Development of a High-Throughput Magnetic Bead DNA Isolation Protocol for Dry Cowpea Leaves

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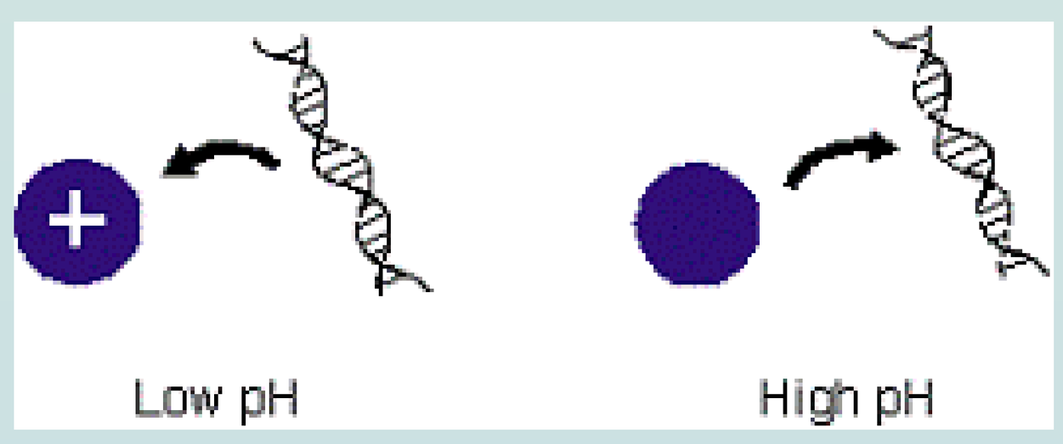
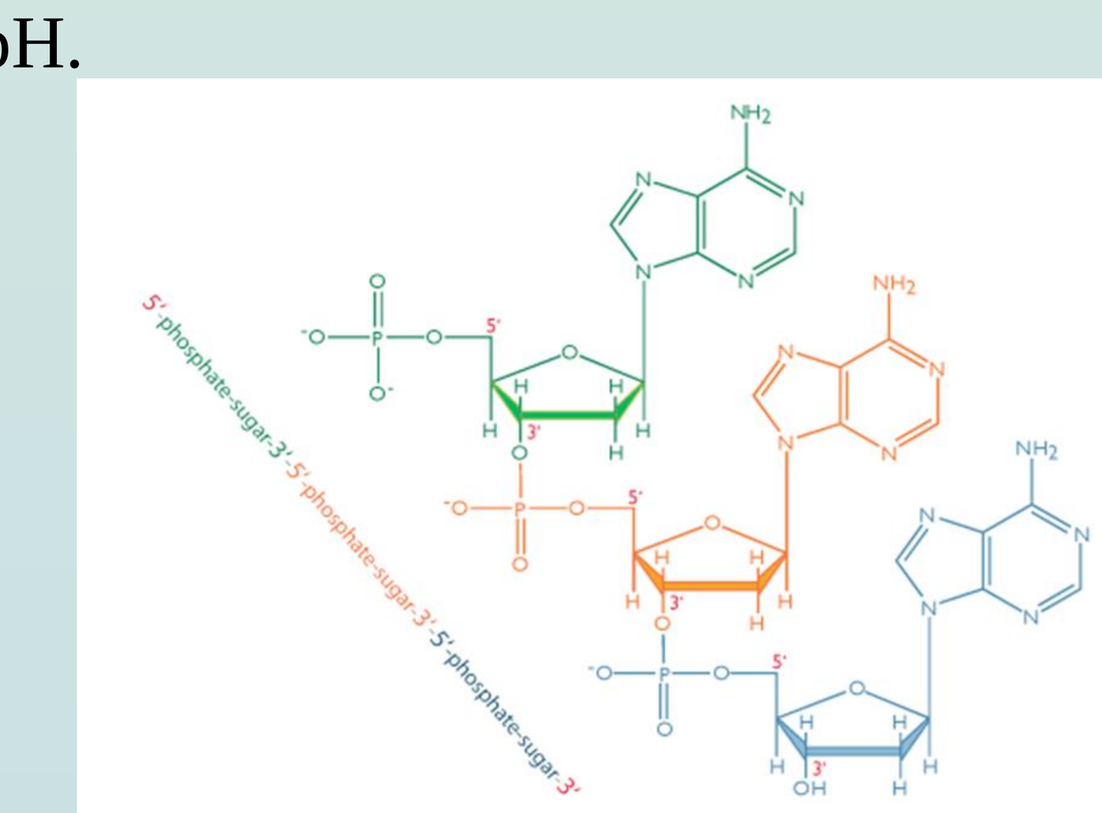
Abstract

New cowpea germplasm must be developed, genotyped, and field-tested to provide West African farmers with varieties exhibiting increased tolerance to biotic and abiotic stress in the face of climate change. Cowpea is a protein-rich staple and source of folate for women and children and is crucial to food security in sub-Saharan Africa. The long-term goal of this research is to accelerate delivery of marker-assisted breeding to West African breeders so they may decrease the breeding cycle and more efficiently develop food security for families in Burkina-Faso, Ghana, Nigeria, and Senegal. The objective of this project is to develop a high-throughput DNA isolation protocol using methods and equipment suitable to West African cowpea breeding programs. By doing so, genotype data of large mapping populations will be made available to expedite mapping and selection of traits. This protocol addresses logistical challenges in processing international cowpea leaf materials through to genotyping. Shipment of transgenic or live plant material from Africa to the U.S. is not authorized, but isolated plant DNA is permitted, for instance. In addition, the cost of reagents is less than predominate DNA extraction kits. The capacity to extract plant DNA on-site will increase the independence and efficiency of collaborations with West African plant breeding programs.

Background

The objective of this research will be achieved by evaluating a commercially available magnetic bead kit adapted to the MiniG tissue homogenizer and V&P Scientific manifold dispenser and vacuum units. These tools increase throughput and automation without the use of expensive robotics. This protocol is competing with a reliable plant DNA kit but attempts to achieve the same workflow and quality at a lower cost. The negatively charged phosphate backbone of DNA is attracted to positively charged magnetic beads at a low pH, and releases them at a high pH.



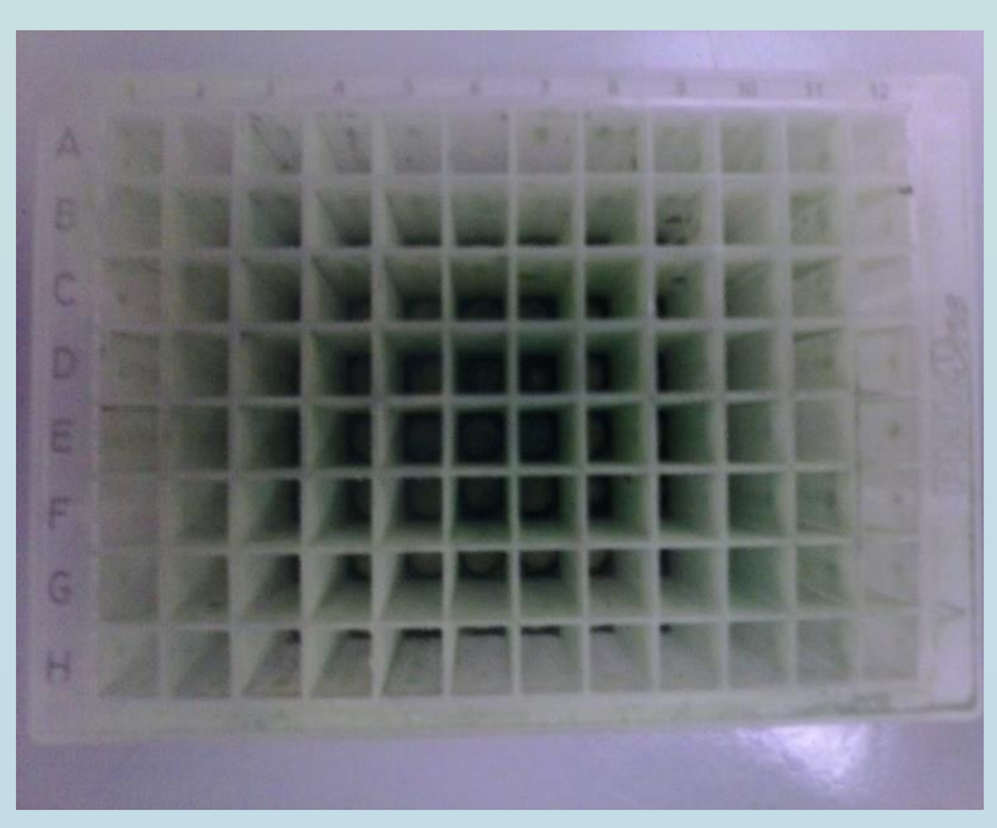


http://cyberbridge.mcb.harvard.edu/dna_1.html

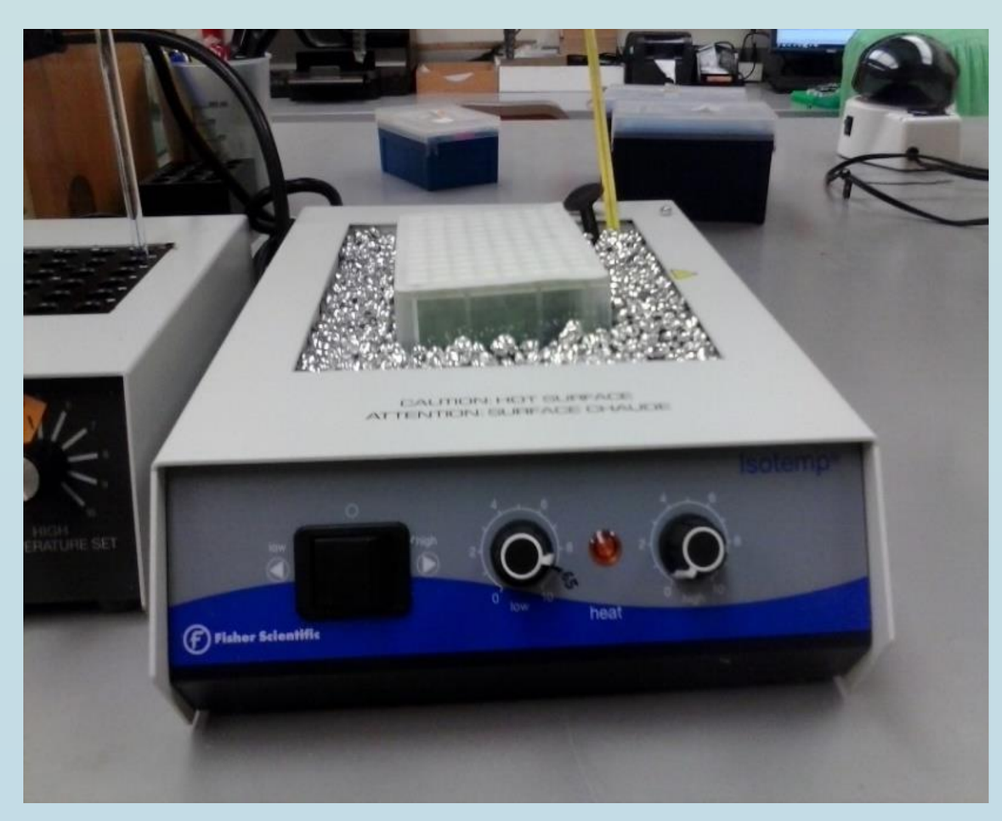
Materials and Methods

Collect & dry leaves

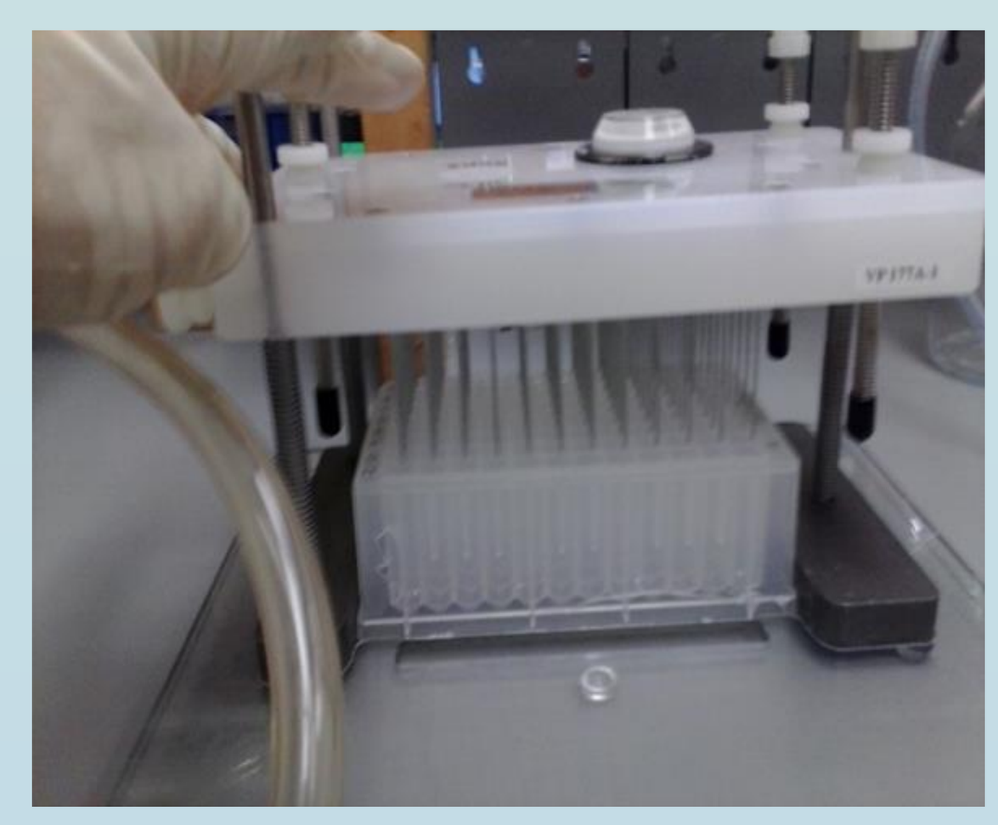
Homogenize tissue



Add lysis buffer



Incubate in dry bath



Bind magnetic beads & aspirate

Perform several washes & elute DNA

1. Collect tissue from young trifoliate leaves and dry them with silica gel. Do not wash leaves with ethanol or water prior to sealing them in a bag with silica gel.
2. Grind dry tissue with the MiniG in a 96-well block.
3. Add lysis buffer and run the plate again in the MiniG for 40 seconds. Incubate the plate in a dry bath at 56° C, not to exceed 60° C.
4. Adjust the pH conditions to pH<6 using the appropriate buffer to bind the magnetic beads with DNA.
5. Perform several washes with ethanol and isopropanol. After each wash, place the 96-well block on a magnetic separation plate and remove supernatant with a vacuum aspirator (V&P Scientific).
6. Adjust pH to pH>8 to release DNA from beads, and elute the final DNA product with 80uL of elution buffer.

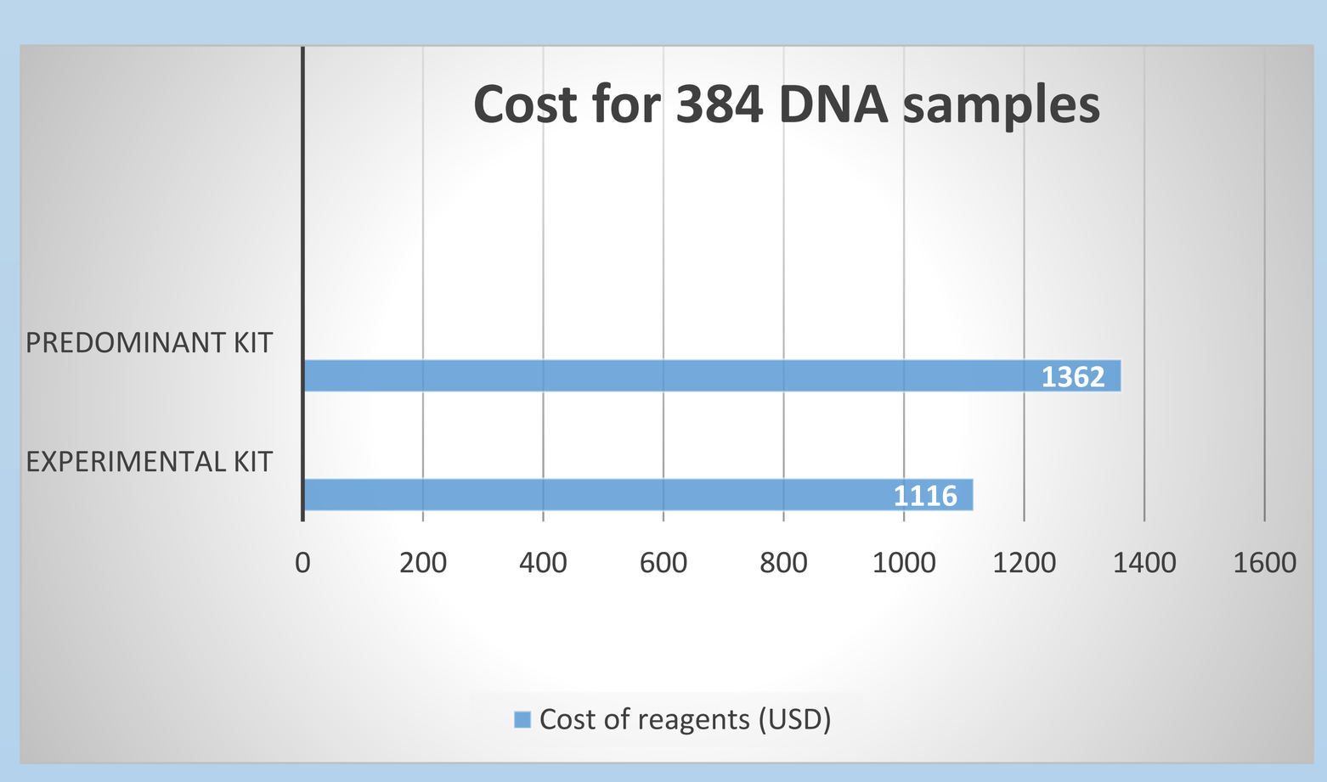
Results

Performance

Reagent	Number genotyped	Number failed	Average % missing
Experimental	76	2	1.25%
Standard Kit	1374	6	1.16%

All of the above samples were genotyped on Illumina iSelect.
A DNA sample *failed* if missing values exceed 20%.

Cost



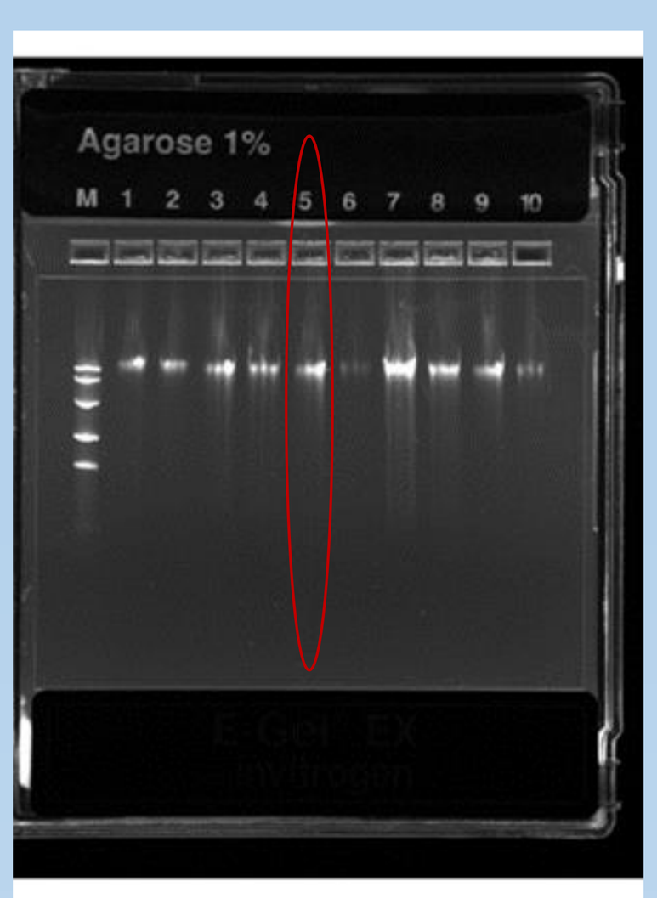
Yield

Global mean	159.5 ng/μL
LSD	53.8 ng/μL

Reagent	Mean
Experimental	173.3 ng/μL A
Standard Kit	111.4 ng/μL B

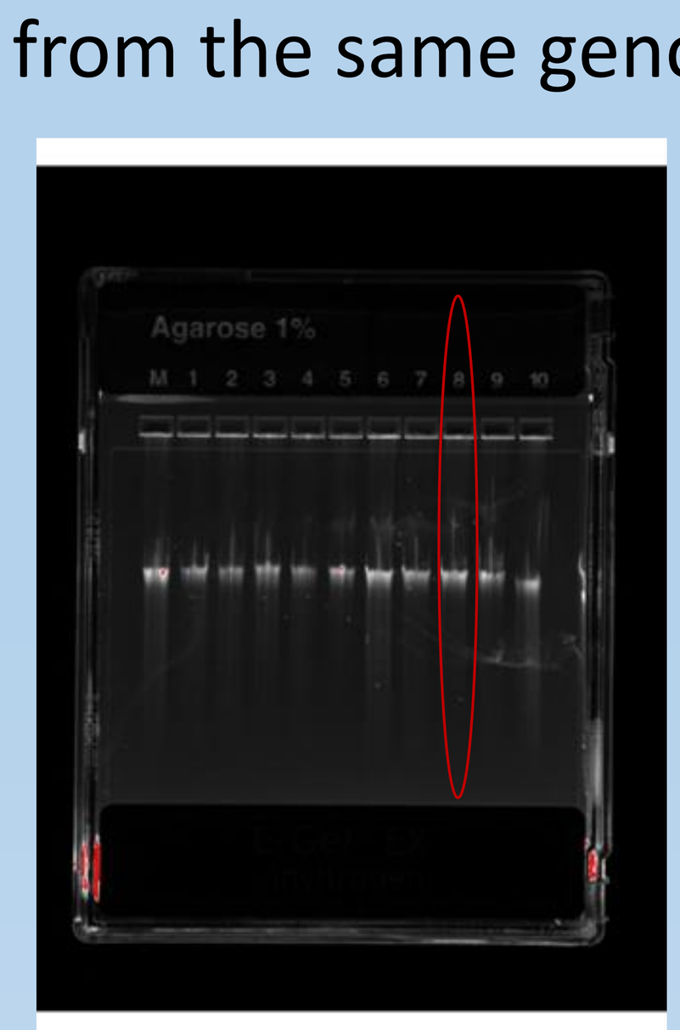
Quality

The bands circled on the gels below are genomic DNA samples from the same genotype isolated by either the Experimental reagent (left) or the Standard kit (right).

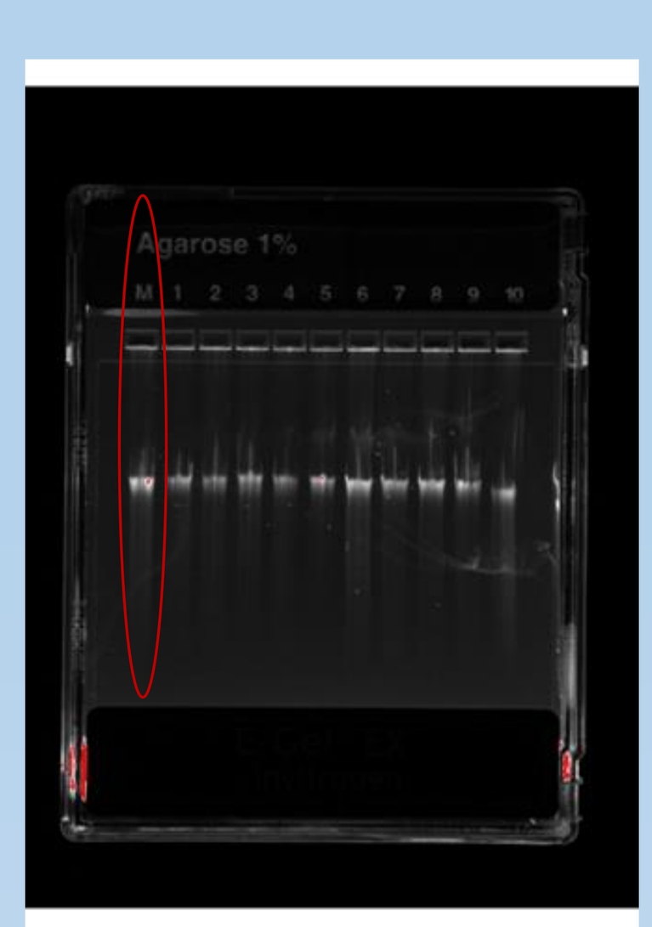


Experimental

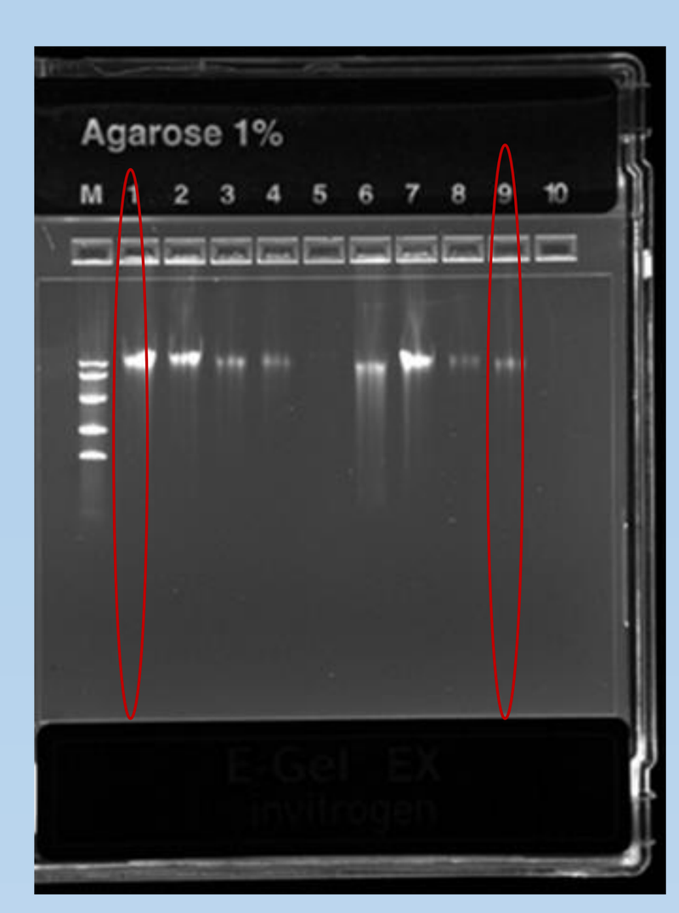
Standard Kit



Experimental



Standard Kit



Experimental

Standard Kit

Conclusion

Magnetic bead DNA isolation can produce 96 cowpea DNA samples in a day at a cost less than common DNA extraction kits. The Standard kit has a run time of approximately three hours for 24 samples, whereas 96 samples can be processed by the Experimental kit in about six hours. The protocol produces high-quality DNA with a concentration range of 70 to 120 ng/μL and applicability to a range of genotyping platforms including Illumina iSelect. The Experimental magnetic bead kit costs 18% less than the predominant Standard kit. There is a slight advantage to the Standard kit in terms of missing data and DNA quality. In light of these results, we conclude that the Experimental magnetic bead kit is comparable to the reliable Standard kit and its implementation can lead to efficiency gains in West African cowpea breeding programs.