

appleton[®]

ISSUE 2



Molecular Biology Reagents

appTAQ Polymerase & Ready-mixes

Reliable, every day PCR

- Amplify amplicons $\leq 6\text{kb}$
- Suitable for routine PCR in genotyping, screening or library construction
- Pre-optimised buffer system with pre-added dNTPs and MgCl_2
- Works under standard or fast cycling conditions
- Clone PCR products into TA vectors
- Amplifies from complex GC- rich or AT-rich genomic DNA templates

appTAQ Polymerase



PCR amplification of a 2kb fragment of the HPRT1 gene using appTAQ Polymerase

Human genomic DNA was serially diluted 20 fold from 100ng to 0.6pg

Competitor N



| 0.6pg | 1.25pg | 0.25ng | 5ng | 100ng |

Code	Description	Size	Price £
ARPO01	appTAQ Polymerase	500 units	110.00
ARPO03	appTAQ Polymerase	2000 units	396.00
ARPO52	appTAQ Mix (2X)	200 reactions	75.00
ARPO62	appTAQ RedMix (2X)	200 reactions	85.00

appTAQ Hot Start Polymerase & Ready-mixes

Ultra Sensitive PCR

- Inactive at ambient temperature for highly specific PCR
- For genotyping, high throughput PCR & low copy number detection
- Optimised buffer system with pre-added dNTPs and MgCl_2
- Amplify products $\leq 6\text{kb}$
- Suitable for difficult templates (low copy number, GC/AT-rich, blood, colony or methylated DNA)
- Works under standard or fast cycling conditions
- Clone PCR products into TA vectors

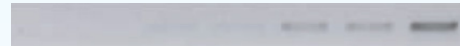
appTAQ Hot Start Polymerase



PCR amplification of a 2kb fragment of the LDHA gene using appTAQ Hot Start Polymerase

Human genomic DNA was serially diluted 2 fold from 100ng to 1.5ng

Competitor T



| 1.5ng | 3.1ng | 6.25ng | 12.5ng | 25ng | 50ng | 100ng |

Code	Description	Size	Price £
ARPO21	appTAQ Hot Start Polymerase	250 units	95.00
ARPO23	appTAQ Hot Start Polymerase	1000 units	342.00
ARPO72	appTAQ Hot Start Mix (2X)	200 reactions	135.00
ARPO82	appTAQ Hot Start RedMix (2X)	200 reactions	135.00

appMEGA Polymerase & Ready-mixes

Long Range Difficult PCR

- Amplify DNA products $\leq 35\text{kb}$ (λ); 25kb (genomic)
- Suitable for next gen re-sequencing, long range, complex, multiplex, colony or crude PCR
- Optimised buffer system with pre-added dNTPs and MgCl_2
- Hot-start enzyme is inactive at ambient temperature for highly specific, processive PCR
- Works under standard or fast cycling conditions
- Clone PCR products into TA vectors

appMEGA Polymerase



PCR amplification of a 25kb fragment of the beta-globin gene using appMEGA Polymerase

Human genomic DNA was serially diluted 2 fold from 100ng to 6.25ng

Competitor K



| 6.25g | 12.5ng | 25ng | 50ng | 100ng |

Code	Description	Size	Price £
ARPO31	appMEGA Polymerase	250 units	135.00
ARPO33	appMEGA Polymerase	1000 units	486.00
ARPO92	appMEGA Mix (2X)	200 reactions	140.00

appHiFi Polymerase

Fast, Sensitive & High fidelity PCR

- 50 fold higher fidelity than appTAQ Polymerase
- Suitable for blunt end cloning, site-directed mutagenesis, and next gen re-sequencing
- Optimised buffer system with pre-added dNTPs and MgCl_2
- Highly processive enzyme amplifies $\leq 10\text{kb}$ from complex GC- or AT-rich templates, colonies or crude samples
- Works under standard or fast cycling conditions

appHiFi Polymerase



PCR amplification of a 5kb fragment of the PGK1 gene using appHiFi Polymerase under fast cycling conditions (<1.5hrs)

Human genomic DNA was serially diluted 2 fold from 100ng to 1.5ng

Competitor F



| 1.5ng | 3.1ng | 6.25ng | 12.5ng | 25ng | 50ng | 100ng |

Code	Description	Size	Price £
ARPO41	appHiFi Polymerase	200 units	100.00
ARPO43	appHiFi Polymerase	1000 units	450.00

Customer review

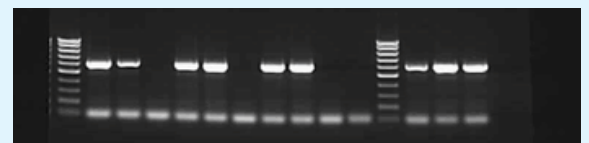
“ I regularly perform marker assisted genotyping by PCR from genomic DNA, so using a Taq mix that is reliable, cheap and simple to utilize is of paramount importance. In that regard, our current 2x ready mix is adequate for this purpose.

Having recently tested the Appleton Woods appTAQ RedMix however, using multiple animal cohorts, I was pleasantly surprised to find that its sensitivity by comparison to our current brand was actually better.

Furthermore, its price for a given number of units is also better than our current supplier's brand.

This has persuaded me to switch to appTAQ RedMix for high throughput genotyping, as there appears to be no down side to making this transition. ”

Laurence Hall
Core Facility Technician
Department of Cardiovascular Sciences
University of Leicester



appTAQ RedMix (2X) More sensitive: 80mSec Exposure



Competitor 2x ready mix: 160mSec Exposure

- ✓ Ideal for absolute gene quantification, gene expression analysis and diagnostic qPCR
- ✓ Pre-optimised mixes deliver fast, specific and sensitive qPCR
- ✓ Robust detection of your low-copy number templates with rapid extension times (low C_T values)
- ✓ Mixes contain a hot start polymerase which is engineered for highly specific qPCR and works in fast or standard thermal cycling conditions
- ✓ Compatible with the majority of real-time thermal cyclers
- ✓ Suitable sample types: complex templates and crude samples

appPROBE qPCR Mixes

- For high performance qPCR using probe-detection technologies like Taqman, Molecular Beacons and Scorpion probes
- Pre-added, optimised levels of $MgCl_2$ and dNTPs for highly reproducible single-gene or multiplex qPCR

Figure 1: Multiplex qPCR of 5 genes using human cDNA and appPROBE High ROX Mix (2X).

The qPCR mix efficiently generates distinct and reproducible traces for each gene under fast cycling conditions.

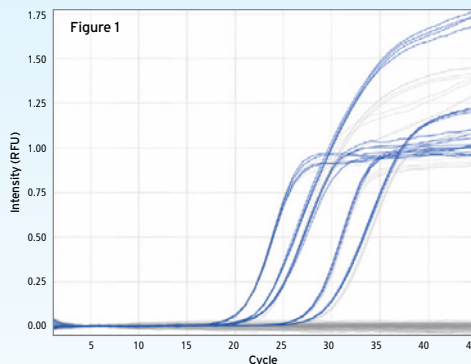
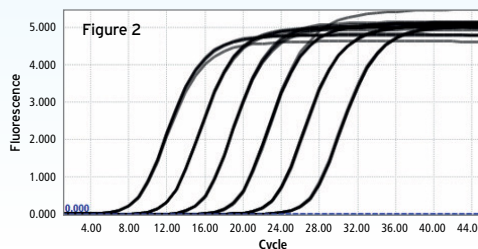


Figure 2: qPCR data traces generated using appPROBE High ROX Mix (2X).

10-fold serial dilutions of mouse beta-actin cDNA were amplified using Taqman probes labelled with Cy5 dye. Four overlapping replicates demonstrate close to 100% efficiency.



appPROBE Low ROX Mix (2X)

Code	Reactions	Price £
ARP302	200	90.00
ARP303	500	225.00
ARP305	5000	2,000.00

appPROBE High ROX Mix (2X)

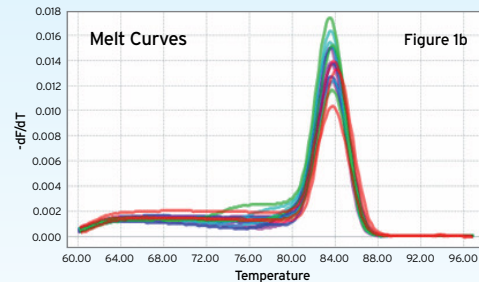
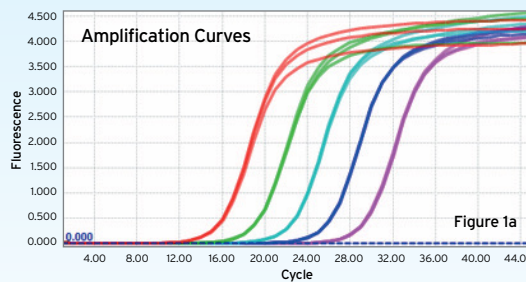
Code	Reactions	Price £
ARP402	200	90.00
ARP403	500	225.00
ARP405	5000	2,000.00

appPROBE No ROX Mix (2X)

Code	Reactions	Price £
ARP502	200	90.00
ARP503	500	225.00
ARP505	5000	2,000.00

appGREEN qPCR Mixes

- For high performance fast, specific and sensitive qPCR using appGREEN (a proprietary intercalating dye which does not interfere with qPCR)
- Pre-added, optimised levels of $MgCl_2$ and dNTPs for highly reproducible single-gene qPCR



appGREEN Low ROX Mix (2X)

Code	Reactions	Price £
ARP102	200	90.00
ARP103	500	225.00
ARP105	5000	2,000.00

appGREEN High ROX Mix (2X)

Code	Reactions	Price £
ARP202	200	90.00
ARP203	500	225.00
ARP205	5000	2,000.00

Figure 1a & 1b : qPCR amplification curves (1a) and corresponding melt curves (1b) of the demo template and kit from a Roche Lightcycler 96 using appGREEN Low ROX Mix (2X).

The equal spacing in Figure 1a demonstrates close to 100% real-time amplification efficiency and overlapping melt curves in Figure 1b demonstrate that the same product was produced from each template dilution.

appGREEN Blue qPCR Mixes

- For high performance fast, specific and sensitive qPCR using appGREEN (a proprietary intercalating dye which does not interfere with qPCR)
- Easy sample visualisation and pipetting using a non-reactive blue dye
- Pre-added, optimised levels of $MgCl_2$ and dNTPs for highly reproducible single-gene qPCR

appGREEN Blue Separate ROX Mix (2X)

Code	Reactions	Price £
ARP142	200	100.00
ARP143	500	240.00
ARP145	5000	2,200.00

appGREEN Blue Low ROX Mix (2X)

Code	Reactions	Price £
ARP122	200	100.00
ARP123	500	240.00
ARP125	5000	2,200.00

appGREEN Blue High ROX Mix (2X)

Code	Reactions	Price £
ARP132	200	100.00
ARP133	500	240.00
ARP135	5000	2,200.00



appGREEN 1-Step RT-qPCR Kits

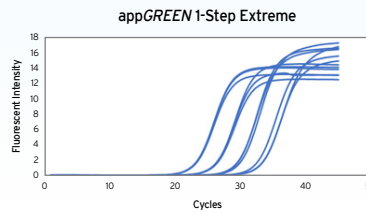
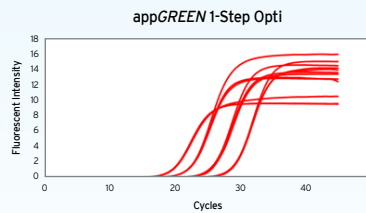
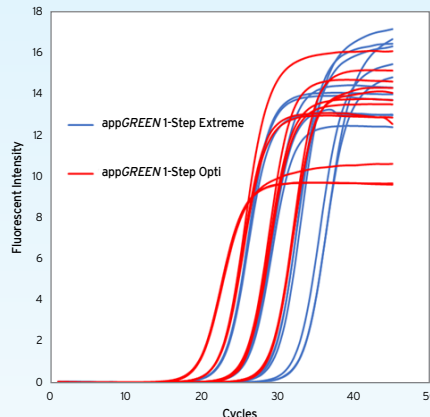
Low or High Copy Number Detection

- For absolute RNA target quantification and relative gene expression analysis
- Extreme kits - reliably detect extremely low copy number targets
- Opti kits - rapidly detect high copy number targets at earlier C_T's
- Highly processive, thermostable reverse transcriptase maximises cDNA yield & extension
- Advanced RNase inhibitor protects your precious RNA targets
- Antibody-mediated hot start polymerase for specificity and easy room temperature set up
- Kits contain an intercalating dye which does not inhibit PCR
- Compatible on all major real-time PCR platforms (see selection guide on page 6)

Figure 1: Comparison of appGREEN 1-Step Extreme and Opti Low ROX Kits, showing the ideal template range of each product.

The GAPDH gene was amplified from a dilution series of total RNA extracted from mouse liver. The appGREEN 1-Step Opti Low ROX kit shows efficient amplification of total RNA in the range 100 ng to 100 pg per reaction.

The appGREEN 1-Step Extreme Low ROX Kit shows efficient amplification of total RNA in the range 10 ng to 10 pg per reaction. Cycling conditions were 45°C for 10 minutes for cDNA synthesis, followed by 95°C for 2min hot start, then 45 cycles of 95°C for 10sec, 60°C for 30sec on a Roche LC480.



appGREEN 1-Step Extreme RT-qPCR Low ROX Kits*

Code	Reactions	Price £
ARP742	200	250.00
ARP743	500	585.00

appGREEN 1-Step Extreme RT-qPCR High ROX Kits*

Code	Reactions	Price £
ARP752	200	250.00
ARP753	500	585.00

* Suitable for starting templates of 1pg-10ng total RNA or >0.01pg mRNA / reaction

appGREEN 1-Step Opti Low ROX RT-qPCR Kits**

Code	Reactions	Price £
ARP792	200	240.00
ARP793	500	575.00

appGREEN 1-Step Opti High ROX RT-qPCR Kits**

Code	Reactions	Price £
ARP802	200	240.00
ARP803	500	575.00

* Suitable for starting templates of 10pg-100ng total RNA or >0.01pg mRNA / reaction

appSCRIPT cDNA Synthesis Kit

Efficient Unbiased Synthesis

- For reproducible and unbiased cDNA synthesis from 5' and 3' ends of mRNAs
- Contains a reverse transcriptase which can synthesize cDNAs up to 9kb
- High cDNA yields from as little as 4pg total RNA or 0.2pg oligo dT mRNA
- The kit contains a buffer which has pre-optimised levels of oligo dT and random hexamers which results in reduced transcript bias for downstream qPCR
- Short protocol (30mins only)

appSCRIPT cDNA Synthesis Kit

Code	Reactions	Price £
ARP601	25	95.00
ARP602	100	342.00
ARP603	250	813.00

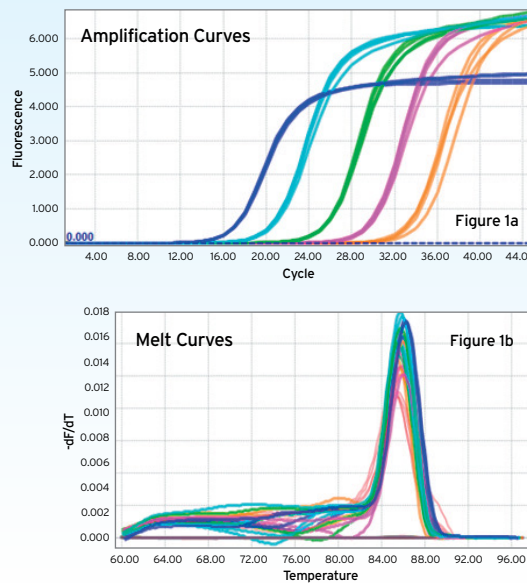


Figure 1a & 1b : qPCR amplification curves (1a) and corresponding melt curves (1b) of serial dilutions of mouse cDNA made using the appSCRIPT cDNA synthesis kit.

A 10 fold serial dilution of mouse total RNA was used for 4 cDNA synthesis reactions. The highest amount of RNA used for cDNA synthesis was 5µg and the lowest 5pg.

Each cDNA synthesis reaction was used as a template for an appGREEN qPCR reaction, amplifying an 80bp fragment of the mouse GAPDH gene. Reactions were incubated for 30 minutes at 42°C. The amplification curves are four 10 fold serial dilutions of cDNA prepared from total mouse RNA. The equal spacing in Figure 1a demonstrates close to 100% amplification efficiency. The overlapping melt curves demonstrate that the same product was produced from each template dilution. appSCRIPT cDNA synthesis kit efficiently produces cDNA from a wide range of RNA concentrations.

Other Appleton products from our range...

Molecular Biology Grade Agarose



- For routine analysis of nucleic acids from 100-1000bp
- High gel strength, suitable for blotting
- Sharp and well defined bands
- Low background
- DNase/RNase free

Code	Pack Size	Price £
AG001	500g	275.00
AG002	100g	57.00

Low Retention Pipette Tips

- Less fluid retention
- Less sample loss
- Less time spent
- Less plastic wasted

SEE WEBSITE FOR DETAILS



appGENE Total RNA Kit

Unparalleled Yields

- Purify consistently high quality total RNA from $\leq 30\text{mg}$ tissue or $10^4\text{-}10^7$ cultured cells
- Market leading yield: $\leq 230\mu\text{g}$ / spin column
- Extract total RNA from a variety of tissues e.g. liver, colon, kidney, spleen, thymus, lung, heart, brain
- Easily process multiple sample types in parallel in ≤ 20 mins
- RNA suitable for RT-PCR, RT-qPCR, Northern blotting, nuclease protection assay, and in vitro translation
- See website for average yields

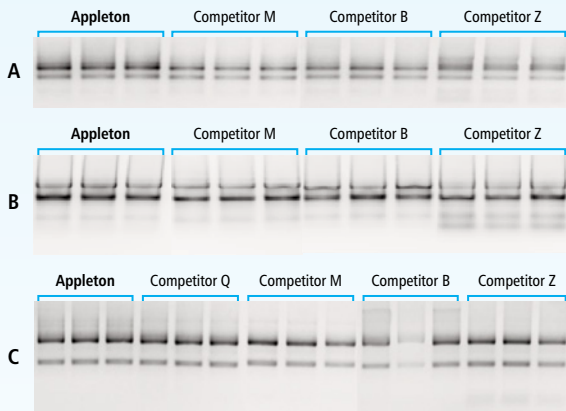


Figure 1: RNA Extraction from Tissue and Cell Cultures using the appGENE Total RNA kit.

Three replicates of total RNA were extracted according to each manufacturer's protocol, with on-column DNase I digestion, if provided. RNA was eluted into a final volume of $50\mu\text{l}$.

- A** - $5\mu\text{l}$ per lane of total RNA from 10mg of liver tissue, diluted 10 fold,
- B** - $5\mu\text{l}$ per lane of total RNA from 10mg of liver tissue, undiluted
- C** - $5\mu\text{l}$ per lane of total RNA from 1×10^6 cells from the A549 cell line

appGENE Total RNA Kit

Code	Size	Price £
ARN015	50 preps	202.40
ARN016	250 preps	867.20

appGENE Gel & PCR Clean-Up Kit

Fast & Reliable DNA Recovery

- Flexible purification of DNA from any enzymatic reaction or agarose gel
- Remove excess primers, unincorporated nucleotides, mineral oil, nucleases, enzyme inhibitors, detergents, restriction enzymes, polymerases, divalent ions, agarose, and ethidium bromide
- Purify DNA fragments 100bp to 10kb with $\geq 99\%$ recovery
- Do PCR clean-up in 5-10 mins / gel extraction in <20 mins
- Purified DNA is suitable for multiple molecular biological applications

appGENE Gel & PCR Clean-Up kit

Code	Size	Price £
ARN025	50 preps	70.40
ARN026	250 preps	298.40

appGENE Genomic DNA Kit

Reliable & Versatile DNA Purification

- Purify consistently high quality genomic, mitochondrial, bacterial, parasite or viral DNA
- Extract from:
 - ✓ $\leq 30\text{mg}$ tissues, insects, hair, rodent tails
 - ✓ $10^3\text{-}10^7$ cell cultures
 - ✓ $\leq 1\text{ml}$ blood
 - ✓ $\leq 5\text{ml}$ physiological fluids
 - ✓ Swabs
 - ✓ $\leq 150\mu\text{l}$ semen
 - ✓ 3ml ($\leq 10^9$ cells) yeast and bacteria
- Maximum yield: $\leq 50\mu\text{g}$ (e.g.liver) / spin column
- Concise sample-specific, pre-optimised protocols for highest yield
- Easily process multiple sample types in parallel in ≤ 25 mins
- DNA suitable for downstream molecular biology, genotyping & pathogen applications
- See website for average yields

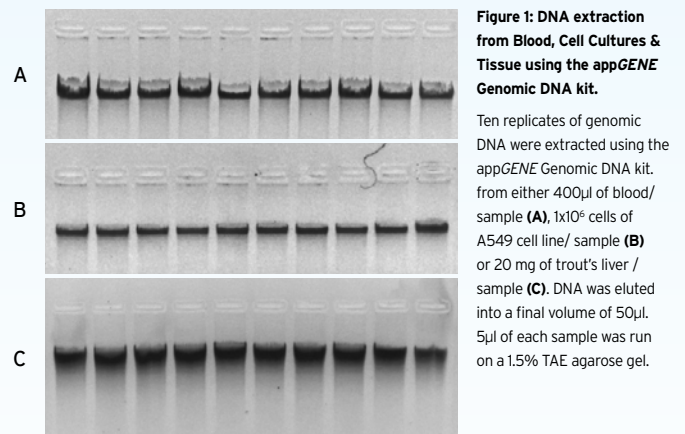


Figure 1: DNA extraction from Blood, Cell Cultures & Tissue using the appGENE Genomic DNA kit.

Ten replicates of genomic DNA were extracted using the appGENE Genomic DNA kit, from either $400\mu\text{l}$ of blood/sample (A), 1×10^6 cells of A549 cell line/sample (B) or 20 mg of trout's liver / sample (C). DNA was eluted into a final volume of $50\mu\text{l}$. $5\mu\text{l}$ of each sample was run on a 1.5% TAE agarose gel.

appGENE Genomic DNA Kit

Code	Size	Price £
ARN020	50 preps	124.00
ARN021	250 preps	559.20

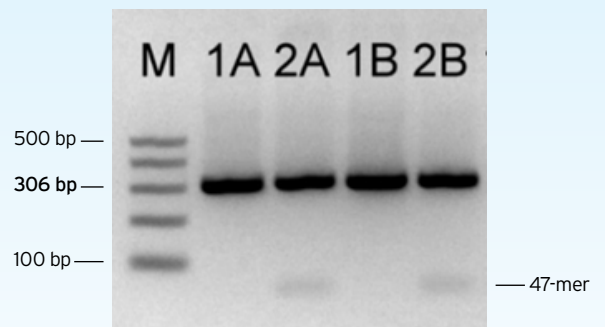


Figure 1: PCR clean-up of primers using the appGENE Gel & PCR Clean-Up kit.

A 306 bp fragment of the human NUMB isoform 4 gene was amplified in duplicate and the PCR reactions were purified using the appGENE Gel & PCR Clean-Up kit. The purified amplicons were eluted into final volumes of $30\mu\text{l}$. $5\mu\text{l}$ of the eluates was loaded onto a 1.5% TAE agarose gel. 47-mer primers were removed.

Lanes:

M - 100-500bp marker **1A & 1B** - after PCR clean-up **2A & 2B** - before PCR clean-up

qPCR Selection Guide

	appGREEN High ROX Mix (2X)	appGREEN Blue High ROX Mix (2X)	appGREEN Low ROX Mix (2X)	appGREEN Blue Low ROX Mix (2X)	appGREEN Blue Separate ROX Mix (2X)	appPROBE High ROX Mix (2X)	appPROBE Low ROX Mix (2X)	appPROBE No ROX Mix (2X)	appGREEN 1-Step Extreme High ROX Kit	appGREEN 1-Step Opti High ROX Kit	appGREEN 1-Step Extreme Low ROX Kit	appGREEN 1-Step Opti Low ROX Kit
Agilent (Stratagene)												
AriaMX		•	•	•		•				•	•	
MX3000P®, MX3005P®, MX4000P®		•	•	•		•				•	•	
Analytik Jena												
qTOWER, qTOWER 2.x		•	•	•		•				•	•	
BMS												
Mic		•	•	•		•				•	•	
Bio-Rad®												
CFX96™, CFX384™		•	•	•		•				•	•	
Chromo4™, MiniOpticon™,		•	•	•		•				•	•	
Opticon™, Opticon™ 2		•	•	•		•				•	•	
iCycler®, iQ™ 5, MyiQ™		•	•	•		•				•	•	
BJS												
Xpress®		•	•	•		•				•	•	
Cepheid®												
SmartCycler®		•	•	•		•				•	•	
Eppendorf												
Mastercycler® ep realplex,		•	•	•		•				•	•	
Mastercycler® ep realplex 2S		•	•	•		•				•	•	
Fluidigm												
BioMark™		•	•	•		•				•	•	
Hain Lifescience												
FluoroCycler® 96		•	•	•		•				•	•	
IT-IS Life Science												
MyGo Pro, MyGo Mini		•	•	•		•				•	•	
PCRmax												
Eco™		•	•	•		•				•	•	
Qiagen (Corbett)												
Rotor-Gene™ 3000, Rotor-Gene™		•	•	•		•				•	•	
6000, Rotor-Gene™ Q		•	•	•		•				•	•	
Roche												
LightCycler® 480, LightCycler® 96,		•	•	•		•				•	•	
LightCycler® Nano		•	•	•		•				•	•	
Takara												
Thermal Cycler Dice® (TP800)		•	•	•		•				•	•	
Techné®												
PrimeQ, Quantica®						•				•	•	
Thermo Fisher (including Applied Biosystems and Life Technologies)												
5700, 7000, 7300, StepOne™,	•	•		•		•				•	•	
StepOne™ plus	•	•				•				•	•	
7500, 7500 FAST, QuantStudio™		•	•	•		•				•	•	
3, 5, 6, 7, 12k Flex, ViiA7™		•	•	•		•				•	•	
7700, 7900, 7900HT, 7900HT FAST	•	•		•		•				•	•	
Piko Real®		•	•	•		•				•	•	

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