Application Note

Authentication of Basmati rice using SSR-PCR and QIAxcel® Advanced

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Introduction

Basmati is one of the most popular types of rice in the world, representing approximately 40% of the dry rice market. Being twice as expensive as non-Basmati rice, it is also often mixed with other types of rice. Approximately 46% of sold Basmati rice is adulterated. The term Basmati is a customer generic name for 15 varieties of rice produced exclusively in India and Pakistan. Nine of the 15 varieties are eligible for zero import duty in the European Union (Regulation (EC) 1549/2004). The 6 other varieties are approved only in India and Pakistan. These two countries also define which varieties can bear the name "Basmati" (Table 1).

Import of Basmati rice into the European Union must comply with several regulations (EC No 1234/2007, 1785/2003 and 972/2006) and requires an import certificate (1). The rice must be analyzed before and sometimes after import. Therefore, analyses that enable identification and quantification of Basmati rice are of great importance. The price of the rice depends on the variety as well as on several physical properties, including the proportion of green grains and broken grains. Various physical methods have been developed for identification based on examining grain dimension, amylose content, grain elongation through ▷

Table 1. Varieties of Basmati rice (reference: the Basmati Code)

Eligible for zero import duty under Regulation (EC) 1549/2004	
Basmati 217	Ranbir Basmati (IET 11348)
Basmati 370	Super Basmati
Basmati 386	Taraori Basmati (HBC-19, Kamal Local)
Kernel Basmati (Basmati Pakistan)	Type – 3 (Dehradun)
Pusa Basmati (IET 10364)	
Other varieties approved in India and Pakistan	
Basmati 198	Kasturi (IET 8580)
Basmati 385	Mahi Suganda
Haryana Basmati	Punjab Basmati (Buani Basmati)
(HKR 228/IET 10367)	

cooking and aroma, but these are time-consuming or unreliable. Microsatellites or simple sequence repeats (SSR) are molecular markers used for identification (2–4). Generally, interpretation of SSR data can be difficult, especially for an admixture of more than 3 individuals. In addition, fragment sizing requires a high degree of accuracy. Consequently, this method can be very time and resource-consuming, especially when performing simplex analyses using conventional gel electrophoresis. The QIAxcel Advanced capillary electrophoresis system can replace conventional gel electrophoresis with an automated process that minimizes manual intervention and errors. Analysis is fast (96 samples in 1 h 30 min) and inexpensive. Additionally, the QIAxcel ScreenGel® software estimates the sizes and areas of the fragments analyzed, rendering complex analysis software unnecessary because interpretation can be done directly with ScreenGel and the Excel® spreadsheets it produces. Using the QIAxcel DNA High Resolution DNA Kit makes an accuracy of 3–5 bp possible, which is crucial for correct sizing of SSRs. We have developed an SSR-PCR protocol for routine analysis of Basmati rice using the QIAxcel Advanced System, where all Basmati as well as other types of rice can be identified and quantified as DNA percentage of the Basmati variety.

Materials and methods

Grains from a range of Basmati and non-Basmati rice samples were selected and homogenized. Homogenized samples were mixed with lysis buffer (Qiagen ATL), heated to 65°C, and treated mechanically by agitation at 1400 rpm. DNA was purified with the QIAsymphony® DSP DNA Mini Kit. DNA amplification by duplex PCR was performed with a set of 8 SSR markers (RM1 + RM72, RM44 + RM55, RM202 + RM241 and RM171 + RM348) using the QIAGEN Multiplex PCR Kit. For some Basmati rice, 2 additional SSR markers were used for more accurate discrimination (data not shown). Size range of the PCR products was 80–400 bp (Figure 1). Capillary electrophoresis was carried out using the QIAxcel DNA High Resolution Kit, QX Alignment Marker 15 bp/600 bp, and QX DNA Size Marker 25–500 bp v2.0. QIAxcel ScreenGel software was used to estimate fragment size and percentage of target rice in the analyzed samples. The type of rice was identified using data from the Excel spreadsheets produced by the software.

Results and discussion

Identification

Analysis of different types of Basmati rice at 8 loci demonstrated that the rice varieties could be identified with high discriminatory power. Results showed characteristic, stable and reproducible DNA fragment profiles for each rice variety (Figure 1). Reproducibility and stability were assessed by repeating the analysis 12 times with a Taraori Basmati rice sample (Figure 2).

Quantification

When non-Basmati rice was detected in a sample, the quantity of Basmati rice DNA was presented as a percentage (Figure 3). This method is based on the comparison of the allele profiles of a known pure-grain sample and the unknown, tested sample. If a new peak was detected above a given threshold level, all possible profiles of all loci were identified. The area under the peak of every marker was calculated as a percentage. The average of all values was considered the quantity of Basmati rice DNA in the tested sample (Figure 4). The overall uncertainty of the analysis was 5.2%. The uncertainty when over 95% of the mixture was Basmati rice was 1.9%, which complies with EU regulations. The quantification had an accuracy of 0.2% and a dispersion of 2.0% based on 133 measurements made on 37 different mixtures of Basmati and non-Basmati varieties.

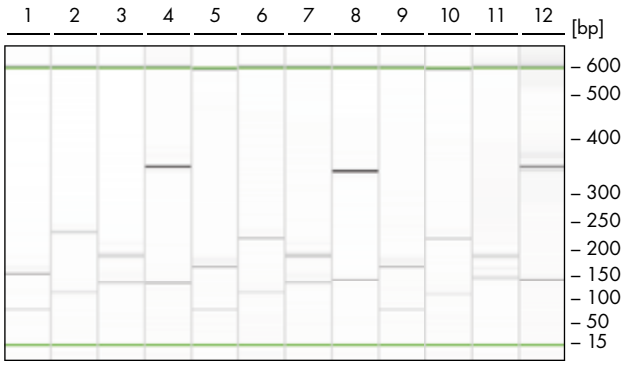


Figure 1. Reproducible and stable profiles of 3 Basmati rice varieties. **Lanes 1–4**: Pusa. **Lanes 5–8**: Taraori. **Lanes 9–12**: Super Basmati. The SSR markers were RM1 + RM72, RM44 + RM55, RM202 + RM241 and RM171 + RM348.

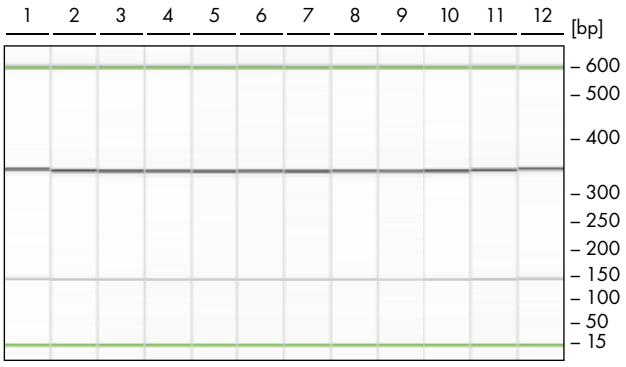


Figure 2. SSR profiles for Basmati rice are reproducible. The Taraori variety was analyzed 12 times with the SSR markers RM171 + RM348 and yielded the same fragment profile.

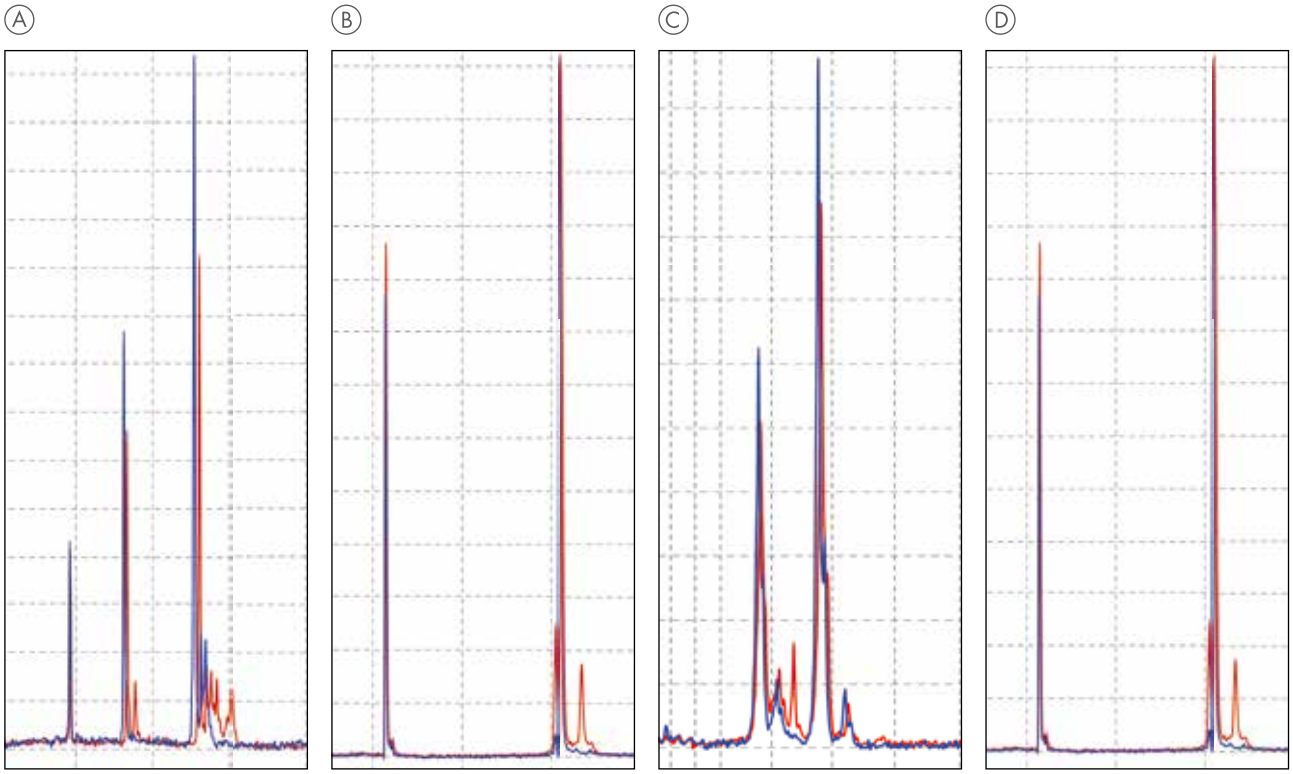
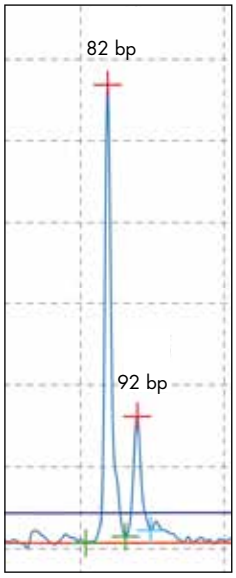


Figure 3. Sensitive detection of non-Basmati rice in a sample. Comparison of pure Taraori grain (blue peak) and a sample containing 75% Taraori and 25% adulterant (red peak). **A**: RM1 + RM72, **B**: RM44 + RM55, **C**: RM202 + RM241, **D**: RM171 + RM348.



#	Size (bp)	NA	NA %
1	15	0.0018099	n/a
2	82	0.0026379	75.86
3	92	0.0008393	24.10
4	600	0.0020555	n/a

Figure 4. Quantification of the RM1 marker in a sample of 75% Taraori Basmati and 25% non-Basmati rice. The 82 bp peak comes from Basmati and the 92 bp peak from the non-Basmati rice. The normalized area percentage (NA %) of each peak represents the quantity of Basmati rice and adulterant, respectively.

Analysis limitations

The identification of rice varieties was based on a database. A sample that generates a profile that is not present in the database cannot be identified. The 8 markers in this study were not sufficient to unambiguously identify all 15 Basmati rice varieties. In ambiguous cases, we used 2 additional markers to identify the variety (data not shown). When a sample consisted of 3 or more rice varieties, the allele patterns became complex (Figure 5), making complete sample identification and quantification difficult or at times, impossible. However, in most cases, identification and quantification could be done by independently analyzing grains that differ in physical characteristics (grain profiles). This method is more time-consuming and expensive, with an uncertainty between 6.5% for samples with >95% Basmati rice content and 22.2% for samples with lower Basmati rice content (average 14.1%).

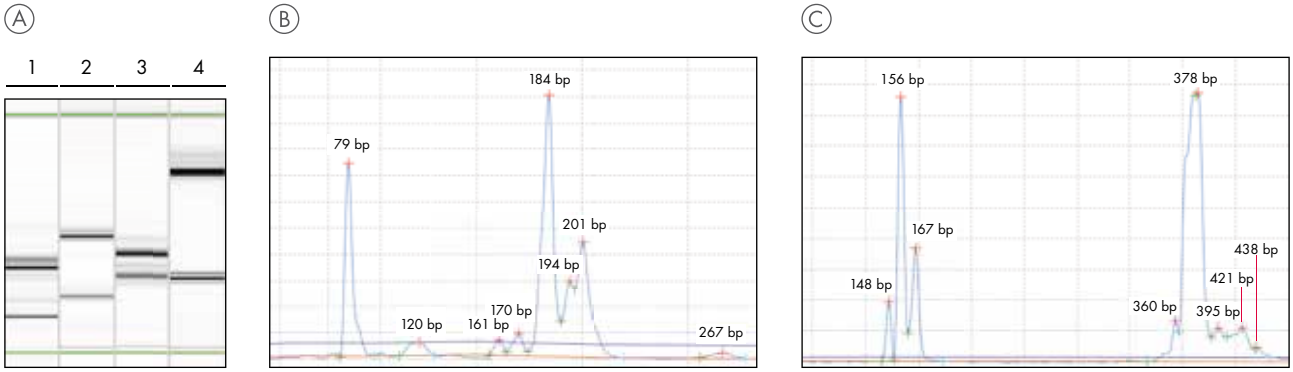


Figure 5. Analysis of a sample containing more than 3 rice varieties. A. **Lane 1**: RM1 + RM72. **Lane 2**: RM44 + RM55. **Lane 3**: RM202 + RM241. **Lane 4**: RM171 + RM348. **B**. Electropherogram of the RM1 and RM72 markers. **C**. Electropherogram of the RM171 and RM348 markers. With such complex allele patterns, identification requires additional analysis of physically different grains present in the sample.

Conclusions

- The SSR-PCR protocol for the QIAxcel Advanced System combined with a SSR marker allele database for rice proved to be accurate, reliable and fast, making this experimental setup a good candidate for routine Basmati rice authentication analysis.
- Identification and quantification of Basmati rice and its adulterants was successfully performed using the QIAxcel High Resolution Kit, which discriminates SSR fragment sizes with an accuracy of 3–5 bp.
- The method is best suited for samples containing 1 or 2 different rice varieties. Analysis of samples with 3 or more different rice types is more complex and requires additional testing.
- The method detects adulterant contents as low as 0.1%, with quantification of up to 99.0% Basmati rice. The overall uncertainty is 5.2%, and only 1.9% for mixtures with a Basmati rice proportion higher than 95%.

References

1. British Retail Consortium (2005) Code of Practice on Basmati Rice. **www.brc.org.uk/Downloads/Basmati_Code.pdf**
2. Archak S., Lakshminarayanareddy, V. and Nagaraju, J. (2007) High-throughput multiplex microsatellite marker assay for detection and quantification of adulteration in Basmati rice (*Oryza sativa*). Electrophoresis **28**, 2396.
3. Vemireddy L.R., Archak, S. and Nagaraju, J. (2007) Capillary electrophoresis is essential for microsatellite marker based detection and quantification of adulteration of Basmati rice (*Oryza sativa*). J Agric Food Chem **55**, 8112.
4. Steele K. (2009) Rice Authentication using SSR and InDel markers. Online presentation. **www.ec.europa.eu/agriculture/analysis/external/basmati/ssr_markers_steele_en.pdf**

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced Instrument	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High-Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
Buffer ATL (4 x 50 ml)	Lysis buffer used in purification of nucleic acids using QIAsymphony DSP Virus/Pathogen kits	939016
QIAsymphony DSP DNA Mini Kit (192)	For 192 preps of 200 µl each. Includes 2 reagent cartridges and enzyme racks and accessories	937236
QIAsymphony SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAGEN Multiplex PCR Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 x 0.85 ml), 5x Q-solution (1 x 2.0 ml), RNase-free water (2 x 1.7 ml)	206143
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25–500 bp (50 µl) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400 and 500 bp; concentration 100 ng/µl	929560

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