crb

PCR RT-PCR qPCR

Application Handbook

All **crb** PCR, RT-PCR, and qPCR products are ISO 13485:2003 and13485:2012 certified as diagnostic grade and in compliance with all regulatory requirements for the design and manufacture of medical devices, as outlined by the International Organization for Standardization (ISO). For technical questions, please email us at support@coderegenesis.com or visit our website at www.coderegenesis.com

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Introduction

PCR (the Polymerase Chain Reaction) is a simple, rapid, and powerful *in vitro* method for amplifying DNA templates. A typical PCR requires primers, DNA Polymerase, a DNA template, dNTPs, and appropriate buffer conditions.

This fundamental technique is widely used in gene cloning, gene expression quantification, mutation detection, DNA sequencing, probe preparation, diagnostics, and forensic analysis. A successful PCR relies on tight parameters such as reaction buffer components, denaturation and annealing temperatures, elongation time, and number of amplification cycles. While the aforementioned parameters are crucial, the key element that dictates successful amplification will often be the DNA Polymerase employed.

Native DNA Polymerase from *Thermus aquaticus* (known as Taq) is widely used for routine PCR, but the enzyme has functional limitations when high fidelity, high speed, and high specificity, are essential requirements. Therefore, many mutant forms of Taq Polymerase have been developed to yield improved performance. Using Error-Prone PCR Technology, scientists at **crb** have generated a comprehensive library of Next Generation DNA Polymerase mutants with superior functionality that are suitable for use in a broad range of PCR applications.

crb also offers ready-to-use PCR MasterMixes containing all of the necessary reagents for successful amplification of a DNA template. To set up a PCR, simply add the DNA template, primers, and nuclease-free H_2O to our proprietary 2X PCR MasterMix. The MasterMixes are also available in a format with loading dye already added for ease of use. This enables immediate analysis of your PCR products by electrophoresis, eliminating the need to add loading dye after amplification. Our MasterMixes provide the following benefits:

-- Saves preparation time by combining DNA Polymerase, dNTPs, and reaction buffer in a ready-to-use format;

-- Decreases the risk of contamination by reducing the number of pipetting steps;

-- Provides consistent performance and results, thereby achieving superior reproducibility.

Notice to Purchasers

crb's products are guaranteed to meet their required quality control standards at the time of shipment. Notice of problematic products must be made to **crb** within 10 days of receipt. This product warranty limits **crb**'s liability to the replacement of the product only.

Technical Support

Technical Support: support@coderegenesis.com

coderegenesis.com

PCR Products

DNA Polymerases

A key requirement for any successful PCR is the availability of thermostable DNA Polymerases that are enzymatically functional at the elevated temperatures (72°C - 95°C) required for template DNA denaturation and primer annealing. Using proprietary engineering, scientists at **crb** have generated a repertoire of thermostable DNA Polymerases that are endowed with enhanced enzymatic functions for diverse PCR applications. As summarized in Table 1, these enzymes have improved robustness, efficiency, specificity, fidelity, and speed, and are capable of amplifying long DNA templates. In addition, **crb**'s Precision™ has been validated as the preferred choice for whole genome sequencing.

Table 1: Comparison of DNA Polymerase Products

Characteristics	DNA Polymerases							
	Bestaq™	Taq	Precision™	Taq Plus	HotStart	TaqFast	Long-Range	Bloodirect
Fidelity *	50X	1X	60X	5X	1X	10X	1X	1X
Processing Efficiency (per min)	3 - 4 kb	1 kb	1 kb	1 kb	1 kb	4 - 6 kb	3 - 4 kb	1 kb
Maximum Template Length	15 kb	6 kb	6 kb	6 kb	6 kb	12 kb	20 kb	2 kb
DNA Product End	Blunt	3'- A	Blunt	3'- A/Blunt	3'- A	Blunt	Blunt	Blunt
Specificity	Standard				High	Standard		
MasterMix Available	Yes	Yes	Yes	Yes	No	Yes	No	Yes
Special Features	All PCR applications	Routine PCR	High fidelity	Improved fidelity	High specificiy	Fast PCR	Suitable for longer amplicons	DNA extraction-free

* Characteristics compared to the Taq polymerase

Table 2: Components of DNA Polymerase Products

	Bestaq™		Taq			Precision™	Taq Plus		HotStart		TaqFast		Long-Range	
Cat. No.	G456	G457	G009	G008	G126	G078	G012	G040	G011	G039	G277	G278	G460	G461
Concentration	5 U/µl		5 U/µl			5 U/µl	5 U/µl		5 U/µl		5 U/µl		5 U/µl	
Volume	50 µl	200 µl	200 µl	1 ml	2 ml	100 µl	50 µl	200 µl	50 µl	200 µl	50 µl	200 µl	50 µl	200 µl
Buffer System [+Mg_{2+}]	5X		10X			5X	10X		10X		5X		5X	
Buffer Volume	1 ml	4 ml	2 ml	10 ml	20 ml	2 ml	1 ml	3 ml	1 ml	3 ml	1 ml	4 ml	1 ml	4 ml
25 mM $MgSO_4$	1 ml		1 ml			1 ml	1 ml		1 ml		1 ml		1 ml	
5X GC Enhancer	1 ml		-			1 ml	-		-		-		-	

Storage Conditions
Store all components at -20°C.

Additional Materials Required, but not provided
dNTP Mix (10 mM): **crb** Cat. No. G010
Opti-DNA Marker: **crb** Cat. No. G106
SafeView™ DNA Stains: **crb** Cat. No. G108
Agarose: **crb** Cat. No. G060-1

Basic PCR Protocol

All thermal cycling conditions shown in this manual are for DNA templates with a predicted primer annealing temperature of approximately 55°C. Please adjust accordingly if primers to be used have different annealing temperatures.

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal conditions (buffer, annealing temperature, DNA Polymerase amount, primers, Mg_{2+}, and template DNA) may vary and need to be optimized for each specific PCR. All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up, and subsequent reaction(s) should be performed in separate areas to avoid cross contamination. The use of "clean" pipettors designated for PCR and aerosol resistant barrier tips are recommended. A negative control reaction (omitting DNA template) should

be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. All PCR buffers contain Mg^{2+} (1.5 mM final concentration). A PCR can be further optimized using the provided 25 mM $MgSO_4$ solution if the initial PCR results are not satisfactory. Add the following components into a **sterile 0.2 ml PCR tube on ice (50 µl reaction volume).**

Table 3: PCR Set-up

Components		**Volume**	**Final Concentration**
DNA Template		~100 ng	**~2 ng/µl**
Forward Primer (10 µM)		1 - **2.5 µl**	200 - 500 nM
Re**verse Primer (10 µM)**		1 - **2.5 µl**	200 - 500 nM
Buffer System [+Mg^{2+}]	10X Buffer	**5 µl**	1X
	5X Buffer	**10 µl**	
5X GC Enhancer (optional) *		**10 µl**	1X
25 mM $MgSO_4$ (optional) **		0 - **3 µl**	1.5 - 3 mM
dNTP Mix (10 mM)		**1 µl**	**200 µM**
DNA Polymerase		0.5 - **1 µl**	-
Nuclease-free H_2O		**Up to 50 µl**	-

* 5X GC Enhancer is recommended for PCR amplification of GC-rich DNA templates. ** Optimal Mg^{2+} concentration is specific to each DNA template-primer set and can only be determined experimentally.

2. Mix tube contents gently and thoroughly, then collect all reagents by a brief centrifugation.

3. Choose an appropriate PCR amplification protocol for different DNA Polymerases as indicated in Table 4.

Table 4: Thermal Cycler Conditions for Different DNA Polymerases

Steps		Temp	Duration	Cycles
Initial Denaturation		94°C	3 mins	1
Denaturation	Bestaq™, Long-Range	94°C	10 secs	30 - 35
	Taq, Taq Plus, HotStart, Precision™		30 secs	
	TaqFast		5 secs	
Annealing	Bestaq™, Long-Range, Taq, Taq Plus, HotStart, Precision™	45°C - 72°C	30 secs	
	TaqFast		15 secs	
Extension	Bestaq™, Long-Range	72°C	3 - 4 kb/min	
	Taq, Taq Plus, HotStart, Precision™		1 kb/min	
	TaqFast		4 - 6 kb/min	
Final Extension		72°C	5 mins	1
Holding		4°C	-	1

4. Final PCR products are analyzed by agarose gel electrophoresis and visualized by SafeView™ (**crb** Cat. No. G108) staining. Opti-DNA Markers (**crb** Cat. No. G106) should be included for size determination.

2X PCR MasterMixes

The ready-to-use PCR MasterMix is a proprietary mixture of high quality DNA Polymerase, dNTPs, Mg^{2+}, and PCR buffer in a 2X concentration. It contains all necessary reagents for DNA amplification. To set up a PCR, simply add DNA template, primers, and nuclease-free H_2O to yield a 1X reaction mix.

PCR MasterMixes denoted Cat. No. GXXX**–**Dye contain loading dye. It allows direct gel electrophoresis of PCR products without the need of adding loading dye to finished PCR products.

Table 5: PCR MasterMix Products

(A set of five PCR MasterMix vials (1.0 ml each) with Mg^{2+}.)

Cat. No.	Product Name	Size
G464	2X PCR Bestaq™ MasterMix	**50 µl X 200 rxns (5 ml)**
G464-Dye	2X PCR Bestaq™ MasterMix with dye	**50 µl X 200 rxns (5 ml)**
G013	2X PCR Taq MasterMix	**50 µl X 200 rxns (5 ml)**
G013-Dye	2X PCR Taq MasterMix with dye	**50 µl X 200 rxns (5 ml)**
G124	2X PCR Precision™ MasterMix	**50 µl X 200 rxns (5 ml)**
G124-Dye	2X PCR Precision™ MasterMix with dye	**50 µl X 200 rxns (5 ml)**
G014	2X PCR Taq Plus MasterMix	**50 µl X 200 rxns (5 ml)**
G014-Dye	2X PCR Taq Plus MasterMix with dye	**50 µl X 200 rxns (5 ml)**
G280	2X PCR TaqFast MasterMix	**50 µl X 200 rxns (5 ml)**
G280-Dye	2X PCR TaqFast MasterMix with dye	**50 µl X 200 rxns (5 ml)**

Storage Conditions

Store at -20°C for long-term storage and at 4°C for up to two weeks.

2X PCR MasterMix Protocol

All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up, and subsequent reaction(s) should be performed in separate areas to avoid cross contamination. The use of "clean" pipettors designated for PCR and aerosol resistant barrier tips are recommended. A negative control reaction (omitting DNA template) should be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. Add the following components into a sterile 0.2 ml PCR tube on ice (50 **µl reaction volume).**

Table 6: 2X PCR MasterMix PCR Set-up

Components	Volume	Final Concentration
2X PCR MasterMix *	**25 µl**	1X
DNA Template	~100 ng	**~2 ng/µl**
Forward Primer (10 µM)	1 - **2.5 µl**	200 - 500 nM
Reverse Primer (10 µM)	1 - **2.5 µl**	200 - 500 nM
Nuclease-free H_2O	**Up to 50 µl**	-

* The 2X PCR MasterMix is available for Bestaq™, Taq, Precision™, Taq Plus, and TaqFast DNA Polymerases.

2. Mix tube contents carefully and thoroughly, then collect all reagents by a brief centrifugation.

3. Choose an appropriate PCR amplification protocol for different DNA Polymerases as indicated in Table 4.

4. Final PCR products are analyzed by agarose gel electrophoresis and visualized by SafeView™ (**crb** Cat. No. G108) staining. Opti-DNA Marker (**crb** Cat. No. G106) should be included for size determination.

Bloodirect DNA Polymerase

Bloodirect DNA Polymerase

crb's Bloodirect DNA Polymerase allows for direct PCR amplification of target templates from fresh or frozen whole blood samples. Bloodirect PCR eliminates the need for DNA extraction and thus greatly reduces the risk of contamination. **crb**'s Bloodirect DNA Polymerase can tolerate up to 20% blood **in a 50 µl reaction.**

Table 7: Components of Bloodirect PCR Products

	Bloodirect DNA Polymerase		Bloodirect 2X PCR MasterMix
Cat. No.	**G462**	**G463**	**G465**
Quantity	**100 µl (100 U, 1 U/µl)**	**400 µl (400 U, 1 U/µl)**	5 ml
Buffer System [+Mg^{2+}]	5X		-
Buffer Volume	1 ml	2 ml	-

Storage Conditions
Store all components at -20°C.

Additional Materials Required, but not provided
dNTP Mix (10 mM): **crb** Cat. No. G010
Opti-DNA Marker: **crb** Cat. No. G106
SafeView ™ DNA Stains: **crb** Cat. No. G108
Agarose: **crb** Cat. No. G060-1

Bloodirect PCR Protocol

All PCR experiments should be assembled in a Nuclease-free environment. In addition, DNA sample preparation, reaction set-up, and subsequent reaction(s) should be performed in separate areas to avoid cross contamination. The use of "clean" pipettors designated for PCR and aerosol resistant barrier tips are recommended. A negative control reaction (omitting DNA template) should be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. Add the following components into a sterile 0.2 ml PCR tube on ice (50 **µl reaction volume).**

Table 8: Bloodirect PCR Set-up

Components	Volume	Final Concentration
5X Bloodirect Buffer [+Mg^{2+}]	10 µl	1X
dNTP Mix (10 mM)	1 µl	200 µM
Bloodirect DNA Polymerase	4 µl	4 U/rxn
Forward Primer (10 µM)	2.5 µl	500 nM
Reverse Primer (10 µM)	2.5 µl	500 nM
Whole Blood	1 - 5 µl	2 - 10%
Nuclease-free H_2O	Up to 50 µl	-

- Preparation of a premix for multiple reactions is recommended to minimize reagent loss and enable accurate pipetting.
- Bloodirect Buffer and 2X MasterMix contains Mg^{2+} (3.0 mM at the final [1X] reaction concentration). Reaction can be further optimized using EDTA or $MgSO_4$ solution (not provided).
- Up to 20% volume of blood can be used.

Table 9: Bloodirect PCR with MasterMix Set-up

Components	Volume	Final Concentration
Bloodirect 2X PCR MasterMix	25 µl	1X
Forward Primer (10 µM)	2.5 µl	500 nM
Reverse Primer (10 µM)	2.5 µl	500 nM
Whole Blood	1 - 5 µl	2 - 10%
Nuclease-free H_2O	Up to 50 µl	-

2. Before adding a blood sample, thoroughly mix components by gently pipetting up and down.

3. Add the blood sample by inserting a pipette tip to the bottom of the tube and slowly dispensing the blood sample. Do not mix the tube contents in order to keep the blood sample and the reaction mix as two separate layers.

4. Use the PCR amplification protocol as indicated in Table 10.

Table 10: Thermal Cycler Conditions for Bloodirect PCR

Steps	Temp	Duration	Cycles
Initial Denaturation	94°C	3 mins	1
Denaturation	94°C	30 secs	30 - 35
Annealing	45°C - 72°C	30 secs	
Extension	72°C	1 kb/min	
Final Extension	72°C	5 mins	1
Holding	4°C	-	1

Difficult PCR

A successful PCR requires optimal conditions with respect to buffer components, pH, annealing temperature, primer length, and the quality of DNA template (purity, GC content and secondary structure). The standard protocols provided in the previous sections are applicable to most PCRs. However, difficulties may arise due to the complexity of DNA templates involved. With years of experience in PCR technology, scientists at **crb** have developed the following advanced protocols and corresponding products to handle the most difficult DNA templates.

Touchdown PCR Strategy

Touchdown PCR is a program that can minimize non-specific DNA priming and promotes primer annealing to target DNA templates at optimal annealing temperatures. The program begins with a high annealing temperature and gradually decreases to lower annealing temperatures every three cycles. The primers annealed at the highest temperature will be the least-permissive to non-specific binding and amplification. Therefore, a touchdown PCR protocol is the choice for optimizing annealing temperature and eliminating non-specific amplifications. Touchdown PCR is very effective at solving over 70% of difficult PCRs that have failed to amplify with standard PCR cycling conditions.

Table 11: Touchdown PCR Cycling Conditions

Steps	Temp	Duration	Cycles
Initial Denaturation	94°C	3 mins	1
Denaturation	94°C	30 secs	
Annealing	65°C	30 secs	3
Extension	72°C	1 kb/min	
Denaturation	94°C	30 secs	
Annealing	60°C	30 secs	3
Extension	72°C	1 kb/min	
Denaturation	94°C	30 secs	
Annealing	55°C	30 secs	30 - 35
Extension	72°C	1 kb/min	
Final Extension	72°C	5 mins	1
Holding	4°C		1

PCR-Sure™ Kit

For a challenging PCR that cannot be resolved with either standard or touchdown PCR protocols, the PCR-Sure™ Kit is the solution to amplify the most difficult DNA templates. The PCR-Sure™ Kit consists of various DNA Polymerases with 12 different reaction buffers to help PCR condition optimization for a particular DNA template. Once an optimal buffer is identified, the buffer (**crb** Cat. No. G065-X) can be ordered separately for large volume PCR application. Together with the touchdown PCR protocol, the PCR-Sure™ kit is

the ultimate solution for the most difficult DNA templates. More conveniently, the MasterMixes included in this kit contain loading dye to allow direct gel electrophoresis of final PCR products.

Table 12: PCR-Sure™ Kit

Cat. No.	Product Name	Size
G065$_1$	PCR-Sure™ Kit	12 X 5 rxns
G065-X$_2$	Individual PCR-Sure™ MasterMix	200 rxns (5 ml)

Kit Component

1. G065: A set of 12 individual PCR M**asterMix at 125 µl each.**
2. G065-X (X: 1~12): An individual PCR MasterMix (5 X 1 ml).

Storage Conditions

Store all components at -20°C.

PCR-Sure™ Protocol

1. There are 12 different PCR MasterMixes designed to provide a broad range of conditions for a specific DNA template. Thus the preparation of a batch mixture containing DNA template, forward primer, reverse primer, and nuclease-free H_2O is recommended to minimize variations caused by pipetting. The PCR set-up for 1 reaction and for 14 reactions (12 reactions for 12 MasterMixes and 2 extra reaction volumes to account for pipetting loss) are as indicated in Table 13.

Table 13: PCR-Sure™ Set-up

Components	Per 1 rxn	Per 14 rxns	Final Concentration
DNA Template	~100 ng	**~1.4 µg**	**~2 ng/µl**
Forward Primer (10 µM)	1 - **2.5 µl**	14 - **35 µl**	200 - 500 nM
Reverse Primer (10 µM)	1 - **2.5 µl**	14 - **35 µl**	200 - 500 nM
Nuclease-free H_2O	**Up to 25 µl**	**Up to 350 µl**	-

2. **Mix all components well and aliquot 25 µl to each of th**e 12 PCR tubes **on ice (50 µl reaction volume). Then add 25 µl of each individual PCR-**Sure™ MasterMix to the 12 PCR tubes.

3. Use the PCR cycling conditions as indicated in Table 11.

Reverse Transcriptases and RT-PCR

Reverse Transcriptases

Reverse Transcriptases (RTases) are used for *in vitro* first-strand cDNA synthesis using RNA as the starting template. Reverse transcription of RNA is often required for PCR, real-time PCR (qPCR), and gene cloning. Two different RTases (OneScript® and OneScript® Plus) are available from **crb**, both differ in operational temperatures used in the first-strand cDNA synthesis. **crb**'s RTases are the best in quality, with guaranteed functionality for any RNA template. Furthermore, both RTases contain an additional fidelity-enhancing subunit, thereby drastically reducing the error rate in reverse transcription. This unique feature allows **crb**'s RTases to outperform most other RTases on the market.

OneScript® RTase

An optimized mutational derivative of native RTase enzyme, the OneScript® represents the best-performing RTase on the market. This enzyme catalyzes the synthesis of complementary DNA strands from single-stranded RNA and DNA templates. Due to a series of mutations introduced within the RNase H domain of this enzyme, there is no detectable RNase H activity associated with the enzyme. The lack of RNase H activity helps to eliminate RNA degradation during first-strand cDNA synthesis, resulting in better yield and length of cDNA synthesized.

OneScript® Plus RTase

OneScript® Plus is a novel recombinant RTase that exhibits much higher efficiency in the first-strand cDNA synthesis from RNA templates with secondary structures and high GC content. OneScript® Plus is engineered to perform under high temperatures (50°C - 55°C) which facilitate the elimination of secondary structures associated with GC-rich RNA templates. Due to this unique feature, OneScript® Plus can synthesize full-length cDNA libraries from RNA templates up to 15 kb in length. In addition, **OneScript® Plus has outstanding proofreading ability**, thus it can be utilized for whole genome sequencing.

Table 14: Components of OneScript® and OneScript® Plus Products

		OneScript®		OneScript® Plus	
Part No.	**Components**	**G231**	**G232**	**G177**	**G237**
RT-1	OneScript® (200 U/µl)	**25 µl**	**100 µl**	-	-
RT-2	OneScript® Plus (200 U/µl)	-	-	**25 µl**	10**0 µl**
RT-7	5X RT Buffer	**150 µl**	**600 µl**	**150 µl**	**600 µl**
Size		25 rxns	100 rxns	25 rxns	100 rxns

Storage Conditions
Store all components at -20°C.

Additional Materials Required, but not provided

RNaseOFF Ribonuclease Inhibitor (40 U/µl):	**crb** Cat. No. G138
dNTP Mix (10 mM):	**crb** Cat. No. G010
Oligo (dT)15 **Primer (10 µM):**	**crb** Cat. No. G140
Random Primers (6N) (10 µM):	**crb** Cat. No. G139

cDNA Synthesis Kit and SuperMix

crb's cDNA Synthesis Kit contains all the materials required for first-strand cDNA synthesis, with the choice of using either Oligo (dT) and/or Random Primers. The Oligo (dT) anneals selectively to the poly (A) tail of mRNAs. Random Primers do not require the presence of poly (A) and can be used for the transcription of mRNA 5'-end regions.

crb's cDNA Synthesis SuperMix is a proprietary mixture of all the materials required for first-strand cDNA synthesis in a 2X concentration. A balanced amount of Oligo (dT) and Random Primers is included in the 2X Reaction Mix.

Table 15: Components of cDNA Synthesis Products

		OneScript® Synthesis Kit		OneScript® Plus Synthesis Kit		OneScript® SuperMix		OneScript® Plus SuperMix	
Part No.	**Components**	**G233**	**G234**	**G235**	**G236**	**G451**	**G452**	**G453**	**G454**
RT-1	OneScript® (200 U/µl)	**25 µl**	**100 µl**	-	-	**25 µl**	**100 µl**	-	-
RT-2	OneScript® Plus (200 U/µl)	-	-	**25 µl**	**100 µl**	-	-	**25 µl**	**100 µl**
RT-3	Oligo (dT) (**10 µM**)	**40 µl**	**160 µl**	**40 µl**	**160 µl**	-	-	-	-
RT-4	Random Primers **(10 µM)**	**40 µl**	**160 µl**	**40 µl**	**160 µl**	-	-	-	-
RT-5	dNTPs (10 mM)	**40 µl**	**160 µl**	**40 µl**	**160 µl**	-	-	-	-
RT-6	RNaseOFF Ribonuclease Inhibitor **(40 U/µl)**	**15 µl**	**60 µl**	**15 µl**	**60 µl**	-	-	-	-
RT-7	5X RT Buffer	**150 µl**	**600 µl**	**150 µl**	**600 µl**	-	-	-	-
RT-8	2X Reaction Mix	-	-	-	-	**300 µl**	1.2 ml	**300 µl**	1.2 ml
RT-0	Nuclease-free H_2O	1 ml	2 ml	1 ml	2 ml	1 ml	2 ml	1 ml	2 ml
Size		25 rxns	100 rxns	25 rxns	100 rxns	25 rxns	100 rxns	25 rxns	100 rxns

Primer Selection

Oligo (dT) are oligonucleotides that anneal to the 3'-poly (A) + mRNA. Therefore, only mRNA or total RNA templates with 3'-poly (A) tails are used in cDNA synthesis.

Random Primers are oligonucleotides that anneal at non-specific sites of RNA templates. Therefore, all forms of RNA can be used in cDNA synthesis. **Gene-Specific Primers** are oligonucleotides that are designed to anneal to the specific site of a target gene.

Storage Conditions

Store all components at -20°C.

2

First-Strand cDNA Synthesis Protocol

cDNA Synthesis with OneScript® or OneScript® Plus

1. Thaw RNA templates and all reagents on ice. Mix each solution gently.

2. Prepare the following reaction mixture on ice.

Table 16a: Part-1 of cDNA Synthesis Set-up

Components	Volume	Final Concentration
Total RNA or poly(A) + mRNA	Variable	1 ng - **2 µg/rxn** 1 pg - 2 ng/rxn
Oligo (dT) (10 µM) **or Random Primers (10 µM)** or Gene-Specific Primer	**1 µl** **1 µl** Variable	**0.5 µM** **0.5 µM** 10 - 15 nM
dNTP Mix (10 mM each)	**1 µl**	**500 µM**
Nuclease-free H_2O	**Up to 14.5 µl**	-

3. Heat mixture to 65°C for 5 mins and incubate on ice for at least 1 min. Collect all components by a brief centrifugation and add the following:

Table 16b: Part-2 of cDNA Synthesis Set-up

Components	Volume	Final Concentration
5X RT Buffer	**4 µl**	1X
RNaseOFF Ribonuclease **Inhibitor (40 U/µl)**	**0.5 µl**	20 U/rxn
OneScript® or OneScript® Plus	**1 µl**	200 U/rxn

4. **Mix components well and collect all components (20 µl) by a brief** centrifugation. Incubate the tube at 25°C for 10 mins if using Random Primers. (Omit this incubation if Oligo (dT) or Gene-Specific Primer is used.)

5. Perform cDNA synthesis by incubating the tube for 50 mins at 42°C for OneScript®, or 50 mins at 50°C for OneScript® Plus.

6. Stop the reaction by heating it at 85°C for 5 mins. Chill on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

cDNA Synthesis with SuperMix

1. Thaw RNA templates and all reagents on ice. Mix each solution gently.

2. Prepare the following reaction mixture on ice.

Table 17a: Part-1 of cDNA Synthesis Set-up with SuperMix

Components	Volume	Final Concentration
Total RNA or poly(A) + mRNA	Variable	1 ng - **2 µg/rxn** 1 pg - 2 ng/rxn
2X Reaction Mix	**10 µl**	1X
Nuclease-free H_2O	**Up to 19 µl**	-

3. Heat mixture to 65 °C for 5 mins and incubate on ice for at least 1 min. Collect all components by a brief centrifugation and add the following:

Table 17: Part-2 of cDNA Synthesis Set-up with SuperMix

Components	Volume	Final Concentration
OneScript® or OneScript® Plus	**1 µl**	200 U/rxn

4. **Mix components well and collect all components (20 µl) by a brief** centrifugation. Incubate the tube at 25 °C for 10 mins. Perform cDNA synthesis by incubating the tube for 50 mins at 42°C for OneScript® or 50 mins at 50°C for OneScript® Plus.

5. Stop the reaction by heating it at 85°C for 5 mins. Chill on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

General Notes

1. *Both poly (A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.*
2. *RNA samples must be free of genomic DNA contamination.*
3. *Unlike Oligo (dT) priming, which requires little optimization, the ratio of Random Primers to RNA is often critical in terms of the average length of cDNA synthesized. A higher ratio of Random Primers to RNA will result in a higher yield of shorter (~500 bp) cDNA, whereas a lower ratio will lead to longer cDNA products.*
4. ***To remove RNA complementary to the cDNA, add 1 µl (2 U) of E. coli RNase H*** *and incubate at 37°C for 20 mins.*

2

One-Step RT-PCR

One-Step RT-PCR

crb's One-Step RT-PCR Kit contains all necessary reagents for both reverse transcription and PCR amplification in a single reaction tube. Specifically, the One-Step RT-PCR Kit combines OneScript® RTase and Bestaq™ DNA Polymerase in a convenient format for highly sensitive and specific RT-PCR with any RNA template. Our proprietary RT-PCR Buffer contains stabilizers and enhancers that optimize the two reactions into a "single step". Together with our specially formulated RT-PCR Buffer, this One-Step RT-PCR Kit offers end-users an efficient, easy to use, and reliable alternative to conventional "two-step" sequential RT-PCR.

Table 19: Components of One-Step RT-PCR Kit

One-Step RT-PCR Kit (Cat. No. G174)		
Part No.	**Components**	
G457	Bestaq™ DNA Polymerase (5 U/μl)	**200 μl**
RT-1	OneScript® (200 U/μl)	**100 μl**
RT-10	2X One-Step RT-PCR Buffer	3 ml
	Size	100 rxns

Storage Conditions
Store all components at -20°C.

One-Step RT-PCR Protocol

1. Thaw RNA templates and all reagents on ice. Mix each solution gently.

2. Prepare the following reaction **mixture on ice (50 μl reaction volume).**

Table 20: One-Step RT-PCR Set-up

Components	Volume	Final Concentration
Total RNA or poly (A) + mRNA	Variable	1 ng - **2 µg/rxn** 1 pg - 2 ng/rxn
2X One-Step RT-PCR Buffer	**25 µl**	1X
OneScript® **(200 U/µl)**	**1 µl**	200 U/rxn
Bestaq™ **DNA Polymerase (5 U/µl)**	**2 µl**	4 U/rxn
Forward Primer (10 µM)	**2.5 µl**	500 nM
Reverse Primer (10 µM)	2.5 **µl**	500 nM
Nuclease-Free H_2O	**Up to 50 µl**	-

3. Mix components well and collect all components by a brief centrifugation.

4. Use the program as indicated in Table 21.

Table 21: Thermal Cycler Conditions for One-Step RT-PCR

Steps	Temp	Duration	Cycles
cDNA Synthesis	42 °C	30 mins	1
Initial Denaturation	94 °C	3 mins	1
Denaturation	94 °C	30 secs	
Annealing	55 °C	30 secs	30 - 35
Extension	72 °C	1 kb/min	
Final Extension	72 °C	5 mins	1
Holding	4 °C	-	1

General Notes

1. *The thermal cycling program listed above is optimized for primers with annealing temperature of 55°C.*
2. *An optional touchdown thermal cycling program can also be used to replace the steps after the cDNA synthesis in the table above. (See Table 11 for more details.)*

5. Final PCR products are analyzed by agarose gel electrophoresis and visualized by SafeView™ (**crb** Cat. No. G108) staining. Opti-DNA Marker (**crb** Cat. No. G106) should be included for size determination.

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Two-Step RT-PCR

crb's Two-Step RT-PCR kits provide all the necessary reagents required for reverse transcription and PCR amplification in two separate steps. The kit offers the flexibility of optimizing different RT-PCR applications with the choice of either OneScript® or OneScript® Plus. The OneScript® Plus makes it possible to efficiently synthesize first-strand cDNA from RNA templates with secondary structures and high GC content.

Table 22: Components of Two-Step RT-PCR Kit

		OneScript® Two-Step RT-PCR Kit		OneScript® Plus Two-Step RT-PCR Kit	
Part No.	**Components**	**G281**	**G282**	**G283**	**G284**
RT-1	OneScript® (200 U/µl)	**25 µl**	**100 µl**	-	-
RT-2	OneScript® Plus (200 U/µl)	-	-	**25 µl**	**100 µl**
RT-8	2X Reaction Mix	**300 µl**	1.2 ml	**300 µl**	1.2 ml
G014	2X PCR Taq Plus MasterMix	1 ml	4 ml	1 ml	4 ml
Size		25 rxns	100 rxns	25 rxns	100 rxns

Please refer to Page 15 for cDNA Synthesis and Page 3 for PCR protocols.

Storage Conditions
Store all components at -20°C.

qPCR Products

BrightGreen qPCR MasterMixes

BrightGreen 2X qPCR MasterMix is designed for quantitative real-time analysis of DNA samples. The components of BrightGreen 2X qPCR MasterMix have been developed for superb performance with respect to sensitivity, signal-to-noise ratio, and complete elimination of primer dimers. In addition, the use of HotStart DNA Polymerases in our MasterMix significantly reduces any non-specific PCR amplification.

BrightGreen 2X qPCR MasterMix Selection Guidelines

Due to the design variations of qPCR instruments from different manufacturers, four BrightGreen 2X qPCR MasterMix formulations optimized for different qPCR instruments on the market are available. Please refer to Table 23 for a general guideline in selecting the appropriate qPCR formulation applicable to your particular instrument model.

Kit Components

Each BrightGreen 2X qPCR MasterMix is a 2X mixture of dNTPs, HotStart DNA Polymerase, Mg_{2+}, fluorescent detection dye, reference dye and proprietary buffer components. Each set of BrightGreen qPCR MasterMix contains 4 vials, each containing 1.25 ml.

Storage Conditions

Store at -20°C and protect from light. Store at 4°C if the MasterMix will be used within 2 weeks.

Table 23: BrightGreen and TaqProbe qPCR MasterMix Selection Guidelines

Cat. No.	Product Name	qPCR Instruments
MasterMix-R	BrightGreen 2X qPCR Master-Mix-ROX	-ABI® 7000, 7300, 7700, 7900, 7900HT, **StepOnePlus™, StepOne™,** OpenArray, **PRISM™ Sequencing Detection Series**
MasterMix-P	TaqProbe 2X qPCR MasterMix-ROX	-Biometra TOptical -Fluidigm BioMark™ -Wafergene SmartChip System -TianLong TL998 System
MasterMix-LR	BrightGreen 2X qPCR Master-Mix-Low ROX	-ABI® **7500 Viia™, QuantStudio** -**BioGene InSyte™** -Illumina Eco -Stratagene® Mx3000, Mx3005, Mx4000 -Analytikjena qTower Series
MasterMix- PL	TaqProbe 2X qPCR MasterMix -Low ROX	
MasterMix-iC	BrightGreen 2X qPCR Master-Mix-iCycler	-BioRad® iCycler®, **iQ™5, MyiQ™**
MasterMix-PC	TaqProbe 2X qPCR MasterMix-iCycler	
MasterMix-S	BrightGreen 2X qPCR MasterMix	-BioRad® CFX96, CF**X384, Chromo4™, CFX Connect™, Opticon 2, MiniOpticon™** -Roche LightCycler® (2.0, 1.5, 480, 1536, Nano) -**MJ Research Opticon™, Opticon™ 2,** Chromo® 4 -Eppendorf® Realplex 4
MasterMix-PS	TaqProbe 2X qPCR MasterMix-no dye	-BioGene **SynChron™** -Corbett Rotor-gene® (3000, 6200, 62H0, 6500, 65H0, 6600) -Eppendorf Mastercycler® realplex (s, 4 , 4s), Pro (S, 384), Nexus (gradient, eco, flat) -Cepheid SmartCycler®, GeneXpert -Enigma® ML -Idaho LightScanner® (24, 32), RapidCycler®2, R.A.P.I.D (LT, LT Food), RAZOR EX, JBAIDS -Qiagen Rotor-**Gene™ (Q, 6000)** -**Takara Dice™** -Thermo Scientific PikoReal -DNA-Technology DT96, DTlite, DT-322 -Bioer LineGene (3310/3320, K FQD-48A, I, II, 9620, 9640, 9660, 9680) -Bioneer Exicycler™
MasterMix-PM	TaqProbe 2X qPCR MasterMix-Multiplex	Any qPCR instrument that supports multiplex reactions

BrightGreen qPCR Protocol

1. Thaw BrightGreen 2X qPCR MasterMix, template DNA, primers and nuclease-free H_2O on ice. Mix each solution well.

2. **Set up the following reaction mixture (20 µl re**action volume).

Table 24: BrightGreen qPCR Set-up

Components	10 µl Reaction	20 µl Reaction	Final Concentration
BrightGreen 2X qPCR MasterMix	**5 µl**	**10 µl**	1X
Forward Primer (10 µM)	**0.3 µl**	**0.6 µl**	300 nM
Reverse Primer (10 **µM)**	**0.3 µl**	**0.6 µl**	300 nM
Template DNA	Variable	Variable	**≤500 ng/rxn**
Nuclease-free H_2O	**Up to 10 µl**	**Up to 20 µl**	-

3. Perform qPCR reactions using the cycling program as indicated in Table 25. **Table 25: BrightGreen qPCR Thermal Cycling Conditions**

Steps	Temp	Duration		Cycles
		Standard	Fast *	
Enzyme Activation	95°C	10 mins	20 secs	Hold
Denaturation	95°C	15 secs	3 secs	40
Annealing/ Extension	60°C	60 secs	30 secs	
Melting Curve	Refer to specific guidelines for instrument used			

* **crb**'s advanced BrightGreen 2X qPCR MasterMix formulation allows it to perform fast qPCR cycling conditions within 30 minutes.

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TaqProbe qPCR MasterMixes

TaqProbe 2X qPCR MasterMix is designed for TaqMan probe-based qPCR analysis of DNA samples. The probe-based qPCR guarantees specific amplification of target sequence only, minimizing any non-specific amplification. The TaqProbe MasterMix-Multiplex (Cat. No.: MasterMix-PM) formulation supports qPCR quantification of up to four target genes simultaneously with consistent performance.

TaqProbe 2X qPCR MasterMix Selection Guidelines

Similar to our proprietary BrightGreen-based qPCR formulations, different TaqProbe 2X qPCR MasterMix formulations are available for different qPCR instruments. Please refer to Table 23 as a general guideline for selecting an appropriate qPCR formulation applicable to your instrument model.

Kit Components

Each TaqProbe 2X qPCR MasterMix is a 2X mixture of dNTPs, HotStart DNA Polymerase, Mg^{2+}, reference dye, and proprietary buffer components. Each set of TaqProbe 2X qPCR MasterMix contains 4 vials, each containing 1.25 ml.

Storage Conditions

Store at -20°C and protect from light. Store at 4°C if the MasterMix will be used within 2 weeks.

TaqProbe qPCR Protocol

1. Thaw TaqProbe 2X qPCR MasterMix, template DNA, primers, probes and nuclease-free H_2O on ice. Mix each solution well.

2. **Set up the following reaction mixture (20 µl reaction volume).**

Table 26: TaqProbe qPCR Set-up

Components	10 µl Reaction	20 µl Reaction	Final Concentration
TaqProbe 2X qPCR MasterMix	**5 µl**	**10 µl**	1X
Forward Primer (10 µM)	**0.3 µl**	**0.6 µl**	300 nM
Reverse Primer (10 µM)	**0.3 µl**	**0.6 µl**	300 nM
Template DNA	Variable	Variable	**≤500 ng/rxn**
TaqMan Probe	Variable	Variable	100 - 300 nM
Nuclease-Free H_2O	**Up to 10 µl**	**Up to 20 µl**	-

3. Perform qPCR reactions using the cycling program as indicated in Table 27. **Table 27: TaqProbe qPCR Thermal Cycling Conditions**

Steps	Temp	Duration		Cycles
		Standard	Fast *	
Taq Activation	95°C	10 mins	10 mins	Hold
Denaturation	95°C	15 secs	3 secs	40
Annealing/ Extension	60°C	60 secs	30 secs	

* **crb**'s advanced TaqProbe 2X qPCR MasterMix formulation allows it to perform fast qPCR cycling conditions within 30 minutes.

PCR Related Products

Opti-DNA Markers

Opti-DNA Markers are molecular weight standards for gel electrophoresis that covers most DNA fragment sizes commonly analyzed. In addition, each DNA marker fragment can be used for the quantification of DNA analyzed with reference point bands of increasing intensity.

dNTP Mix

Biotechnology grade and suitable for all PCR, first-strand cDNA synthesis, and qPCR applications.

SafeView™ and SafeView Plus™ Nucleic Acid Stains

SafeView™ products represent a new and biologically safe class of nucleic acid stains for the visualization of double-stranded DNA, single-stranded DNA, and RNA in agarose gels. The dyes are an alternative to commonly used ethidium bromide, which is both toxic and mutagenic. SafeView™ products are non-carcinogenic as indicated by the Ames-test. SafeView Plus™ has higher sensitivity and enhanced performance in comparison to SafeView™.

Agarose

Agarose, derived from agar, is a polymeric cross-linked polysaccharide that is mainly used in gel electrophoresis. The lattice bonds formed in the agarose gel allow DNA of different sizes to travel at different speeds down the gel.