

# Better PCR Reagents

For accuracy, sensitivity, and speed



# Smart Amplification Starts with the Right Tools

Agilent's family of PCR reagents and kits began with -Stratagene's enzyme expertise - and continues to develop, providing a wide range of high-fidelity, specialty and routine enzymes built to meet virtually every amplification need.

Agilent's PCR portfolio solution goes beyond the products, offering you an experienced technical support team for help in designing assays, optimizing protocols, and troubleshooting programs.

# Better PCR



#### The ArchaeMaxx Polymerase-Enhancing Factor

ArchaeMaxx overcomes the stalling of proofreading enzymes when dUTP is present within the template or PCR reaction.

- Exclusively found in Agilent's high-fidelity DNA polymerases
- Functions as a dUTPase, converting poisonous dUTP into harmless dUMP
- Increases PCR yield and polymerase length amplification capability



#### The Importance of High-Fidelity

High-fidelity PCR enzymes are valuable for minimizing the introduction of amplification generated errors in products utilized in downstream processes.

- Ideal for cloning, sequencing, and expression studies
- Reduces the number of clones that must be sequenced, improving overall TAT to results
- Obtains a higher frequency of error free constructs and accurate consensus sequences



#### **Fusion Technology**

The processivity of Agilent's premium enzymes are dramatically increased by fusing the DNA polymerase with a high-affinity double-stranded DNA binding domain.

- Shorter extension times through faster nucleotide incorporation, so faster PCR cycling times
- Serves to better anchor the DNA polymerase
- Prevents early dissociation from the DNA template



#### **Hot Start Formulation**

Hot start formulation provides higher specificity, reduced background and enhanced yield.



#### **Master Mix Format**

Master mix format provides added convenience and allows high-throughput setup.

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## **DNA Polymerase Selection Guide**

	PCR Enzyme	Page	Fidelity	Speed	Yield	Target Lenght (genomic DNA)	Sensitivity
PCR -	<i>PfuUltra</i> II Fusion HotStart DNA Polymerase Engineered to be the highest fidelity and fastest polymerase available	6	1 error/2.5 million bp	15 sec/kb		0-19 kb	
	Herculase II Fusion DNA Polymerase High-fidelity polymerase for difficult targets. Provides superior yields over a broad range of targets. Economical enough for routine use	8	1 error/770,000 bp	15 sec/kb		"0-12kb 12-23 kb (optimized)"	
oifficult/GC Ric	<i>PfuUltra</i> High-Fidelity DNA Polymerase AD Engineered for high-fidelity	13	1 error/2.25 million bp	1 min/kb		19 kb (optimized)	
High-Fidelity & D	<i>PfuTurbo</i> DNA Polymerase AD First high-fidelity polymerase to include the ArchaeMaxx Polymerase-Enhancing factor	13	1 error/770,000 bp	1 min/kb		19 kb (optimized)	
	Cloned Pfu DNA Polymerase AD Cloned to ensure ultrapure manufacturing of Pfu	13	1 error/770,000 bp	2 min/kb		"1 kb 5 kb (optimized)"	
	<i>Pfu</i> <b>DNA Polymerase</b> Stratagene introduced the first thermophilic proofreading polymerase	13	1 error/770,000 bp	2 min/kb		(up to 1 kb)	
ty Enzymes	<b>PfuTurbo Cx HotStart DNA Polymerase</b> The only high-fidelity polymerase that can read through dUTP in the template and extending strand	13	1 error/770,000 bp	1 min/kb		0-10 kb	
Special	PicoMaxx High-Fidelity PCR System Most sensitive polymerase offered	10	2x Taq	1 min/kb		0-6 kb	
Routine Enzymes	<i>Taq</i> DNA Polymerase First thermophilic PCR enzyme	-		1 min/kb		"1 kb 4 kb (optimized)"	

Blunt or 3'-A Ends	ArchaeMaxx MAXX Advantage	Enzyme Only	HotStart	Master Mix	PCR Enzyme
		100 U 500 U 1000U 5000 U	100 U 500 U 1000 U 5000 U	100 rxn 400 rxn	
Blunt	MAXX		(40 rxn) (200 rxn) 600670 6 00672 (400 rxn) 600674	600850	<i>PfuUltra</i> II Fusion HotStart DNA Polymerase Engineered to be the highest fidelity and fastest polymerase available
Blunt	MAXX	(40 rxn) 600675 (200 rxn) 600677 (400 rxn) 600679			Herculase II Fusion DNA Polymerase High-fidelity polymerase for difficult targets. Provides superior yields over a broad range of targets. Economical enough for routine use
Blunt	MAXX	600385 600387 600389	600390 600392 600394	600630	PfuUltra High-Fidelity DNA Polymerase AD Engineered for high-fidelity
Blunt	MAXX	600255 600257	600320 600322 600324		PfuTurbo DNA Polymerase AD     First high-fidelity polymerase to include the       ArchaeMaxx Polymerase-Enhancing factor     40
Blunt		600353 600355 600357			Cloned Pfu DNA Polymerase AD Cloned to ensure ultrapure manufacturing of Pfu
Blunt		600135			<b>Pfu DNA Polymerase</b> Stratagene introduced the first thermophilic proofreading polymerase
Blunt	Alternative uracil resistance ( <i>Pfu</i> mutation)		600410     600412       600414     600414		PfuTurbo Cx HotStart DNA Polymerase     The only high-fidelity polymerase that can     read through dUTP in the template and     extending strand
Mixed	MAXX		600422		PicoMaxx High-Fidelity PCR System
З'-А					Taq DNA Polymerase Image: Comparison of the second secon

## **High-Fidelity & Difficult/GC-Rich PCR**

### PfuUltra II Fusion HotStart DNA Polymerase

#### Extreme accuracy - 1 error/2.5 million bp

When accuracy is critical for your downstream applications, such as cloning, protein crystallization studies, and sequencing, PfuUltra II is the industry standard for high-fidelity PCR. PfuUltra II features a unique genetically engineered mutant of Pfu DNA polymerase, the ArchaeMaxx Polymerase-Enhancing Factor, fusion technology, and hot start formulation.

- Highest fidelity for the most reliable, error-free PCR
- 70-80% quicker time to results
- Accurately amplifies targets up to 19 kb gDNA
- Average error rate 3x lower than PfuTurbo & 20x lower than Taq DNA polymerase



Ordering Information	
Size	Agilent p.n.
40 rxn	600670
200 rxn	600672
400 rxn	600674

Agilent specialists performed a study to see how the specificity claims from both Agilent and competing brand enzymes compared to the results generated after running the thermal cycling reactions according to the manufacturer protocols. Agilent and competitor polymerases were used to PCR amplify a 2.6 kb DNA target from genomic DNA. Amplicons were then analyzed directly on the TapeStation instrument with D5000 reagents and Screen tapes.

The results indicate that the Agilent DNA Polymerases show higher specificity than competing brands.

Superior Specificity with PfuUltra II Fusion Polymerase than other HotStart DNA Polymerases



*PfuUltra* II Fusion exhibits increased specificity over comparative polymerases

2.6 kb target amplified according to respective enzyme conditions. Lane A1. Lane B1, Agilent PfuUltra II Fusion. Lane C1, Competitor T. Lane D1, Competitor T.

**Note**: Multiple arrowheads highlighting nonspecific amplification in competitor samples, lanes C1 and D1.

#### **High Specificity Hot Start Formulation**

PfuUltra II is formulated with hot start antibodies that neutralize both polymerase and exonuclease activities during reaction setup, thereby enhancing specificity and enabling automated workflows.

#### **Convenient Master Mix Format**

A High-throughput PCR reaction can be easily set up with PfuUltra II HotStart PCR Master Mix since it tolerates room temperature assembly up to 24 hours prior to thermal cycling without any impact on performance.



*PfuUltra* II amplifies genomic targets up to 19 kb in length under faster cycling conditions – 15 sec/kb (most fusion enzymes are limited to 6 kb target amplification).



Fidelity was measured using Agilent's validated and reference fidelity assay. (Accuracy = 1/error rate).

"High-Fidelity PCR & Difficult/GC-Rich DNA Targets? Better PCR Solutions."

## Herculase® II Fusion DNA Polymerase

#### Superior yield for routine PCR applications and difficult/ GC-rich targets

Herculase II, with its unique formulation and exclusive ArchaeMaxx Polymerase-Enhancing Factor, addresses your need for robust yields with all sample types. For mid-length (1.7 kb) or long (6 kb) DNA fragments, Herculase II produces as much as 10x greater yield with an extension rate as fast as 15 sec/kb.

- Superior yield for routine PCR applications & difficult/GC-rich targets
- Fast cycling time with 15 sec/kb extension rate
- Accurately amplifies targets up to 23 kb gDNA
- Preserves Pfu fidelity 1 error/770,000 bp
- High sensitivity for amplification of low amounts of DNA - important for precious and archival samples

MAXX	HF	*
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Ordering Information	
Size	Agilent p.n.
40 rxn	600675
200 rxn	600677
400 rxn	600679

Agilent specialists performed a study to see how the specificity claims from both Agilent and competing brand enzymes compared to the results generated after running the thermal cycling reactions according to the manufacturer protocols. Agilent and competitor polymerases were used to PCR amplify a 2.6 kb DNA target from genomic DNA. Amplicons were then analyzed directly on the TapeStation instrument with D5000 reagents and Screen tapes.

The results indicate that the Agilent DNA Polymerases show higher specificity than competing brands.

Superior Specificity with Herculase II Fusion Polymerase than other non-HotStart DNA Polymerases



Herculase II Fusion exhibits increased specificity over comparative polymerases:

A1AT gene, 2.6 kb target amplified according to respective enzyme conditions. Lane A1, Ladder. Lane B1, Agilent Herculase II Fusion. Lane C1, Competitor N. Lane D1, Competitor B.

**Note**: Multiple arrowheads highlighting nonspecific amplification in competitor samples, lanes C1 and D1.

#### **Robust Performance on Difficult and GC-Rich Targets**

Amplification of templates with high GC-content often leads to the formation of secondary structures, such as hairpins, causing the DNA polymerase activities to stall and results in incomplete and non-specific amplification.

Herculase II helps you overcome challenging PCR with the combination of exclusive enzyme improvement additives and an optimized buffer system, allowing you to easily amplify targets with GC-content as high as 84%.

#### High Sensitivity with Low Target Abundance

Herculase II amplifies DNA fragments over a wide range of template lengths with great sensitivity, enabling you to detect limited amounts of DNA. Herculase II successfully amplifies 3.9 kb fragments of the Ha1AT gene from as little as 1 ng of template DNA.



Herculase II produced superior yields of a 6 kb fragment in amplifications employing human genomic DNA at extension rates of 15, 30, 45, and 60 sec/kb.

Herculase II easily amplified GC-rich fragments from human genomic DNA: IGFB (79% GC); FMR1 (84% GC); HTR (65% GC); MMZ5 (68% GC).

#### "Have you tried "Perfect Match"?

Enhance your enzymes to further increase specificity and yield See page 12"

## **Specialty Enzymes**

### PfuTurbo II Cx HotStart DNA Polymerase

#### Overcome uracil stalling

*PfuTurbo* Cx is formulated with a unique mutant of Pfu DNA polymerase that completely overcomes uracil stalling without sacrificing fidelity.

*PfuTurbo* Cx reads through dUTP located in both the template strand and extending strand.

- World's only high-fidelity polymerase that efficiently reads through dUTP
- Great for bi-sulfite sequencing, UNG decontamination protocols & DNA methylation studies
- Amplifies templates 5-10 kb or with high GC-content
- DMSO provided separately to assist in optimizing PCR condition

## FF 🔥

Ordering Information	
Size	Agilent p.n.
100 U	600410
500 U	600412
1000 U	600414

#### PfuTurbo Cx maintains fidelity

Thermostable DNA Polymerase	Error Rate number	% of mutated 1 kb PCR products
<i>PfuTurbo</i> Cx HotStart DNA Polymerase	1.3 x 10 <sup>-6</sup>	2.6
PfuTurbo DNA Polymerase	1.3 x 10 <sup>-6</sup>	2.6
Taq DNA Polymerase	8.0 x 10 <sup>-6</sup>	16

## PicoMaxx High-Fidelity PCR System

#### Low copy number target amplification in complex samples

PicoMaxx confers the sensitivity to detect low copy number targets and the robustness to withstand the effects of PCR inhibitors without the need for extensive purification and subsequent DNA loss.

- Robust amplification of targets up to 10 kb gDNA
- High-specificity hot start increases enzyme sensitivity



Ordering Information - Various starting gDNA template qty (ng)			
Size	Agilent p.n.		
500 U	600422		



Amplification of a 3.9 kb  $\alpha\text{-}1$  anti-trypsin at various starting gDNA template qty (ng).

## **Other PCR Enzymes**

#### PfuUltra High-Fidelity DNA Polymerase

 Low error rate (1 error/2.25 million bp) without the speed and robustness of PfuUltra II

#### PfuTurbo DNA Polymerase

- Low error rate (1 error/770,000 bp)

#### Cloned Pfu DNA Polymerase

- Proofreading with  $3' \rightarrow 5'$  exonuclease activity
- Cloned version eliminates smearing and unwanted background artifacts







## **PCR Enhancers**

#### Perfect Match PCR Enhancer

- Reduces PCR artifacts by increasing specificity
- Destabilizes mismatched primer-template complexes

#### Taq Extender PCR Additive

- Improves length, yield and reliability of PCR with Taq DNA polymerase
- Enhances the efficiency of template extension by Taq DNA polymerase

## **cDNA Synthesis-Reverse Transcriptases**

### AccuScript High-Fidelity Reverse Transcriptase

#### Highest reverse transcription accuracy

AccuScript reduces errors during cDNA synthesis with 3.7x more accuracy than other commercially available RTs and 6.6x more accuracy than Avian Myeloblastosis Virus Reverse Transcriptase (AMV RT).

- Moloney Murine Leukemia Virus Reverse Transcriptase derivative MMLV-RT combined with proofreading 3'-5' exonuclease, reducinges errors
- High yields of full-length cDNA up to 20 kb



Ordering Information		
Product	Size	Agilent p.n.
AccuScript Reverse Transcriptase	50 rxn	600089
AccuScript High Fidelity 1st Strand cDNA Synthesis Kit	50 rxn	200820

#### Accuracy of RT-PCR Is Affected by the Fidelity of Both RT and PCR Enzymes

In this table, we illustrate a situation where an RNA template is reverse transcribed with AccuScript<sup>™</sup> RT or SuperScript<sup>®</sup> II RT and then a 1-kb portion is PCR amplified for 20 duplications using different PCR enzymes. We predict mutation frequencies using the equation MF = ER × bp × d, where MF is the mutation frequency, ER is the error rate, bp is the length of the target, and d is the number of template doublings (106-fold amplification equals 20 doublings). Using *PfuUltra<sup>™</sup>* DNA polymerase, the percentage of 1 kb cDNAs that are expected to contain errors is 2.5%, where the contribution from the RT is 1.6% (1.6 x 10-5/base x 1000 bases), and the contribution from *PfuUltra* enzyme is 0.9%. In contrast, if the same RT-PCR reaction was carried out with SuperScript RT II and a *Taq* DNA polymerase blend, 6.4% of 1-kb cDNAs are expected to contain Platinum<sup>®</sup> *Taq* HiFi polymerase generated mutations. The overall MF in this case would be almost 7x higher, at 18%.



Full-Lenght cDNA up to 20kb and great RT-PCR yields. A 0.61 kb region corresponding to the 5' end of the 20.8 kb human nebulin gene was successfully amplified, indicating the complete reverse transcription of the human nebulin gene.



AccuScript<sup>™</sup> system maximizes fidelity and yield. AccuScript<sup>™</sup> RT-PCR kit delivers high yields across a wide range of amplicon sizes.

Enzymes		Error Rate	(x10 <sup>-8</sup> ± SD)	Predicted Percent Mutant Clones
RT	PCR Enzymes	RT <sup>1</sup>	PCR	RT + PCR
AccuScript <sup>™</sup> RT	PfuUltra™ DNA Polymerase	16.1 ± 3.9	0.43 ± 0.04	2.5%
AccuScript <sup>™</sup> RT	PicoMaxx <sup>™</sup> DNA Polymerase	16.1 ± 3.9	4.0 ± 1.3	9.6%
SuperScript <sup>®</sup> RT	Platinum® Taq HiFi DNA Polymerase	64.1 ± 5.9	5.8 ± 0.3	18%

## AffinityScript Multiple Temperature RT

#### Enzyme activity at a broad range of temperatures from 37-55 °C

AffinityScript ensures reverse transcription through GC-rich sequences with high cDNA yields, whether priming at room temperature with random hexamers or at stringent temperatures to enhance specificity.

- High affinity to primer/template complexes
- Detects low input RNA

Ordering Information	
Size	Agilent p.n.
50 rxn	600107
200 rxn	600109



## **DNA Cloning**

## StrataClone PCR Cloning Kit

#### Fast and reliable cloning

The StrataClone PCR Cloning Kit allows high-efficiency, 5-minute cloning of PCR products, using the DNA rejoining activity of DNA topoisomerase I and the DNA recombination activity of Cre recombinase.

- No PCR clean-up required
- Clone both long and short amplicons
- Available for both blunt-end and UA cloning
- High efficiency yields

>95% insert positive clones with StrataClone Ultra Blunt PCR Cloning Kit

Ordering Information	
Product	Agilent p.n.
PCR Cloning	240205
Blunt PCR Cloning	240207



## **DNA Purification**

### RecoverEase DNA Isolation for genomic DNA isolation

Ordering Information	
Size	Agilent p.n.
15 rxn	720203
30 rxn	720202

### StrataCoolers & Plastics

#### StrataCooler LP Benchtop Cooler

Provides the most protection for your enzymes whether in your freezer or on your benchtop.

- Maintains -15° C for at least 2 hours.
- Includes adapters for use with 0.5 mL tubes
- Eliminates ice chips & potential contamination



Ordering Information	
Product	Agilent p.n.
StrataCooler Cryo Preservation Module	400005
StrataCooler LP Benchtop Cooler, Blue	401349

#### StrataCooler Cryo Preservation Module

Enables controlled freezing (0.4-0.6° C/min) of cells to significantly increase survival rates (up to 90%).

- Convenient and controlled freezing
- Overall survival rates of 80-90%
- Avoids expense of methanol-based cooling bath



Ordering Information	
Product	Agilent p.n.
96-well plate, 25 pack	401333
Adhesive sealing films, 100-pack	410186

## **Agilent Custom Manufacturing Servicess**

#### Produces ready-to-use highly purified DNA

Whether you need bulk packaging, custom formulations, higher-level documentation, or want to establish an OEM relationship, Agilent's dedicated team of experts will help you find the fastest, most cost- effective way to reach your goals.



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Product	Product Size	Catalog Number	Page Number
High-Fidelity & Difficult PCR			
PfuUltra II Fusion HotStart DNA Polymerase	40 rxn	600670	6
	200 rxn	600672	
	400 rxn	600674	
PfuUltra II HotStart Master Mix	100 rxn	600850	б
	400 rxn	600852	
Herculase II Fusion DNA Polymerase	40 rxn	600675	8
	200 rxn	600677	
	400 rxn	600679	
PfuUltra High-Fidelity DNA Polymerase	100 U	600380	10
	500 U	600382	
PfuUltra HotStart DNA Polymerase	1000 U	600394	13
PfuUltra High-Fidelity DNA Polymerase AD	100 U	600385	13
	500 U	600387	
	1000 U	600389	
PfuTurbo DNA Polymerase	100 U	600250	13
	500 U	600252	
	1000 U	600254	
PfuTurbo HotStart DNA Polymerase	500 U	600322	13
PfuTurbo HotStart PCR Master Mix	100 rxn	600600	13
Cloned Pfu DNA Polymerase	100 U	600153	13
	500 U	600154	
	1000 U	600159	
Cloned Pfu DNA Polymerase AD	500 U	600355	13
Pfu DNA Polymerase	100 U	600135	13
	1000 U	600140	
High-Fidelity & Difficult PCR			

Product	Product Size	Catalog Number	Page Number
Specialty Enzymes			
PfuTurbo Cx Hotstart DNA Polymerase	100 U	600410	10
	500 U	600412	
	1000 U	600414	
StrataClone PCR Cloning Kits	20 rxn	240205	11
StrataClone Blunt PCR Cloning kit	20 rxn	240207	11
PicoMaxx High-Fidelity PCR System	500 U	600422	11
RT-PCR			
AccuScript High-Fidelity Reverse Transcriptase	50 rxn	600089	14
AccuScript High-Fidelity 1st Strand cDNA Synthesis Kit	50 rxn	200820	-
AffinityScript One-Step RT-PCR Kit	100 rxn	600188	14
AffinityScript Multiple Temperature cDNA Synthesis Kit	50 rxn	200436	-
AffinityScript Multiple Temperature Reverse Transcriptase	50 rxn	600107	14
AffinityScript qPCR cDNA Synthesis Kit	50 rxn	600559	-
Purification			
RecoverEase Kits	15 rxn	720203	15
	30 rxn	720202	
DNA Extraction Kit	1 kit	200600	
Plastics & Labware			
StrataCooler Cryo Preservation Module	1 module	400005	16
StrataCooler LP Benchtop Cooler, Blue	1 cooler	401349	16
96-Well Plates	25 pack	401333	18
Adhesive Sealing Films	100 pieces	410186	18
Additional Products			
Perfect Match PCR Enhancer	100 U	600129	
Taq Extender PCR Additive	1000 U	600148	
Deoxynucleotide Mix, PCR Grade	400 µL	200415	
Rnase Block	4000 U	300151	
	16000 U	300152	
Absolutely RNA Miniprep Kit	50 preps	400800	
Absolutely RNA Nanoprep Kit	50 preps	400753	
Absolutely RNA Microprep Kit	50 preps	400805	
QuikChange Lightning Kit	10 rxn	210518	
IPTG	1 g	300127	
Amp Tabs Ampicillin Tablets	200 x 25 mg	300021	

#### Take advantage of web-only promotions: www.agilent.com/en/promotions-discounts-special-offers

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