Addendum: qPCR of DNA Isolated with CTAB, DNeasy®, and Synergy™ Methods

A follow up to the "**Evaluation of the Synergy™ Rapid Plant DNA Isolation Chemistry"** study.

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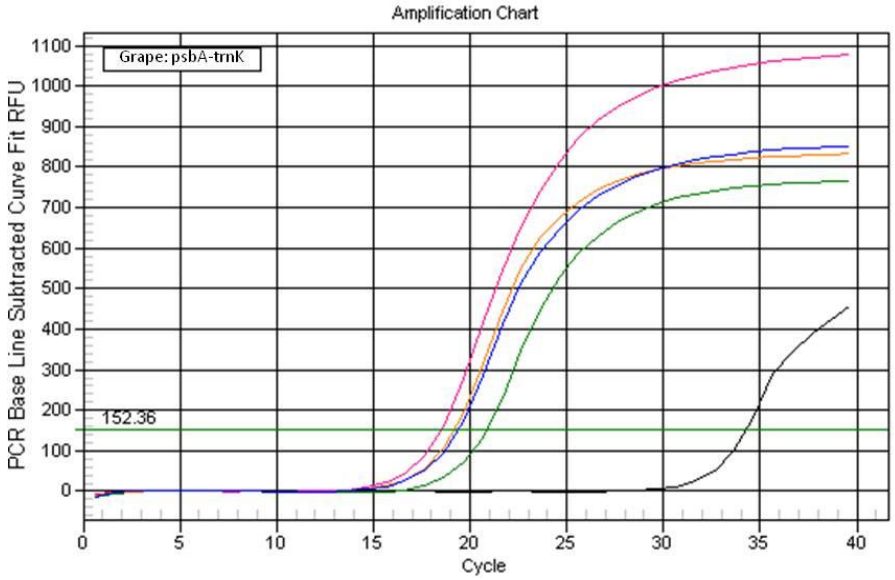
A recent study by OPS Diagnostics examined and compared several methods of isolating DNA from plants (link). Parameters examined were DNA yield, purity (as measured by 260/280 and 260/230 ratios), and whether the DNA would amplify by standard PCR. The study concluded that the Synergy™ Plant DNA Extraction Kit is equal to or out performs a traditional CTAB method and DNeasy® kit for DNA isolation from several plants tested, including rye, rice, corn, sorghum, soybean, wheat, and rape seed. This addendum supplements the previous study by examining plant DNA isolated by the same methods and its subsequent analysis by qPCR. The objective is to assess the relative effect of each DNA isolation method on quantitation.

A standard CTAB method and two commercial DNA isolation kits (DNeasy® Plant DNA Isolation Kit and Synergy™ Plant DNA Extraction Kit) were used to isolate DNA from grape, rice, and sorghum (link). Both the CTAB and DNeasy® methods yield DNA in TE buffer following alcohol precipitation. The Synergy™ Plant DNA Extraction Kit yielded two sample types, namely lysate from the initial purification step and DNA resuspended in TE buffer following alcohol precipitation.

PCR primers and conditions used for qPCR were described previously (link). The reaction was performed on a Bio-Rad Qi5 Real Time PCR System using SYBR Green detection. A SYBR Green cocktail (iTaq Universal SYBR Green Supermix) from Bio-Rad (#172-5120) was used for the reactions (see note).

Results:

Grape: Chloroplast psbA-trnK Intergenic Spacer (HQ108323.1)



Key: **Black Line** = Negative Control, **Pink** = Synergy™ Lysate, **Blue** = Synergy™ Alcohol Precipitated, **Green** = CTAB, **Orange** = DNeasy®

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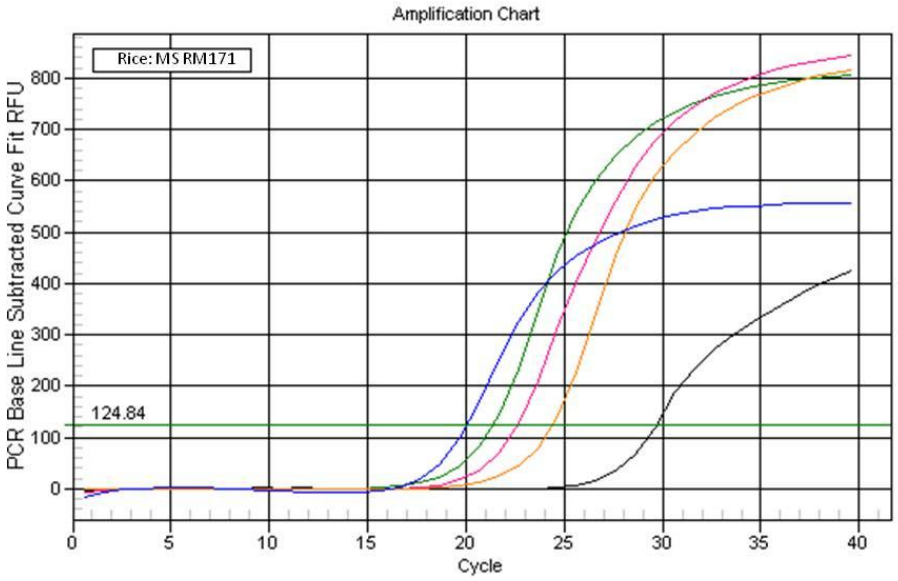
Rice: Microsatellite Sequence RM171 (http://gramene.org)



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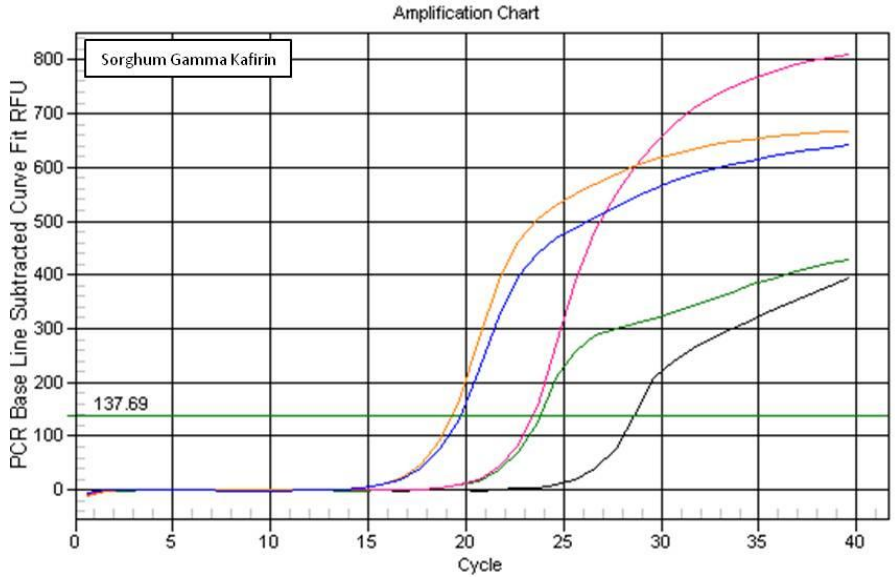
Sorghum: Gamma Kafirin, Promoter MML 4 Region (AY294252.1)



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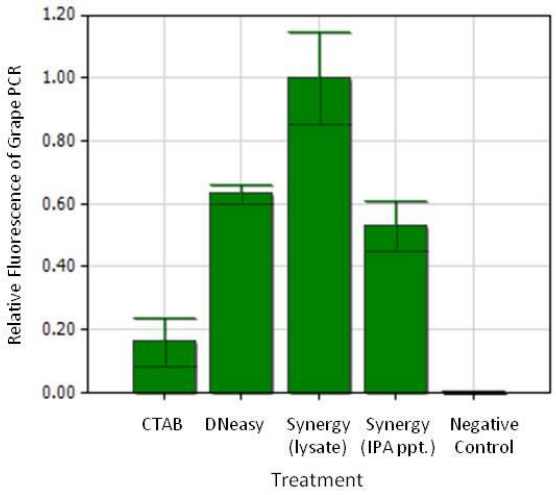
Discussion

The plant kingdom is an incredibly heterogeneous group of organisms, which is illustrated in the diverse, species-specific biochemical composition of plants. In molecular biology, the species-to-species variation in polysaccharide and polyphenol content alone complicates the process of isolating DNA from plants. These biochemicals frequently contaminate DNA preparations and often negatively affect downstream manipulations of plant DNA. The qPCR reactions with grape, rice and sorghum samples clearly reflect the diversity of plants and the graphs illustrate how the differences in biochemistry can affect individual reactions. Furthermore, the methods evaluated in this addendum for isolating DNA from different plant tissues demonstrate that not all methods produce the same results; there is not any one method that shines brightest for all plant species.

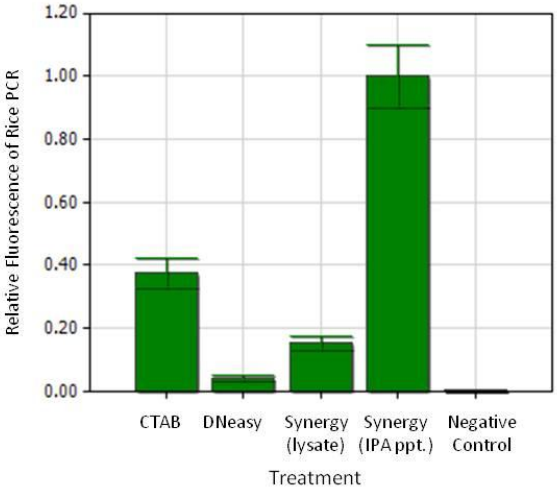
Relative fluorescence of the grape leaf PCR product shows not only that all four reactions were successful, but also that the specific DNA isolation method used clearly impacted results. Synergy™ lysate generated the greatest fluorescence, while DNeasy® and Synergy™ with IPA precipitation followed. Using a traditional CTAB method generated the least signal. When compared to the starting concentration of DNA in each preparation, the relative fluorescence is in order. The concentrations of DNA (ng/ml), in decreasing order, were 36 (Synergy™ lysate), 25 (DNeasy®), 18 (Synergy™ IPA), and 17 (CTAB). With the exception of CTAB, which had low relative fluorescence, the remaining fluorescent signals are very much in proportion with the starting concentration of DNA. Thus, with grape leaf, DNeasy®, Synergy™ IPA and Synergy™ lysate are comparable methods. This is significant as grape is high in polyphenolics and is often a difficult source of DNA. Interestingly, the CTAB method gave less sensitive results though DNA was present in greater concentrations as measured by fluorescence.

Relative Fluorescence of Grape qPCR



The relative fluorescence levels of the rice leaf qPCR suggest significant differences between DNA preparations. Unlike grape, the Synergy™ IPA precipitated sample generated the most robust signal, while the DNeasy® sample was the least robust. The degree of fluorescence, however, correlates well with the order of DNA concentrations. Synergy™ IPA precipitated was the most concentrated (1283 ng/ml), followed by CTAB (659), Synergy™ lysate (235), and DNeasy® (119). Though the order of fluorescence and DNA concentration are correct, the relative level of signal is not strictly proportional in all cases.

Relative Fluorescence of Rice qPCR

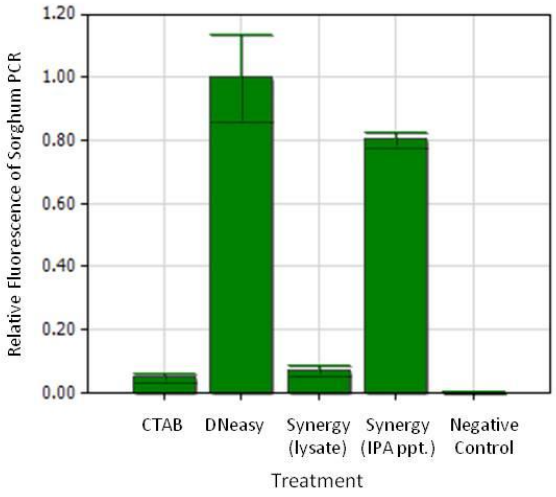


It should be noted that the final concentrations of DNA is highest with Synergy™ IPA precipitated and CTAB (as both have a final precipitation and resuspension step), while DNeasy® requires elution with a large volume of buffer while Synergy™ lysate is the largest volume and typically most dilute. Synergy™ IPA precipitated is concentrated from about 250 µl down to 20 µl. CTAB preparations are resuspended in 20 µl, while DNeasy® DNA is eluted into 200 µl. Consequently, all starting concentrations would be expected to be different.

The set of sorghum qPCRs differ significantly from grape and rice. Clearly, DNeasy® and Synergy™ IPA precipitated reactions demonstrate very strong amplification reactions. However, the starting DNA concentrations do not correlate to the order of relative fluorescence. Starting concentrations (ng/ml) were 776, 27, 237, and 879 for CTAB, DNeasy®, Synergy™ lysate and Synergy™ IPA precipitated, respectively. There is no correlation between relative fluorescence and initial DNA concentration.

A closer examination of previous PCR results for the sorghum gamma kafirin promoter shows that the primer set used for this amplification may exhibit significant non-specific priming. Indeed, PCR in the original study (link), which used the same DNA preparations, showed significant primer dimers or non-specific product from the Synergy™ IPA precipitated sample, yet very low yields from the DNeasy® sample. Consequently, results may be more of a factor of the primer set than DNA isolation method.

Relative Fluorescence of Sorghum qPCR



Conclusion

DNA isolated using CTAB, DNeasy® and Synergy™ methods (lysate and precipitated) generated relative fluorescence for two of the three plant species investigated. Grape and rice displayed fluorescence very much in association with the concentrations within the DNA preparations. This association suggests that all methods produce DNA that can be amplified and yield results relative to the concentration in the tube. Overall yield of DNA between the methods is very different however, as is the time for sample preparation. It must be emphasized that for both rice and grape, Synergy™ lysate was prepared in 5 minutes, as opposed to 90 minutes for DNeasy® and several hours for CTAB. The sorghum qPCR set does not reflect the initial concentrations of DNA in the four preparations. It is believed that non-specific priming may be causing significant background fluorescence. The sorghum reaction requires additional investigation.

Note: qPCR reaction was performed in 25 µl reactions using 12.5 µl iTaq Universal SYBR Green Supermix (Bio-Rad #172-5120) and 12.5 µl of a DNA/primer cocktail (1 ul DNA, 1 µl each of forward and reverse primers (100 pmol/µl), and 47 µl water).