HiGenoMB™ Unzipping Genes

HIMEDIA®

Product Information

HiPer®GMO Detection Teaching Kit

Product Code: HTBM035

Number of experiments that can be performed: 5

Duration of Experiment:
Protocol: 2 hours
Agarose Gel Electrophoresis: 45 minutes

Storage Instructions:

- The kit is stable for 6 months from the date of manufacture
- Store 2X PCR Master Mix, Template DNAs, 50bp DNA Ladder and Primer mixes at -20°C
- Store 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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HIMEDIA
For life is precious

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Aim:

To detect GMO (Genetically Modified Organism) by PCR

Introduction:

DNA-based methods for GMO (Genetically Modified Organism) detection and identification are based mainly on the use of the polymerase chain reaction (PCR). PCR has the advantage that it can be used to screen a sample for the presence of GMOs by using primers that target sequences that are commonly found in a variety of different GMOs. The amplified product can then be detected to determine whether or not DNA originating from a GMO is present in a sample.

Principle:

GMO or genetically modified organism is called a "Transgenic Organism" whose genetic material has been altered using genetic engineering techniques by the addition of genetic material from a different unrelated organism. PCR based GMO detection methods can be designed to detect any relevant transgenic sequences based on the specific information desired. This method is effective with a broad array of sample types and can provide definitive quantification of GMOs since analysis is performed directly at the DNA level. Target sequences only found in the GMO are amplified using primers that have been designed to specifically bind them during the PCR reaction. The resulting PCR product can be detected through agarose gel electrophoresis.

Twovery prominent genetic elements in GMO-associated plants are 35S promoter of the cauliflower mosaic virus (CaMV-35S) and the terminator of the nopaline synthase gene (nos) of *Agrobacterium tumefaciens*. These DNA sequences are widely incorporated in the GM crops.35S promoter is used to drive expression of the glyphosate resistance gene or Bt gene in crops.

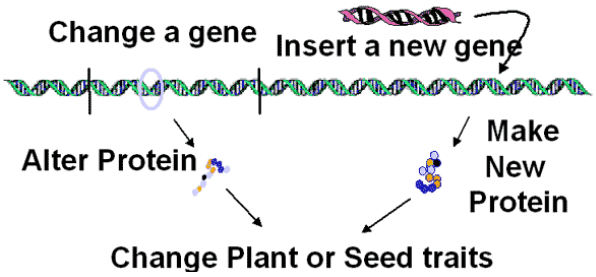


Fig 1: The DNA of GMO is altered by insertion of genetic material from a different unrelated organism.

As a control to check the integrity of the plant DNA extracted from GM crops PCR is done to amplify a section of the chloroplast gene, rbcL that is common to higher plants. This control sequence is amplified in the same reaction tube along with the target sequence or in separate tubes.

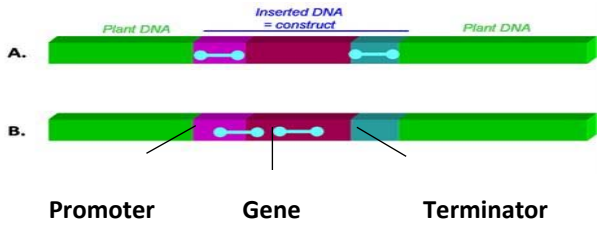


Fig 2: In a GMO different genetic regions are targeted at the DNA level e.g. promoter, gene, and terminator.

HiPer® GMO Detection Teaching Kit is designed for the detection of specific sequences of 35S gene and *nos* terminator gene from a GMO sample. Two PCR reactions are performed for each sample. One primer set amplifies the 35S promoter from cauliflower mosaic virus. The presence of a 35S product indicates the presence of a transgene. The second primer set amplifies a fragment of rbcL gene (control) along with *nos* terminator.

Kit Contents:

The kit can be used to detect GMO sample using specific primers.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			5 expts	
1	MBT061	2X PCR Master Mix	0.6 ml	-20^{0}C
2	*TKC406	Template DNA 1	0.060 ml	-20^{0}C
3	*TKC407	Template DNA 2	0.060 ml	-20^{0}C
4	*TKC408	Primer Mix 1	0.050 ml	-20^{0}C
5	*TKC409	Primer Mix 2	0.050 ml	-20^{0}C
6	ML024	Molecular Biology Grade Water	1 ml	RT
7	*MBT084	50 bp DNA Ladder	0.020 ml	-20^{0}C
8	ML015	6X Gel Loading Buffer	0.1 ml	2-8^{0}C
9	MB002	Agarose	6 g	RT
10	ML016	50X TAE	60 ml	RT
11	CG282	Polypropylene Tubes, 0.2 ml (PCR Tubes)	20 Nos	RT

*** Always give a quick spin before opening the vial as the liquid material may stick to the wall or to the cap of the vial.**

Materials Required But Not Provided:

Glasswares: Measuring cylinder, Beaker
Reagents: Ethidium bromide (10 mg/ml), Distilled Water
Other requirements: Thermocycler, Electrophoresis apparatus, UV Transilluminator, Vortex Mixer, Micropipettes, Tips, Adhesive tape, Microwave/ Hotplate/ Burner, Crushed ice

Storage:

HiPer® GMO Detection Teaching Kit is stable for 6 months from the date of manufacture without showing any reduction in performance. On receipt, store 2X PCR Master Mix, Template DNAs, 50bp DNA Ladder and Primer Mixes at -20ºC and 6X Gel Loading Buffer should be stored at 2-8ºC. Other reagents can be stored at room temperature (15-25ºC).

Important Instructions:

- Read the entire procedure carefully before starting the experiment.
- Before use, all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Centrifuge the components briefly once thawed.
- The 50bp DNA ladder supplied in the kit is ready to use and can be directly loaded onto the agarose gel.

Procedure:

1) **Preparation of master mix for PCR**

Take four PCR tubes, label them and add the following ingredients in mentioned order:

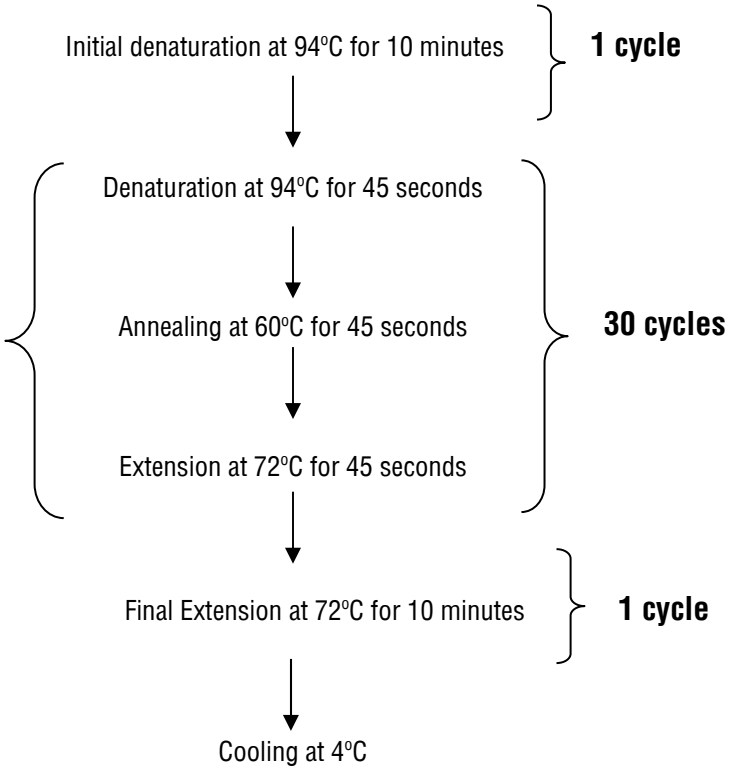
	Test Sample 1	Test Sample 2	Test Sample 3	Test Sample 4
Molecular Biology Grade Water	16 µl	16 µl	16 µl	16 µl
2X PCR Master mix	25 µl	25 µl	25 µl	25 µl
Template DNA 1	5 µl	5 µl	-	-
Template DNA 2	-	-	5 µl	5 µl
Primer Mix 1	4 µl	-	-	4 µl
Primer Mix 2	-	4 µl	4 µl	-
Total volume	**50µl**	**50µl**	**50µl**	**50µl**

2) Tap the tubes for 1 – 2 seconds to mix the contents thoroughly.

3) Place the tubes in the thermocycler block and set the program to get DNA amplification.

PCR Amplification Cycle:

Carry out the amplification in a thermocycler for 30 cycles using the following reaction conditions.



Agarose Gel Electrophoresis:

Preparation of 1X TAE: To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water*. Mix well before use.

* Molecular biology grade water is recommended (Product code: ML024).

Preparation of agarose gel: To prepare 50 ml of 2% agarose gel, add 1 g agarose to 50ml of 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate by swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure).

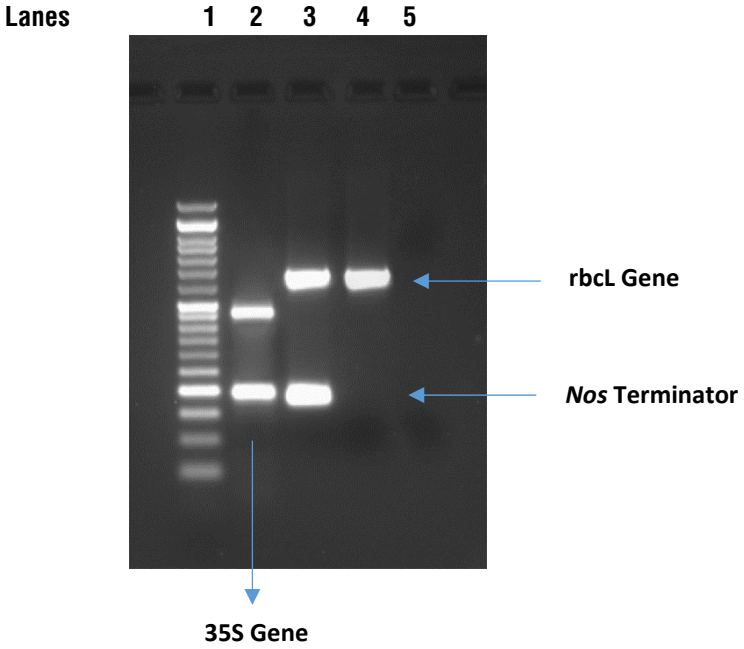
NOTE: Preparation of 2% gel will take more time than the regular 0.8% gel. Care should be taken while melting. Continuous boiling is not recommended during the preparation. Make sure that melted agarose solution appear clear and transparent devoid of any suspended particles of agarose.
Allow the solution to cool down to about 55-60ºC. Add 0.5µl Ethidium bromide (10mg/ml), mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

Loading of the DNA samples: Load 3 µl of ready to use DNA ladder into the first well. Add 2 µl of 6X Gel loading buffer to 10 µl of PCR product. Load the PCR samples into the following wells.

Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black-Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

Observation and Result:

After the completion of PCR, perform agarose gel electrophoresis. Compare the amplified product obtained from the four test samples.



Lane 1: 50 bp Ladder
Lane 2: Test Sample 1
Lane 3: Test Sample 2
Lane 4: Test Sample 3
Lane 5: Test Sample 4

Interpretation:

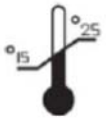
In this experiment, a duplex PCR and a uniplex PCR is set for the purpose of amplifying three different genes for two samples. In the duplex PCR reaction, one primer set will amplify a 685 bp product of rbcL for both GMO and non-GMO samples and the other primer set will amplify a 183 bp product of *nos* terminator for only GMO sample. In the uniplex PCR reaction one primer set will simultaneously amplify a 195 bp and a 490 bp product of 35S promoter for only GMO sample.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Non –specific/ spurious bands observed	Template DNA damaged	Minimize damage to template DNA by avoiding vortexing or vigorous mixing
2	No or poor amplification yield	Template may be degraded, enzymes may have been inactive	Store the kit at -20°C and avoid repeated freeze thaw. Also keep all the materials in ice while performing the experiment
		Thermocycler operation or program improper	Ensure proper functioning of Thermocycler. Run positive control with every reaction
		Inadequate mixing of the reaction tube	Mix the reaction mixture using a micropipette, avoid air bubble
3	Smearing of the product	DNA degraded	Work in sterile conditions to avoid contamination. Avoid vigorous mixing of the DNA samples

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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