PCR and It's Practical Applications

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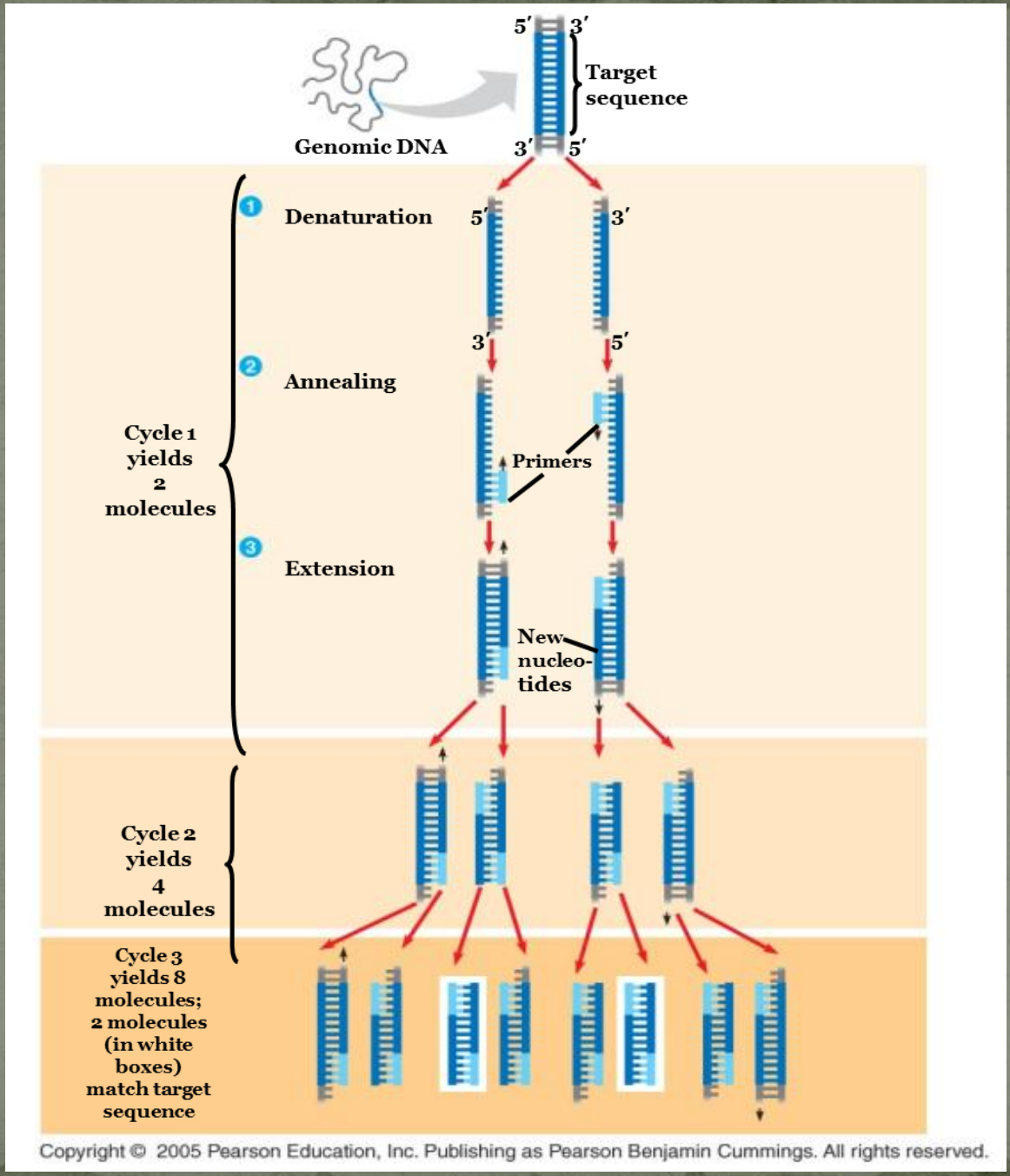
Brief History of Polymerase Chain Reaction (PCR)

- Developed in 1983 by Kary Mullis
  - Novel Idea: to use short complementary sequences (primers) to amplify a small region of a targeted sequence of DNA
  - The practical application came later with the discovery of a thermophilic bacterium known as *Thermus aqauticus*. The heat stable DNA polymerase was used for the PCR reaction
    - 72°C functions optimally
- Thermo cycling machine developed by Mullis' Lab (Cetus Corp of California)

PCR: The Basics

- Three Steps:
  - Heating (Denaturing)
  - Cooling (Annealing of Primers)
  - Extension (Replication of DNA)
- Constituents of the PCR Reaction
  - **Buffer**
  - **Nuclease free Water**
  - ***Taq* Polymerase**
  - **$MgCl_2$**
  - **DNA**
  - **dNTPs** (deoxynucleotide triphosphates)
  - **Primers** (Forward and Reverse)



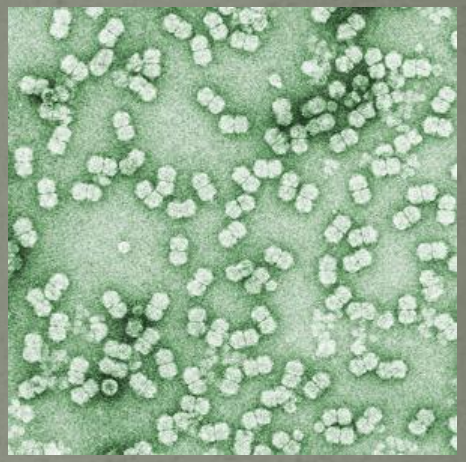
Use of the asymmetric polymerase chain reaction and DNA sequencing to determine genetic variability of the bean golden mosaic geminivirus in the Dominican Republic.

Robert L. Gilbertson, Maria R. Rojas, David R. Russel, and Douglas P. Maxwell

Journal of General Virology, 1991, 72, 2843-2848

Introduction

- Geminiviruses: plant viruses characterized by their shape and their ssDNA
  - Two Subgroups (I and II)
  - Subgroup II
    - Transmitted by *Bemisia tabaci*
    - Affects the common bean plant
      - Bean Golden Mosaic Virus
        - Bipartite Genome: Two DNA Components!
        - High genetic diversity



http://www.flickr.com/photos/ajc1/3306747852/#/

Purpose

- “Because of the diverse nature of geminiviruses that can infect the common bean, there is a need to develop rapid and specific methods for their detection and differentiation”
- Four Different Strains of Bean Golden Mosaic Virus (BGMV)
  - Puerto Rico
  - Guatemala
  - Dominican Republic
  - Brazil

Introduction (cont.)

- DNA Sequence Data on all of the BGMVs for this study (databases)
- Highly Variable region of DNA on Component of DNA-B
  - 300-400 BP
- This can be used two distinguish the different strains of the BGMV from different geographic regions.

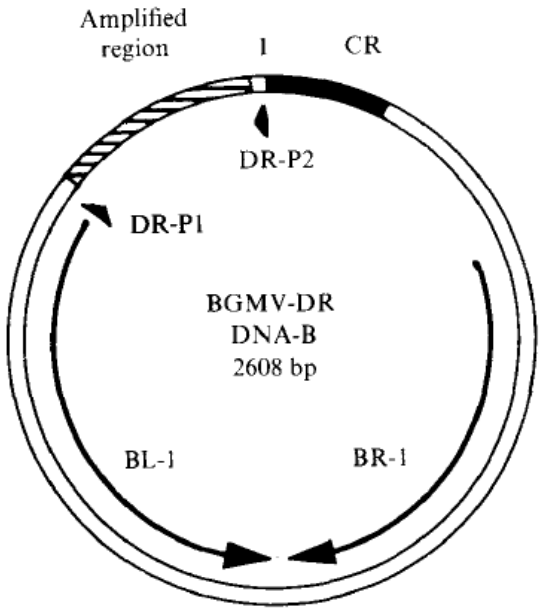


Fig. 1. Diagrammatic representation of the genomic organization of the DNA-B component of BGMV-DR showing the positions of the common region (CR), the two open reading frames (BR-1 and BL-1) and the region amplified for evaluation of genetic variability of BGMV-DR isolates [for the purposes of this figure, the circularized DNA-B insert of pDRB1 (Gilbertson *et al.*, 1991*b*) is shown, with nt 1 corresponding to the first nucleotide of the common region]. The locations at which amplification primers (DR-P1, viral-sense; DR-P2, complementary-sense) anneal to BGMV-DR DNA-B are shown by the arrowheads.

Experimental Design

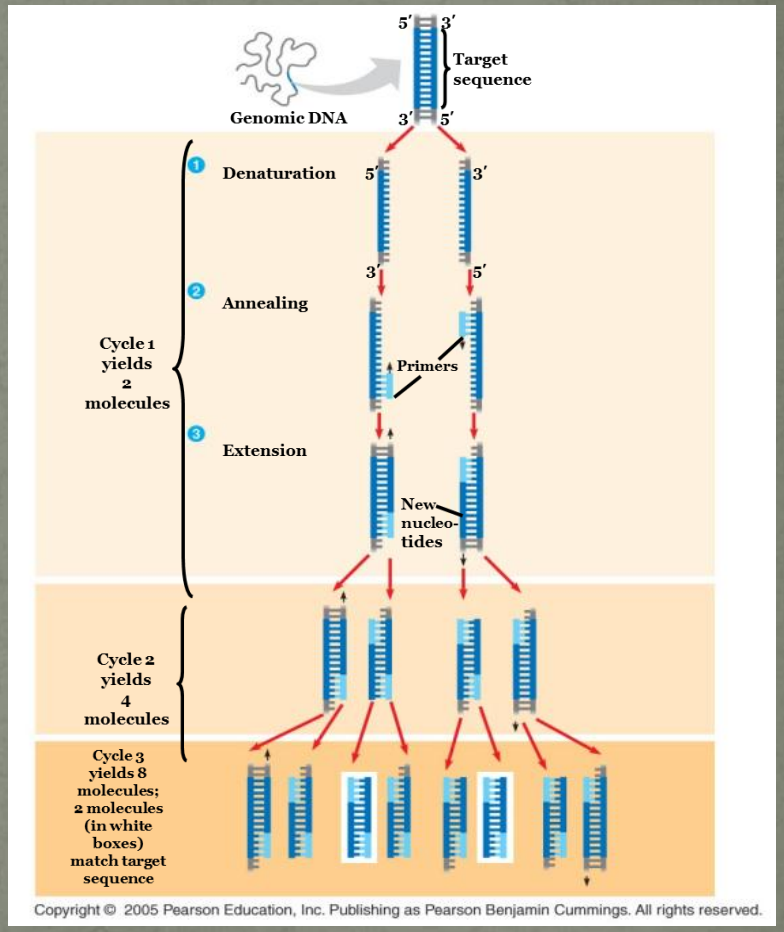
- The authors proposed "to use the DNA sequence of the hypervariable region to determine whether the BGMV-DR infectious clones are representative of BGMV isolates in the Dominican Republic and to assess variability among the field isolates of BGMV."
- Basic Procedures:
  - DNA Extraction
  - PCR
  - Asymmetric PCR
  - DNA Sequencing

Methods

- Original field isolates of BGMV from the Dominican Republic had the DNA-B component cloned and inserted into the recombinant plasmid pDRB1
- 5 Samples from different locations were taken within the Dominican Republic
- DNA was extracted from the plant materials
- PCR of the hypervariable region
  - Primers were designed using the sequence present in the pDRB1 plasmid
  - Asymmetric PCR of hypervariable region

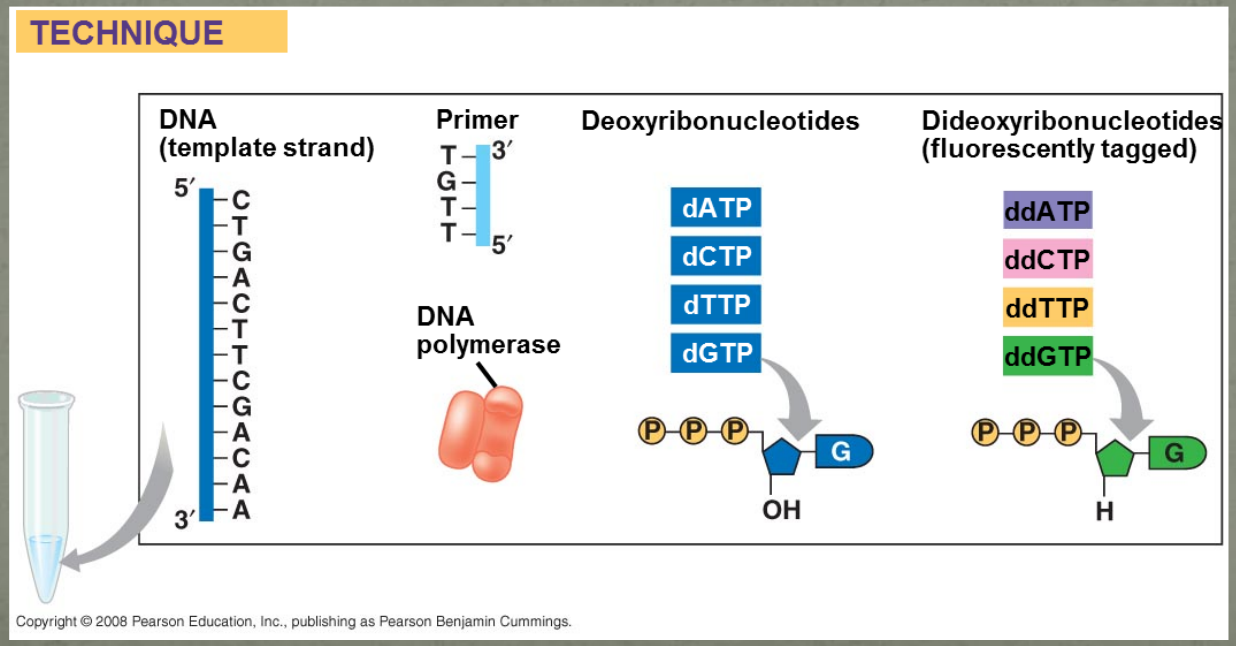
Asymmetric PCR

- Similar reaction set-up as in the normal PCR reaction.
- Only difference is in the primer components
  - 50:1 ratio of the reverse primer to the forward
  - Creates a large quantity of single stranded DNA

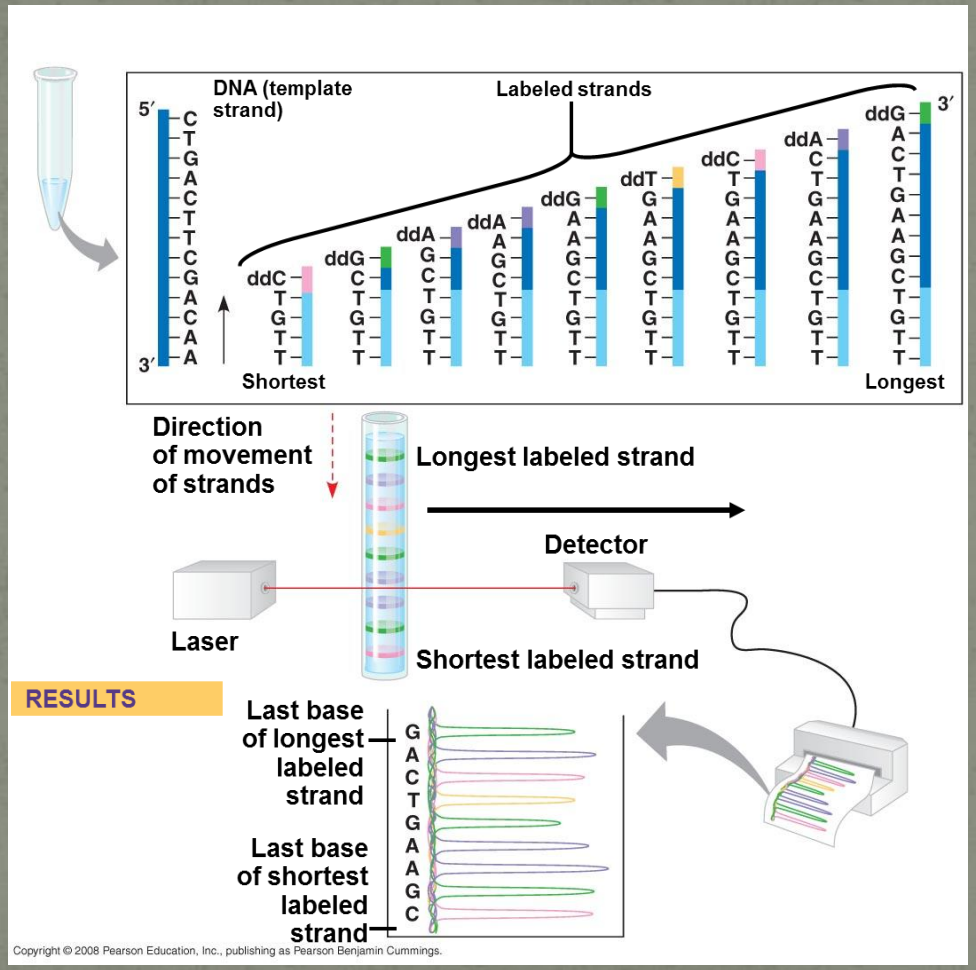


DNA Sequencing

- Dideoxynucleotide Chain Termination Method
  - Template Strand (A-PCR)
  - Labeled and non-labeled nucleotides
  - Primer (same for PCR Reaction)

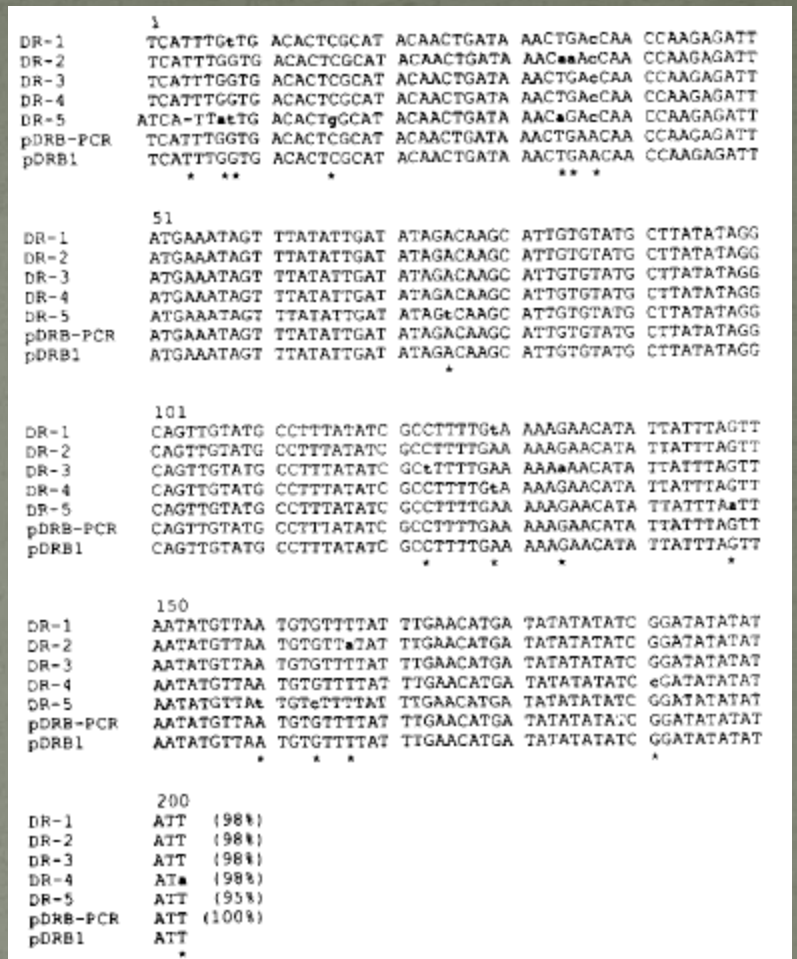


DNA Sequencing (cont.)



Results

- Figure 2 (Right) shows the DNA sequences produced after using the Dideoxynucleotide Chain Termination Method



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                1
DR-1        TCATTTGtTG ACACTCGCAT ACAACTGATA AACTGAcCAA CCAAGAGATT
DR-2        TCATTTGGTG ACACTCGCAT ACAACTGATA AACaaAcCAA CCAAGAGATT
DR-3        TCATTTGGTG ACACTCGCAT ACAACTGATA AACTGAcCAA CCAAGAGATT
DR-4        TCATTTGGTG ACACTCGCAT ACAACTGATA AACTGAcCAA CCAAGAGATT
DR-5       ATCA-TTatTG ACACTgGCAT ACAACTGATA AACaGAcCAA CCAAGAGATT
pDRB-PCR    TCATTTGGTG ACACTCGCAT ACAACTGATA AACTGAACAA CCAAGAGATT
pDRB1       TCATTTGGTG ACACTCGCAT ACAACTGATA AACTGAACAA CCAAGAGATT
                 *  **       *                      **  *

               51
DR-1        ATGAAATAGT TTATATTGAT ATAGACAAGC ATTGTGTATG CTTATATAGG
DR-2        ATGAAATAGT TTATATTGAT ATAGACAAGC ATTGTGTATG CTTATATAGG
DR-3        ATGAAATAGT TTATATTGAT ATAGACAAGC ATTGTGTATG CTTATATAGG
DR-4        ATGAAATAGT TTATATTGAT ATAGACAAGC ATTGTGTATG CTTATATAGG
DR-5        ATGAAATAGT TTATATTGAT ATAGtCAAGC ATTGTGTATG CTTATATAGG
pDRB-PCR    ATGAAATAGT TTATATTGAT ATAGACAAGC ATTGTGTATG CTTATATAGG
pDRB1       ATGAAATAGT TTATATTGAT ATAGACAAGC ATTGTGTATG CTTATATAGG
                                              *

              101
DR-1        CAGTTGTATG CCTTTATATC GCCTTTTGtA AAAGAACATA TTATTTAGTT
DR-2        CAGTTGTATG CCTTTATATC GCCTTTTGAA AAAGAACATA TTATTTAGTT
DR-3        CAGTTGTATG CCTTTATATC GCtTTTTGAA AAAaAACATA TTATTTAGTT
DR-4        CAGTTGTATG CCTTTATATC GCCTTTTGtA AAAGAACATA TTATTTAGTT
DR-5        CAGTTGTATG CCTTTATATC GCCTTTTGAA AAAGAACATA TTATTTAaTT
pDRB-PCR    CAGTTGTATG CCTTTATATC GCCTTTTGAA AAAGAACATA TTATTTAGTT
pDRB1       CAGTTGTATG CCTTTATATC GCCTTTTGAA AAAGAACATA TTATTTAGTT
                                   *    *    *           *

              150
DR-1        AATATGTTAA TGTGTTTTAT TTGAACATGA TATATATATC GGATATATAT
DR-2        AATATGTTAA TGTGTTaTAT TTGAACATGA TATATATATC GGATATATAT
DR-3        AATATGTTAA TGTGTTTTAT TTGAACATGA TATATATATC GGATATATAT
DR-4        AATATGTTAA TGTGTTTTAT TTGAACATGA TATATATATC eGATATATAT
DR-5        AATATGTTAt TGTeTTTTAT TTGAACATGA TATATATATC GGATATATAT
pDRB-PCR    AATATGTTAA TGTGTTTTAT TTGAACATGA TATATATATC GGATATATAT
pDRB1       AATATGTTAA TGTGTTTTAT TTGAACATGA TATATATATC GGATATATAT
                     *    *  *                        *

              200
DR-1        ATT  (98%)
DR-2        ATT  (98%)
DR-3        ATT  (98%)
DR-4        ATa  (98%)
DR-5        ATT  (95%)
pDRB-PCR    ATT (100%)
pDRB1       ATT
            *
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Fig. 2. Alignment of the DNA sequences of the amplified hypervariable region of five BGMV-infected bean field samples from the Dominican Republic and from the cloned BGMV-DR DNA-B (pDRB-PCR), with the previously determined hypervariable region sequence of the BGMV-DR component DNA-B (nt 2279 to 2481 of pDRB1). Variations in nucleotide sequence of the amplified fragments from the pDRB1 sequence are shown as bold lower-case letters, and the positions of these variations with respect to pDRB1 are indicated by an asterisk below the pDRB1 sequence. The percentage sequence identities of the amplified fragment with the pDRB1 sequence are shown in parentheses after the sequence of each fragment.

Conclusions

- The pDRB1 clones are representative of the BGMV isolates collected from the Dominican Republic.
- This approach could be used in future studies to detect as well as identify different strains of geminiviruses.
  - Characterization of the wide range of geminiviruses
- Finally, the authors provide other cases where this method of virus detection could be beneficial.

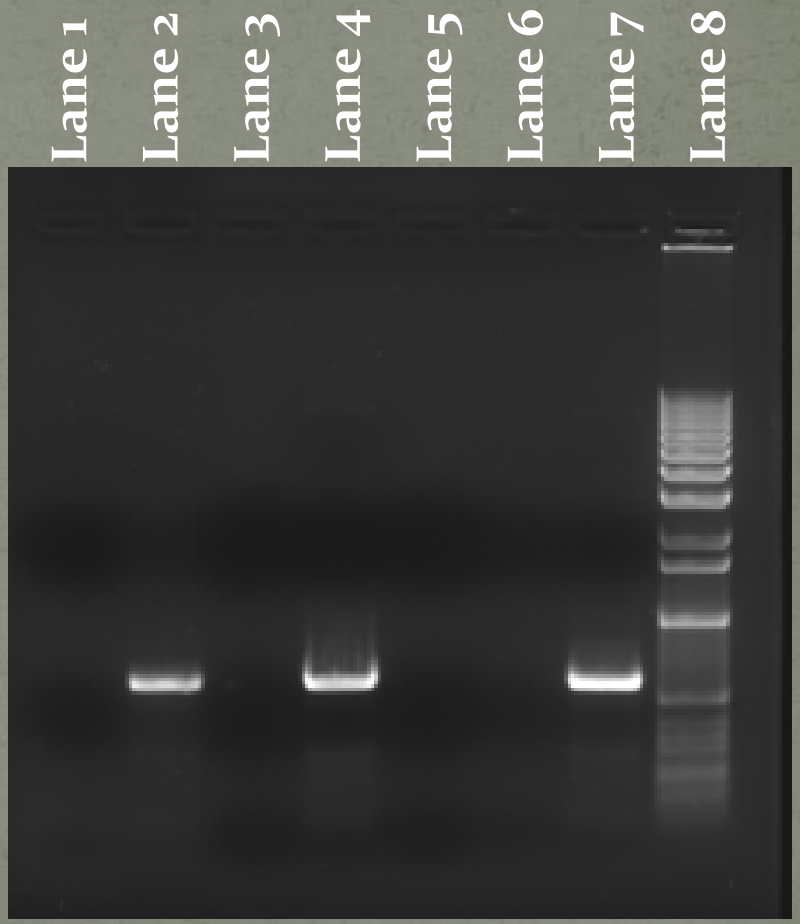
References

- Campbell, N., & Reece, J. (2008). *Biology*. San Francisco, CA: Pearson.
- Gilbertson, R., Rojas, M., Russel, D., and Maxwell D. Use of the asymmetric polymerase chain reaction and DNA sequencing to determine genetic variability of the bean golden mosaic geminivirus in the Dominican Republic. Journal of General Virology, 1991, 72, 2843-2848.
- Bartlett, JS, and Sterling, D. A Short History of the Polymerase Chain Reaction. *Methods in Molecular Biology,* Vol. 226 3-6.

PCR Results of Putative Transgenic Plants at PSH

Gene: Gus
Explant: Jatropha

Lane Number	2	4	7
Intensity (I-%)	54.56	69.7	72.55

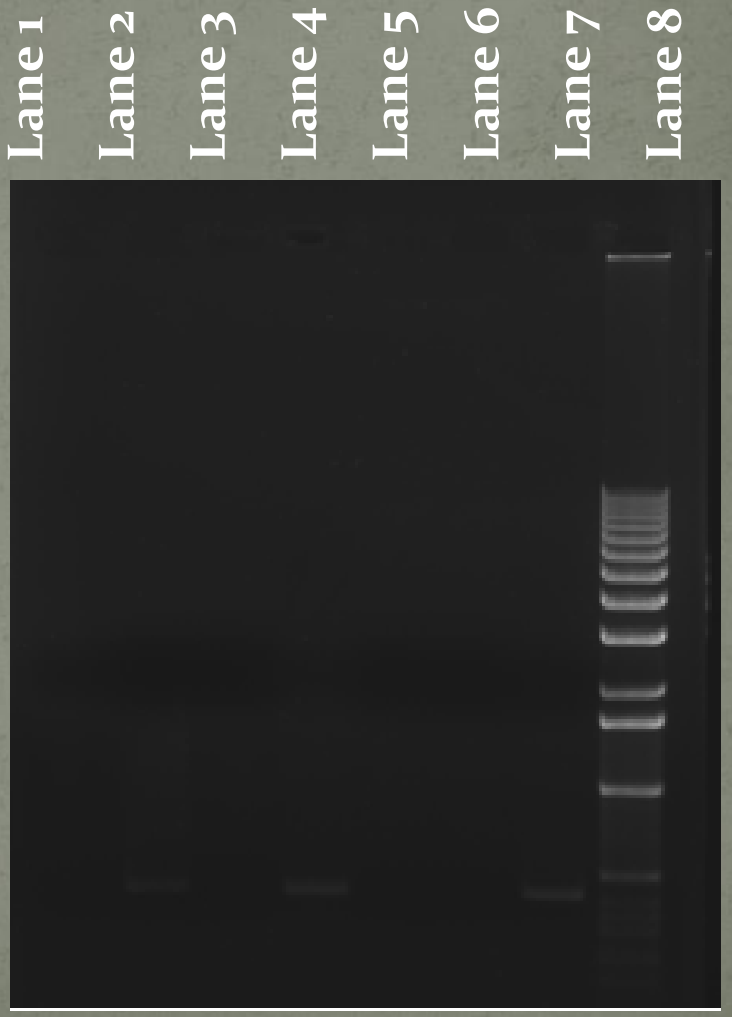


PCR Results of Putative Transgenic Plants

Gene: SAMdc
Explant: Tomato

Lane Number	2	4	7
Intensity (I-%)	-	-	-

Intensities were not enough to be read by the *BioSpectrum* imaging system.



Extra Information

Table 1. *Percentage nucleotide sequence identities between the hypervariable regions* of BGMV isolates and BDMV*

	Nucleotide sequence identity (%)†				
Geminivirus isolate	BGMV-DR	BGMV-GA	BGMV-PR	BGMV-BZ	BDMV
BGMV-DR	–	86	75	46	42
BGMV-GA		–	70	44	44
BGMV-PR			–	52	50
BGMV-BZ				—	44
BDMV					–

* The hypervariable region is the intergenic region of component DNA-B that lies between the initiation codon of open reading frame BL-1 and the beginning of the common region.

† Numbers are the direct percentage nucleotide sequence identities rounded to the nearest whole number. All comparisons were made using the GCG program GAP.