

Basmati Verifiler™ Kit

(P/N BV81001)

User Manual

Version 1.0

For identification of authorized* Basmati cultivars and
detection of adulteration

**Authorized by Governments of India and Pakistan*

December 2007

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Aiming for the Best

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Patents and Licensing:

This product is covered by Indian patents 260/MAS/2002 and 662/CHE/2006; USA patents 10/357,488 and 11/406,257 and International patents PCT/IN06/00254. All patents pending

LABINDIA Contact Info:

Gurgaon: Tel: 91-124-284 3000; Fax: 91-124-284 3398/99
labindiaab_gurgaon@labindia.com

Thane: Tel: 91-22-2598 6288/6262; Fax: 91-22-2533 5940
labindiaab_mumbai@labindia.com

Kolkata: Tel: 91-33-2466 1396/3362; Fax: 91-33-2466 1352
labindiaab_kolkata@labindia.com

Chennai: Tel: 91-44-2432 7437/0352; Fax: 91-33-2434 6328
labindiaab_chennai@labindia.com

Bangalore: Tel: 91-80-2344 9143; Fax: 91-80-2344 9147
labindiaab_bangalore@labindia.com

Hyderabad: Tel: 91-40-5550 4133; Fax: 91-40-2331 3312
labindiaab_hyderabad@labindia.com

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Product Overview

Basmati rice commands a special place among all aromatic rice cultivars on account of the extra-long and slender nature of the grain, soft and fluffy texture of the cooked rice, and its pleasant distinct aroma. Cultivation of authentic Basmati rice is confined to the Indo-Gangetic regions of the Indian sub-continent, and genuine Basmati traits are best defined by the *Traditional Basmati* varieties that have been cultivated by farmers since centuries. *Traditional Basmati* rice is not only in great demand in the domestic markets, but is also seen in menus of connoisseurs worldwide. To satisfy the growing demand for Basmati rice, plant breeders have bred novel Basmati varieties that are suitable for intensive cultivation and these varieties are known as *Evolved Basmati* varieties. In addition, there are relatively inferior, non-aromatic non-*Basmati* long grain rice varieties in the market.¹

Globally, the annual Basmati export market is valued at US \$1 billion and is reported to be on the rise (www.apeda.com; www.epb.gov.pk). The export of Basmati rice is increasing at the rate of 12% annually with India exporting two-thirds of the total Basmati exported to Europe and the Middle East. Consumer-preference brings higher returns for *Traditional Basmati* varieties, leading to generation of brand equity. The difficulty in differentiating *Traditional Basmati* grains from the usual adulterants, and the steep price difference between *Traditional Basmati* and the other Basmati types in the market often results in adulteration.^{2,3} The adulteration of *Traditional Basmati* grains also affects the exporting countries in terms of diminished interest in the brands and consequently the Basmati trade. Hence, to protect the interests of consumers

identification of genuine Basmati rice samples and devaluation of adulterated samples is vital. There are few testing methods available whose applicability for routine and large-scale use is demonstrated in practical terms. That brings us to the employment of DNA marker based approaches similar to forensic DNA fingerprinting. The **Basmati Verifiler™ Kit** amplifies 8 independent microsatellite loci in a single PCR reaction. The **Basmati Verifiler™ Kit** is based on a high throughput “single-tube assay” method followed by analysis on a capillary based genotyping platform for the unambiguous identification of different Basmati cultivars and detection of adulteration. Specific microsatellite profiles of loci for all major Basmati varieties facilitate unique identification of genuine Basmati from non-Basmati possible.

For 1, 2 and 3, please see Appendix.

Kit Contents




The Basmati Verifiler™ Kit contains sufficient reagents for performing **100 assays** with final reaction volume of 10µl each.

Catalogue #	Component	Volume
123-RB	Basmati Verifiler™ 10X Buffer	120µl
123-MC	Basmati Verifiler™ Magnesium Chloride	100µl
123-DNTP	Basmati Verifiler™ 10X dNTP Mix	120µl
123-PM	Basmati Verifiler™ Primer Mix	180µl
123-TAQ	Basmati Verifiler™ PCR Enzyme	22µl
123-CTRL	Control DNA (15ng/ul)	40µl
123-CD	Basmati Information CD	1 no.

Basmati information CD contains Basmati Panel and Bin set information that need to be imported in GeneMapper® Software (Applied Biosystems) for the analysis of capillary electrophoresis results.

Storage Conditions

All components of Basmati Verifiler™ Kit except CD should be stored at -20°C.

-  Store and use the positive control DNA provided with the kit separately to avoid possible contamination.
-  Aliquot the Primer Mix into smaller volumes on receiving the kit depending on the usage, to avoid degradation due to repeated freeze thawing, which may result in degradation.
-  The fluorescent dyes attached to the primers are light-sensitive. Therefore, take precaution to protect the Basmati Verifiler™ Primer Mix from light especially when not in use.

Product Use Limitations

- The Basmati Verifiler™ Kit has been exclusively developed, designed, and is sold for the use in DNA profiling and profile based assays in authorized Basmati rice varieties.
- The kit is not to be used for any other diagnostics (human or otherwise) applications or for any other kind of application.
- Proper care should be taken while handling the contents of the kit.
- Good Molecular Biology Laboratory Practices should be adhered to while using the kit.

Product Warranty

LABINDIA guarantees the performance of all the components of the kit. The purchaser has to restrict the use of the kit to the recommended applications only. Should any component fail to perform satisfactorily due to reasons other than misuse or mishandling, LABINDIA's liability will be limited to replacement of the failed component or the kit at its own discretion.

LABINDIA reserves the right to change, alter, or modify any component to enhance the performance and design.

Safety Information



Care should be taken to handle, store and discard the components as well as the waste in accordance to the laboratory and community safety principles.

Technical Assistance

Although the Basmati Verifiler™ Kit has been developed for easy application of routine DNA profiling of Basmati varieties, LABINDIA has set up a technical team with extensive practical and theoretical expertise in molecular biology and equipment handling. If you have any questions or experience any difficulties regarding the Basmati Verifiler™ Kit in particular or any other products offered by LABINDIA, please do not hesitate to contact us.



LABINDIA APPLICATION SUPPORT

Dr. Anupama Gaur

9871591527

gaura@labindia.com

anugaur11@yahoo.com

Ms. Alpana Razdan

9811368862

razdana@labindia.com

Dr. Atima Agarwal

9312834838

agarwala@labindia.com

Work-Bench

Set up a dedicated work area in the laboratory for Basmati profiling assays in order to obtain accurate and reproducible results. Use disposable gloves, sterile fresh tips and tubes, calibrated micro-pipettes, and maintain the miscellaneous items such as calculator, permanent marker pen, racks, bottles etc. at the PCR Setup work area. Results of the kit depend upon the precision and consistency of handling.

Reagents and Equipment to be Supplied by the User

For PCR amplification

- ③ PCR grade water (All other reagents for PCR amplification are supplied in the kit)
- ③ GeneAmp® PCR System 9700, Applied Biosystems (AB P/N N805-0001)
- ③ MicroAmp Reaction Tubes 0.2-ml, Applied Biosystems (AB P/N N801-0540)
- ③ Microcentrifuge (Any known commercial vendor)
- ③ Micro pipettes, Pipet tips (sterile, disposable, hydrophobic filter-plugged) (Any commercial vendor)
- ③ Vortexer (Any commercial vendor)
- ③ Microcentrifuge tubes 0.2 ml, 1.5 ml, 2 ml (Any commercial vendor)

For capillary electrophoresis

- ③ Applied Biosystems multi-capillary Genetic Analyzers e.g. 3130 or 3730

- ③ DS-33 Matrix standards, Applied Biosystems (AB P/N 4345833)
- ③ GS 500 (LIZ) size standard, Applied Biosystems (AB P/N 4322682)
- ③ POP 7 (AB P/N 4352759)
- ③ Hi Di Formamide, Applied Biosystems (AB P/N 4311320)
- ③ PCR Grade Water, any major supplier or MQ water
- ③ MicroAmp™ 96 Well Trays for Tubes with Caps, Applied Biosystems (AB P/N N801-0541)
- ③ MicroAmp Reaction Tubes with Caps, 0.2-ml, Applied Biosystems (AB P/N N801-0540)
- ③ MicroAmp Reaction Tubes (8 tubes/strip), Applied Biosystems (AB P/N N801-0580)
- ③ MicroAmp Caps (8 caps/strip), Applied Biosystems (AB P/N N801-0535)
- ③ MicroAmp 96-Well Tray/Retainer Set, Applied Biosystems (AB P/N 403081)
- ③ MicroAmp 96-Well Base, Applied Biosystems (AB P/N N801-0531)
- ③ MicroAmp Optical 96-Well Reaction Plate, Applied Biosystems (AB P/N N801-0560)

Basmati Verifiler™ Principle

Various Basmati varieties belong to a narrow genetic pool of rice genotypes. They share most of the traits, especially aroma and grain elongation. Any deviation in the quality traits will make a variety, *non-Basmati*. *Traditional Basmati* and elite *evolved Basmati* varieties rule the market due to their superior quality. Even in the absence of the perceptible differences among genuine Basmati and possible adulterants, DNA markers can efficiently differentiate between the varieties.¹

The Basmati Verifiler™ Kit employs microsatellite DNA markers identified and validated specifically for the Basmati rice assays.² The Basmati Verifiler™ Kit makes use of a set of eight microsatellite markers (Table 1); the use of multiple markers provides accuracy and reliability to the assay.

Table 1 Microsatellite marker panel employed for multiplex assay and expected allele sizes for all Basmati varieties.

Locus	Repeat	Chr	Expected Allele sizes (bp)	Fluorescent Label
RM 1	(GA) ₂₆	1	74, 100, 106, 108	6 FAM
RM 72	(TAT) ₅ C(ATT) ₁₅	8	151,160, 172, 175	6 FAM
RM171	(GATG) ₅	10	335, 343	6 FAM
RM 202	(GA) ₃₀	11	161, 164, 182	VIC
RM 241	(GA) ₃₁	4	128, 131, 142	VIC
RM 44	(GA) ₁₆	8	101, 107, 110	NED
RM 55	(GA) ₁₇	3	217, 227, 232	NED
RM 348	(CAG) ₇	4	128,137	NED

The microsatellite loci were selected after extensive screening of around 350 microsatellite loci (sequence source: www.gramene.org). All these 350 loci were tested on six *Traditional Basmati* varieties; nine *Evolved Basmati* varieties; two non-*Basmati* long grain rice varieties and a short grain variety (Table 2). The selected loci ensure amplification of a single allele as well as high discrimination power to differentiate between different known *Basmati* and non-*Basmati* varieties.²

For 1 and 2 please see Appendix.

Table 2 List of Basmati varieties and adulterants used in the validation study.²

Variety	Source	Type	Pedigree
Basmati370	I P	TB	Selection
Dehradun Basmati	I	TB (Type 3)	Selection
Ranbir Basmati	I	TB (IET11348)	Selection
Taraori Basmati	I	TB (Karnal local, HBC 19)	Selection
Basmati386	I	TB	Selection
Basmati217	I	TB	Selection
Kernel Basmati	P	EB	CM7/Basmati370, 1968
Basmati385	P	EB	TN1/Basmati370, 1985
Super Basmati	P, I	EB	Basmati320/IR661, 1996
Basmati198	P	EB	Basmati370/TN1, 1972
Pusa Basmati	I	EB	Pusa150/Karnal Local, 1989
Punjab Basmati	I	EB	Sona/Basmati370, 1982
Kasturi	I	EB	Basmati370/CR88-17-1-5, 1989
Mahi Sugandha	I	EB	BK79/Basmati370, 1995
Haryana Basmati	I	EB	Sona/Basmati370, 1991
Sharbati*	-	NB	Selection from a UP landrace
IR64*	-	NB	IR5657-33-2-1/IR2061-465-1-5-5, 1985
Jaya**	-	NB	TN1/T141, 1967

I: Basmati variety approved originally by India; P: Basmati variety approved originally by Pakistan

- Non Aromatic, long grain; ** Non Aromatic, short grain
TB: Traditional Basmati; EB: Evolved Basmati; NB: non-Basmati

Fluorescent Dye Technology with multi-component analysis

The Basmati Verifiler Kit™ uses a five-dye fluorescent system for automated DNA analysis. Five dye technology helps in accommodating more loci in a single injection, thus providing more flexibility in terms of option of adding more Basmati Verifiler™ Kit User Manual

markers in future. The spectral range of these five dyes gives better separation of PCR products. Each of the fluorescent dyes used in the kit emits its maximum fluorescence at a specific wavelength. 6-FAM emits at the shortest wavelength and is displayed as blue; followed by the VIC dye (green), NED dye (yellow), PET dye (red) and LIZ dye (orange). Although their fluorescence maxima appear at different wavelengths, there is some overlap in the emission spectra between the dyes (Figure 1). However, multi-component analysis effectively corrects for this spectral overlap.

For 2 please see Appendix.

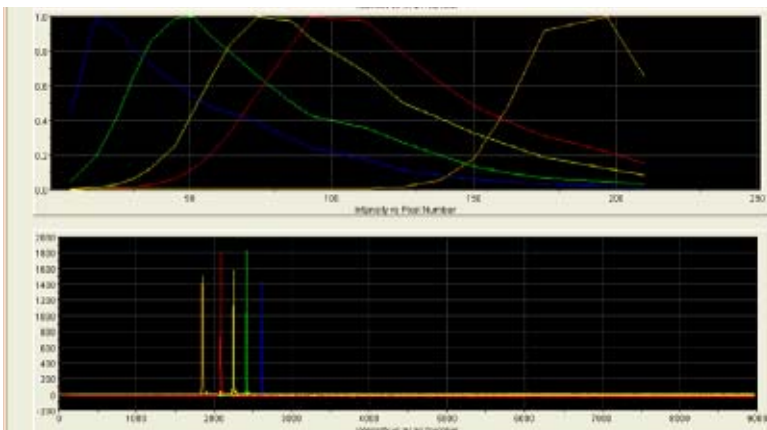




Fig 1. Separation of 5 dyes and their spectral overlap correction.

Basmati Verifiler™ Protocol

Preparing for the multiplex PCR

Master Mix: Prepare the Master Mix by combining PCR-grade water (user supplied), Basmati Verifiler™ 10X Buffer, Basmati Verifiler™ Magnesium Chloride, Basmati Verifiler™ 10X dNTP Mix, Basmati Verifiler™ Primer Mix, Basmati Verifiler™ PCR Enzyme as mentioned below:

 The fluorescent dyes attached to the primers are light-sensitive. Protect the Basmati Verifiler™ Primer Mix from light when not in use. Also protect the fluorescently labeled PCR products from light.

 The Basmati Verifiler™ PCR Enzyme provided in the kit may cause eye and skin irritation. It may cause discomfort if swallowed or inhaled. Wear appropriate protective eye wear, clothing, and gloves.

Vortex the following reagents for 5 sec

Basmati Verifiler™ 10X Buffer

Basmati Verifiler™ MgCl₂

Basmati Verifiler™ 10X dNTP Mix

Basmati Verifiler™ Primer Mix

- ③ Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.
- ③ Select a clean, unused tube for the Master Mix preparation
- ③ Determine the total number of samples, including controls and calculate the required amount of components as shown:

Number of samples X 1 µl of Basmati Verifiler™ 10X
PCR Buffer

Number of samples X 0.8 µl of Basmati Verifiler™

MgCl₂

Number of samples X 1 µl Basmati Verifiler™ 10X dNTP

Mix

Number of samples X 1.6 µl Basmati Verifiler™ Primer

Mix

Number of samples X 0.2 µl Basmati Verifiler™ PCR

Enzyme

Number of samples X 3.4 µl of PCR grade water

- ③ Vortex the Master Mix at medium speed for 5 sec
- ③ Dispense 8 µl of Master Mix per PCR tube containing 2 µl of DNA at a recommended concentration of 15 ng per µl
- ③ To prepare a positive control, vortex the Basmati Verifiler™ Control DNA tube (supplied with the kit), spin the tube briefly in a microcentrifuge to remove any liquid from the cap; add 2 µl of Basmati Control DNA to the Positive Control Tube
- ③ To prepare a negative control, add 2 µl of PCR grade water (user supplied) instead of DNA.
- ③ The final volume in each PCR tube is 10 µl.

Performing PCR

For the best results use either of the following thermal cyclers to amplify loci using the Basmati Verifiler™ kit:




_ GeneAmp® PCR System 9700

_ GeneAmp® PCR System 9600

- ⚠ If using the GeneAmp PCR System 9700, select the 9600 Emulation Mode.

Table 2 PCR Cycle Conditions

Initial Denaturation	Denature	Anneal	Extend	Final Extension	Final Step
Hold	Cycle (30 cycles)			Hold	Hold
94°C 10 min	95°C 45sec	55°C 90 sec	72°C 60 sec	60°C 30 min	4°C forever

-  If leaving the amplified products in the thermal cycler for more than 18 hours, set the final step to HOLD at 4°C – 25°C forever. The final step can be held anywhere in this range.
-  To store amplified product for <1 week, store at 2 to 6°C. To store amplified product for >1 week, store at –15 to –25°C.
-  Protect the amplified products from light.

Instrument requirement for performing GeneScan

The Basmati Verifiler™ Kit should be analyzed on any one of the Applied Biosystems multi-capillary Genetic Analyzers e.g. 3130/3130x/ or 3730/3730x/.

Calibrate the instrument before starting the experiment:

- ③ Spatial calibration
- ③ Spectral calibration for Dye set G5 (using DS 33 matrix standards) on 36 cm array

The instructions related to Instrument operation including spatial and spectral calibrations are contained in *Instrument User Manual* supplied with the instrument (Applied Biosystems).



The Basmati Verifiler™ Kit has been optimized and validated with the above mentioned parameters. For best results and confidence always carry out in-house validation using the kit. Please contact Labindia's Application Support team for more information.

Preparing the Instrument for the run

Before proceeding with the run, the following instrument setups need to be checked:

- ③ Set up the GeneMapper® Software by importing panels and bin sets provided in the Basmati Information CD accompanying the kit, create analysis methods.
- ③ Create an instrument protocol for running fragment analysis specifying the Dye set G5, type of polymer and array length used (follow user manual of the instrument).
- ③ Create a Result Group (following the instructions given in the instrument's user manual).

Preparing samples for GeneScan

Prepare the *formamide size standard* mixture for each individual sample or prepare in bulk for all samples in the run. The size standard to be used with Basmati Verifiler™ Kit is GeneScan 500 LIZ® (Applied Biosystems).

1. To prepare each sample, add the following in a micro-centrifuge tube. Prepare the appropriate volume of loading mix according to the number of samples being processed.

Genescan-500 LIZ size standard (Applied Biosystems)	0.30 µl
Hi-Di™ Formamide (Applied Biosystems)	12.0 µl

2. Vortex the tube(s) to mix.
3. Spin the tube(s) briefly.

Loading the samples

1. Dispense 10.5 μl of the *formamide size standard* mixture into each well .
2. Add 10 μl of the formamide to each ***blank well***.
3. Dilute the PCR amplified products by adding 10 μl PCR grade water, and load 0.5 μl of the diluted sample into each well.
4. Cover the reaction plate either Reservoir septa (Applied Biosystems), or a 96-well plate septa. Denature the samples in a thermal cycler at 95°C for 5 minutes; chill on ice 3 minutes.
5. Briefly spin the reaction plate in a centrifuge to ensure that the contents of each well are collected at the bottom.

Preparing the Plate Assembly (According to Applied Biosystems recommendations)

1. Arrange the plate on the plate base and snap the plate retainer onto the plate and plate base.
2. Verify that the holes of the plate retainer and the septa strip are properly aligned. If not, reassemble the plate assembly.



Damage to the array tips will occur if the plate retainer and septa strip holes do not align correctly.

Running module


1. Select the appropriate Run Module for doing Fragment Analysis as mentioned in the Instrument

- User manual (Applied Biosystems) for 36 cm array using POP 7 for Dye set G5.
2. Prepare the Plate Record on the Genetic analyzer prior to the run as per the Instrument User's Manual.
 3. Link the plate on the auto-sampler to the plate record.
 4. **START** the run.

Data Analysis and Interpretation

Data Interpretation

The Basmati Verifiler™ Kit components and the run parameters have been optimized to adequately amplify approximately 30 ng of sample DNA. The PCR cycle number and amplification conditions have been specified to produce low peak heights by using samples with a template DNA concentration of ~30 ng (rice genomic DNA). Thus, the overall sensitivity of the assay has been adjusted to avoid or minimize stochastic effects. Labindia has successfully typed samples with ~30 ng of template DNA concentration.

 Individual laboratories may find it useful to determine an appropriate minimum peak height threshold based on their own results/instruments using low amounts of template DNA.

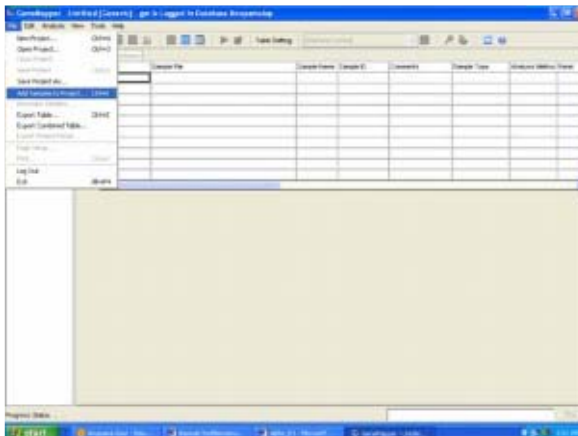
Detection of Adulteration

The multiplex profiles of the most of the exported Basmati varieties has been determined (Refer the Panel and Binset given in the accompanying CD). The protocol assumes **a)** equivalent contribution of DNA from the grain mixtures and **b)** faithful representation of the samples. Table 1 gives the allele bins corresponding to important Basmati varieties.

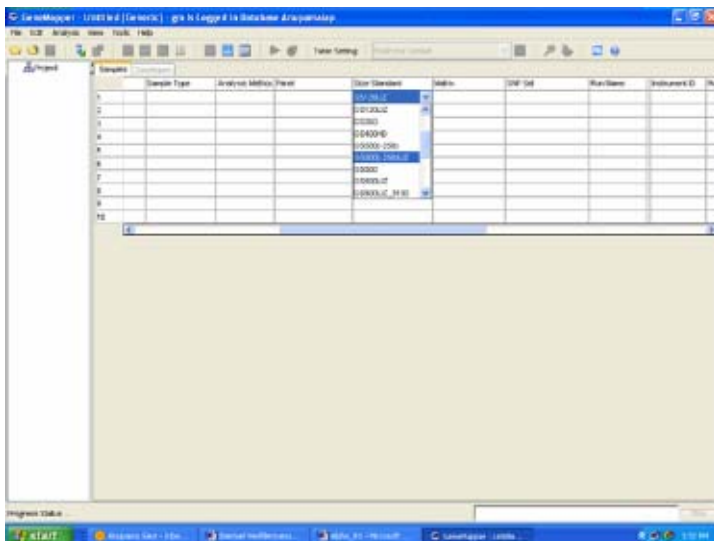
GeneMapper® Software Analysis

To detect adulteration the data has to be analyzed using **GeneMapper® Software (Applied Biosystems)** with the Basmati Panel and Binset (supplied in the CD with the kit) in the following steps:

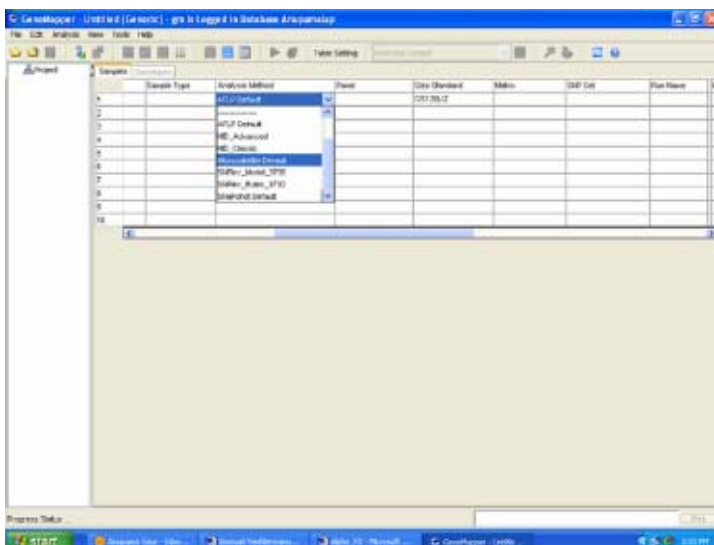
1. Open GeneMapper® Software and add the sample files.



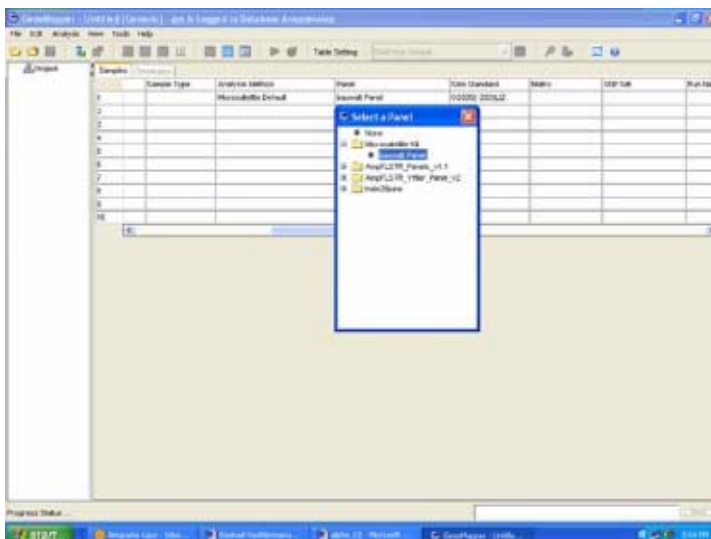
2. Select size standard as GS 500 LIZ from the dropdown menu of the size standard column.



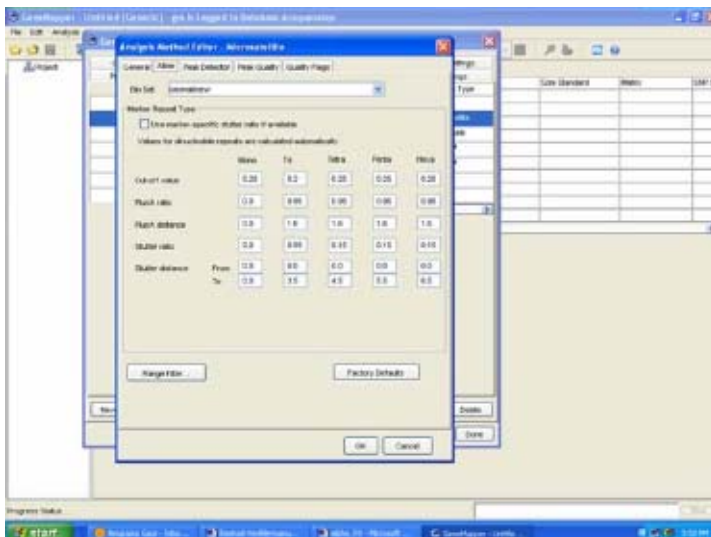
3. Select Analysis method as microsatellite default.



4. Import Basmati Panel (provided in the Information CD) in the panel window.



- In the microsatellite analysis method, select Bin Set as Basmati (provided in the Information CD).



6. Perform the analysis.
7. Open Genotypes table to view the allele calls
8. Identify the Basmati variety by comparing with the known allele profile

Quantification of Adulteration

Adulteration in Traditional Basmati (TB) with Evolved Basmati (EB) or Non Basmati (NB) varieties can be readily detected based on the unique Basmati allele profile (Table 1) using their corresponding peak areas for calculations. The Basmati Verifiler™ Kit consistently amplifies a single allele of eight robust microsatellite markers per variety for each locus and thus, is an efficient tool to distinguish all the Indian and Pakistani notified Basmati varieties among themselves and also from putative adulterants. Traditional Basmati varieties can be differentiated from their long grain putative adulterants by simply looking at the deviations from their specific profile (Table 1). The fraction of adulterant, if any, in the sample can be measured by quantifying the amplification product. The quantity of the amplification product (a peak) is computed by determining the corresponding peak ***area, as reported in the Genemapper™ Software results for the samples.***

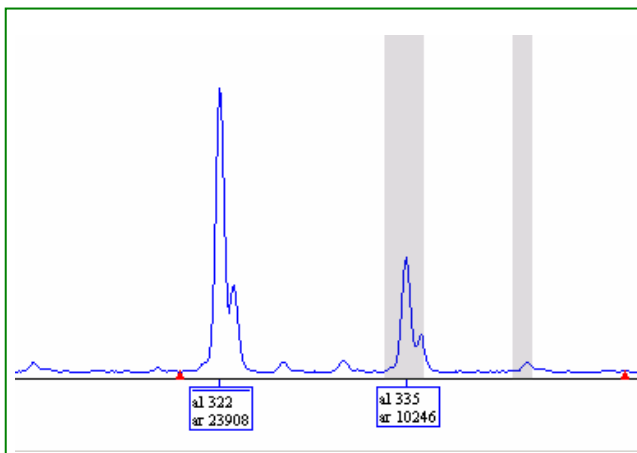
For quantitation of adulteration, only those loci which are showing actual polymorphism are taken for peak area calculation. In most of the cases, alleles of RM72 and RM348 are recommended to be used since these loci show greater stability and reproducibility of the amplification intensity. The peak profiles for all eight loci are compared with the basmati profile chart (Table 1). Peak area of the expected allele (usually of the genuine Basmati variety) is

labeled as **A** and peak area(s) of additional allele(s) (usually of the putative adulterant) as **B**. The adulteration (expressed in percentage) is determined as:

$$\text{Adulteration \%} = \left[\frac{B}{A + B} \right] \times 100$$

Please see references 2 and 3. By achieving greater accuracy through the use of improved dye chemistry and resolution of alleles, it is now possible to quantitate adulteration without referring to standard curves. However, to pursue standard curve based quantitation method, follow the procedure given in reference 2.

Following example will show adulteration calculation using the formula:



•**Fig 2: Allele profile of sample at Locus RM171.**

- Allele A (335 bp) has a peak area of 10246, therefore A=10246
- Allele B (322 bp) has a peak area of 23908, therefore B=23908
- Another minor allele has less than detectable area based on the fixed parameters.

Hence adulteration at locus RM171:

$$\bullet \text{Adulteration} = \left[\frac{23908}{23908 + 10246} \right] \times 100$$

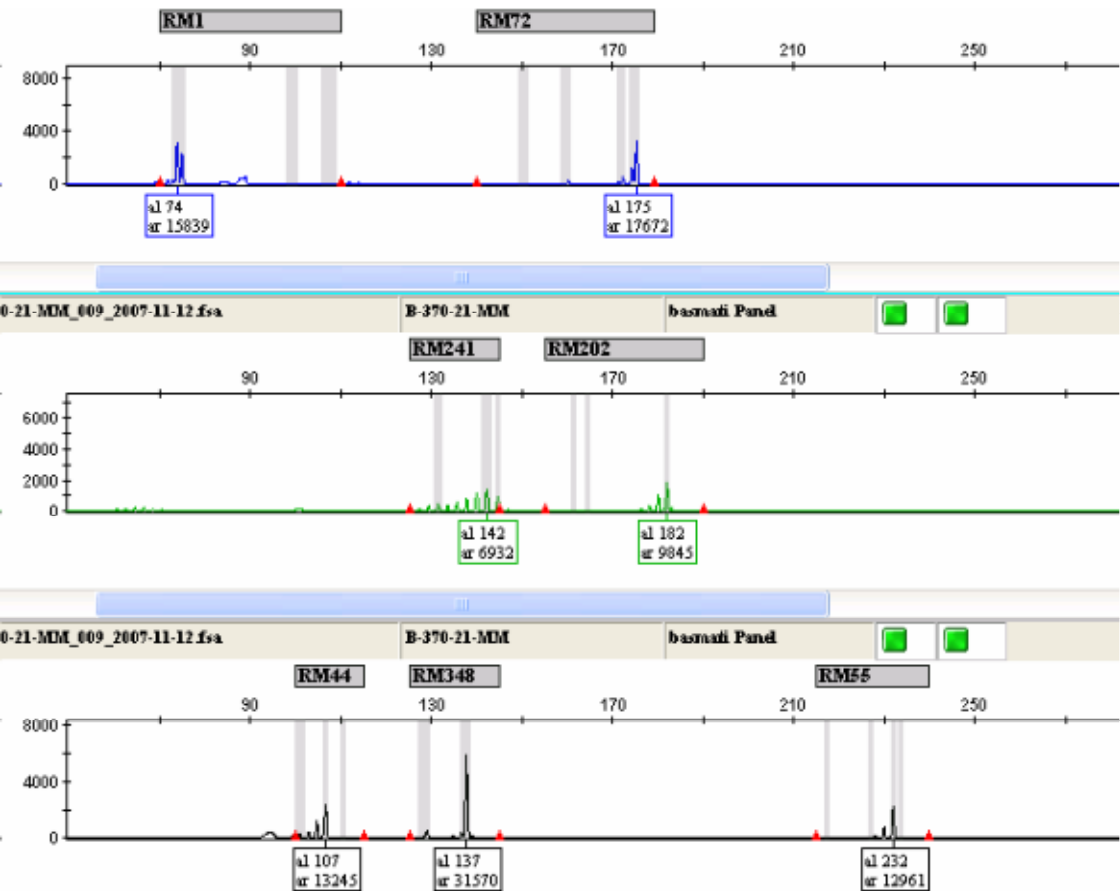
Adulterant in sample = 70%

For any further assistance for data analysis and interpretation please contact Application support. For

GeneMapper® Software related queries please check the User manual.

Annexure-1

The Basmati Verifiler™ Kit Positive Control DNA Profile.



Troubleshooting Guide

Observations	Causes	Recommendations
Faint or no signal from Basmati control DNA and DNA test samples at all the loci	Incorrect volume or absence of reagent of PCR master mix	Repeat PCR amplification
	Inactivation of Basmati PCR enzyme	Make sure that initial denaturation at 95 °C at 10 min is given
	Basmati PCR Buffer mix was not vortexed before aliquoting	Vortex the mixture and use
	Thermal cycling conditions were not proper	Check the thermal cycling conditions and repeat PCR
	PCR product for injection not sufficient	Repeat injection with recommended amount of Product
	Degradation of Hi Di Formamide	Use fresh Hi Di and repeat
Positive amplification from Basmati control DNA but no profile in test sample	Quantity of DNA in test sample not sufficient	Quantitate and take 30ng DNA and repeat amplification
	Test sample might be degraded	Check the quality by running on agarose, and if degraded amplify using more DNA or reisolate DNA
	Test sample might be having inhibitors	Quantitate DNA , wash or reprecipitate and repeat test
Some but not all alleles are visible in electropherogram	Test sample might be degraded	Check the quality by running on agarose, and if degraded amplify using more DNA or reisolate DNA
	Test sample might be having inhibitors	Quantitate DNA , wash or reprecipitate and repeat test
Extra Peaks visible when DNA is known to contain sample from single source	Denaturation was not proper before injection	Denature at 95 °C for 5 min before loading

Appendix

DNA Extraction and Quantitation

Many techniques are available for plant DNA extraction. One can choose depending upon the resources available. The most efficient homebrew method for Basmati DNA extraction is the CTAB method.⁴ Among the different kits available commercially for DNA extraction, QIAGEN DNeasy® Plant Mini Kit (Cat.# 69104) can also be used for Basmati DNA extraction.



Regardless of DNA extraction method used, all samples being processed together must be handled carefully to avoid contamination.

DNA Storage

Extracted DNA can be stored in Tris-EDTA buffer (TE) for up to 6 months at -20°C.

DNA Quantitation

Quantitation of DNA is important prior to PCR amplification as insufficient or excess template DNA leads to erroneous results. The template DNA for all samples should be ~30 ng, so that the peak areas can be compared with the kit control DNA and off-scaling of peaks during analysis can be avoided. One can check an aliquot of the isolated DNA on agarose gel along with a known sized ladder and also quantify using spectrophotometer or fluorimeter. DNA quality is also important as it influences PCR amplification. Lack of amplification or partial amplification is mainly due to degradation of DNA. Before setting up reaction it is always recommended to check the quality of DNA.

References

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3. Reddy VLN, Archak S and Nagraju J (2007). Capillary Electrophoresis Is Essential for Microsatellite Marker Based Detection and Quantification of Adulteration of Basmati Rice (*Oryza sativa*). *Journal of Agricultural and Food Chemistry* 55:8112-8117.
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Notes

