

## FAQs for the Basmati Verifiler™ Kit

### Chemistry & Protocol FAQs

**Q: What is the part number of the Basmati Verifiler™ Kit?**

**A:** BV81001

**Q: What reagents, instrument and software do I need to implement the Basmati Verifiler Kit in my lab?**

**A: Reagents:** PCR grade water from any vendor and the following products from Applied Biosystems (AB): 5-dye matrix or spectral standards (DS-33) to configure the instrument for 5-dye analysis for Filter set G5, GS500 LIZ size standard for sizing and Hi-Di Formamide.

**Instrument:** The Basmati Verifiler Kit is validated for electrophoresis on the AB 3130 and 3730 series instruments.

**Software:** GeneMapper® software v3.1 or higher for data analysis.

**Q: What are the correct storage conditions for the kit?**

**A:** The kits are shipped frozen and should be maintained at –20°C. Once opened for use, it is recommended that control DNA provided in the kit should be stored separately at –20°C.

**Q: Do we need to add more Taq to improve the PCR reaction for difficult samples?**

**A:** The Basmati Verifiler kit has been optimized to yield results with balanced peak heights with 30-40 ng of DNA. Increasing the amount of Taq is not necessary and will not improve the performance of the assay.

**Q: Which loci can be analyzed by the Basmati Verifiler™ Kit?**

**A:** The Basmati Verifiler™ Kit employs an 8-plex assay and can be used to analyze the following loci: RM1, RM72, RM171, RM202, RM241, RM44, RM55 and RM348.

**Q: What is the recommended protocol for isolating DNA from rice samples?**

**A:** Laboratories may use any standardized protocol for isolating DNA available resources. We have found Kang HW et al., 1998 (References, below) to be the most effective method to isolate DNA from Basmati rice. The QIAGEN DNeasy® Plant Mini Kit (Cat # 69104) can also be used for Basmati DNA extraction.

**Q: What are the specifications for the sample DNA to be tested for optimal results with Basmati Verifiler Kit?**

**A:** The Basmati Verifiler Kit has been optimized to amplify approximately 30 – 40 ng of sample DNA reliably. The PCR cycle number and amplification conditions have been specified to produce balanced peak heights for a sample containing 30 ng of rice genomic DNA. Labindia has successfully typed samples containing 30 – 40 ng DNA.

**Q: How can extraction/purification methodology impact results from samples?**

**A:** The presence of PCR inhibitors that are co-extracted with sample DNA or degradation in sample DNA can result in allele dropout. Degradation or inhibition in sample DNA can be confirmed with results obtained with positive control DNA.

**Q: What indicators of degradation and/or inhibition should I look for in sample data?**

**A:** In such samples, the ability to amplify longer amplicons is diminished relative to the ability to amplify the shorter amplicons. Samples containing inhibitors may exhibit partial profiles similar to those generated from degraded samples, resulting in complete loss of all alleles or resulting in drop-out or imbalance at select loci.

**Q: What precautions should be taken during setting-up an amplification reaction with Basmati Verifiler™ Kit?**

**A:** --Once opened for use, it is recommended that control DNA provided in the kit should be stored separately at  $-20^{\circ}\text{C}$ .  
--All the kit components except the enzyme should be vortexed and spin briefly before setting-up a reaction.  
--Care should be taken not to contaminate the reagents from the kit.  
--DNA from the samples and positive control DNA should be added by a separate pipette in a different area.  
--Amount of DNA per sample should be as per the kit specifications.

**References**

Kang HW, Yong GC, Yoon UC and Eun MY (1998). A Rapid DNA extraction method for RFLP and PCR analysis from a single dry seed. *Plant Molecular Biology Reporter* 16:1-9.