

FOOD STANDARDS AGENCY INFORMATION BULLETIN ON METHODS OF ANALYSIS AND SAMPLING FOR FOODSTUFFS

This Bulletin is issued by the Food Standards Agency to Public Analysts, to other analysts working in the food sector and to others with an interest in the sector. Its principal purpose is to act as an electronic consultation forum on methods of analysis and sampling for foodstuffs proposed for inclusion in EU Regulations and Directives, or on topics to be discussed in the organisation such as the Codex Alimentarius Commission. Other topics, e.g. forthcoming collaborative trials to validate specific methods of analysis, will be covered from time to time.

This Bulletin may be regarded as the successor to the MAFF Information Bulletin for Public Analysts on EEC Methods of Analysis and Sampling for Foodstuffs. ***However, unlike that Bulletin, it will only be issued in electronic form.*** It will be issued in pdf format downloadable from the FSA Website.

It should be regarded as somewhat less formal than the previous Bulletin. Comments are invited on any items included in the Bulletin, but only *via* a conventional email approach rather than through a Bulletin Board approach. It is hoped that this will not only elicit comments but also develop discussion between recipients; comments will therefore be copied to all Bulletin recipients.

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Contents

Cumulative Index for the Food Standards Agency Information Bulletin on Methods of Analysis and Sampling for Foodstuffs

See separate index.

Report on the inter-laboratory trial of the microsatellite method for the identification of certain basmati rice varieties

The Food Standards Agency has carried out the above qualitative collaborative trial. The results are given below.

Comments on the results from this trial may be sent to:

Mark Woolfe (mark.woolfe@foodstandards.gsi.gov.uk)

Thank you.

REPORT ON THE INTER-LABORATORY TRIAL OF THE MICROSATELLITE METHOD FOR THE IDENTIFICATION OF CERTAIN BASMATI RICE VARIETIES.

Summary

Eleven laboratories participated in the trial. The laboratories were provided with a DNA microsatellite standard operating procedure (SOP), eleven DNA extracts of Basmati and non-Basmati rice varieties, and five unknown commercial rice samples sold as 'Basmati'. Even though participant labs were using different capillary sequencers, dyes, and in some cases different DNA Taq polymerase, there was a large measure of agreement on the alleles obtained for the DNA extracts of the eleven standards. On the unknown samples where the sample was predominantly one variety, there was good agreement between the labs on identifying the unknown variety. Where the sample was a more complex mixture, identification was more difficult. However, even in complex cases it was possible to distinguish the group of Basmati varieties that were present rather than the individual variety. This would still be sufficient to determine compliance with Commission Regulation 1549/04 as regards the presence of non-listed varieties. However, identification of rice varieties in commercial samples with a mix of the more than two varieties would require the use of single grain assay.

As a result of the feedback from the participating labs, improvements to the SOP are recommended in this report. These include optimised conditions for Beckman Coulter and ABI capillary sequencers, replacement of one or two of the markers in the database with new ones, which give better differentiation between Basmati and non-Basmati varieties. It is recommended that the use of an internal reference sizing standard, and a reference collection of authentic rice varieties to be held by institutions such as IRMM Geel, would strengthen the SOP.

The trial was carried out under the Agency's Food Authenticity Programme. Further information can be obtained from Dr Mark Woolfe, Food Standards Agency, Enforcement Division, Room 425, Aviation House, 125 Kingsway, London WC2B 6NH. Tel +44 (0) 207 276 8176. e-mail: mark.woolfe@foodstandards.gsi.gov.uk

Introduction

1. The DNA microsatellite method has been developed to assist in the checking of consignments of Basmati rice imported into the EU. Commission Regulation 1549/04 grants a refund concession on nine Basmati varieties - Basmati 370, Dehradun (Type 3), Basmati 217, Taraori, Ranbir Basmati, Kernel, Basmati 386, Pusa Basmati and Super Basmati. The objective of the trial was to verify that the method could be used in a number of laboratories i.e. that labs were proficient in using the method. Also whether the standard operating procedure (SOP) developed was robust and fit for purpose.

Materials and method provided for the trial

2. A list of participating laboratories is given in Annex 1. The SOP (Annex 2) for the DNA microsatellite method, based on work funded and evaluated by the Food Standards Agency, was circulated to the participating laboratories. The SOP explains the basis of the method and gives detailed instructions on how to carry it out. Dried DNA extracts of known rice varieties (Table 1) were provided by the University of Wales, Bangor and sent to each laboratory. The extracts required rehydration before use in the PCR cyclers. As each laboratory received the same DNA extract, they should determine similar alleles.

Table 1 Freeze-dried DNA extracts of varieties used in the trial

Basmati 198	Basmati 385
Basmati 386	Dehradun (Type 3)
Mugad Sugandha	Pusa Basmati
Pusa Sugandha	Pakistan 386
Sherbati	Taraori (HBC 19)
Yamini (CSR-30)	

3. In addition, 10g of five unknown commercial samples of milled white rice labelled as 'Basmati' were also sent. Participants were asked to record all the alleles obtained from the standards and samples, and identify which varieties are present in the unknown samples.

Results and Discussion

Sizing of alleles in the standards

4. Annex 3 gives all the laboratories' results for the 11 standards. Where different dyes were used on different makes of capillary sequencer, the allele size was adjusted, so that all the laboratories' results can be compared. Comparing the size of the alleles (number of

repeat units) obtained from the different primer markers is the basis of identification of rice varieties. The capillary sequencer automatically assigns a size in base pairs (bp) to the sample allele by comparing it with known size standards added to the mixture at fixed intervals (usually 20bp), and calculating its size using an algorithm. Differences in sizes up to ± 3 bp from one laboratory to another can be as a result of all or some of the following variables:

- i) Rounding up or down of the size
- ii) Using different dyes attached to the primers
- iii) Using or not using tailed primers
- iv) Not changing the software (algorithm) for the type of dye used
- v) Variations between one run and another
- vi) Choosing which peaks to report in stutter peaks

5. Although there seems to be a high level of failed PCR reactions in some laboratories, there is a high degree of agreement in the alleles obtained on the 11 standards. Apart from one or two cases, most laboratories obtained alleles within ± 2 bp of each other and the consensus allele in Annex B of the SOP. RM 252 was considered a difficult marker to size accurately because it gives a “stutter” (or multiple) peak. It is normal to have a few PCR failures in the trial, and the experienced labs normally repeat PCR runs several times. Further clarification may be needed in the SOP to reduce the failure rate, and also alternatives to the Taq polymerase recommended in the SOP, as well as optimised conditions for PCR.

6. The allele tables in Annex 3 also show that there some larger difference in sizes between the standards sent out and those reported in Annex B of the SOP, e.g. RM252 and RM 282 in Pusa Basmati, RM 44 in Basmati 385, RM 282 in Sherbati. In certain cases the standards sent out were not exactly the same ones as reported in Annex B, which were chosen as consensus alleles from several authentic standards. This indicates the importance of having agreed standards in the database. One recommendation would be that an institution such as IRMM, Geel hold agreed standards (even leaf DNA could be used) from the producing countries India and Pakistan. However, even using agreed standards, it must be recognised that the same variety grown in different locations and in different seasons may differ in allele size with one or two of the markers, as there is a natural process of mutation of microsatellites.

Identification of the unknown samples

7. Participating laboratories were required to extract DNA from the rice samples according to the SOP, and identify the varieties present from the PCR alleles using the eight markers in the SOP. The alleles obtained and the varieties identified by each laboratory are recorded in Annex 4. As these are commercial samples purchased on the UK market, their exact identity is not known.

8. As most of the rice samples gave multiple alleles for the some of the markers, this indicated that more than one variety was present. This makes varietal identification more difficult. In order to try and identify which varieties were present, the University of Wales, Bangor also conducted single grain analysis on the samples. Eight single grains of rice were chosen at random, each one extracted and the alleles determined as detailed in the SOP with the 8 markers. The results of these determinations from 11 labs are shown in Annex 5. In addition, Table 1 below summarises the identification of the varieties in samples 1-5 by both bulk analysis by 10 labs and the single grain analyses. In some cases it was difficult to identify even the single grain variety as it did not match exactly the database of alleles. Also statistically, more than eight grains of rice should be used, but for the purposes of this trial the single grain analysis was carried out to find the predominant varieties present in the unknown mixtures.

9. Table 1 shows that where there is a consignment with one variety, then this is relatively simple to identify. Thus all labs were able to identify sample 1 as Super Basmati, and all eight individual grains confirmed this variety. Samples 2, 3 and 5 appeared to contain at least 3 different varieties, and because the resultant allele pattern is therefore difficult to interpret, and only single grain analysis was able to give an indication of the main varieties. Even so, nine labs were able to identify varieties from the Kernel group of Basmatitis in sample 2, but only two labs gave sample 5 as a mix of the Basmati 370 and Kernel groups, although a further eight labs identified the 370 group of Basmatitis. Sample 4 gave 7 out of eight grains identical to the Pusa standard circulated to labs (note this had two alleles difference with the SOP profile), and one grain, which was closest to Sherbati. Only five labs clearly identified Pusa, but 2 further labs listed Pusa Basmati as one of several varieties identified.

10. The analysis of the samples again indicated that most labs concurred with the allele profile found. However mixtures of varieties make identification difficult, and a lab would

need to resort to single grain analysis to be able to identify the varieties separately. In addition, identification would improve with more experience, as some labs have only used this method for the first time.

11. For the purposes of enforcing the EC Regulation, which require single varieties, it would be evident that a mixture was present which included non-Basmati varieties. The SOP permits identification especially if a single variety is present and hence is fit for purpose. However, the trial has also highlighted the need to optimise the SOP for different makes of capillary sequencers, as well as replace one or two of the markers (RM 252 in particular), which might improve identification of varieties a little better than those in the detailed in the SOP.

Table 1. Identification of Unknown Samples and Single Grain Analysis

Sample No.	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Single Grain Analysis (No/8) [*]
1	Super Bas.	Super Bas.	Super /Punjab Bas.	Super Bas.	Super Bas.	Super Bas.	Super Bas	Super Bas	Super Bas.	Super Bas.	Super	Super Bas. (8)
2	Kernel Yamini	Taraori Bas 386	Taraori, Bas386, Kernel Pusa	Yamini	Kernel	Yamini	Taraori 386, Kernel, Pusa	Taraori 386 or Yamini	Taraori, 386, Pusa Sugand. Kernel	Kernel Sherbati	Taraori + ?	Taraori/386 (4) Sherbati/Pak 386 (2) Bas 385?? (1), Ranbir ?? (1)
3	Super Bas.	Type 3 Taraori	Bas 370	Super Yamini	Yamini	Bas 386 Kernel	Type 3 Yamini	Type 3	Taraori Super Bas 386 Kernel	Yamini	Taraori Super	Yamini (3) Super (4) Pusa (1)
4	Kernel	Pusa	Taraori/ 386/ Kernel/ Pusa/ Bas.370	Kernel Pusa	Pusa Sherbati	Not Ident.	Taraori/ 386 Kernel/Pusa	Pusa	386/ Taraori/Ker nel	Taraori Sherbati	Pusa Taraori	Pusa (7) Sherbati (1)
5	Bas 217	Bas 370/ 217	Bas 370	Bas 217	Bas 217/ Kernel	Bas 370/ Bas 217	Bas 370 Bas 217 Ranbir	Bas 198 Type 3	Type 3/ Bas 370 Bas 217 / Pak 385	Super Yamini Type 3	Taraori Bas 370	Taraori Group (3) Bas 370 Group (4) Bas 385 ? (1)
* The number of grains out the 8 analysed where the variety occurred.												

Laboratories Participating in the Trial

Central Science Lab – York, UK

Eurofins Ltd (Medigenomix GmbH) – Martinsried, Germany

Reading Scientific Services Ltd - Reading, UK

Campden and Chorleywood Food Research Association - Chipping Campden, UK

NIAB – Cambridge, UK

LGC – Teddington, UK

EC Joint Research Centre, IRMM – Geel, Belgium

University of Wales – Bangor, UK

Canton Consumer Laboratory – Neuchatel, Switzerland

United Riceland – New Delhi, India

Centre for DNA Finger printing and Diagnostics – Hyderabad, India

PROTOCOL FOR THE IDENTIFICATION OF SELECTED BASMATI RICE VARIETIES

Food Standards Agency, London, and
CAZS, University of Wales, Bangor

February 2005

PRINCIPLE

1. Commission Regulation 1549/04 grants a refund concession on 9 Basmati varieties – Basmati 370, Dehradun (Type 3), Basmati 217, Taraori, Ranbir Basmati, Kernel, Basmati 386, Pusa Basmati and Super Basmati. Eight microsatellite markers have been chosen as an initial DNA test to establish whether those varieties are present in a consignment of rice.
2. Varieties can be identified because they have characteristic genotype profiles (a set of alleles) when tested with all eight markers. Examination of a consignment with these markers following the standard operating procedures in Annex A can prove that the above permitted varieties are present alone or mixed. The test can also detect the presence of other unlisted varieties in the consignment. The examination is not, however, designed to distinguish definitively between all of the permitted varieties. The genetic profiles for Basmati 370 and Basmati 217 are similar, as are those for Kernel and Basmati 386.
3. The screen is qualitative, i.e. it can detect whether a consignment contains a pure variety or a mixture, and it can indicate the main component variety. If a quantitative measure of the amount of unlisted varieties present in a mixture is required, an additional quantitative test procedure should be followed (FSA, 2004).

SAMPLING

4. Sub-samples (1 kg) must be taken from the bulk consignment.
5. Sub-samples must be further reduced down to give a representative sample (1 g) for DNA extraction. The whole 1 kg sub-sample is poured into a funnel and collected in rotating array of 50 ml tubes (Greiner Bio One), one of which is chosen at random. The entire contents (weighing between 70 – 100 g) are milled to fine powder in a coffee-grinder (Bosch MKM 6000) and used for duplicate DNA extractions, each from 1 g of the powder.
6. DNA extracts from single grains are also made for each sample. The grains must be taken at random from the mixed 1 kg sample by pouring the whole sample into a tray and dividing the grains into two and discarding one half, the process is repeated with alternate left and right halves discarded until only 7 grains remain. Each grain is used for a single DNA extract.

MICROSATELLITE SCREEN

7. The listed Basmati varieties, unlisted approved Basmati varieties and a selection of potential adulterant varieties have specific profiles based on different sized alleles produced with the 8 microsatellites (Annex B). By colour-coding alleles, the genotype profiles can be readily compared with those of known variety standards. Some varieties can only be distinguished from each other by one locus (e.g. Yamini and Super differ only for RM339). This information enables verification of a sub-sample, if the profiles of all bulk and single grain extracts match that of a Basmati variety standard. It must be noted that the test cannot detect new varieties for which standards are not available, and hence the Table of alleles and the primers used may have to be updated in the future.

STANDARD OPERATING PROCEDURES**SOP1- STANDARD OPERATING PROCEDURE FOR EXTRACTING DNA FROM RICE****Introduction**

DNA is extracted from rice using the commercially-available Nucleon Phytopure Genomic DNA Extraction Kit (Tepnel Life Sciences PLC, Manchester, UK). Bulk samples are extracted in duplicate. When each sub-sample is used for duplicate bulk extracts, 7 single grains extracts and one blank (water extractions done according to the single grain method), the SL-8510 kit contains sufficient reagents for two sub-samples, and the SL-8511 kit contains sufficient reagents for 15. Note that all plastic-ware and steel balls for single-grain grinding must be sterilised before use.

Procedural variation

No procedural variation is permitted and all steps must be done exactly as described. If for any reason a procedural variation is made then it must be documented in full and the appropriate authority notified.

Preparation of TE buffer

10 x TE buffer is prepared to a concentration of 200 mM tris-HCl (adjusted to pH 8) and 20 mM EDTA. It is diluted 1:9 with distilled with water to give the TE buffer (1 x) used though out the protocol.

DNA Extraction Procedure for bulk samples

1. Grind 20 g of the rice sample to a fine powder in a coffee-bean grinder (Bosch MKM 6000, or similar), and discard it to minimise cross-contamination between samples. Grind the remaining sample and store the powder in a labelled polythene bag. Dry the grinder with pressurised air to remove all dust, swab it with ethanol, and make sure it is dry before the next sample.
2. Weigh approx. 1 g (± 0.05 g) of ground rice into sterile 15 ml centrifuge tubes (Greiner Bio One) labelled A and B to identify duplicates.
3. Add 4.0 ml of Reagent 1 from the Nucleon Phytopure Plant DNA Extraction kit
4. Add 1.4 ml of Reagent 2 from the Nucleon Phytopure Plant DNA Extraction kit
5. Vortex thoroughly to mix, approx 15 seconds
6. Incubate at 65 °C in a controlled-temperature water-bath or heat block for 10 mins, then cool on ice for 20 mins
7. Transfer 1.8 ml from the top to a 2 ml microcentrifuge tube (Eppendorf) and centrifuge at 13000 g for 15 min at 4 °C
8. Transfer 0.5 ml of the supernatant to a fresh microcentrifuge tube and place on ice for 20 mins
9. Add 0.5 ml of chloroform (stored at -20 °C)
10. Add 0.1ml of resuspended Nucleon Phytopure DNA extraction resin
11. Vortex for 15 seconds

12. Place on a rotating platform shaker (IKA-VIBRAZ-VXR, Janke and Kunkel, or similar) for 10 min at room temperature, to mix
13. Centrifuge at 16000 g for 15 min
14. Transfer 0.5 ml of the upper phase (containing DNA) to a fresh microcentrifuge tube
15. Add 0.5 ml of propan-2-ol (stored at -20 °C) and gently invert tube 10 times, to mix
16. Centrifuge at 16000 g for 15 min to pellet DNA
17. Decant supernatant and add 0.5 ml 70 % (v/v) ethanol
18. Centrifuge at 16000 g for 5 min at room temperature to pellet DNA
19. Decant supernatant and air dry pellet for 10 min
20. Resuspend DNA in 0.5 ml TE buffer and allow to rehydrate for 1 hour
21. Store DNA at 4 °C (for up to 1 month) or at -20 °C (for more than one month) in the labelled microcentrifuge tube.

DNA Extraction Procedure for single grains

The protocol for single seed extracts is the same as for bulk samples (above), except for modifications at the steps indicated.

- 1-2. Grind up 7 single grains simultaneously in sterile 2 ml tubes (Eppendorf) with steel balls in a TissueLyser (Qiagen Ltd, Crawley, West Sussex, UK) mill for 2 min at 50 Hz.
3. Add 0.9 ml of Reagent 1 from the Nucleon Phytopure Plant DNA Extraction kit to the tube containing the ground grain and steel ball.
4. Add 0.3 ml of Reagent 2 from the Nucleon Phytopure Plant DNA Extraction kit.
20. Resuspend DNA in 0.12 ml TE buffer and allow to rehydrate for 1 hour.

Estimation of DNA concentration

DNA concentration of extracts is estimated with the PicoGreen dsDNA quantification kit (PicoGreen, Molecular Probes, Leiden, The Netherlands) following the manufacturer's recommended protocol. Samples can be diluted in TE buffer, if necessary, to give stocks for PCR of approximately 2-5 ng μ l⁻¹.

SOP2 - STANDARD OPERATING PROCEDURE FOR SCREENING RICE DNA WITH MICROSATELLITE MARKERS

Introduction

PCR with microsatellite markers is carried out following the method of Chen et al. (1997) with minor modifications. For variety analysis, 8 different microsatellites are used. The method has been adapted to incorporate an M13 tailed primer with fluorescent label, following the protocols of Oetting et al. (1995) and Scheuke (2000). The M13 tailed primer makes multiple locus testing more cost effective because the same dye-labelled tailed primer can be used with many microsatellites. The amplified DNA fragment spans the repeat sequence between two specific primers, but includes an additional tail sequence attached to a Beckman Coulter WellRED dye. The microsatellites are: RM201, RM44, RM252, RM212, RM263, RM110, RM282 and RM339 (Table 1). PCR products from 4 different microsatellites are pooled and two pools are used for fragment analysis of one extract.

Table 1. Primer sequences for eight microsatellite loci used for variety genotype profiling. Recommended combinations for two pools of 4 loci, with appropriate Beckman Coulter WellRED dyes (in brackets), are indicated. The M13- tail sequence (CACGACGTTGTAAAACGAC) is added to the 5' end of each forward primer.

Marker	Forward Primer	Reverse Primer	Repeat Motif	Pool (dye)
RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	(GA) ₁₇	1 (D4)
RM44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	(GA) ₁₆	1 (D4)
RM252	TTCTGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	(CT) ₁₉	2 (D3)
RM212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	(CT) ₂₄	2 (D4)
RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	(CT) ₃₄	1 (D3)
RM110	TCGAAGCCATCCACCAACGAAG	TCCGTACGCCGACGAGGTCGAG	(GA) ₁₅	2 (D3)
RM282	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTTGCAGCAAG	(GA) ₁₅	1 (D3)
RM339	GTAATCGATGCTGTGGGAAG	GAGTCATGTGATAGCCGATATG	(CTT) ₈ CCT (CTT) ₅	2 (D4)

Procedural variation

No procedural variation is permitted and all steps must be done exactly as described. If for any reason a procedural variation is made then it must be documented in full and the appropriate authority notified.

Materials

Primers

Sixteen unlabelled primers are required (table 1), synthesised by MWG (Germany). Two M13-tail primers, 5'-labelled with Beckman Coulter WellRED dyes D3 and D4 (PA-linked dye labels) are also required, synthesised by Proligo (France). The dyes give different coloured peaks so that fragments of the same size from different loci can be distinguished.

Thermal-cycling conditions

Microsatellite amplification is conducted in a MJ Research PTC-100 96-well programmable heat/cool block, but a similar PCR machine can be used. Thermoprime hot-start master mix (ABgene, Epsom, Surrey, UK) is used to prevent unwanted reactions before the start of the cycle. 96-well plates (Greiner Bio One) or strips of 0.5 ml tubes (Low Profile Thermo-Strip, ABgene) are used for PRC reactions and for pooling of products. All plastic ware and water are exposed to UV light before use. De-ionised and filtered water is used throughout (ELGA Ultra Water Purification System with PALL disposable filter, or similar). An initial incubation at 95 °C serves both to denature the DNA template and to activate the Taq DNA polymerase enzyme. A final extension step for one hour reduces the size of +A peaks.

Fragment analysis

Fragment separation and detection is carried out using a Beckman Coulter CEQ 8000 Genetic Analysis System (Beckman Coulter, High Wycombe, UK) and associated reagents and plastic ware supplied by Beckman Coulter. An ABI capillary sequencer can also be used with appropriate modifications for dye labels, although fragment sizing may be slightly different, but the difference between alleles should be the same. Directly-labelled primers may also be used. The sizing should be checked against authentic standards of rice varieties.

Procedure

1. For each DNA extract and a blank (control) set up 8 PCR reactions with the following components (μl, per well or tube) using the primer combinations in Table 1:

Extract (5 ng μl ⁻¹)	1.00
Forward primer (2 μM)	0.13
Reverse primer (20 μM)	0.13
M13-tail primer dye-labelled (D3 or D4; 20 μM)	0.13
Thermoprime Hot Start mix with 2.5 mM MgCl ₂ (ABgene)	6.50
Water	5.20
Total volume	13.09

2. Subject each reaction mixture to the following PCR cycling conditions: 15 min at 94 °C followed by 35 cycles of {1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C}; then a final extension for 60 min at 60 °C. Up to 96 reactions can be conducted simultaneously.
3. Following PCR the reactions can be stored at 4 °C for up to one week.
4. PCR products are diluted in de-ionised formamide, according to the recommended dilutions for WellRED dyes (Beckman Coulter). Diluted PCR products are pooled (2 pools, each containing 4 reactions) and Beckman Coulter 400 bp size standard is added according to the manufacturer's instructions.
5. Fragments are separated using the 'Frag 3' method of the CEQ 8000.
6. Fragment peaks are scored by the CEQ 8000 software and confirmed by eye. 'Stutter peaks', a characteristic of microsatellite amplification, are not used for analysis.

Identification of Rice Varieties

Rice varieties can be identified by comparing the genotype allele profile obtained in the above procedure with those in Annex B. To help identification of varieties, the alleles have been colour coded so that the varieties listed in Regulation 1549/04 have as similar colours as possible, and no red coloured alleles occur with the 9 varieties. The easiest way to identify the varieties is record the alleles in the same order as in Annex 2 i.e. RM 201, 44, 252, 212, 263, 110, 282, 339, and colour code them according to the list of alleles and colour codes at the base of Annex 2. The sequence of colours can be matched against those in the Annex 2, and the variety identified.

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GENOTYPE PROFILES

ANNEX B

Genotype profiles are colour-coded alleles from 8 rice microsatellites, with nucleotide sizes (rounded to the nearest whole nucleotide) detected using CEQ 8000. The use of colours is arbitrary, but light blue was chosen for predominant alleles in the Bas 370 group.

	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Varieties listed in Commission Regulation 1549/04								
Basmati 370	162	127	252	134	177	170	149	200
Dehra Dun (Type 3)	162	127	254	134	181	170	149	200
Basmati 217	162	127	254	134	177	170	149	200
Ranbir	162	127	244	134	177	170	149	200
Taraori	162	131	264	134	181	170	149	200
Basmati 386	162	131	258	134	181	170	149	200
Kernel	162	131	260	134	181	170	149	200
Pusa	162	131	254	134	181	170	157	200
Super	162	127	260	134	181	170	149	204
Other approved Basmati varieties*								
Basmati 198	162	127	254	152	177	170	149	200
Basmati 385	162	131	252	152	181	170	149	200
Kasturi	162	121	254	132	220	170	149	166
Haryana Basmati	162	121	238	152	177	150	149	166
Mahi Sugandha	176	121	256	152	177	156	155	166
Punjab Basmati	162	127	256	152	177	156	155	200
Non-approved varieties								
Basmati 2000	162	131	254	152	181	170	149	204
Shaheen Basmati	162	131	254	152	181	170	157	200
Sherbati	178	121	238	130	202	174	160	166
Mugad Sugandha	178	121	238	132	202	176	157	166
Pak 386	178	121	238	130	220	174	155	166
Superfine	178	131	238	132	202	174	157	166
Pusa Sugandha	162	121	238	132	181	170	157	178
Yamini	162	127	260	134	181	170	149	200
List of alleles, colour codes								
	162	121	238	130	177	150	149	166
	176	127	244	132	181	156	155	178
	178	131	252	134	202	170	157	200
			254	152	220	174	160	204
			256			176		
			258					
			260					
			264					

*Varieties not included in Regulation 1549/04 but recognised by the FSA to use the name Basmati.

RESULTS OF STANDARDS

ANNEX 3

Dehradun (Type 3)	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
<i>Consensus from SOP</i>	162	127	254	134	181	170	149	200
LAB 1	162	131 127	254	134 132	181	170 174	149	200 166
LAB 2	159	125	248	131	179	169	147	199
LAB 3	162	127	N	134 152	181	170	149	200 166
LAB 4	162	127	254	134	181	170	149	200
LAB 5	162	127 131	254 238	134 152	181	170	149	
LAB 6	159	125	251	132	179	167	145	199
LAB 7	161	125	252	132	180	170	148	200
LAB 8	160	126 124	251 249	132 130	178 176 177	167 166	146 146	199 (196)
LAB 9 (Corrected)	162	127	255	134	181	170	149	200
LAB 10 (Corrected)	162	127	253	134	181	170	149	200
LAB 11(Corrected)	162	127	254	134	181	170	149	200

Taraori	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
<i>Consensus alleles from SOP</i>	162	131	264	134	181	170	149	200
LAB 1	162	131	260 264	134 132	181	170 174	149	200
LAB 2	159	129	254	131	179	170	147	199
LAB 3	162	121	252	134	181	170	149	200
LAB 4	162	131	264 260	134	181	170	149	200
LAB 5	162	131	264 -260	134	181	170	149	200
LAB 6	160	125	255	132	179	167	144	199
LAB 7	161	129	258	132	180	170	148	200 178
LAB 8	160 158	129 127	255 257 253	132	178 177 176	167 166	145 146	199 (196)
LAB 9 (corrected)	162	131	261	134	181	170	149	200
LAB 10 (corrected)	162	131	258	134	181	170	149	200
LAB 11(corrected)	162	131	260	134	181	170	149	200

Basmati 386 <i>Consensus alleles from SOP</i>	RM201 162	RM44 131	RM252 258	RM212 134	RM263 181	RM110 170	RM282 149	RM339 200
LAB 1	N	131	258 264	130 134	181	170 174	149	200
LAB 2	159	129	254	131	N	169	147	199
LAB 3	162	121	N	134	181	170	149	200
LAB 4	162	131	262 / 264 260 258	134	181	170	149	200
LAB 5	162	131 -258	264	134	181	170	149	200
LAB 6	160	129	256	132		167	145	
LAB 7	161	129	256	132	181	170	148	200
LAB 8	160 158 159	127 129	256 258 253	132 130	178 177 176	167 166	145 146	199
LAB 9 (corrected)	162	131	259	134	181	170	149	200
LAB 10 (corrected)	162	131	258	134	181	170	149	200
LAB 11(corrected)	162	131	259	134	181	170	149	200

Pusa Basmati	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
<i>Consensus alleles from SOP</i>	162	131	254	134	181	170	157	200
LAB 1	162 178	131	254 256 252	134 152	181	170 174	157	200 166
LAB 2	159	N	N	131	N	169	155	199
LAB 3	162 178	121	N	134 152	181	170	157 149	200
LAB 4	162 (176)	131	280	134	181	170	157	200
LAB 5	162	131 121	280 (276) 254	134 152	181	170	157	200
LAB 6	160	129	N	132	179	167	N	199
LAB 7	161	128	276	132 151	181	170	157	200 166
LAB 8	160	129	276 278	132	178 176	167 166	153 154	199
LAB 9 (Corrected)	162 180	131 121	280	134 152	181	170	157	200 164
LAB 10 (Corrected)	162	131	275	134	181	170	157	200
LAB 11 (Corrected)	162 178	131	279	134	181	170	157	200

Basmati 198	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Consensus alleles from SOP	162	127	254	152	177	170	149	200
LAB 1	162	127	254	152	177	170 174	149	200
LAB 2	N	N	N	150	N	169	N	N
LAB 3	162	127	N	134 152	181	170	149	200
LAB 4	162	127	254	152	177	170	149	200
LAB 5	162	127	254	152	177	170	149	200
LAB 6				132				199
LAB 7	161	125	252	151 130		170	148	200 178
LAB 8	160 158	126 124	249 251	150 148	174 173 172	167 166	145 146	199
LAB 9 (Corrected)	162	127	255	152	177	170	149	200
LAB 10 (Corrected)	161	127	N	152	177	170	149	200
LAB 11(Corrected)	162	N	254	N	181 177	170	N	200

Basmati 385	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Consensus from SOP	162	127	254	152	181	170	149	200
LAB 1	162 178	131 127 121	252 254 260,238	152 134 130	181	170	149	200 166
LAB 2	159	129	250 248 246	130 127	N	169	147	150
LAB 3	162 178	121	238 252	152 134	181	170	149	200 166
LAB 4	162	131	254 238	152	181	170	149	200
LAB 5	162	131	254 (252) 238	152 134	181	170	149 154	200
LAB 6	160		250	150	179	167	145	199
LAB 7	161	129	250	151 128 132	181	170	148	200 166
LAB 8	160	130 128	249 251 247	150 148	178 176 177	167 166	145 146	199
LAB 9 (corrected)	162	131 121	255 238	152 134 130	181	170	149 155	200 164 203
LAB 10 (corrected)	162	131	252 238	152	181	170	149	200
LAB 11 (corrected)	162	131	254	152	181	170	149	200

Sherbati	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Consensus alleles from SOP	178	121	238	130	202	174	160	166
LAB 1	178 162	131 121 127	238 242	130 132 134	202	174 176 170,156	155	166 200
LAB 2	175	119	N	127	198	173	153	165
LAB 3	178 162	127 131	238	130	202 181	174	155	166
LAB 4	178	121	260 238	130	202	174 176	156	166
LAB 5	178 162	121 129 131	238 260 -258	130	202	174	155	166
LAB 6	176	N	235	128	200	171	N	164
LAB 7	177 161	120	236	128 132	201	174	155	166
LAB 8	176 174	120 118	235 233	128	199 197 198	171 170 172	151 152	164
LAB 9 (corrected)	180	121	238 261	130 134	202	174 176 170	155	164
LAB 10 (corrected)	179	121	258 215	130	201	175	155	164
LAB 11 (corrected)	178 162	121	238 260	130	202 181	174	155	165 200

Mugad Sugandha	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Consensus alleles from SOP	178	121	238	132	202	176	157	166
LAB 1	178	121	238	132	202	176	157 155	166
LAB 2	173	119	234	129	N	175	155	N
LAB 3	172	127 131	238	132	202 181	176	157	166
LAB 4	178 176	121	238	132	202	176	157 155	166
LAB 5	176 -178	121 131	238	132	202	176	157 155	166
LAB 6	N	119	235	130	N	173	154	164
LAB 7	177 176	120	236	130	201	176	157	166
LAB 8	174 176 172	120 118	235 233 231	130 128 129	199 197 198	173 171 172	153 151 (154+152)	164
LAB 9 (Corrected)	178 180	121	238	132	202	176	157 155	164
LAB 10 (Corrected)	177	121	238	132	197	154	157	164
LAB 11(Corrected)	176	N	238	132	202 181	176	157	165

Pak 386	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Consensus alleles from SOP	178	121	238	130	220	174	155	166
LAB 1	178 162	121 127	238 260	130 152	220 202	174	155 149	166 204
LAB 2	N	N	N	129	N	N	N	N
LAB 3	178 162	127	238	130	179 202 220	174 176	155 149	166
LAB 4	178 176	121	238	130	220 202	176 174	155	166
LAB 5	178	121	238	130 132	220	174	155	166
LAB 6	177	119	235	128		171	152	164
LAB 7	177	120	236 268 270	128 151	217	175	155	166
LAB 8	176 174	120 118	235 233	128 127	222 (weak) 199 (weak)	171 169 170	151 152	164
LAB 9 (Corrected)	180	121	238	130	221 202	174 176	155 149	164
LAB 10 (Corrected)	179	121	238	129	218	175	155 149	164
LAB 11 (Corrected)	178	121	238	130	220 181	174	155	165

Pusa Sugandha	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Consensus from SOP	162	121	238	132	181	170	157	178
LAB 1	162 178	121	238 256	132 134 152	181	170 174 176	157	166 178 200
LAB 2	159	119	234	129	179	169	155	177
LAB 3	162	121 127 131	238	132	181	170 176	157	178 166
LAB 4	162	121	238	132	181	176	157	178
LAB 5	162	121	238	132	181	170 176	157	
LAB 6		119	235	130	179	167		176
LAB 7	161	120	236	130	180	170 176	157	178
LAB 8	160	120 118	235 233	130 128	178 177 176	167 166	153 154 151	176
LAB 9 (corrected)	162 180	121	238	132	181	170 176	157	177 164
LAB 10 (corrected)	161	121	239	131	181	171	157	177
LAB 11(corrected)	162	121	238	132	181	170	157	177

Yamini	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339
Consensus alleles from SOP	162	127	260	134	181	170	149	200
LAB 1	162	131	260	134 132	181	170 174	149	200 178
LAB 2	N	N	N	N	N	N	N	175
LAB 3	162	127	N	132 134 130	181	170	149	N
LAB 4	162	131	260	134	181	170	149	200
LAB 5	162	131	260 -258	134	181	170	149	200
LAB 6	N	N	N	132	N	N	N	199
LAB 7	161	129	256	132	N	175	148	200
LAB 8	160 158	128 130	255 253 257	132 130	178 176 177	167 166	145 146	199
LAB 9 (corrected)	162	131	263	134	181	170	149	200
LAB 10 (corrected)	161	N	N	134	N	171	149	200
LAB 11 (corrected)	162	127	259	134	181	N	N	200

N – denotes failed PCR or no allele

RESULTS OF UNKNOWN SAMPLES

ANNEX 4

FSA Sample 1	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339	Suggested variety
LAB 1	162	121 127	238 260	134 152	181	170	149	204 178	Super Basmati
LAB 2	159		254	131	179	169	146	202	Super basmati
LAB 3	162	127	252 238	134 152	181	170 156	149	204 200 166	Super Basmati Punjab Basmati
LAB 4	162	131 127	260 258	134	181	170	149	204 200	Super
LAB 5	162	127	260 258	134	181	170 156	149 157 155	204	Super
LAB 6	161	124	258	132	179	167	145	202	Super basmati
LAB 7	161	125 120	256	132 151	181	170	148	203 200	Super (ignoring the RM252 results)
LAB 8	160 159	125 123 129	258 255	132 130	179 178	167 166	145 146	202 199	Taraori or Basmati386 Or Yamini
LAB 9	162	127	259 261	134	181	170	149	203	Super Basmati
LAB 10	162	127	258	134	181	170	149	203	Super Basmati
			238	136		177		164	
			227						
LAB 11	162 178	N	260	135 152	180 202	170 157	149 155	205 202	Super Basmati

FSA Sample 2	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339	Suggested variety
LAB 1	162 178	131 121 127	260 238	134 152 132	181 177	170 174	149	200 166	Kernel (Yamini)
LAB 2	159	129	254	131	179	169	147	199	Taraori or Bas 386
LAB 3	162 178	131 127 121	238 252	134 152	181 202	170 156	149 157	200 166	Taraori, Basmati386, Kernel, Pusa Basmati Not clear
LAB 4	174 162	131 127 121	260 238 234	134 132 130	202 181 177	184 / 176 170 156	157 155 149	204 200 166/163	Yamini
LAB 5	162 178	131 121 127	260 238 246	134 152 130	181 202 177	170 174	149 155	200 166	Kernel
LAB 6	161	124	256	132	178	167	145	199	Yamini
LAB 7	161 177 175	129 120	256 236	132 151	181 200	170 175 177	148 157 155	200 166	Taraori, Bas 386, Kernal or Pusa with Shaheen basmati as adulterant ()
LAB 8	160 158	130 126 128	256 258	132 130	178 177	167 166	145	199 198	Taraori or Basmati386 Or Yamini

LAB 9	162 178	131 127 121	259 261 238	134	181	170	149	200 164	Taraori/Bas386/PS/ Kernel
LAB 10	162 179 168	131 121	258 238 216	134 136	181 201	170 156 175	149 155 157	200 164	Kernel Sherbati
LAB 11	162 176 178	N	260 258 238	135 152 131	180 202 221	170 157 176	149 157 154	200 167	Taraori + ?

FSA Sample 3	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339	
LAB 1	No Signal	127 131 121	260 254 238	134 152 132	181	170	149	204 200 166	Super Basmati
LAB 2	159	125	252	131	179	169	147	202 199	Dehradun / (Type 3)/Taraori
LAB 3	162 178	127 131	252 238	134 152	181	170	177 181	200 204	Basmati370 Not clear
LAB 4	162	131 127	280 260 258 /252	134	181 177	170	157 149	204 200 166	Super and Yamini
LAB 5	162	127 131	260 262 280/254	134 152	181	170	149 157 155	204 200	Yamini
LAB 6	160	126	256	132	179	167	145	199	Basmati 386 or Kernel
LAB 7	161 177	125 129	258 252	132 151 128/130	180 177	170	148 157	200 203	Type 3 or Yamini with large number of other rice as possible adulterants (ignoring the RM252 results)
LAB 8	160 159	124 125	256 258 251	132 130	179 178	167 166	145 146	202 199	Dehradun
LAB 9	162	127 131	259 261	134 152	181	170	149	200 203	Taraori/Super Bas/Bas386 /Kernel
LAB 10	162	127	258	134	181	170	149	200	Yamini
	179	131	238	136	173	179	155	203	
		133	217				157	164	
LAB 11	162 178	N	260 238	135 152	181 202	170 167	149 157	202 205	Taraori + Super

FSA Sample 4	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339	Suggested variety
LAB 1	162	131	260 256 238	134 132	181	170 174	No Signal	200 166	Kernel
LAB 2	159	129	254	131	179	170	155	199	Pusa Basmati
LAB 3	162	131 127	238 252	134	181	170 156	157 149	200 166 204	Taraori, Basmati386, Kernel, Pusa Basmati Basmati370
LAB 4	162	131 127	280 260 254	152 134	181	180 174 170	157 149	204 200 166	Kernel and Pusa
LAB 5	162 178	131 121	280 238 276	134 130	181 202	170 174	157 155	200 166	Similar to Pusa, with Sherbati
LAB 6	160	128	258	132	179	167	153	199	Cannot identify variety
LAB 7	161 177	129 125	256 274 276	132 128	180 177	170	157 148	200 203	Taraori, Bas 386, Kernal or Pusa with Yamini as adulterant (ignoring RM252)
LAB 8	160 159	128 129 126	276 274 272	132 130	178 177 176	167 166 165	153 145 151	199 164 196	Pusa Basmati
LAB 9	162	131	278 259	134	181	170	149 157	200	Bas386/Taraori/Kernel
LAB 10	162	131	258	134	181	170	157	200	Taraori
	168	121	238	136	173	154	149	164	Sherbati
	179		276		201	177	159	203	
LAB 11	162	N	279	135	180	170	157	202	Pusa
	178		260				149	167	Taraori
			238						

FSA Sample 5	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339	Suggested variety
LAB 1	162	127 131	254 260	134 132	177 181	170 174	149	200 166	Basmati 217
LAB 2	159	125	250	131	175	169	147	199	Basmati 370 / Basmati 217
LAB 3	162 187	127 131	252	134 152	147	170 156	149	200 204	Basmati370 Not clear
LAB 4	162	131 127	260 254 252	134	183 181 177	170	149	204 200	Basmati 217
LAB 5	162	127 131	260 262 280/254	134 152	181 177	170	149 157 155	200	Bas 217 / kernel
LAB 6	160	124	252	132	175	167	145	199	Basmati 370 or 217
LAB 7	161 177 175	125 129	252	132 128	177 181 200	170 177 175	148	200	Bas 370, Bas 217 or Ranbir with many other rice as possible adulterant (ignoring RM252)
LAB 8	160 159	126 124	249 251	132 130	174 173 172	167 166	145 146	199 196 198	Basmati198 (from RM263) Dehradun from RM212
LAB 9	162	127 131	255 253	134	177 181	20	149	200	Type3/Bas370/Bas217 /Bas385
LAB 10	162 145	127 133	254 258 238 227	134 136	177 181	171 175 179	149 155	19 200 164 203 161 158	Super Basmati Yamini Dehradun
LAB 11	162	N	254	135	177	170	149	202	Taraori
	178		260	132	180	157	155	167	Bas 370
			236		202				

RESULTS OF SINGLE GRAIN ANALYSES

ANNEX 5

Samples	RM201	RM44	RM252	RM212	RM263	RM110	RM282	RM339	Rice Variety
1 x166	162	127	260(258)	134	181	170	149;157?	204	Super
1 x167	162	127	260(258)	134	181	170	149;157?	204	Super
1 x168	162	127	260(258)	134	181	170	149;157?	204	Super
1 x169	162	127	260(258)	134	181	170	149;155?	204	Super
1 x170	162	127	260(258)	134	181	170	149;155?	204	Super
1 x171	162	127	260(258)	134	181	170	149;155?	204	Super
1 x172	162	127	258	134	181	170	149;155?	204	(super)
1 x173	162	127	260	134	181	156;170	149	204	Super
2 x174	162	131	260(258)	134	181	170	149	200	Kernel/386/ Taraori
2 x175	162;176	131;121	238;258 (256)	152;134	181	170	149;155?	200	Segregating (Bas 385?)
2 x176	178;162	121;127?	238;260 (258)	134	202;181	174	155;149?	166	Sherbati/Pak 386
2 x177	162	131	260(258)	134	181	170	149	200	Kernel/386/ Taraori
2 x178	162	131	260(258)	134	181	170	149;155?	200	Kernel/386 Taraori
2 x179	178	121;127?	260(258)	130	202;181	174	155;149?	166	Sherbati
2 x180	162	131	260(258)	134	181	170	149	200	Kernel/386/ Taraori
2 x181	162	127;131?	246	134	177;181?	170	149	200	(Ranbir) or (Yamini)
3 x182	162	127	260(258)	134	181	170	149;155?	204	Super
3 x183	162	131	280(276); 260(258)	134	181	170	157	200	Pusa
3 x184	162	127	254	152;134	181	170	149;157?	204	Super ?
3 x185	162	131	262(260)	134	181	170	149	200	Yamini
3 x186	162	127	260(258)	134	181	170	149;155?	204	Super
3 x187	162	131	260(258)	134	181	170	149;155?	200	Yamini
3 x188	162	127	260(258)	134	181	170	149;155?	204	Super
3 x189	162	131	262(260)	134	181	170	149	200	Yamini

4 x190	162	131	276(274)	134	181	170	157?	200	Pusa
4 x191	178;162?	121;131	238;280	130	202;181	174	155	166	Sherbati
4 x192	162	131	280(276)	134	181	170	157	200	Pusa
4 x193	162	131	280(276)	134	181	170	157	200	Pusa
4 x194	162	131	276(274)	134	181	170	157	200	Pusa
4 x195	162	131	280(278)	134	181	170	157	200	Pusa
4 x196	162	131	278(274/276)	134	181	170	157	200	Pusa
4 x197	162	131	280(276)	134	181	170	157?	200	Pusa
5 x198	162	131	260	134	181	170	149;157	200	Kernel/Taraori
5 x199	162	127	254	134	177	170	149;155?	200	Basmati 370 group
5 x200	162	127	254	134	177	170	149;155?	200	Basmati 370 group
5 x201	162	127	254	134	177	170	149;155?	200	Basmati 370 group
5 x202	162	131	244;254	152;134	181	170	149;155?	200	(Basmati 385)
5 x203	162	127	260(258)	134	181	170	149	200	Kernel/Taraori
5 x204	162	127	254	134	177	170	149;155?	200	Basmati 370 group
5 x205	162	131	260	134	181	170	149	200	Kernel/Taraori
RM252 - peak furthest right has been scored (and tallest peak added in brackets) Results in brackets are the closest match, but not identical									