**Analysis of Bollgard II cotton seeds for "the absence of terminator gene i.e. a patented embryogenesis deactivation system in Mahyco bollgard cotton hybrid seeds containing *cry1Ac* and *cry2Ab* genes."**

Report submitted to Maharashtra Hybrid Seeds Company Limited (MAHYCO)

Study performed at the **Department of Genetics, University of Delhi South Campus.**

**Objective**: The purpose of the present study was to ascertain the presence/absence of *cre* recombinase gene using molecular approaches in Bt cotton hybrid and its parents provided by the Maharashtra Hybrid Seed Company Limited (MAHYCO). Absence of the above gene, which is an integral component of the "terminator technology", would in turn, indicate the absence of the "terminator genes" in the supplied germplasm.

**Plant material**: Hybrid line MRC-7326 (Bollgard II or BG-II) and its parent lines (P1 and P2) were provided by MAHYCO. Ten seeds of each line were sown in autoclaved 1:1 mix of soil and soilrite. The plants were maintained in a containment greenhouse. Genomic DNA from an untransformed cotton plant was used as negative control for the analysis. Genomic DNA of a *cre* gene transformed cotton plant and the pPZP200 35S*cre* binary plasmid DNA were used as positive controls.

**Genomic DNA isolation**: The genomic DNA was isolated from the first true leaf of two randomly selected seedlings each of the two parent lines P1, P2, the hybrid Bollgard II and the controls. DNA was extracted using Plant DNeasy Mini Kit from QIAGEN. The representative DNA samples were labeled as P1.1 & P1.2, P2.1 & P2.2 and BG1 & BG2 for parent 1, parent2 and bollgard II hybrid respectively.

**Amplification of *cre* gene**:

The following primer available in the lab were used for the study

CRE F2: 5'-CAG CAA CAT TTG GGC CAG CTA-3'

CRE R2: 5'-TCT CTA CAC CTG CGG TGC TAA-3'

The primers amplify an amplicon of size 368 bp specific to the *cre* gene. Optimized conditions for each amplification included ~100 ng of genomic DNA template (plasmid control was used at lower concentration ~25 ng), 12.5 pmoles of each primer, 200µM of each dNTP, 1 unit of Taq DNA polymerase (Invitrogen), the supplied reaction buffer at 1x concentration and supplied $MgCl_2$ (working concentration 1.5 mM) in a final reaction volume of 25 µl. Cycling parameters were as follows: initial denaturation at 94 $^0$C, 5 min followed by 30 cycles comprising 94 $^0$C, 30 sec; 55 $^0$C, 30 sec; 72 $^0$C, 45 sec. A final extension at 72 $^0$C for 7 min was given. Two independent PCR analyses were performed. PCR products were electrophoresed on a 1% agarose gel with appropriate markers to determine size of the amplified products.

**Results:**

In both PCRs, **no amplification specific to the *cre* gene was observed for the Mahyco samples** i.e. Parent1, Parent2 & Bollgard-II hybrid and the untransformed cotton control genomic DNA (Lanes P1.1, P1.2, P2.1, P2.2, BG1, BG2 and UT. Fig 1 and 2). In the *cre*-cotton genomic DNA and the *cre* gene harboring plasmid DNA (marked as cre+ and plasmid ctrl respectively in Fig 1 and 2), a specific band corresponding to the size (368bp) of the expected amplicon was observed.

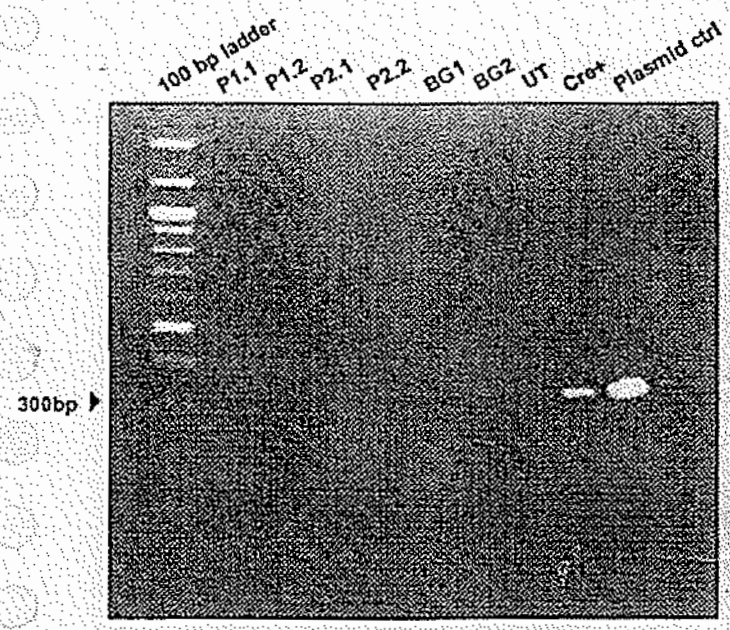


Fig 1

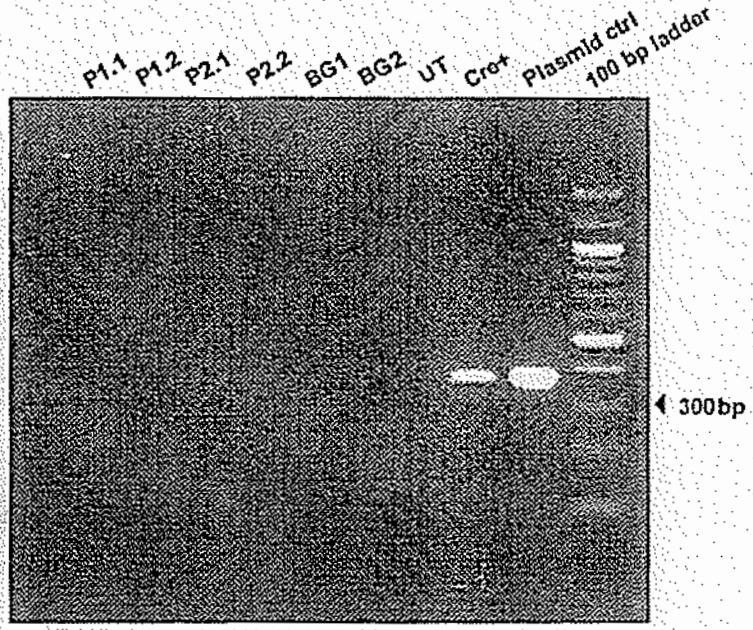


Fig 2

**Conclusion**:

The absence of any *cre* gene specific amplification using *cre* primers conclusively proves the absence of *cre* gene in the parents P1 & P2 and their hybrid Bollgard-II (MRC-7326 of Mahyco). Absence of the *cre* gene in turn shows that the lines (P1, P2 and BG-II) are devoid of "terminator technology" where *cre* gene is a component.

Sanjeev Kumar
Scientist-Cotton group

Deepak Pental
Director-CGMCP
CGMCP
NDDB Project
Dept. of Genetics

# ANNEXURE 6.7

Additional study to establish absence of embryogenesis deactivator gene

August 29, 2005

Dr. M. K. Sharma
General Manager
Maharashtra Hybrid Seeds Company Limited
4E/15, Ashok Center, III Floor
Jhandewalan Extension
New Delhi

Dear Sir,
With reference to your letter MKS/1133/2005, please find enclosed the analysis report on "presence/absence of terminator gene.....in Mahyco Bollgard II hybrid cotton seeds" carried out at the Department of Genetics, University of Delhi South Campus under the supervision of Prof Deepak Pental.

Please let us know if any further testing of the plant material is required.
Sincerely,

Sanjeev Kumar
Research Scientist
Cotton Project
Department of Genetics
UDSC
New Delhi

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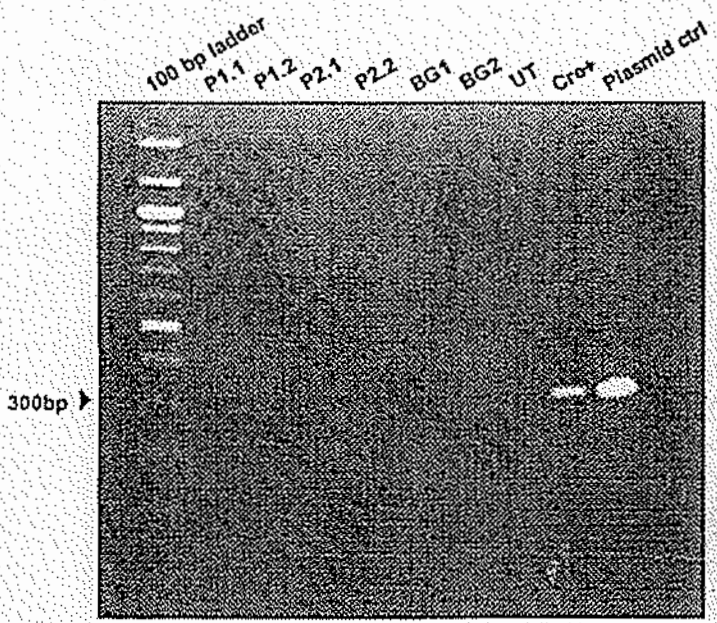


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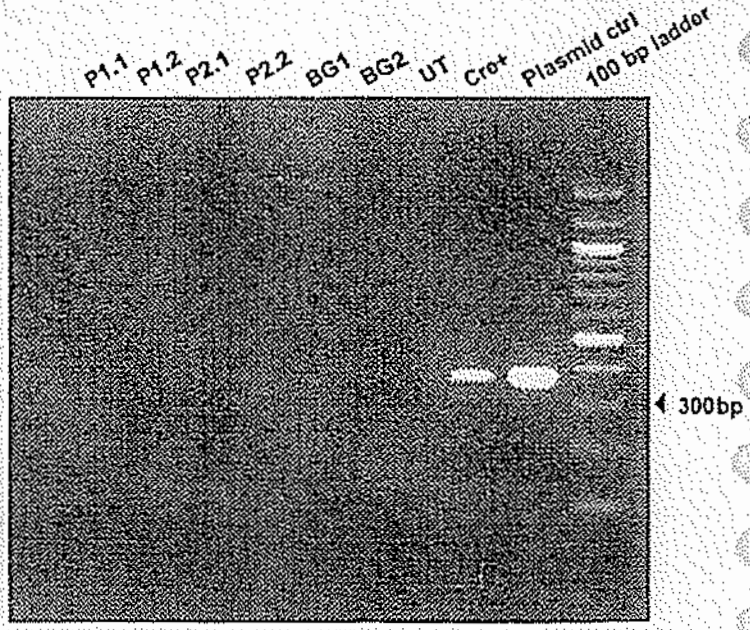


Fig 2