TaqPath[™] ProAmp[™] Master Mixes USER GUIDE

Genotyping and copy number variation PCR workflows

Publication Number MAN0015758 Revision A.0



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Revision history. Pub. No. MAN0015758

Revision	Date	Description
A.0	02 September 2016	New document.

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Product information

Product description

Applied Biosystems[™] TaqPath[™] ProAmp[™] Master Mixes are optimized for genotyping and copy number (CNV) experiments that use samples extracted from various human or animal sources, including blood cards, buccal swabs, and whole blood.

TaqPath[™] ProAmp[™] Master Mixes are formulated for:

- Extended shelf life and benchtop stability
- Enhanced specificity and inhibitor tolerance during PCR for:
 - Samples with stubborn inhibitors
 - Samples from crude lysates (with most genotyping assays)

Note: For copy number assays, crude lysates are not recommended because the input DNA concentration and quality cannot be determined accurately.

See Table 4 for compatible genotyping and copy number TaqMan® Assays.

TaqPath[™] ProAmp[™] Master Mixes are supplied at a 2X concentration and contain:

- Dual-Lock[™] Taq DNA Polymerase
- Heat-labile uracil-DNA glycosylase (UNG)
- dNTPs with dUTP
- Passive reference dye (ROX[™] –*or* MUSTANG PURPLE[™])
- Optimized buffer components

For more information about the components of TaqPath[™] ProAmp[™] Master Mixes, see "Master mix components" on page 17.

Table 1 Recommended master mix formulation for single- and multiplex reactions

PCR option ^[1]	Compatible reporter dyes	Recommended master mix formulation		
Singleplex (1 probe)		TaqPath [™] ProAmp [™] Master Mix with ROX [™]		
Multiplex (2–3 probes)	FAM , VIC , ADT	TaqPath ProAmp Master Mix with ROX		
		TaqPath [™] ProAmp [™] Multiplex Master Mix with MUSTANG PURPLE [™]		
Multiplex (> 3 probes)	ex (≥ 3 probes) JUN [™] plus FAM [™] , VIC [™] , ABY [™]	Note: See your instrument user guide about additional calibration that may be required for use of MUSTANG PURPLE [™] dye, including any additional spectral calibration plates that may be sold separately.		

^[1] For detailed information about multiplex reactions, see the *TaqMan[®] Multiplex PCR Optimization User Guide* (Pub. No. MAN0010189).

Contents and storage

TaqPath [™] ProAmp [™] Master Mix	TaqPath [™] ProAmp [™] Multiplex Master Mix	Amount	Storage	
with ROX [™]	with MUSTANG PURPLE [™]			
A30865	A30868	1 × 1 mL		
A30866	A30869	1 × 10 mL		
A30871	A30873	2 × 10 mL	2-8°C	
A30867	A30870	1 × 50 mL		
A30872	A30874	2 × 50 mL		

Table 2 TaqPath[™] ProAmp[™] Master Mix formulations

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Table 3 Recommended DNA isolation kits

Kit	Cat. No.
MagMAX [™] DNA Multi-Sample Ultra Kit	A25597
	A25598
DNA Extract All Reagents Kit	4402599

 Table 4
 Compatible TaqMan[®] Assays

Assay collection	Source
TaqMan [®] SNP Genotyping Assays	thermofisher.com/taqmansnp
TaqMan [®] Drug Metabolism Genotyping Assays	thermofisher.com/taqmandme
TaqMan [®] Copy Number Assays	thermofisher.com/taqmancnv

Item	Source				
Real-time PCR instrument, one of the following:					
QuantStudio [™] 3 or 5 Real-Time PCR System					
QuantStudio [™] 6 / QuantStudio [™] 7 Flex Real-Time PCR System					
QuantStudio [™] 12K Flex Real-Time PCR System					
QuantStudio [™] Dx Real-Time PCR Instrument	Contact your local				
ViiA [™] 7 Real-Time PCR System	sales office				
ViiA [™] 7 Dx Real-Time PCR Instrument					
7500 Fast Real-Time PCR System					
StepOne [™] or StepOnePlus [™] Real-Time PCR System ^[1,2]					
Equipment					
Centrifuge with plate adapter	MLS				
Microcentrifuge	MLS				
Single- or multi-channel pipettes (electronic or manual)	MLS				
Laboratory mixer (vortex or equivalent)	MLS				
Tubes, plates, and other consumables					
Plastics consumables	thermofisher.com/ plastics				
Aerosol-resistant barrier pipette tips	MLS				
Disposable gloves	MLS				

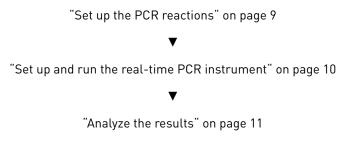
Table 5 Other materials and equipment required for PCR reactions

[1] The StepOne[™] and StepOnePlus[™] Systems are not compatible with TaqPath[™] ProAmp[™] Multiplex Master Mix.
 [2] The StepOne[™] Real-Time PCR System is not compatible with the requirements for copy number experiments.



Perform PCR for genotyping experiments

Workflow: Genotyping experiments



Procedural guidelines

Guidelines for input DNA	 Use a compatible DNA isolation kit (see Table 3 on page 6). All wells that use the same assay must contain similar amounts of sample DNA. For genotyping experiments, use a final DNA concentration of at least 0.2 ng/µL
	for each reaction.
	• For crude lysates, sample volume can be up to 25% of the total reaction volume. Use the DNA Extract All Reagents Kit (Cat. No. 4402599) for crude lysate preparation.
Guidelines for PCR reactions	• For reaction volumes that are different from those detailed, scale all components proportionally and include 10% overage.
	 For genotyping experiments, no template control (NTC) reactions are required to call the genotype. NTC reactions can also be used to identify PCR contamination.
	NTC reactions contain all reaction components (master mix, assays, water) except DNA sample.

Set up the PCR reactions

1. Prepare the required number of reactions according to the following table, plus 10% overage.

Table 6	PCR reactions	for	aonotynina	ovporimonte
Table 0	FUNTEACTIONS	101	genotyping	experiments

	Volume per reaction				
Component	5 μL (384-well)	10 µL (96-well Fast)	25 µL (96-well Std)		
TaqPath [™] ProAmp [™] Master Mix	2.5 µL	5.0 µL	12.5 µL		
TaqMan [®] SNP Genotyping Assay ^[1] (20X)	0.25 µL	0.5 µL	1.25 µL		
Genomic DNA - <i>or</i> - NTC ^[2]	up to 2.25 µL	up to 4.5 µL	up to 11.25 µL		
Nuclease-Free Water	to 5 µL total	to 10 µL total	to 25 µL total		
Total volume	5 µL	10 µL	25 µL		

^[1] For Research Use Only. Not for use in diagnostic procedures.

^[2] For crude lysates, sample volume can be up to 25% of the total reaction volume.

- **2.** Mix the components thoroughly, then centrifuge briefly to spin down the contents and eliminate air bubbles.
- 3. Add the appropriate volume of each reaction to each well of an optical plate.
- **4.** Seal the plate with an optical adhesive cover, then centrifuge briefly to spin down the contents and eliminate air bubbles.

(Optional) Store the reaction plate for up to 72 hours at room temperature.

TaqPath[™] ProAmp[™] Master Mixes User Guide



Set up and run the real-time PCR instrument

- 1. Load the reaction plate in the real-time PCR instrument.
- **2.** Set the appropriate experiment settings and PCR thermal cycling conditions. Select genotyping as the experiment type, and select the correct passive reference for the master mix formulation.

Note: Heat-labile UNG is active during the reaction setup and is completely inactivated during the first ramp to the 95°C hold step.

Note: Fast cycling is not recommended for TaqMan[®] Drug Metabolism Genotyping Assays.

Step	Temperature	Time	Cycles
Pre-Read	60°C	30 seconds	
Initial denature / Enzyme activation	95°C	5 minutes Hold	
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	60 seconds ^[1]	40
Post-Read	60°C	30 seconds	Hold

 Table 7
 Genotyping experiments: standard cycling

^[1] For Drug Metabolizing Enzyme (DME) assays, set duration to 90 seconds.

Table 8 Genotyping experiments: fast cycling

Step	Temperature	Time ^[1]	Cycles	
Pre-Read	60°C	30 seconds		
Initial denature / Enzyme activation	95°C	5 minutes	Hold	
Denature	95°C	5 seconds	- 40	
Anneal / Extend	60°C	30 seconds		
Post-Read	60°C	30 seconds	Hold	

^[1] Optional cycling for Fast 96-well or 384-well plates only.

- 3. Set the reaction volume appropriate for the reaction plate.
- 4. Start the run.

(Optional) After PCR, store the reaction plate protected from light:

- Up to 24 hours at room temperature.
- Up to 72 hours at 4°C.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your assay and real-time PCR instrument.

Analyze allelic discrimination plots for genotyping data using any of the following tools:

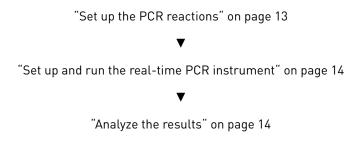
Software	Resource	
Thermo Fisher Cloud GT Analysis module	thermofisher.com/us/en/home/cloud.html	
TaqMan [®] Genotyper software	<i>TaqMan[®] Genotyper Software Getting Started Guide</i> (Pub. No. 4448637)	
Real-time PCR system analysis software	See "Related documentation" on page 22	

For general information about SNP genotyping experiments and analysis, see the *TaqMan*[®] *SNP Genotyping Assays User Guide* (Pub. No. MAN0009593).



Perform PCR for copy number experiments

Workflow: Copy number experiments



Procedural guidelines

Guidelines for input DNA	 Use a compatible DNA isolation kit (see Table 3 on page 6). Use purified gDNA as the DNA input; we do not recommend using crude lysate for copy number (CNV) experiments. All wells that use the same assay must contain similar amounts of sample DNA. For copy number experiments, use a final purified DNA concentration of 1 ng/µL for each reaction.
Guidelines for	 For copy number experiments, we recommend four replicates of each reaction. For reaction volumes that are different from those detailed, scale all components proportionally and include 10% overage. NTC reactions can be used to identify PCR contamination.
PCR reactions	NTC reactions contain all reaction components (master mix, assays, water) except DNA sample. These reactions should not result in amplification.

3

Set up the PCR reactions

1. Prepare the required number of reactions according to the following table, plus 10% overage.

Table 9	PCR	reactions	for	сору	number	experiments

	Volume per reaction		
Component	10 μL (384-well)	20 μL (96-well Std)	
TaqPath [™] ProAmp [™] Master Mix	5.0 µL	10.0 µL	
TaqMan [®] Copy Number Assay ^[1] (20X)	0.5 µL	1.0 µL	
TaqMan [®] Copy Number Reference Assay ^[1] (20X)	0.5 µL	1.0 µL	
Genomic DNA - <i>or</i> - NTC	up to 4 µL	up to 8 µL	
Nuclease-Free Water	to 10 µL total	to 20 µL total	
Total volume	10 µL	20 µL	

^[1] For Research Use Only. Not for use in diagnostic procedures.

- **2.** Mix the components thoroughly, then centrifuge briefly to spin down the contents and eliminate air bubbles.
- 3. Add the appropriate volume of each reaction to each well of an optical plate.
- **4.** Seal the plate with an optical adhesive cover, then centrifuge briefly to spin down the contents and eliminate air bubbles.

(Optional) Store the reaction plate for up to 72 hours at room temperature.



Set up and run the real-time PCR instrument

- 1. Load the reaction plate in the real-time PCR instrument.
- **2.** Set the appropriate experiment settings and PCR thermal cycling conditions. Select standard curve as the experiment type, and select the correct passive reference for the master mix formulation.

Note: Heat-labile UNG is active during the reaction setup and is completely inactivated during the first ramp to the 95°C hold step.

Step	Temperature	Time	Cycles
Initial denature / Enzyme activation	95°C	10 minutes	Hold
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	60 seconds	40

 Table 10
 Copy number experiments: standard cycling

- **3**. Set the reaction volume appropriate for the reaction plate.
- 4. Start the run.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your assay and real-time PCR instrument.

We recommend using the following options in the analysis settings:

- Select the auto-baseline setting.
- Manually set the threshold values.

For copy number experiments, set the thresholds for the reference assay and the copy number target assay to the same value.

Master mix formulation	Recommended threshold value
TaqPath [™] ProAmp [™] Master Mix	0.2
TaqPath [™] ProAmp [™] Multiplex Master Mix	0.8

Analyze copy number data using CopyCaller[™] Software; see the *CopyCaller[™]* Software v2.0 User Guide (Pub. No. 4400042).

For general information about copy number experiments and analysis, see the *TaqMan*[®] *Copy Number Assays Protocol* (Pub. No. 4397425).



Troubleshooting

Observation	Possible cause	Recommended action
Small particles during reaction mix setup	The particles are components of the master mix.	No action is required. The particles do not interfere with the reaction and do not affect physical apparatus, including robotics.
Amplification plot shows a high level of noise	The incorrect dye is set as the passive reference in the run file.	Change the passive reference to the correct dye, then reanalyze the data. • TaqPath [™] ProAmp [™] Master Mix—ROX [™] • TaqPath [™] ProAmp [™] Multiplex Master Mix— MUSTANG PURPLE [™]
Unexpected results for genotyping experiments using crude lysates	The assay that was used for the genotyping experiment is not compatible with crude lysates.	Use a purified DNA sample input for use with the chosen genotyping assay.
High NTC signal for genotyping experiments	Some genotyping assays have a significant NTC signal that is not due to amplification; rather, it is a property of the assay. In the absence of DNA template, some dye cleavage may occur.	No action is required. The signal does not contribute to or interfere with the genotype cluster signal or analysis.



Observation	Possible cause	Recommended action
Amplification curves for samples appear as sigmoidal curves	When automatic baseline is used, the software selects baseline cycle values that are too narrow.	For copy number experiments: When analyzing the data, change the analysis setting to allow manual adjustment, then set the Baseline Start Cycle to 3 and the Baseline End Cycle to 15.
		Set the Amplification Plot's graph type to linear. If amplification appears to begin earlier than cycle 15, adjust the Baseline End Cycle to 2 cycles prior to the start of amplification. For example, if the amplification appears to begin at cycle 13, set the Start Cycle to 3 and End Cycle to 11.
The C _t is low Note: Related observations are false positives or high NTC signal.	When automatic baseline is used, the software selects baseline cycle values that are too narrow.	For copy number experiments: When analyzing the data, change the analysis setting to allow manual adjustment, then set the Baseline Start Cycle to 3 and the Baseline End Cycle to 15. Evaluate the multi- component plot to ensure that the C _t signal represents a true amplification and not part of the background signal noise.



Supplemental information

Master mix components

Dual-Lock [™] Taq DNA Polymerase	This master mix contains Dual-Lock [™] Taq DNA Polymerase that uses a combination of two hot-start mechanisms to control its activity. By providing tight control over Taq activation, this dual hot-start approach helps to prevent undesirable early activity of the polymerase at low temperatures that can lead to nonspecific amplification.
	The polymerase is provided in an inactive state to automate the hot-start PCR and allow flexibility in the reaction setup, including pre-mixing of PCR reagents at room temperature. The polymerase is activated after a brief hold step at 95°C.
Heat-labile uracil- N-glycosylase (UNG)	This master mix contains heat-labile uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)). Heat-labile UNG prevents reamplification of carryover PCR products.
	Treatment with UNG degrades dU-containing PCR carryover products and misprimed, nonspecific DNA molecules. UNG acts on single- and double-stranded dU-containing DNA by hydrolyzing uracil-glycosidic bonds at dU-containing DNA sites. The enzyme causes the release of uracil and creates an alkali-sensitive apyrimidic site in the DNA. Apyrimidic sites block replication by DNA polymerases. The enzyme has no activity on RNA or dT-containing DNA.
	UNG enzymatic activity occurs during the PCR reaction setup at room temperature; an activation step before thermal cycling is not necessary. Unlike standard UNG, heat-labile UNG is completely inactivated during the first ramp to the high- temperature step for template denaturation and polymerase activation.
	PCR products from reactions that include heat-labile UNG are stable for up to 72 hours post-amplification.
dUTP	This master mix includes dUTP to enable uracil-N-glycosylase (UNG) activity and maintain optimal PCR results.
MUSTANG PURPLE [™] dye	MUSTANG PURPLE [™] dye can act as a passive reference (absorption 647 nm; emission 654 nm) to provide an internal reference for normalizing the reporter-dye signal during data analysis for multiplex reactions. Normalization corrects for fluorescence fluctuations due to changes in concentration or volume.
	For multiplex reactions, MUSTANG PURPLE TM dye can be used instead of the typical ROX TM reference dye. This replacement allows the use of JUN TM dye which is detected by the same channel as ROX TM dye.
	For more information about multiplex reactions, see the <i>TaqMan[®] Multiplex PCR Optimization User Guide</i> (Pub. No. MAN0010189).

Best practices for PCR and RT-PCR experiments

Good laboratory	When preparing samples for PCR or RT-PCR amplification:
practices for PCR and RT-PCR	 Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or during sample preparation).
	 Change gloves whenever you suspect that they are contaminated.
	• Maintain separate areas and dedicated equipment and supplies for:
	 Sample preparation and reaction setup.
	 Amplification and analysis of products.
	• Do not bring amplified products into the reaction setup area.
	• Open and close all sample tubes carefully. Avoid splashing or spraying samples.
	• Keep reactions and components capped as much as possible.
	• Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
	 Clean lab benches and equipment periodically with 10% bleach solution or DNAZap[™] Solutions (Cat. No. AM9890).
Use UNG to prevent false- positive	Carryover amplicons can result in false-positive amplification during PCR. Use a master mix that contains heat-labile uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)) to degrade many contaminating carryover amplicons.
amplification	UNG enzymatic activity occurs during the PCR reaction setup at room temperature; an activation step before thermal cycling is not necessary. Unlike standard UNG, heat-labile UNG is completely inactivated during the first ramp to the high- temperature step for template denaturation and polymerase activation.
	To ensure the desired UNG activity:
	 Use PCR components and thermal cycling conditions as specified.
	UNG-containing master mixes incorporate the optimal concentration of UNG to prevent cross-contamination while not affecting real-time PCR performance.
	• Do not attempt to use UNG-containing master mixes in subsequent amplification of dU-containing PCR products, such as in nested-PCR protocols. The UNG will degrade the dU-containing PCR products, preventing further amplification.
	Although treatment with UNG can degrade or eliminate large numbers of carryover PCR products, use good laboratory practices to minimize cross-contamination from non-dU-containing PCR products or other samples.
Detect fluorescent contaminants	Fluorescent contaminants can generate false positive results. To help detect these contaminants, we recommend including a No-Amplification Control reaction that contains sample, but no master mix.
	After PCR, if the absolute fluorescence of the No-Amplification Control is greater than the fluorescence of the no template control (NTC), fluorescent contaminants may be present in the sample or in the heat block of the real-time PCR instrument.

Safety





WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
- www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
 World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
 www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
TaqPath [™] ProAmp [™] Master Mixes Quick Reference	100040972
TaqMan [®] SNP Genotyping Assays User Guide	MAN0009593
TaqMan [®] Copy Number Assays Protocol	4397425
Instrument documentation	
QuantStudio [™] Design and Analysis desktop Software User Guide	MAN0010408
QuantStudio [™] 6 and 7 Flex Real-Time PCR System Software Getting Started Guide	4489822
<i>QuantStudio[™] 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide</i>	4470050
Applied Biosystems [™] ViiA [™] 7 Real-Time PCR System Getting Started Guide	4441434
Applied Biosystems [™] 7500/7500 Fast Real-Time PCR System Getting Started Guide: Genotyping Experiments	4387784
Applied Biosystems [™] 7500/7500 Fast Real-Time PCR System Getting Started Guide: Standard Curve Experiments	4387779
Applied Biosystems [™] StepOne [™] and StepOnePlus [™] Real-Time PCR Systems Genotyping Experiments Getting Started Guide	4376786
Data analysis	
Thermo Fisher Cloud GT Analysis module	See thermofisher.com/us/ en/home/cloud.html
TaqMan [®] Genotyper Software Getting Started Guide	4448637
TaqMan [®] SNP Genotyping Assays User Guide	MAN0009593
CopyCaller [™] Software v2.0 User Guide	4400042

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- Product documentation, including:
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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