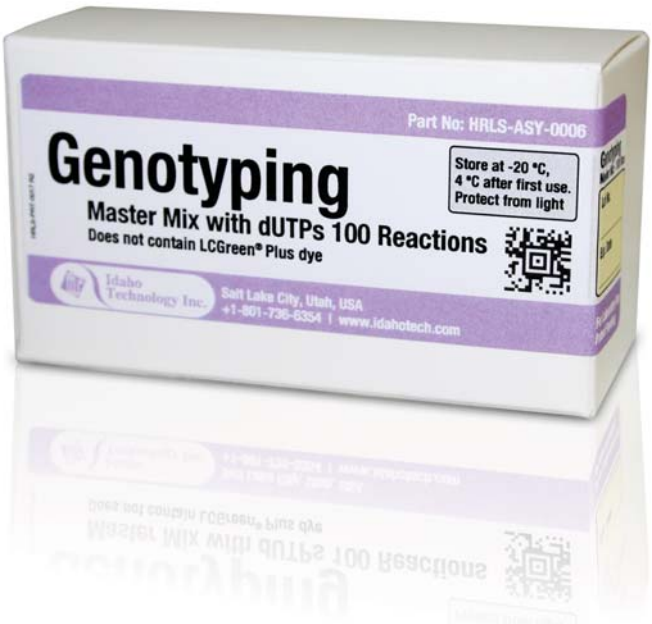
100 Reactions

Genotyping Master Mix

User's Guide



Designed for Real-Time PCR and Hi-Res Melting™ Instruments

Idaho Technology Inc.

Package Contents

Quantity	Description
1 x 100 reactions	2.5X Master Mix
1.5 mL	Reagent grade water
1	*Genotyping Master Mix User's Guide*

NOTICE TO PURCHASER: DISCLAIMER OF LICENSE

This product is compatible for use in real-time PCR methodologies, including dsDNA-binding dye processes claimed in patents owned by Roche Molecular Systems, Inc., F. Hoffmann-La Roche Ltd. No right to perform any of these patented processes is conveyed, expressly, by implication or by estoppel to the purchaser by the purchase of this product. A license to use these patented processes for the purchaser's own internal research accompanies the purchase of certain reagents of Applied Biosystems and other licensed suppliers, or is available from Applied Biosystems. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Genotyping Master Mix User's Guide, 100 Reactions
Part No. HRLS-PRT-0028 Rev 01 4/08

About the Genotyping Master Mix

The Genotyping Master Mix from Idaho Technology is a pre-optimized Master Mix specially designed for genotyping experiments not requiring an enzyme with exonuclease activity. This Master Mix is compatible with technologies like SimpleProbes™, where the genotype is determined via melting temperature of the SimpleProbe. This product is intended for laboratory use only.

Idaho Technology's Genotyping Master Mix includes Taq polymerase, a dNTP/UTP mix, magnesium chloride, and bovine serum albumin (BSA) and is formulated at a 2.5X concentration. Enzyme preactivation (i.e., high temperature, hot start, incubation) is not required before thermocycling.

Equipment needed but not provided:
White-well PCR plates*, Roche LightCycler® capillaries
Sealing film*
Pipettes (1–10 µL, 200 µL)
Plate or capillary centrifuge
Thermocycler: Idaho Technology R.A.P.I.D.® LT, Roche LightCycler, standard heat block cycler
Idaho Technology LightScanner®

*See **Appendix A** for recommended PCR plates and sealing film.

Storage/Stability

The Genotyping Master Mix is stable for at least 6 months when stored at –20ºC. Once thawed it may be stored for 1 week at 4ºC without substantial loss in genotyping sensitivity, or it can be refrozen up to three times over 4 weeks.

Reaction Setup

Thaw the frozen Master Mix solution on ice. Mix thoroughly before using.

Setup

Preparation of PCR mix for **one** 10 µL reaction (8 µL template-free PCR mix + 2 µL DNA). These volumes can be scaled up as desired.

Component	Volume (µL)	Final Concentration	Example for 10 Reactions (µL)
2.5X Master Mix	4	1X	4 x 10 = 40
10X Primer/probe mix	1	1X	1 x 10 = 10
Water	3	N/A	3 x 10 = 30
Final Volume	8	N/A	80

Mix the reagent gently but thoroughly before dispensing (e.g., vortex, pipette up and down, and spin).

Note: Aliquot 8 µL of the template-free PCR mix into each reaction vessel. Next, add 2 µL of template DNA (ITI recommends 10–100 ng total) directly into the aliquoted PCR mix to bring the total reaction volume to 10 µL.

If amplifying reactions on a thermal block-based instrument, load the plate as follows:

- Aliquot **20 µL** of mineral oil into each well.
- Aliquot template-free PCR mix into each well (8 µL per well) directly into oil.
- Add DNA template to each well (2 µL per well) directly into oil.
- Cover plate with sealing film.
- Centrifuge 1–2 min. at 2500 rpm.

Typical Rapid PCR Air Thermal Cycling Protocol

This amplification protocol can work for many PCR products.

Program: Denature Cycles: 1

Step	Target Temp. (ºC)	Hold Time (sec.)	Ramp rate (ºC/sec.)	Fluorescence Acquisition Mode
Initial denature	95	30	20	None

Program: Amplification Cycles: 45

Step	Target Temp. (ºC)	Hold Time (sec.)	Ramp rate (ºC/ sec.)	Fluorescence Acquisition Mode
Denature	95	2	20	None
Anneal	57	10	20	None
Extension	72	10	20	None

Typical Melt Protocol—Rapid Air Thermal Cycler

This melting protocol can work for many PCR products.

Program: Melt Cycles: 1

Step	Target Temp. (ºC)	Hold Time (sec.)	Ramp rate (ºC/sec.)	Fluorescence Acquisition Mode
Denature	94	10	20	None
Anneal	40	120	20	None
Melt	80	0	0.1	Continuous
Cool down	40	60	20	None

Typical PCR Protocol for Thermal Block Instruments

This amplification protocol can work for many PCR products.

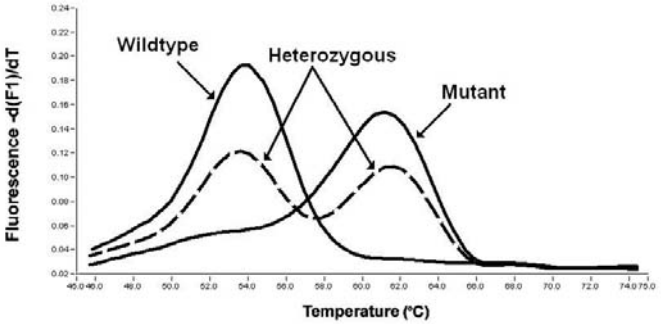
Block Thermocycling Conditions			
No. of Cycles	Temp. (°C)	Time (sec.)	Step
1 (optional)	94	120	Initial Template Denaturation
40–45	94	30	Denaturation
	62	30	Annealing
	72	10	Extension
1	94	30	Final denature
1	25	30	Reanneal

Typical LightScanner Run Protocol

This melting protocol can work for many PCR products.

LightScanner Melting Settings	
Start Temperature	40°C
End Temperature	94°C
Hold Temperature	37°C
Exposure	Auto

Typical Melt Peaks



APPENDIX A:
Compatible PCR Plates, Sealing Films, and Mineral Oils for the LightScanner

CAUTION: Use only white-well plates with the LightScanner. Clear plates allow fluorescence bleed over from well to well, and black plates quench fluorescence and increase noise.

Note: Compatibility list is subject to change. Check ITI Web site for updates and additional information.

Manufacture	96-Well Plate	384-Well Plate	Sealing Film
ABgene www.abgene.com			AB-1170[b]
Axygen www.axygen.com			UC-500[b]
Bio-Rad www.bio-rad.com	HSP-9665	HSP-3865	223-9444[b, c]
Applied Biosystems www.appliedbiosystems.com			4311971[b]
Phenix www.phenixresearch.com	MPC-96HS4-WW	MPC-384HS4-WW	LMT-RT2[b] LMT-RT5[b]
4titude® www.4ti.co.uk www.bioke.com	4ti-0961	4ti-0385	
Eppendorf www.eppendorf.com	951022067 USA[a] 0030 132.556 Intl.[a]		
Nunc™ www.nuncbrand.com			232702[b] 235307[b]

[a]Compatible with the Applied Biosystems GeneAmp 9700 Thermal Cycler.

[b]Film may remain intact during melting analysis. (Please note that use of this film does not eliminate the need for an oil overlay.)

[c]Available by phone order only.

Compatible Mineral Oils

Important: The use of mineral oil is required to prevent sample evaporation in the LightScanner.

Manufacture	Catalog No.	Mineral Oil
Sigma www.sigmaaldrich.com	M5904	Mineral Oil Light for Molecular Biology
Fisher www.fishersci.com	0121-1	Mineral Oil Light
Alfa Aesar www.alfa.com	31911	Mineral Oil
USB www.usbweb.com	71600	Ultra Pure Mineral Oil—Molecular Biological Grade

Notes

Notes

390 Wakara Way, Salt Lake City, Utah 84108 USA
+1-801-736-6354 | www.idahotech.com

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