Project Report on,

Verification of SOP titled "PCR Protocol for a Bt cotton transgenic event, MLS 9124 contamination".

Submitted to,
Metahelix Life Sciences Pvt. Ltd.

On,
April 18, 2008

By,

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Verification of SOP titled "PCR Protocol for a Bt cotton transgenic event, MLS 9124 contamination".

Objective

- PCR amplification using the given primers "Event Specific 923, 926" and "Genome Specific 229, 230" on the given template DNA named as Cotton 9124 (Positive control), 9124 (1%), 9124 (0.1%), 9124 (0.01%), 9124 (Negative control).

Method

Polymerase Chain Reaction (PCR) was carried out according to the protocol (Table 1, 2, 3, 4) given by Metahelix on the DNA named as Cotton 9124 (Positive control), 9124 (1%), 9124 (0.1%), 9124 (0.01%), 9124 (Negative control).

PCR reaction was carried out on Eppendorf ep thermal cyclers at Avesthagen Laboratories.

No	Reagent/Sample	Final Concentration	Volumes (µl)
1	Water	1 X	16
2	Primer 923 (10 µM)	0.5 µM	2.5
3	Primer 926 (10 µM)	0.5 µM	2.5
4	HotStart PCR Master Mix (2X)	1 X	25
5	Template DNA (50 ng/µl)	4 ng/µl	4
	Total		**50**

Table 1: PCR components for primers named as Event Specific 923, 926 by metahelix.

No	Reagent/Sample	Final Concentration	Volumes (µl)
1	Water	1 X	7.2
2	Primer 229 (10 µM)	0.5 µM	1
3	Primer 230 (10 µM)	0.5 µM	1
4	HotStart PCR Master Mix (2X)	1 X	10
5	Template DNA (50 ng/µl)	2 ng/µl	0.8
	Total		**20**

Table 2: PCR components for primers named as Genome Specific 229, 230 by metahelix.

Step	Temperature ^{o}C	Time (min:sec)	Cycles
1	94	15:00	1
2	94	0:30	1
3	68	0:25	1
4	72	1:00	1
5		Back to 2	40
6	72	5:00	1
7	4	Storage	

Table 3: PCR thermal condition for primers named as Event Specific 923, 926

Step	Temperature ^{o}C	Time (min:sec)	Cycles
1	94	15:00	1
2	94	0:30	1
3	60	0:25	1
4	72	1:00	1
5		Back to 2	40
6	72	5:00	1
7	4	Storage	

Table 4: PCR thermal condition for primers named as Genome Specific 229, 230

Results

PCR product was loaded on to 1.4% agarose gel and run with 1X TAE buffer for 30 minutes at 100V. The gel was stained with Ethidium Bromide and visualized on Biorad gel document system (Fig 1).

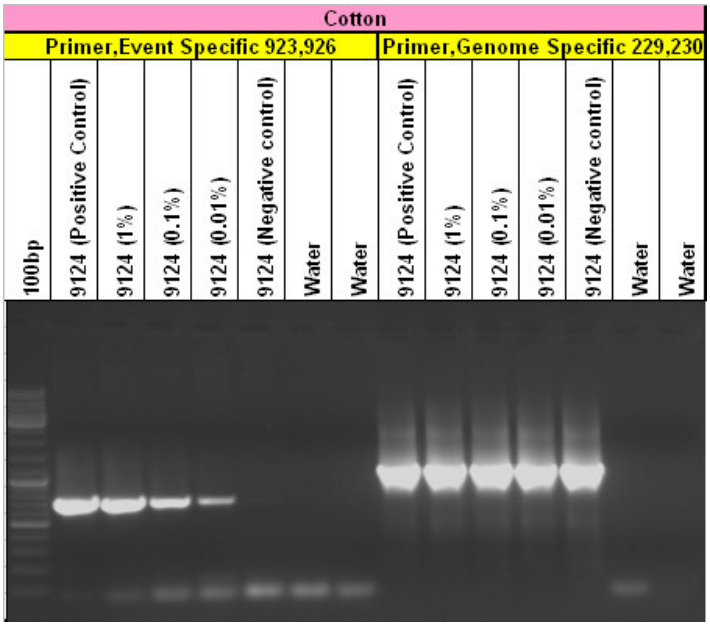

Fig 1: PCR amplification pattern for primers named as Event Specific 923, 926 and Genome Specific 229, 230

Conclusion:

1) Bands could be seen in all the DNA samples amplified by Genome Specific 229, 230 primers including the sample named as 9124 (ctrl) (Fig 1).

2) Bands could be seen in all the DNA samples amplified by Event Specific 923, 926 primers except in the sample named as 9124 (ctrl) (Fig 1).

Disclaimer:

AQUAS (Avesthagen Quality Agriculture Services Pvt. Ltd) is accredited by NABL (National Accreditation Board for Testing and calibration Laboratories, DST, Govt of India) to conduct GMO (Genetically Modified Organism) Testing at its laboratory of biological samples by PCR-based methods using the Genetic-ID suite of methods. The verification of SOP provided by Metahelix Life Sciences Pvt Ltd. for this project is not covered within the scope of AQUAS's accreditation by NABL.

AQUAS

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April 16, 2008.

Subject: Verification of SOP titled "PCR Protocol for a Bt cotton transgenic event, MLS 9124 contamination".

This is to state that the PCR amplification of DNA samples provided by Metahelix Life Sciences Private Limited, Bangalore has been carried out successfully by AQUAS. Band intensities varied in a manner proportional to the dilution of the event described on the samples provided by Metahelix Life Sciences Private Limited, Bangalore.

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