# Use of the Agilent 2100 Bioanalyzer for Basmati Rice Authenticity Testing

Application

**Food**


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## Abstract:

**Ensuring integrity of raw food materials, ingredients, and products is both a product quality and regulatory compliance concern. Food suppliers and manufacturers may suffer economic and legal damages if proven to be supplying incorrectly labeled products. For example, EU Commission 1549/04 grants lower import tax on nine basmati rice varieties. A quick and cost-effective analytical method utilizing the Agilent 2100 bioanalyzer and DNA 1000 assay is shown as an alternative method to establish the authenticity of basmati rice products and to estimate the level of some varieties of non-basmati rice in ground rice products.**


## Introduction

The integrity of raw food materials, ingredients, and products must be maintained to ensure that they meet appropriate quality and legislative requirements. Food ingredient suppliers, manufacturers, and retailers can face legal action if proven to be supplying materials or products that are incorrectly labeled due to substitution or contamination.

One food area which has been under the spotlight in recent years is the supply of basmati rice to the UK from India and Pakistan. In Europe, Commission Regulation 1549/04 grants a lower import tax on nine basmati varieties: Basmati 370, Dehradun (Type 3), Basmati 217, Taraori, Ranbir Basmati, Kernel, Basmati 386, Pusa Basmati, and Super Basmati. Other basmati rice varieties approved by India, Pakistan, and the UK include Basmati 198, Basmati 385, Haryana Basmati, Kasturi, Mahi Suganda, and Punjab Basmati. In a Code of Practice developed by Indian, Pakistani, and UK industry and enforcement organizations that came into effect for products packed and labeled after January 2006, the level of non-basmati rice in a basmati rice product must not exceed 7% (see www.riceassociation.org.uk).

In order to check the supply of basmati rice, a DNA variety testing method using PCR amplification of eight rice microsatellite sequences has been developed for UK compliance (www.foodstandards.gov.uk/multimedia/pdfs/fsis4704basmati.pdf). During 2003, the UK Food Standards Agency carried out a surveillance exercise on basmati rice products using this method and revealed that 74% of them contained > 7% non-basmati varieties.

This study evaluates the use of the Agilent 2100 bioanalyzer to differentiate approved and non-approved varieties using three primer sets and to estimate the level of non-basmati using reference rice admixtures.

## Experimental

### Method

All reference basmati rice samples were obtained from the UK Food Standards Agency via the University of Wales (Bangor, UK ). Other samples were from in-house basmati rice sample collections.

PCR amplification was performed using either a PE9600 or PE2400 PCR machine (Applied Biosystems).

### Extraction

DNA was extracted from ground rice grains using Qiagen's DNeasy Plant Mini Kit or Promega's Maxwell 16 automated DNA extractor

### PCR

DNA extracts were diluted 1 to 1 in sterile distilled water (SDW) to produce template DNA prior to use in PCRs. Amplification was performed in 25-µL PCRs containing 1x Amplitaq Gold PCR buffer (Applied Biosystems), 60 nM of each primer, 200 nM dNTPs, 3 mM $MgCl_2$, 0.05 U/µL of Ampli-Taq Gold (Applied Biosystems), and 2 µL of template DNA.

| Marker | Forward Primer |
|---|---|
| RM201 | CTC gTT TAT TAC CTA CAg TAC C |
| RM212 | CCA CTT TCA gCT ACT ACC Ag |
| RM339 | GTA ATC gAT gCT gTg ggA Ag |
| **Marker** | **Reverse Primer** |
| RM201 | CTA CCT CCT TTC TAg ACC gAT A |
| RM212 | CAC CCA TTT gTC TCT CAT TAT g |
| RM339 | gAg TCA TgT gAT AgC CgA TAT g |

Amplification profiles (95 °C for 15 minutes [denaturation]; 50 cycles of: 95 °C for 1 minute, 60 °C for 1 minute [amplification]; 72 °C for 10 minutes [final extension]) were used in all PCR reactions. DNA amplification was confirmed by separating PCR products using the Agilent 2100 bioanalyzer.

### Capillary Gel Electrophoresis on 2100 Bioanalyzer

Reagents were prepared following manufacturers' instructions. Batches (~500 µL) of gel matrix (used to fill LabChip capillaries) were prepared as required or at 4 weekly intervals. All reagents were stored at 4 °C and allowed to reach room temperature for 1 hour before use. PCR products (1 µL)

**Table 1. Analysis of Authenticated Basmati Rice Varieties Using Three Microsatellite Primer Sets**

| Rice variety | List of microsatellite amplification product sizes obtained with primer set* | | |
|---|---|---|---|
| | RM201 | RM212 | RM339 |
| Varieties listed in Commission Regulation 1549/04 | | | |
| Basmati 370 | 162 (**162**) | 134 (**139**) | 200 (**193**) |
| Dehra Dun (Type 3) | 162 | 134 (**139**) | 200 (**195**) |
| Basmati 217 | 162 | 134 | 200 |
| Ranbir | 162 | 134 | 200 |
| Taraori | 162 | 134 (**139**) | 200 (**195**) |
| Basmati 386 | 162 | 134 (**138**) | 200 (**195**) |
| Kernel | 162 | 134 | 200 |
| Pusa | 162 | 134 (**139**) | 200 (**194**) |
| Super | 162 | 134 (**140**) | 204 (**196**) |
| Other varieties approved as basmati by UK Food Standards Agency | | | |
| Basmati 198 | 162 | 152 | 200 |
| Basmati 385 | 162 | 152 | 200 |
| Kasturi | 162 | 132 | 166 |
| Haryana Basmati | 162 | 152 | 166 |
| Mahi Sugandha | 176 | 152 | 166 |
| Punjab Basmati | 162 | 152 | 200 |
| Non-approved varieties | | | |
| Basmati 2000 | 162 | 152 | 204 |
| Shaheen Basmati | 162 | 152 | 200 |
| Sherbati | 178 (**176**) | 130 (**135**) | 166 (**167**) |
| Mugad Sugandha | 178 | 132 | 166 |
| Pak 386 | 178 | 130 (**135**) | 166 (**167**) |
| Superfine | 178 | 132 | 166 |
| Pusa Sugandha | 162 | 132 | 178 |
| Yamini | 162 | 134 | 200 |

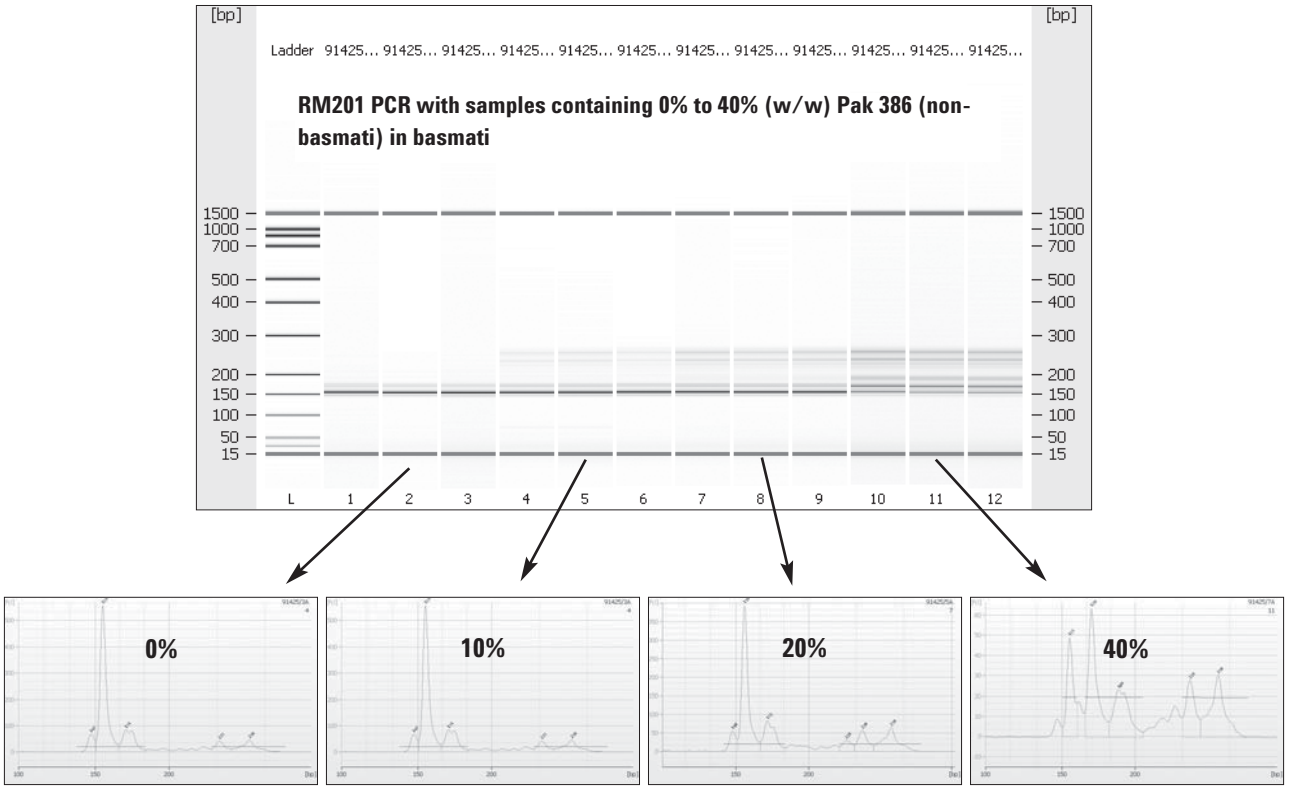
*These are from the FSA method developed by the University of Wales, Bangor. Actual size of fragments determined by the bioanalyzer using a DNA 500 chip kit are shown in brackets. The variation in bioanalyzer-determined fragment sizes can be about 5%. Shaded cells show how the varieties can be grouped using the three primer sets with analysis performed on the bioanalyzer.

were loaded directly onto prepared Series I DNA 500/DNA 1000 or Series II DNA 1000 labchips. All analysis was performed on the 2100 bioanalyzer, as per the manufacturers' instructions.

## Results and Discussion

The different-sized PCR products generated when these three primer sets are applied to the different varieties are easily resolved on the bioanalyzer using the DNA 500 or DNA 1000 chip. RM 212 primers produce a 139 bp product with varieties listed in Commission Regulation 1549/04 and a 154 bp product with FSA-approved varieties apart from Kasturi and a few non-basmati varieties. The other primer sets enable separation of these varieties apart from the Yamini variety, which produces fragments similarly sized to the EC-approved varieties for all three primer sets. This variety is also difficult to distinguish from approved varieties using the standard method. The other FSA-approved basmati varieties could be distinguished from the EC-approved varieties but not from Basmati 2000 or Basmati 386 using these microsatellite primer sets. Use of further microsatellites that give PCR products that can be separated on the bioanalyzer will give improved differentiation of non-basmati rice varieties.

**a) Primer set RM201**



**b) Primer set RM339**

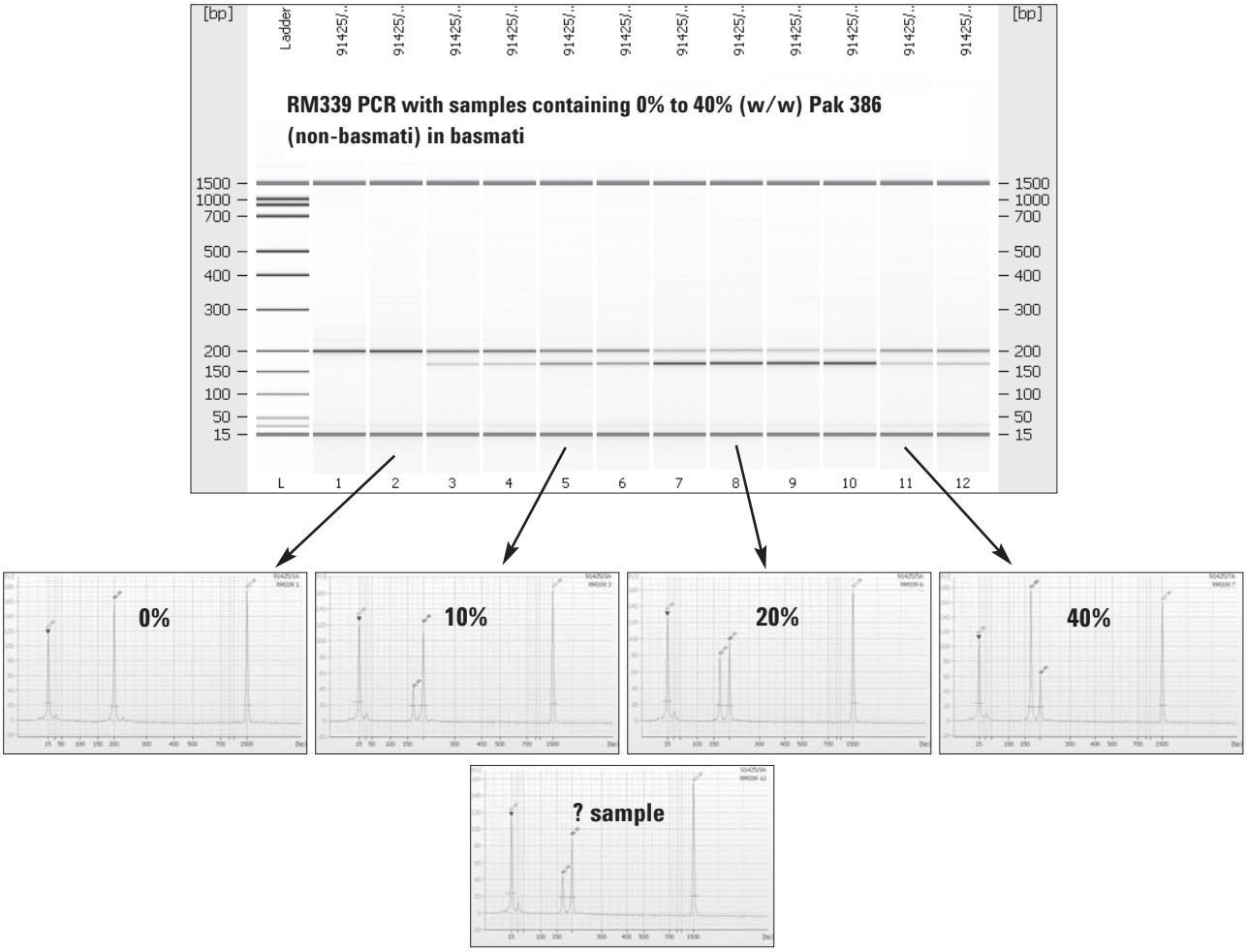



**Figure 1. Analysis of non-basmati/basmati rice admixtures using two microsatellite primer sets. Estimation of percentage non-basmati variety (Pak 386) in an unknown sample.**

**Table 2. Experimental Summary**

| % Pak 386 in basmati rice | Primer RM201 mean ratio 156 bp/170 bp PCR fragments (n = 3) | Primer RM339 mean ratio 199 bp/169 bp PCR fragments (n = 2) |
|---|---|---|
| 0 | 9.6 | 0 |
| 10 | 3.5 | 3.1 |
| 20 | 2.7 | 1.1 |
| 40 | 0.6 | 0.4 |
| 50 | 0.7 | 0.3 |
| Unknown sample estimated to contain between 10–20% Pak 386 | 3.1 | 2.1 |

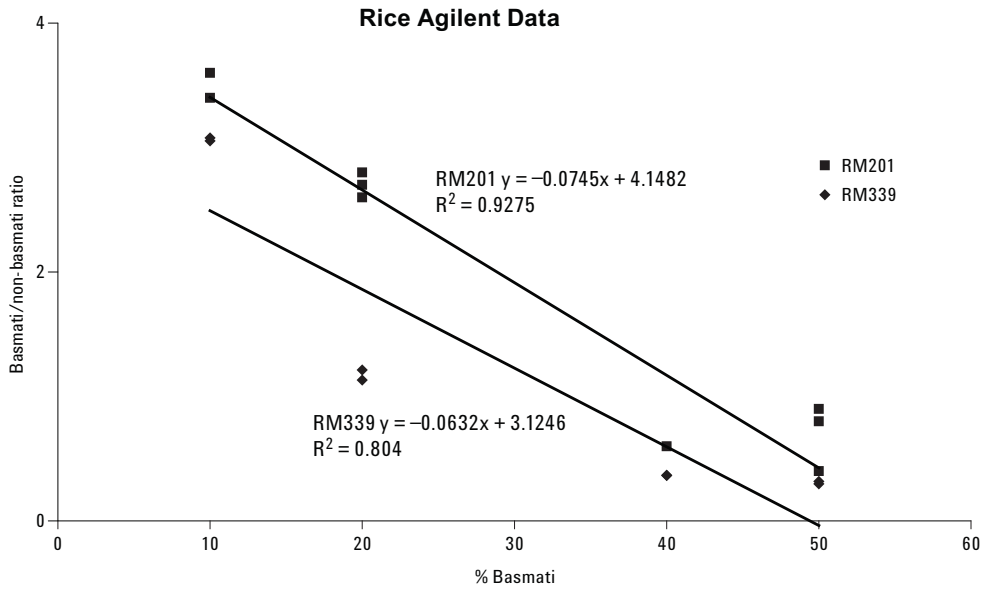



**Figure 2. Standard curves based on the PCR product concentration ratios.**

Both the RM201 and RM339 primer sets (Figure 1 and Table 2) could be used to produced separate and measurable PCR products with the PAK 386 basmati rice admixtures. The RM201 primers set gave a 170 bp product generated from the basmati rice and a 156 bp product from the Pak 386 variety, whereas RM339 primers gave 199 bp and 169 bp products. RM281 also gave other larger PCR products, which seemed to correlate with the presence of Pak 386 in the admixture. Standard curves (Figure 2) were produced using the PCR product concentration ratios. An unknown admixture sample was also analyzed and estimated to contain between 10 and 20% non-basmati rice in basmati.

Results show that the bioanalyzer can be used to estimate the level of non-basmati rice (Pak 386) in a basmati rice which produces different sized microsatellite PCR products from the Pak 386.

## Conclusions

The Agilent 2100 bioanalyzer can be used as a quick and cost-effective alternative to establish the authenticity of basmati rice products and to estimate the level of some varieties of non-basmati rice. It should be feasible to develop further primer sets that would allow the bioanalyzer to be used to identify individual varieties. Simple manual and automated DNA extraction followed by fast PCR and post-PCR analysis on the bioanalyzer would allow rapid screening of rice materials prior to export from India and Pakistan and also allow enforcement bodies to efficiently test for microsatellite markers from non-approved basmati rice varieties in imported products.

## Acknowledgements

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## For More Information

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