







Basmati Verifiler™ Kit - Quick Reference Card

Intended Use:

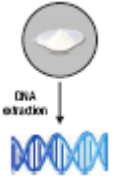


The kit enables unambiguous detection and quantification of adulteration in Basmati rice samples based on multiplex panel of microsatellites. The kit provides all the reagents necessary for single tube amplification of all the eight microsatellites.




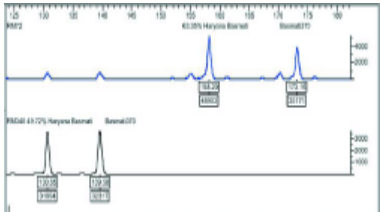
Kit Contents

-  Basmati Verifiler™ 10X Reaction Buffer (one tube, 120µl)
-  Basmati Verifiler™ Magnesium Chloride (25mM) (one tube, 100µl)
-  Basmati Verifiler™ 10X dNTP Mix (one tube, 120µl)
-  Basmati Verifiler™ Primer Mix (one tube, 180µl)
-  Basmati Verifiler™ PCR Enzyme (5U/µl) (one tube, 22µl)
-  Basmati Verifiler™ Control DNA (15ng/µl) (one tube, 40µl)

The reagents provided are sufficient for 100 tests at 10µL reaction volume.

Procedure

1	<p>Isolate DNA from rice samples to be tested</p> 	<p>DNA can be isolated using Qiagen DNeasy Plant Mini Kit (Cat.No.69104) or by following CTAB method (see manual for more details). Estimate the quantity of DNA.</p>
2	<p>Make the working stock of DNA</p> 	<p>The assay is optimized for use with 30ng of DNA. Adjust the concentration of the isolated DNA to 15ng/uL.</p>
3	<p>Prepare PCR Master Mix</p> 	<ol style="list-style-type: none"> 1. Number of samples X 3.4µl of PCR grade water. 2. Number of samples X 1µl of Basmati Verifiler™ 10X Reaction Buffer. 3. Number of samples X 0.8µl of Basmati Verifiler™ Magnesium Chloride. 4. Number of samples X 1µl 10X Basmati Verifiler™ 10X dNTP Mix. 5. Number of samples X 1.6µl Basmati Verifiler™ Primer Mix. 6. Number of samples X 0.2µl Basmati Verifiler™ PCR Enzyme.

4	<p>Mix the PCR Master Mix by vortexing and spin it briefly. Aliquot 8µl of Master Mix per sample.</p> 																						
5	<p>Prepare Samples for PCR</p> 	<table border="1"> <thead> <tr> <th data-bbox="683 411 954 443">If preparing...</th> <th data-bbox="954 411 1455 443">Then combine...</th> </tr> </thead> <tbody> <tr> <td data-bbox="683 443 954 506">Negative Control</td> <td data-bbox="954 443 1455 506"> <ul style="list-style-type: none"> 8µl PCR Master Mix 2µl nuclease free water </td> </tr> <tr> <td data-bbox="683 506 954 600">Positive Control</td> <td data-bbox="954 506 1455 600"> <ul style="list-style-type: none"> 8µl PCR Master Mix 2µl of Basmati 370 positive control DNA (15ng/µl) </td> </tr> <tr> <td data-bbox="683 600 954 695">Test Samples</td> <td data-bbox="954 600 1455 695"> <ul style="list-style-type: none"> 8µl PCR master mix 2µl of working stock of DNA from sample </td> </tr> </tbody> </table> <p>Cap the tubes or seal the 96-well tray, then place them in the thermal cycler.</p>	If preparing...	Then combine...	Negative Control	<ul style="list-style-type: none"> 8µl PCR Master Mix 2µl nuclease free water 	Positive Control	<ul style="list-style-type: none"> 8µl PCR Master Mix 2µl of Basmati 370 positive control DNA (15ng/µl) 	Test Samples	<ul style="list-style-type: none"> 8µl PCR master mix 2µl of working stock of DNA from sample 													
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6	<p>Perform PCR</p> 	<p>a. Program the thermal cycler as follows:</p> <table border="1"> <thead> <tr> <th data-bbox="691 825 824 888" rowspan="2">Denature</th> <th colspan="3" data-bbox="824 825 1187 856">30 Cycles</th> <th data-bbox="1187 825 1320 888" rowspan="2">Final Extension</th> <th data-bbox="1320 825 1446 888" rowspan="2">Final Step</th> </tr> <tr> <th data-bbox="824 856 927 888">Melt</th> <th data-bbox="927 856 1049 888">Anneal</th> <th data-bbox="1049 856 1187 888">Extend</th> </tr> </thead> <tbody> <tr> <td data-bbox="691 888 824 919">Hold</td> <td colspan="3" data-bbox="824 888 1187 919">Cycle</td> <td data-bbox="1187 888 1320 919">Hold</td> <td data-bbox="1320 888 1446 919">Hold</td> </tr> <tr> <td data-bbox="691 919 824 1014">95°C 10 min</td> <td data-bbox="824 919 927 1014">94°C 45sec</td> <td data-bbox="927 919 1049 1014">55°C 90 sec</td> <td data-bbox="1049 919 1187 1014">72°C 60 sec</td> <td data-bbox="1187 919 1320 1014">60°C 30 min</td> <td data-bbox="1320 919 1446 1014">4°C forever</td> </tr> </tbody> </table> <p>b. Set the reaction volume for thermal cycling at 10 µl, then start the run. c. Add 10 µl of nuclease free water to the amplified products to obtain a 1:1 dilution. d. Store the PCR products at -15°C to -25°C until loaded.</p>	Denature	30 Cycles			Final Extension	Final Step	Melt	Anneal	Extend	Hold	Cycle			Hold	Hold	95°C 10 min	94°C 45sec	55°C 90 sec	72°C 60 sec	60°C 30 min	4°C forever
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7	<p>Perform GeneScan</p> 	<p>Prepare the Master Mix of Hi Di formamide and GeneScan 500 LIZ size standard :</p> <ol style="list-style-type: none"> Number of samples X 10.2µl of Hi Di Formamide Number of samples X 0.3µl of GS 500 LIZ Add 0.5µL of diluted PCR product to 10.5µL of above master mix for loading. Denature the samples by placing them at 95°C for 5 min. and place on ice immediately. Give the samples a short and quick spin to bring the liquid to the bottom of the tubes. The samples should be stored at 4°C until ready to load on the instrument. Select Dye Set G5 on the instrument according to manufacturer's defined instrument protocol and run the samples. 																					

Note: For Research Use Only. Not for use in diagnostic procedures.

Not for re-sale.

The patent for the product are pending under one or more patent nos. UPSTO 10/357 and PCT/IN06/00254.

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