

# OPTIMIZATION OF THE AUTOMATED MAGNESIL® ONE SYSTEM FOR USE WITH INVADER® ASSAYS



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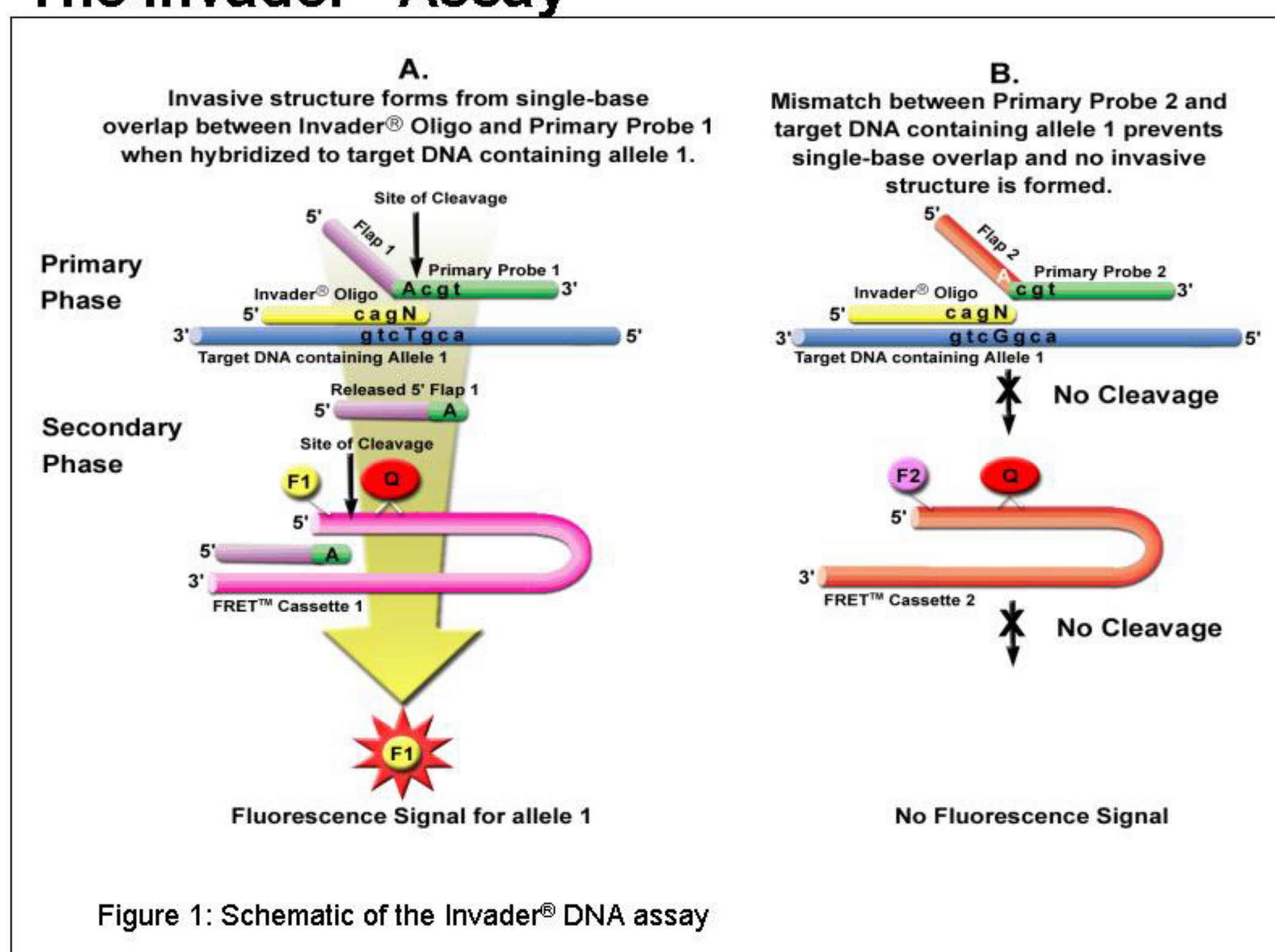
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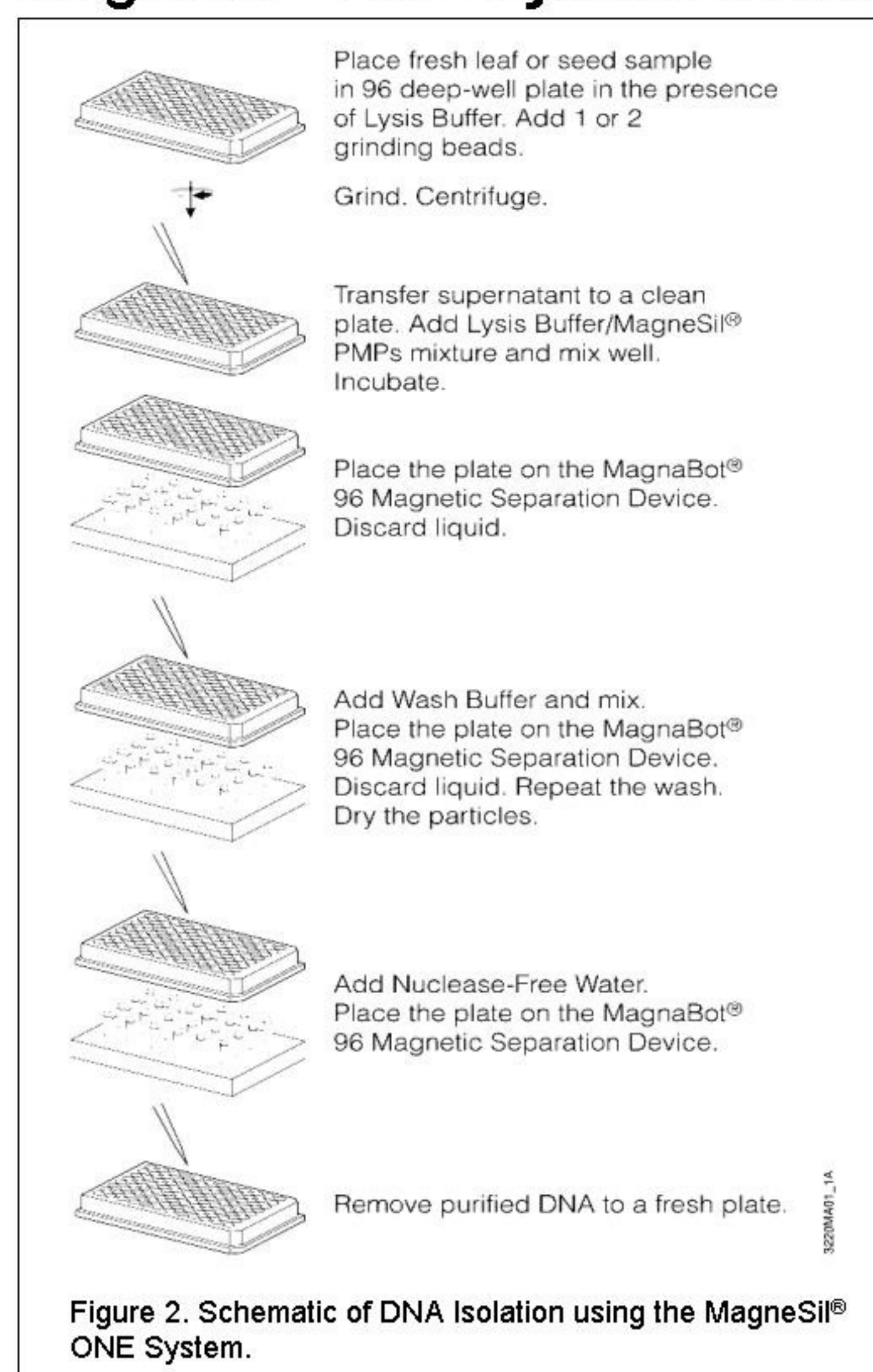
## Abstract

The coupling of genomic DNA purification with SNP interrogation or transgene detection is an integral part of any genotyping technology. Promega and Third Wave Technologies collaborated to provide a streamlined integration between MagneSil® plant DNA isolation and the Invader® technology for SNP and transgene detection. Two magnetic DNA isolation systems were evaluated for use with the Invader® assay. The Wizard® Magnetic Plant DNA isolation system and MagneSil® ONE system are both automated methods using different but compatible chemistries for use on 96 well robotic platforms. Twenty-four variations of the two methods were performed on soybean and corn leaf material, and the isolated DNA was tested in several Invader® assays. Dramatic signal generation differences were seen between the twenty-four variations. Three variants were chosen from the original twenty-four and several varieties of corn and soy were extracted and then tested with Invader® assays. The results showed the greatest compatibility with the MagneSil® ONE system and the Invader® products.

## The Invader® Assay



## MagneSil® ONE System Method



## MagneSil® ONE System

The MagneSil® ONE System MD1370 is a method using paramagnetic particles to isolate purified DNA for highly sensitive assays including Third Wave Technology's Invader® Platform. This system differs from Promega's Wizard® Magnetic 96 DNA Plant System FF3760, designed for PCR applications, in the use of a single detergent lysis buffer and non-porous magnetic particles that bind DNA quantitatively, purifying high quality for Invader Assays. This protocol was developed using a Beckman Biomek® FX robot, and can be adapted to other robotic workstations.

## Invader® assay Results

Nopaline synthase Invader® assay with corn gDNA

Twenty-two variants of Promega's Wizard® Plant DNA Isolation and MagneSil® ONE Kits were performed on both corn and soybean seedlings. The extracts were quantitated using PicoGreen (Molecular Probes, Eugene, OR) and then normalized so that either 80ng of corn gDNA or 35ng soybean gDNA was used. All extracts were used in the Cauliflower Mosaic Virus (CaMV 35S promoter) and Nopaline Synthase terminator (*nos*) Invader® reactions. Conventional variety corn and soy should be represented by internal control signal but not by transgenic (*nos* or CaMV) signal. Therefore, procedures E and R are not desirable. The graph in Figure 4 shows a graph of the conventional variety corn when run in the *nos* Invader® reaction.

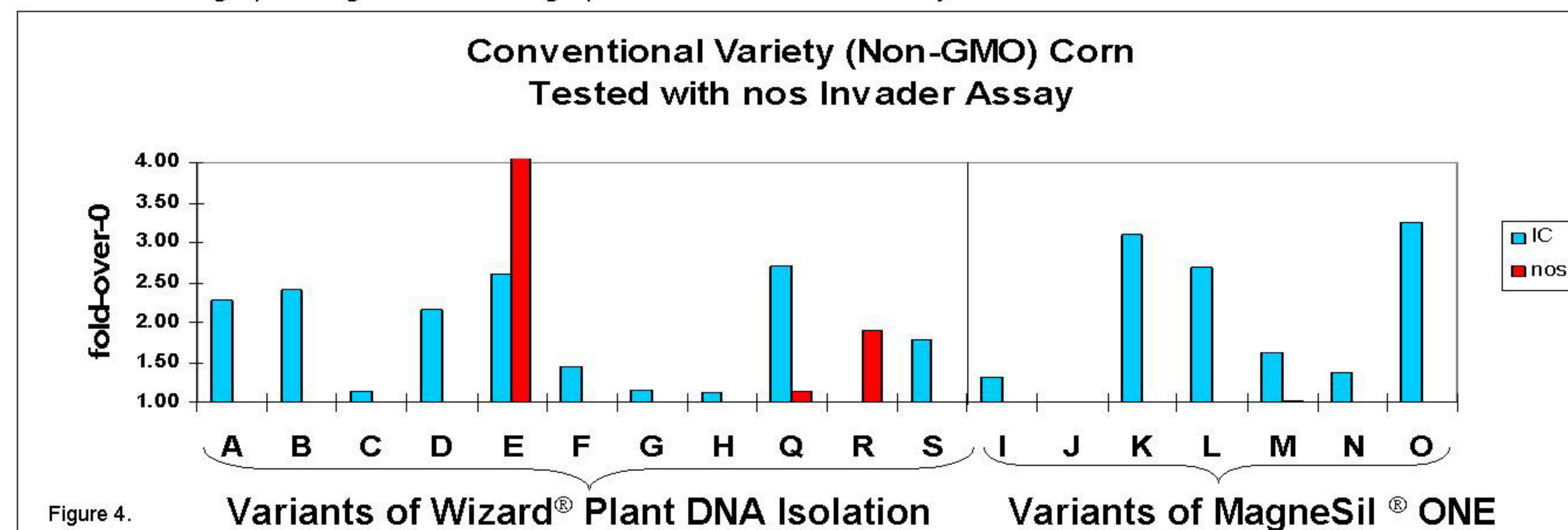


Figure 4. The extracts from variations of the sample prep methods as they performed in the Invader® assay. Blue bars indicate internal control signal, red bars indicate the presence of nopaline synthase. Fold-over-0 is the sample signal divided by the background signal.

## Conclusion

Starting with corn and soybean leaf material, we created twenty-two variants of the Wizard® and the MagneSil® ONE prep methods. We tested these extracts with two Invader® assays, and chose the three best procedures. The three best performing extraction procedures were all variants of the MagneSil® ONE prep method. We tested these three variants further by testing them with more varieties of corn, soy, tomato and pepper plants. We tested cotyledon and leaf material from the tomato and pepper plants (results not shown), and we tested seedling and adult corn and soy leaves. The data pointed us to Procedure K (MagneSil® ONE System) as the overall best performing variant. Details of this method can be obtained from Promega Technical Services: 1-800-356-9526 or [techserv@promega.com](mailto:techserv@promega.com)

## Sample Prep Yield Results

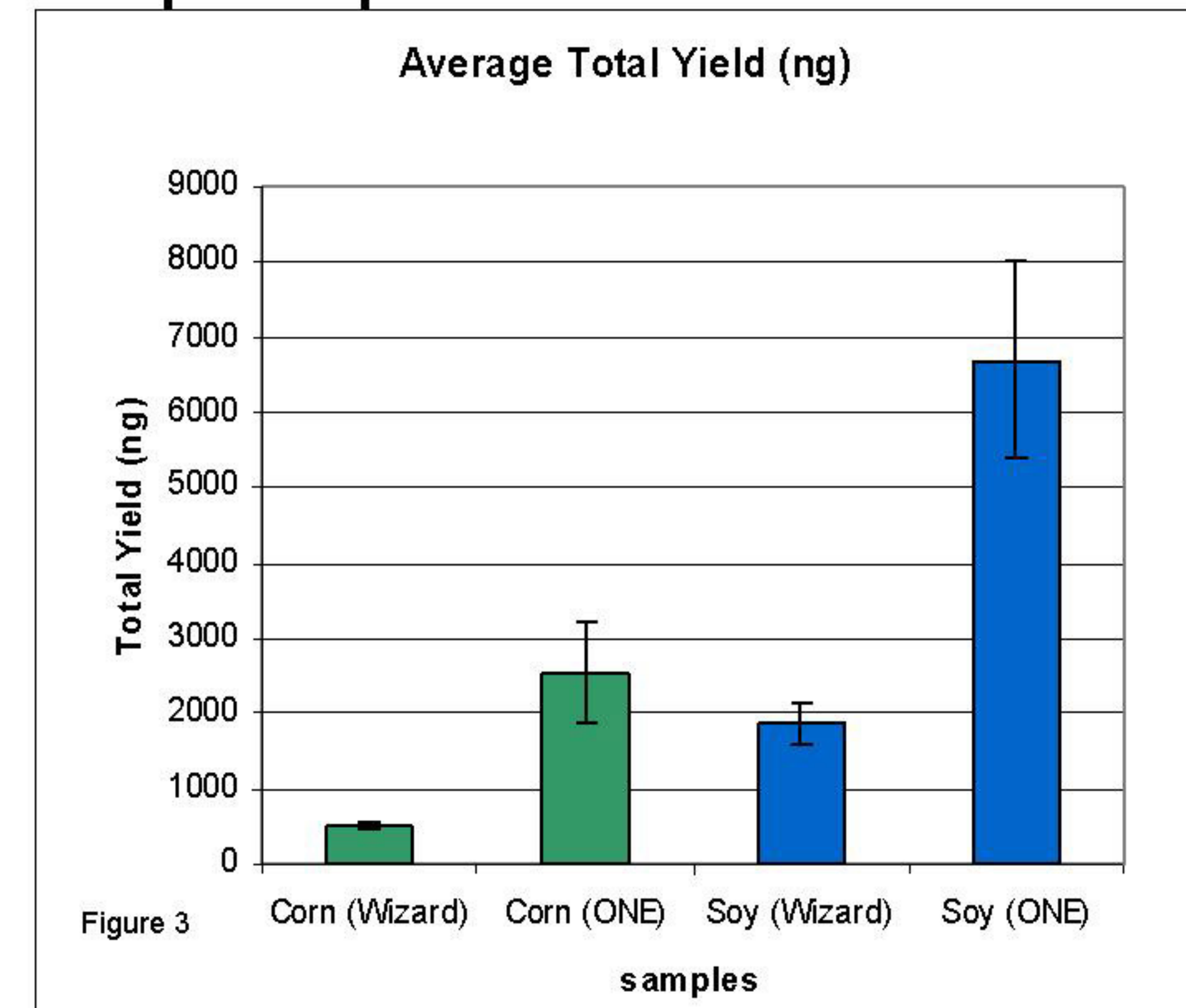


Figure 3. Graph showing the average total yield for the Wizard® and MagneSil® ONE sample prep methods for corn and soy as determined by PicoGreen® (Molecular Probes, Eugene, OR).

## CaMV Invader® assay with soy gDNA

Variants K, L and O of the MagneSil® ONE Kit were the chosen as the best performing sample prep variants. Additional Varieties of corn and soy were grown and extracted using variants K, O and L. Figure 5 shows the results from the soy extracts tested with the Cauliflower Mosaic Virus Invader® assay. The CV (Conventional variety) should have CaMV signal equal to background signal generation. The RR (RoundUp Ready) samples should have CaMV signal. Procedures K (MagneSil® ONE System), O (+RNase A) and L (+DTT) show similar results.

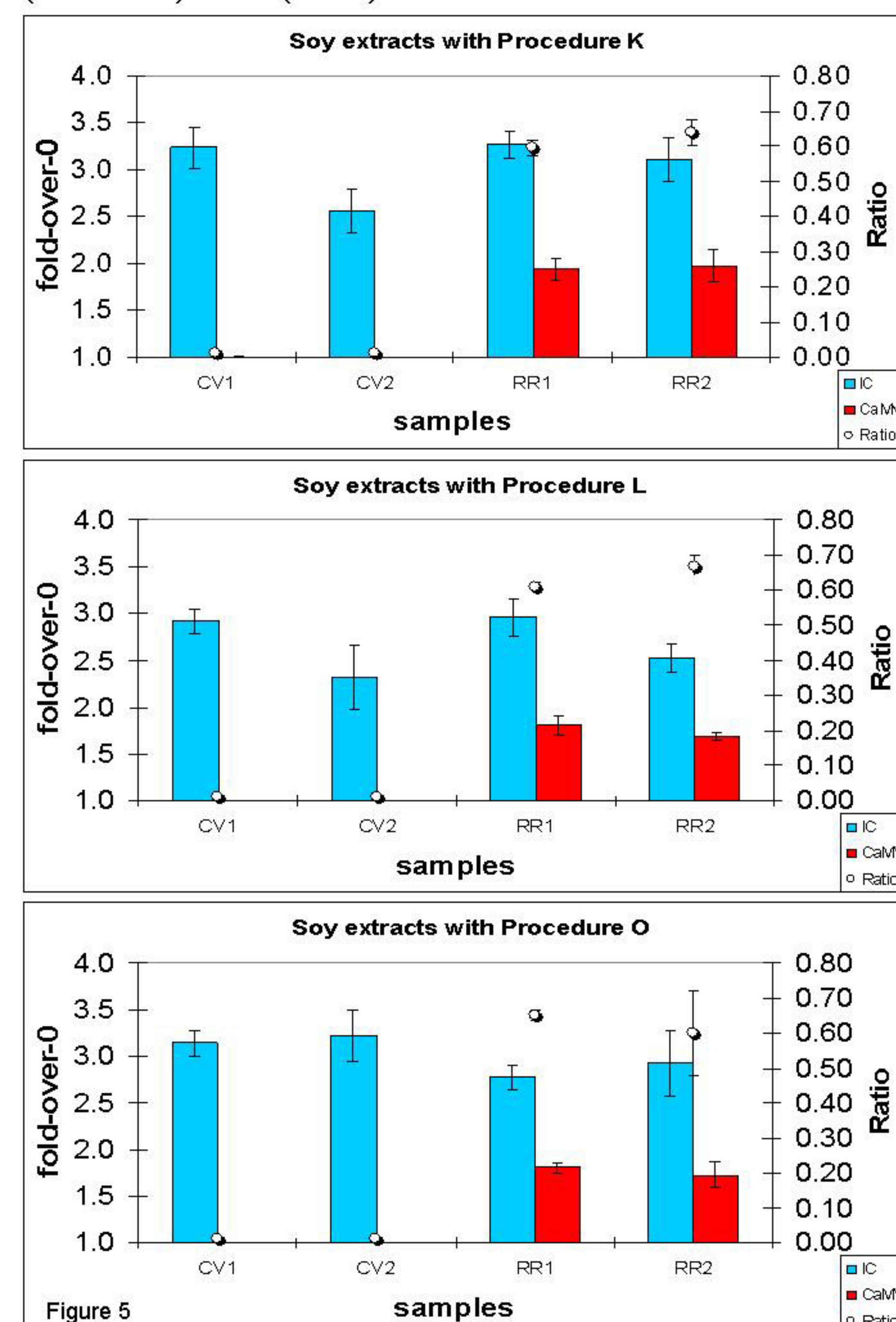


Figure 5. Cauliflower Mosaic Virus (CaMV) Invader® assay tested with soy extracts using variants K, L and O. Ratio calculation is the (fold-over-0 signal of the transgene-1)/(fold-over-0 signal of the Internal Control -1).

U.S. Patent Nos. 6,027,945 and 6,368,800, Australian Pat No 732756 and other patents and patents pending.  
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The Invader® assay is covered by U.S. Patent Nos. 5,846,717; 6,090,543; 6,001,567; 5,985,557; 5,994,069; 6,090,543; 6,348,314; 6,458,535, and various pending applications in the US and internationally.  
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