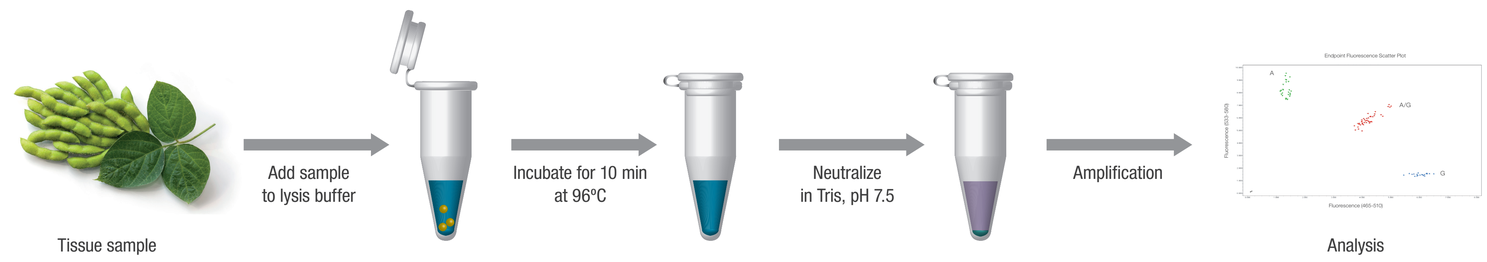


**Fig. 1 Overview of workflow.** The amplification and SNP genotyping analyses were done using the Roche LightCycler 480 II platform and software.

HRM genotyping was performed using Bioline SensiFAST HRM Kit on 1µl of a 1/5 dilution of same plant extracts in a 4µl total reaction volume.

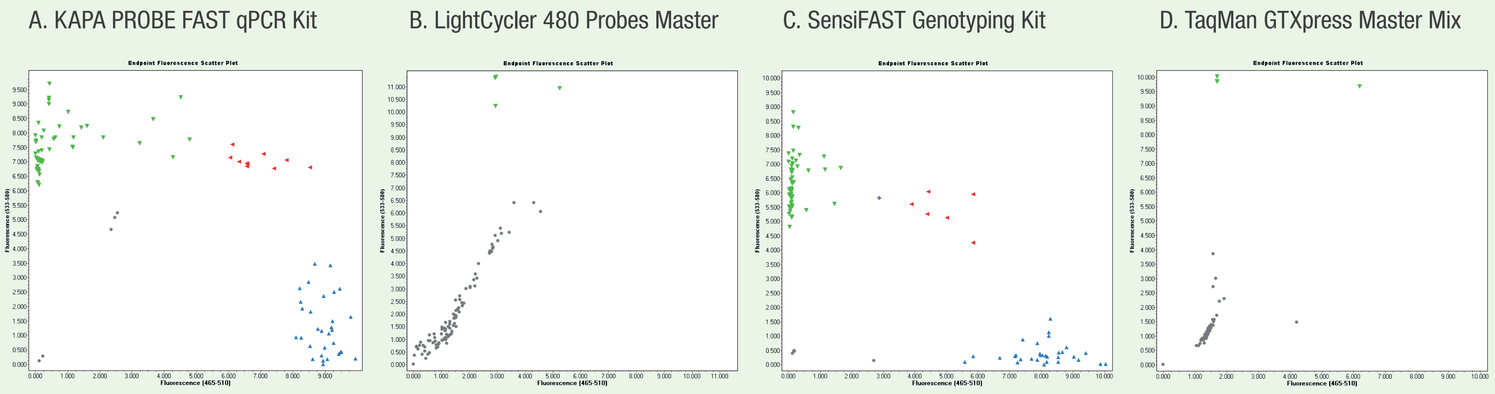


**Fig. 2 Allelic discrimination plots.** The crude samples were split between the four genotyping kits (A-D) and run together in a 384 well plate, included four positive controls and four NTC. The results illustrating the defined clustering of the SensiFAST Genotyping Kit, resulting in higher confidence calling.

## RESULTS

The crude samples using the KAPA PROBE FAST qPCR Kit generated indistinct clusters and the crude samples using LightCycler 480 Probes Master and TaqMan GTXpress Master Mix failed to amplify (Fig. 2). However, all crude samples amplified using Bioline SensiFAST Genotyping Kit and the genotypes were called correctly as were samples for the SensiFAST HRM Kit (data not shown).

## CONCLUSION

We present a rapid, fully automatable DNA extraction method for use on soybean leaves and seeds, which does not involve any manual grinding of leaves or centrifugation steps. When combined with SensiFAST Genotyping Kit or SensiFAST HRM Kit from Bioline, this method allows for a fast and cost-effective way to genotype soybean using either probes or high-resolution melt assays. As minimal optimization is required to achieve these fast, reproducible and accurate results, SensiFAST Genotyping Kit in particular is ideal for all large-scale, high-throughput genotyping projects.

1. Bernardo R. *Crop Sci.* **48(5)**: 1649–1664 (2008).
2. Hartung J.S., *et al. Phytopath.* **86**: 95–101 (1996).
3. Dong C., *et al BMC Plant Bio.* **9**:143 (2009).

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