

Fig.1 (a): Assay designing for Standard PCR assays for MON810

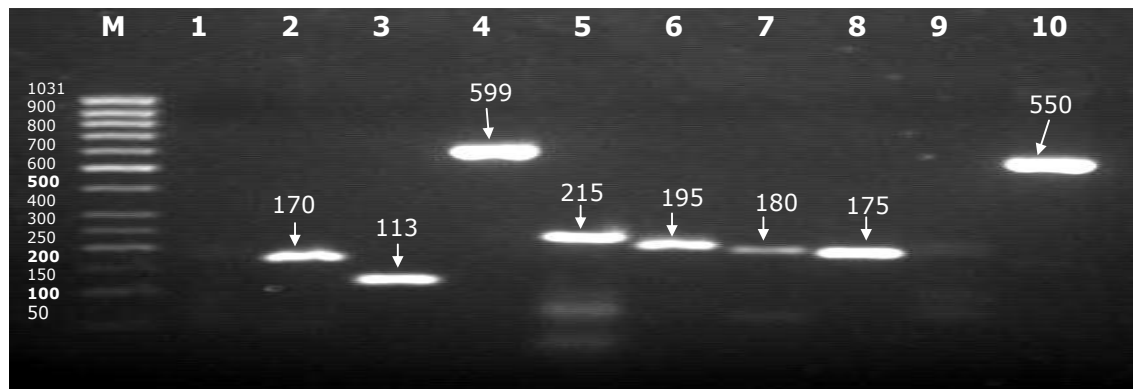


Fig.1 (b): Molecular detection of MON810 - Standard PCR assays.

Lane M: 50bp marker, Lane 1: Env. Control, Lane 2: *cry1Ab* event specific (maize genome - P-e35S), Lane 3: *cry1Ab* construct specific (*hsp-cry1Ab*), Lane 4: gene specific (*cry1Ab*), Lane 5: *npt-II*, Lane 6: P-e35S, Lane 7: T-nos, Lane 8: *hmgA*, Lane 9: Neg. control, Lane 10: Pos. control (Chl. t-RNA).

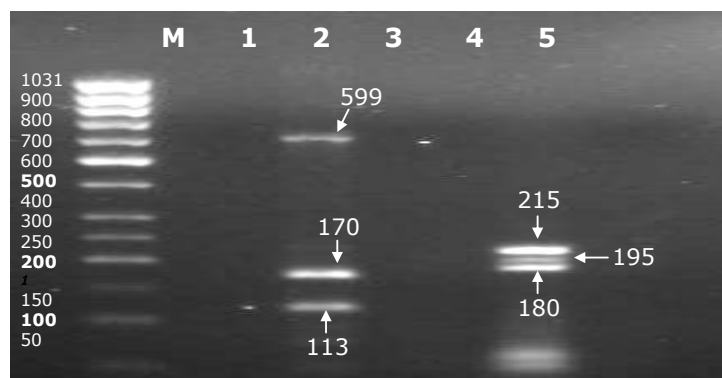


Fig. 2: Multiplex PCR assays for MON810.

Lane M: 50bp marker, Lane 1: Env. Control, Lane 2: Multiplex PCR assay for *cry1Ab* transgene with gene (*cry1Ab*), event (maize genome - P-e35S) and construct (*hsp-cry1Ab*) specificity, Lane 4: Multiplexing for GMO specific sequences (*npt-II*, P-e35S & T-nos), Lane 3 & 5: Their respective Neg. Controls.

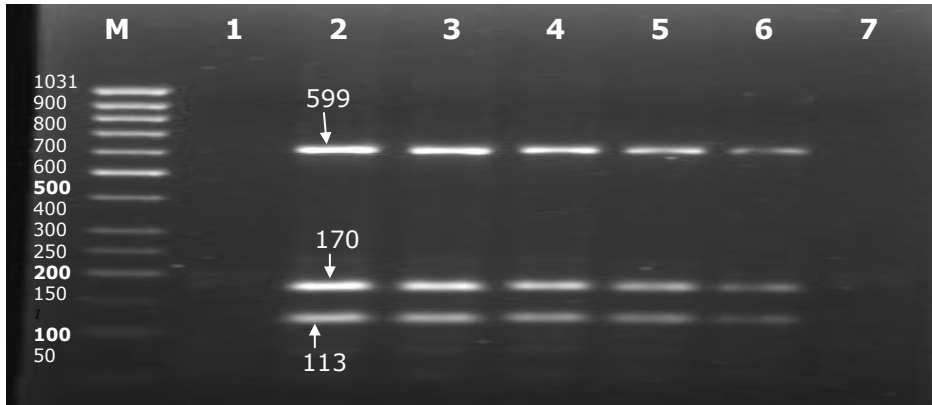


Fig. 3: LOD with Multiplexing for MON810 - gene (*cry1Ab*), event (maize genome - P-e35S) and construct (*hsp-cry1Ab*) specific assays.

Lane M: 50bp marker, Lane 1: Env. Control, Lane 2-7: Multiplex PCR assay for *cry1Ab* transgene construct with gene (*cry1Ab*), event (maize genome - P-e35S) and construct (*hsp-cry1Ab*) specificity, with 5%, 2%, 1%, 0.5%, 0.1% & 0.0% MON810 CRM respectively.

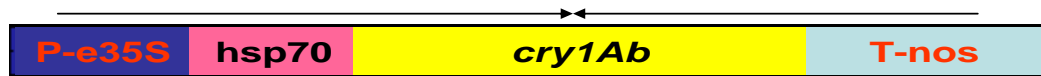


Fig. 4 (a)

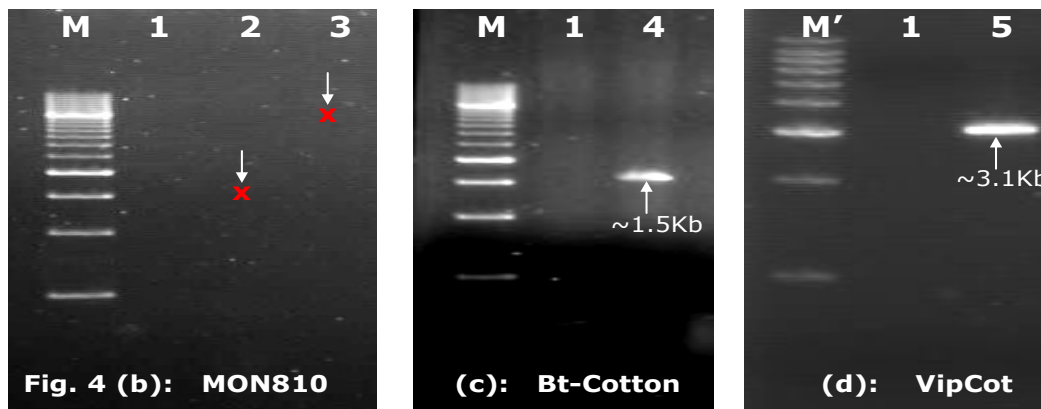


Fig. 4: Universal longrun construct specific PCR assays for MON810, Bt-cotton and VipCot.

Lane M: 0.5Kb ladder, Lane M': 1.0Kb ladder, Lane 1: Env. Control, Lane 2 & 3: Longrun PCR assay utilizing forward primer of P-e35S and reverse primer of T-nos using template DNA from Mon810, Lane 4: Bt-cotton (Bollgard I), Lane 5: VipCot – *vip-S* bearing transgenic cotton.

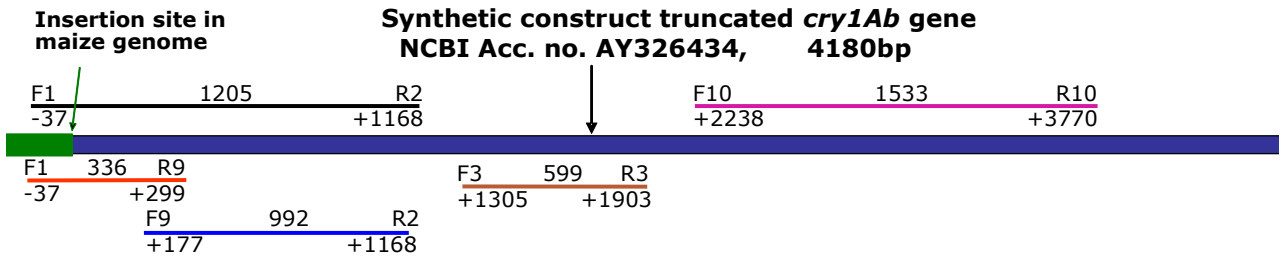


Fig. 5(a): Schematic representation of construct specific PCR assay.

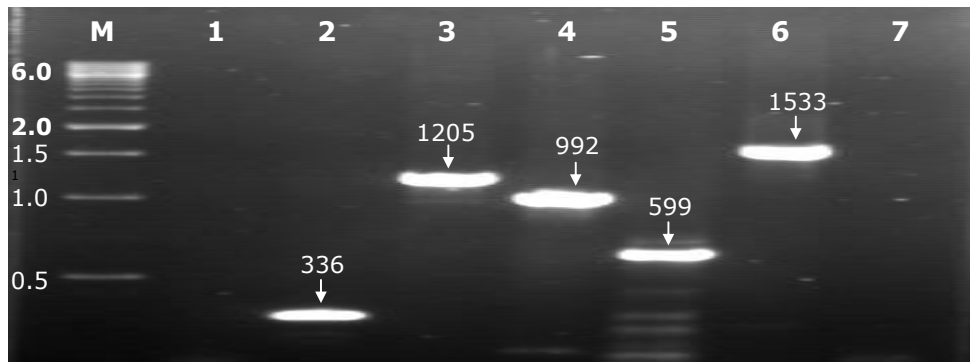


Fig. 5(b): Event & Construct-specific PCR assay for MON810 – to confirm the insert integrity and stability of transgene.

Lane M: 0.5Kb ladder, Lane 1: Env. Control, Lane 2: Amplification of adjoining sequences of maize genome & P-e35S (event specific), Lane 3: maize genome & *cry1Ab* transgene (construct specific), Lane 4: P-e35S & *cry1Ab* transgene (construct specific), Lane 5: *cry1Ab* (gene specific), Lane 6: *cry1Ab* – maize genome (event specific), Lane 7: Neg. Control.

[I: AY326434](#). Reports Synthetic constru...[gi:37524000]

[Features](#) [Sequence](#)

LOCUS	AY326434	4180 bp	DNA	linear	SYN 01-MAY-2006
DEFINITION	Synthetic construct truncated CRYIA(b) (<i>cryIA(b)</i>) gene, partial cds.				
ACCESSION	AY326434				
	promoter	1..328	/note="35S promoter from CaMV"		
	intron	323..1134	/note="HSP70"		
	gene	1135..3591	/gene="cryIA(b) "		
	source	3581..4180	/organism="Zea mays"		
			/mol_type="genomic DNA"		

Fig. 5(c): Conformational proof of construct-specific amplicons.

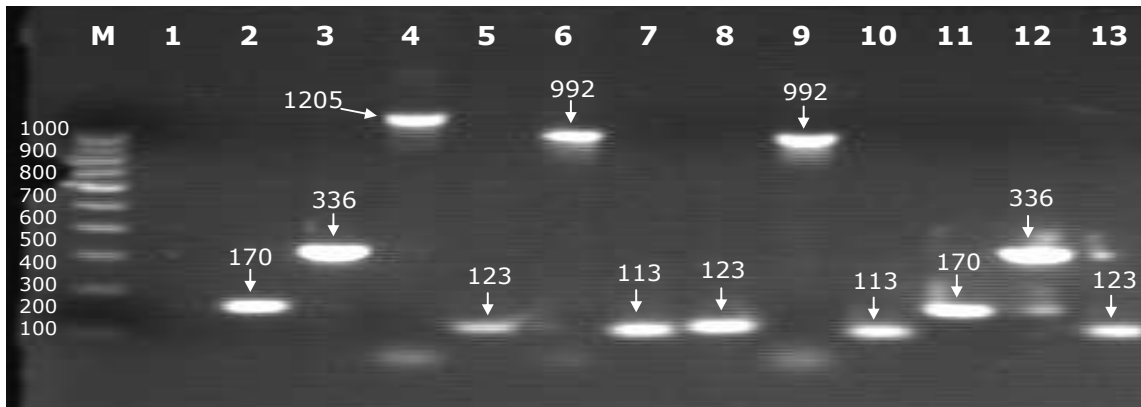


Fig. 6: Nested PCR assay for MON810 to confirm the transgene insert integrity.

Lane M: 100bp ladder, Lane 1: Env. Control, Lane 2-13: Amplification as given below –

Template – (1205bp) amplicon of construct-specific PCR assay

2. Maize genome F1 + P-e35S R1 - 170bp
3. Maize genome F1 + P-e35S R9 - 336bp
4. Maize genome F1 + cry1Ab R2 - 1205bp
5. P-e35S F9 & R9 - 123bp
6. P-e35S F9 + cry1Ab R2 - 992bp
7. hsp70 F2 + cry1Ab R2 - 113bp

Template – (992bp) amplicon of construct-specific PCR assay

8. P-e35S F9 & R9 - 123bp
9. P-e35S F9 + cry1Ab R2 - 992bp
10. hsp70 F2 + cry1Ab R2 - 113bp

Template – (336bp) amplicon of construct-specific PCR assay

11. Maize genome F1 + P-e35S R1 - 170bp
12. Maize genome F1 + P-e35S R9 - 336bp
13. P-e35S F9 & R9 - 123bp

Table 1. Primer sets used in this assay

Primer pair	NCBI Acc. No.	Sequence (5'–3')	Amplicon (bp)	Ref.	
Event specific (maize genome – P-e35S)	AY326434	F1= 5'- tcg aag gac gaa gga ctc taa cg -3' R1= 5'- tcc atc ttt ggg acc act gtc g - 3'	170	8-9	
Construct specific (hsp70 – cry1Ab)		F2= 5'-gat gcc ttc tcc cta gtg ttg a-3' R2= 5'-gga tgc act cgt tga tgt ttg-3'	113		
Gene specific (<i>cry1Ab</i>)		F3= 5'- cct ggg cct cgt gga cat cat ct -3' R3= 5'- tcg ggt agg tgc ggg agt cgt agt -3'	599	This Study	
GMO specific	<i>npt II</i> gene	AF269238	F4= 5'- ctc acc ttg ctc ctg ccg aga - 3' R4= 5'- cgc ctt gag cct ggc gaa cag - 3'	215	11-12
	P-e35S	V00141	F5= 5'- gct cct aca aat gcc atc a - 3' R5= 5'- gat agt ggg att gtg cgt ca - 3'	195	
T-nos	V00087	F6= 5'- gaa tcc tgt tgc cgg tct tg - 3' R6= 5'- tta tcc tag ttt gcg cgc ta - 3'	180		
<i>hmgA</i> – maize specific	AJ131373	F7= 5'-gaa atc cct gag cga gtc ggt a-3' R7= 5'-gcg atg gcc ttg ttg tac tcg a-3'	175	13	
<i>chloroplast t RNA</i> gene – plant specific	Z00044, X15901	F8= 5'- cga aat cgg tag acg cta cg - 3' R8= 5'- ggg gat aga ggg act tga ac - 3'	550	12, 14	
Additional primers used for structural stability analysis					
P-e35S	V00141	F9= 5'- cca cgt ctt caa agc aag tgg - 3' R9= 5'- tcc tct cca aat gaa atg aac ttc c-3'	123	12	
<i>cry1Ab</i>	AY326434	F10= 5'- cgc agg ccc ttc aac atc ggt atc-3' R10= 5'- ggt cgg cgc cca aca aca aga -3'	1533	This Study	

Table 2. Calculation of 'GMO' genome copy for MON810 in a 100ng target DNA to establish limit of detection (LOD).

S. No.	MON810 CRM (%)	'GMO' genome copies for MON810
1	5.0	1930
2	2.0	772
3	1.0	386
4	0.5	193
5	0.1	38
6	0.0	0.0

- *Zea mays* nuclear DNA content¹⁹ is reported to be 4.75-5.63 pg/2C, thus 1C value would be $5.19/2 = 2.59\text{pg}$.
- So, number of 'GMO' genome copies in 100ng of 100% MON810 DNA will be $100\text{ng} / 2.59\text{pg} = 38,610$

Table 3.

Sequence details of cry1Ab transgene cassette, initially used in making of MON810⁶.

S.No.	Element – name	Size (Kb)
1.	P-e35S	0.62
2.	hsp70	0.80
3.	<i>cry1Ab</i> transgene	3.50
4.	T-nos	0.24
5.	<i>npt-II</i> gene	0.79