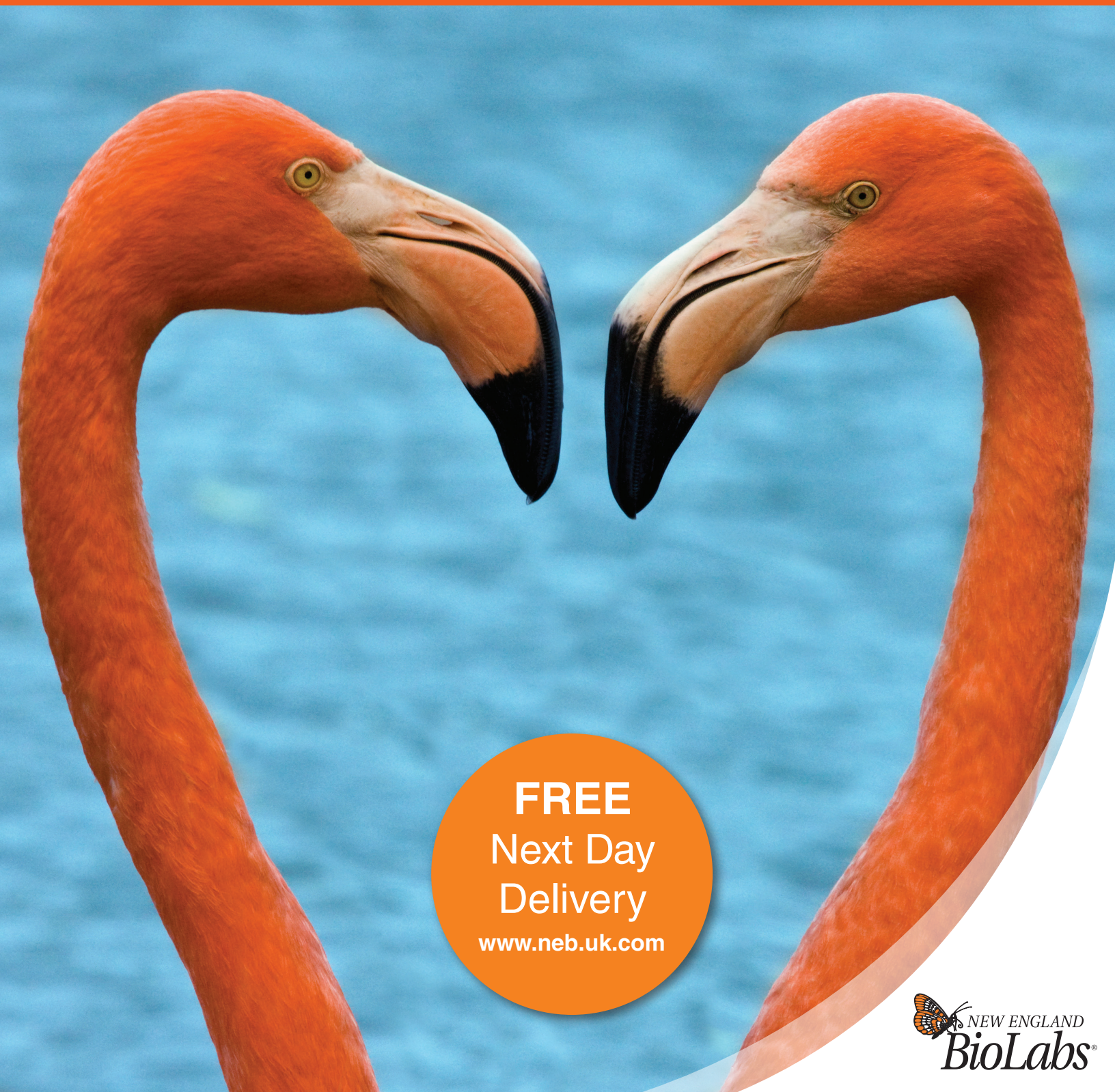
One*Taq* DNA Polymerase

ENZYMES & KITS FOR PCR



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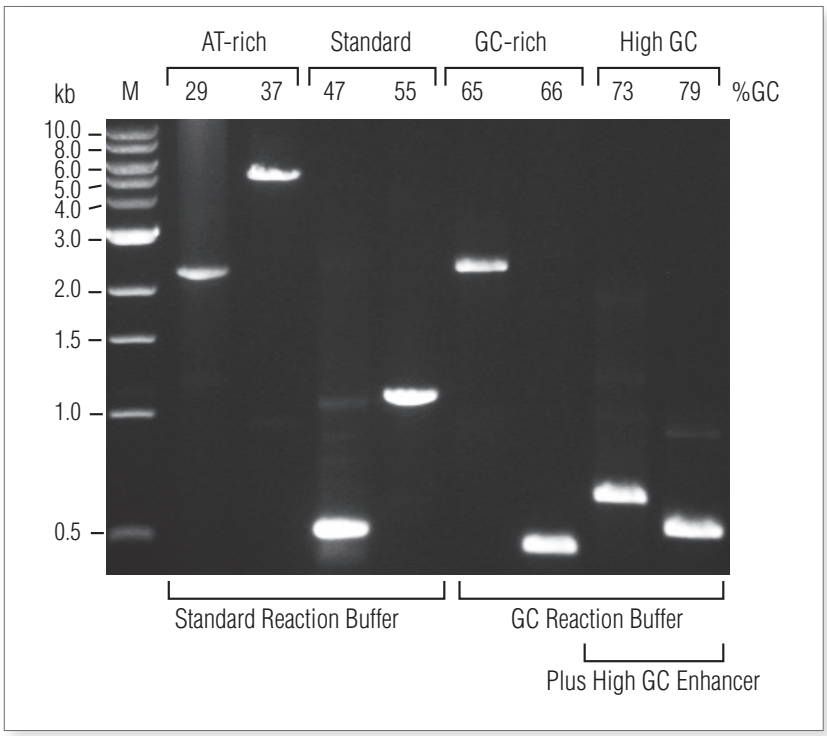
NEW ENGLAND
BioLabs®

One*Taq* DNA Polymerase
One*Taq* Hot Start DNA Polymerase

An optimized blend of *Taq* and Deep Vent$_R$ DNA polymerases, One*Taq* and One*Taq* Hot Start DNA Polymerases offer robust amplification across a wide range of templates. The 3´→5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robustness of *Taq*. Additionally, One*Taq* Reaction Buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.

Both One*Taq* and One*Taq* Hot Start DNA Polymerases are available in master mix and Quick-Load master mix formats. Master mixes include dNTPs, $MgCl_2$ and other buffers and stabilizers. Quick-Load master mixes also include two tracking dyes for use with downstream visualization (i.e., agarose gels).

Achieve robust amplification for routine, AT- and GC-rich templates with One*Taq*



Amplification of a selection of sequences with varying AT and GC content from human and C. elegans genomic DNA using OneTaq DNA Polymerase. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).

One*Taq* Buffer Recommendations

AMPLICON % GC BUFFER	RECOMMENDED DEFAULT BUFFER	OPTIMIZATION NOTES
< 50% GC	One*Taq* Standard Reaction Buffer	Adjust annealing temperature, primer/template concentration, etc. if needed.
50–65% GC	One*Taq* Standard Reaction Buffer	One*Taq* GC Reaction Buffer can be used to enhance performance of difficult amplicons.
> 65% GC	One*Taq* GC Reaction Buffer	One*Taq* GC Reaction Buffer with 10–20% One*Taq* High GC Enhancer can be used to enhance performance of difficult amplicons.

POLYMERASE DETAILS

Extension Rate . . . . . 1 kb/min
Amplicon Size . . . . . ≤ 6 kb
Fidelity . . . . . 2X *Taq*
Units/50 µl rxn . . . . . 1.25 units
Resulting Ends . . . . . 3´ A/Blunt
3´→5´ Exonuclease Activity . . . . . Yes
5´→3´ Exonuclease Activity . . . . . Yes
Supplied Buffer . . . One*Taq* Std Rxn Buffer, One*Taq* GC Rxn Buffer
Supplied Enhancer One*Taq* High GC Enhancer
Compatible w/Other Buffers . . . with Reduced Activity Profile

Advantages

- Exceptional performance in endpoint PCR across a wide range of templates
- Robust yields with minimal optimization
- Convenient product formats (stand-alone enzyme, master mixes, and Quick-Load® formats)
- Hot start version allows room temperature reaction setup and does not require a separate activation step
- Compatible with standard *Taq* protocols

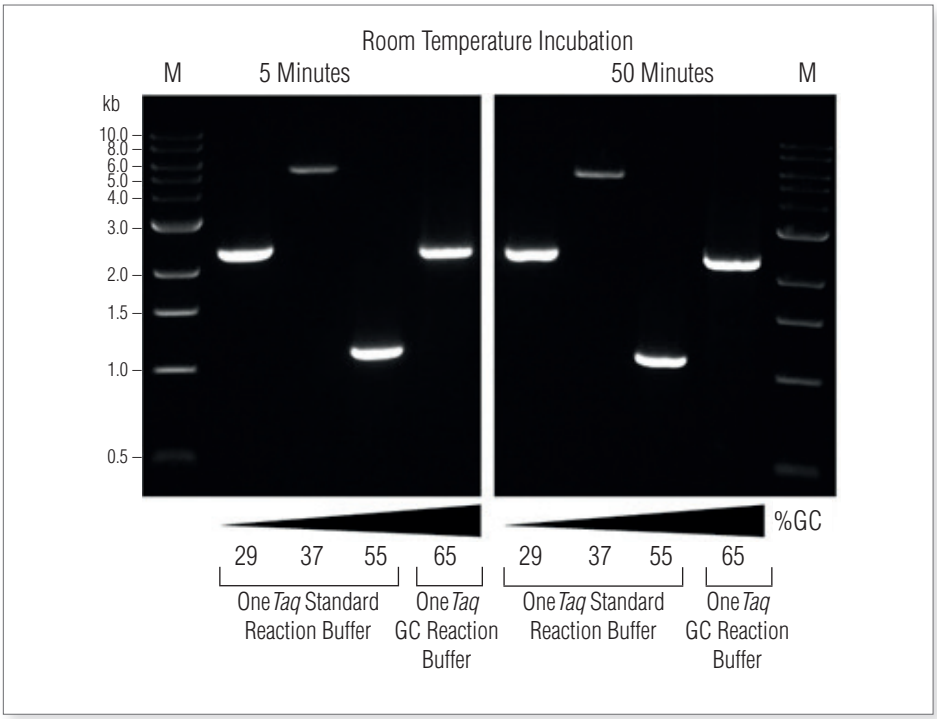
Applications

High sensitivity PCR . . . . . Yes
High throughput PCR . . . . . Yes
Routine PCR . . . . . Yes
SNP Detection . . . . . Yes
T/A, U/A Cloning . . . . . Yes
GC-rich PCR . . . . . Yes
Hot Start PCR (NEB #M0481) . . . . . Yes
Primer extension . . . . . Yes
Colony PCR . . . . . Yes
Long PCR (up to ~6 kb genomic) . . . . . Yes

One*Taq* Hot Start DNA Polymerase allows room temperature reaction setup with no separate activation step

In contrast to chemically-modified or antibody-based hot start polymerases, NEB's One*Taq* Hot Start utilizes aptamer technology. This aptamer/inhibitor binds to the polymerase through non-covalent interactions, blocking polymerase activity at temperatures below 45°C. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. One*Taq* Hot Start does not require a separate high temperature incubation step to activate the enzyme and can be used in typical *Taq*-based cycling protocols. This ultimately shortens reaction times and increases ease of use.

Extended room temperature incubation does not affect performance of One*Taq* Hot Start DNA Polymerase



Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using OneTaq Hot Start DNA Polymerase. The presence or absence of an extended room temperature incubation does not affect performance. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB# N3232).

Table 1: Recommended time for enzyme activation of commercially available Hot Start *Taq* products

MANUFACTURER	ENZYME	ACTIVATION STEP*	HOT START FORM
Applied BioSystems	AmpliTaq Gold® 360	10´, 95°C	Modified
Invitrogen	Platinum® *Taq*	30´´–2´, 94°C	Ab
Promega	GoTaq® Hot Start	2´, 94–95°C	Ab
Qiagen	HotStarTaq	15´, 95°C	Modified
Roche	FastStart Taq	4´, 95°C	Modified
Sigma	JumpStart™ Taq	1´, 94°C	Ab
Thermo Fisher	Thermo-Start *Taq*	15´, 95°C	Modified
NEB	One*Taq*	None	Aptamer

** May include initial denaturation step.*

PRODUCT FORMATS

Hot Start Available . . . . . Yes
- Activation Required . . . . . No
Master Mix Available . . . . . Yes
Direct Gel-loading Available . . . . . Yes
PCR Kit Available . . . . . No

Master Mix Formulations

Both One*Taq* and One*Taq* Hot Start DNA Polymerases are available in master mix and Quick-Load master mix formats. Master mix formulations include dNTPs, $MgCl_2$ and other buffers and stabilizers. The Quick-Load master mix formulations also include two tracking dyes for use with downstream visualization (i.e. agarose gels). With these convenient formats, the addition of primers and template are all that is required for robust amplification.

Master mix format contains inert tracking dye for easy and direct loading of PCR products onto gels.

Use your One*Taq* PCR products in Sanger sequencing

The dye in One*Taq* Quick-Load 2X Master Mix buffer doesn't interfere with Sanger sequencing. Prepare your samples with the fast and easy Exo-CIP Rapid PCR Cleanup Kit (#E1050) and proceed directly into Sanger sequencing.

Visit www.neb.com/One*Taq* for more information.

Robust Colony PCR from Multiple *E. coli* Strains using One*Taq* Quick-Load Master Mixes

Yan Xu, Ph.D., New England Biolabs, Inc.

Introduction

Colony PCR is a commonly used method to quickly screen for plasmids containing a desired insert directly from bacterial colonies. This method eliminates the need to culture individual colonies and prepare plasmid DNA before analysis. However, the presence of bacterial cell contents and culture media in colony PCR reactions often results in polymerase inhibition. A robust polymerase is required to perform colony PCR with high efficiency in many different bacterial strains.

One*Taq* DNA Polymerase, an optimized blend of *Taq* and Deep Vent$_R$™ DNA polymerases, has been formulated for robust yields with minimal optimization. This robustness makes One*Taq* ideal for use in demanding applications, such as colony PCR.

Furthermore, the One*Taq* Quick-Load Master Mix product format increases the ease-of-use for colony PCR. The master mix formulation contains dNTPs, $MgCl_2$, buffer components and stabilizers, as well as two commonly used tracking dyes for DNA gels. On a 1% agarose gel in 1X TBE, Xylene Cyanol FF migrates at ~4 kb and Tartrazine migrates at ~10 bp. Both dyes are present in concentrations that do not mask any co-migrating DNA bands.

General Protocol

1. Transform ligation mix or other plasmid-containing reaction mixture into the desired bacterial strain, and incubate agar plates overnight at the appropriate temperature.
2. Set up 50 µl reactions as follows:

One*Taq* Master Mix	25 µl
PCR primer	200 nM
H_2O	to 50 µl

Note: *If OneTaq Hot Start Quick-Load 2X Master Mix is used, reactions can be set up at room temperature. If OneTaq Quick-Load 2X Master Mix is used, reactions should be set up on ice.*

3. Use a sterile toothpick to pick up individual colonies and dip into each reaction tube.
4. As soon as the solution looks cloudy, remove the toothpick. To create a stock of each individual colony either:

   a.) Dip the toothpick into 3 ml growth media with appropriate antibiotics and culture overnight.
   or
   b.) Streak the toothpick onto another agar plate containing the appropriate antibiotics and grow overnight.

5. Transfer reactions to a PCR cycler, and perform PCR following the guidelines below for cycling conditions:

Initial denaturation:	
94°C	2 minutes
30 cycles:	
94°C	15–30 seconds
45–68°C	15–60 seconds
68°C	1 minute per kb
Final hold:	
68°C	5–10 minutes
10°C	hold

6. Load 4-6 µl of each PCR reaction directly onto an agarose gel, alongside an appropriate DNA ladder.

Materials

- Well-isolated bacterial colonies, ideally 1-2 mm in diameter
- Sterile toothpicks or pipette tips
- Additional agar plate, or culture tubes with growth media for retention of original colonies.
- One*Taq* Quick-Load 2X Master Mix with Standard Buffer (M0486) or One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer (M0488)*
- Sterile H_2O
- PCR primers

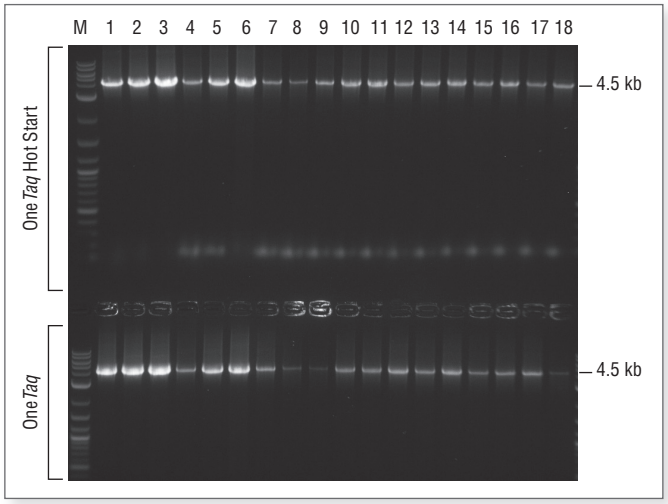
**For amplicons with a GC content over 65% GC, OneTaq Quick-Load 2X Master Mix with GC Buffer or OneTaq Hot Start Quick-Load 2X Master Mix with GC Buffer may be used.*

Results

Colony PCR was performed in 2 separate experiments using the protocol described above, with the following colonies:

1. Colonies obtained from transformation of a plasmid with a 4.5 kb insert into 18 different *E. coli* strains. Amplification of the plasmid insert was achieved in each case. One*Taq* Quick-Load 2X Master Mix with Standard Buffer and One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer were used.

   Colony PCR of a 4.5 kb insert using One*Taq* and One*Taq* Hot Start Quick-Load 2X Master Mixes with Standard Buffer and 18 different *E. coli* strains

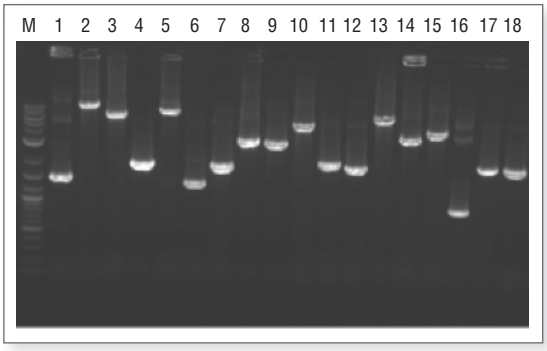


Reactions were set up according to the protocol and analyzed by agarose electrophoresis. Marker M is the 1 kb DNA Ladder (NEB #N3232)

Lane	Name	NEB #	Lane	Name	NEB #	Lane	Name	NEB #
1	NEB 10-beta	C3019	7	Lemo21(DE3)	C2528	13	T7 Express lysY	C3010
2	NEB 5-alpha	C2987	8	NiCo21(DE3)	C2529	14	T7 Express	C2566
3	NEB 5-alpha F´Iq	C2992	9	NEB Express I^q	C3037	15	T7 Express Crystal	C3022
4	dam-/dcm-	C2925	10	NEB Express	C2523	16	SHuffle® Express	C3028
5	NEB Turbo	C2984	11	T7 Express I^q	C3016	17	SHuffle T7 Express lysY	C3030
6	BL21(DE3)	C2527	12	T7 Express lysY/I^q	C3013	18	SHuffle T7 Express	C3029

2. Colonies from *E. coli* library clones with inserts ranging from 0.8 kb to 10 kb. One*Taq* Quick-Load 2X Master Mix with Standard Buffer was used, and results illustrate the robustness of the One*Taq* Quick-Load 2X Master Mix in this application.

   Colony PCR of library clones with inserts of 0.8 kb – 10 kb, using One*Taq* Quick-Load 2X Master Mix



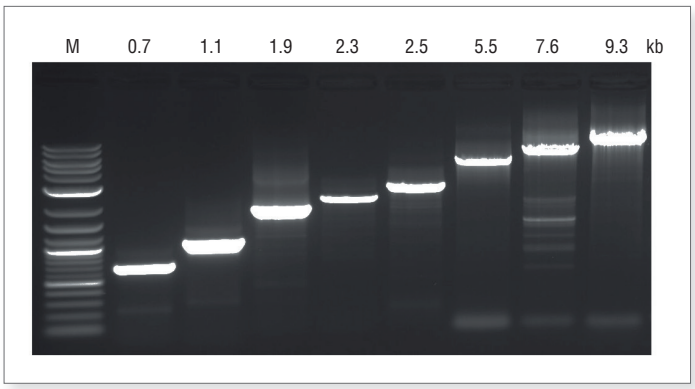
Summary

One*Taq* and One*Taq* Hot Start Quick-Load Master Mixes provide reliable performance in colony PCR, and are compatible with multiple *E. coli* strains. Reliable performance has been seen with amplicons up to 10 kb. The Quick-Load format offers additional convenience by enabling direct loading of the PCR reaction onto an agarose gel for analysis. Lastly, the Hot Start formulation provides additional functionality by reducing interference from primer-dimers and secondary amplification products.

One*Taq* One-Step RT-PCR Kit

The One*Taq* One-Step RT-PCR Kit offers sensitive and robust end-point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers, resulting in a streamlined RT-PCR protocol and reaction setup.

Efficient RT-PCR with templates of different lengths.

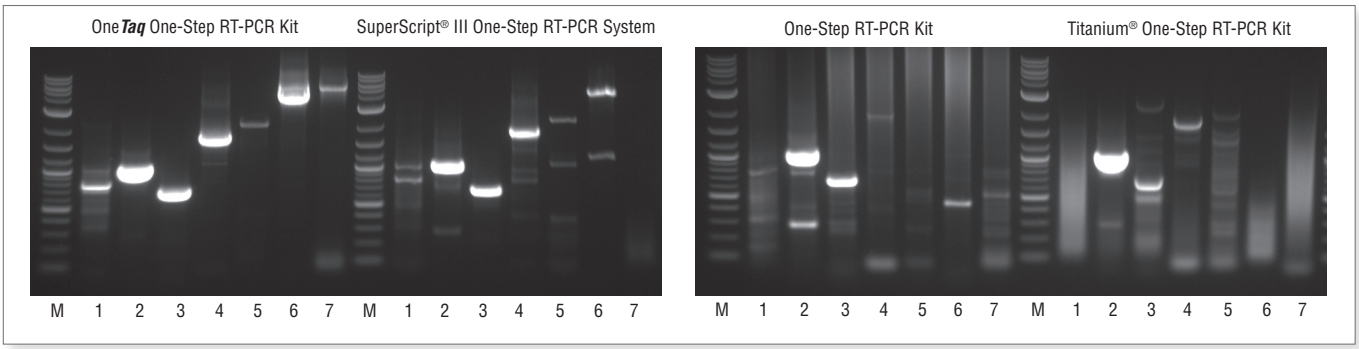


About 100 ng of Jurkat total RNA was used in 50 µl reactions following the standard protocol. Target sizes are indicated above the gel. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).

Advantages

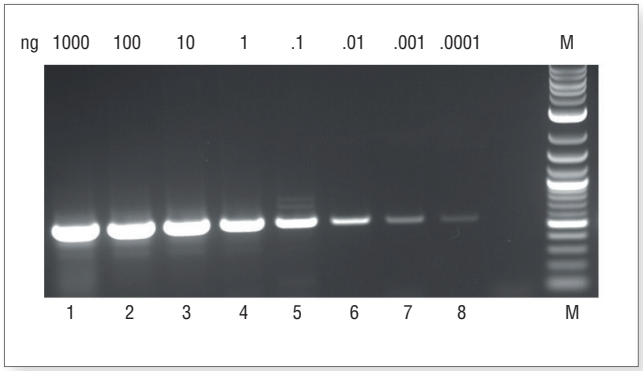
- Save time by combining cDNA synthesis and PCR in a single reaction
- Detect at little as 0.1 pg of a GAPDH target
- Robust amplification of amplicons from 100 bp to 9 kb
- Faster protocols with less hands-on time
- Quick-Load Reaction Mix allows instant gel loading

One*Taq* One-Step RT-PCR Kit offers superior performance over a broad range of template lengths.



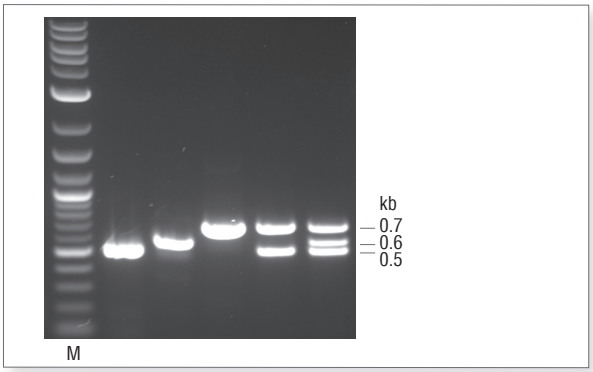
10 ng Jurkat total RNA (Lane 1: 0.8 kb, Lane 2: 1.0 kb, Lane 3: 0.7 kb, Lane 4: 1.9 kb, Lane 5: 2.3 kb, Lane 6: 4.8 kb, Lane 7: 5.5 kb) was used in 50 µl reactions, following manufacturers' recommended conditions, with the following One-Step RT-PCR Kits: OneTaq One-Step RT-PCR Kit (NEB #E5315), SuperScript III One-Step RT-PCR System (Life Technologies, Inc.), One-Step RT-PCR Kit (Qiagen®), Titanium One-Step RT-PCR Kit (Clontech). After 40 cycles 6 µl was loaded on an agarose gel. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).

Detect RNA as low as 0.1 pg.



Serial dilution of HeLa total RNA (Amount of RNA used is indicated above the gel.) was used in 50 µl reactions following the Standard Protocol. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).

Amenable to multiplexing.



10 ng Jurkat total RNA was used in 50 µl reactions following the Standard Protocol. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).

Ordering Information

PRODUCT	NEB #	SIZE
One*Taq* DNA Polymerase	M0480S M0480L M0480X	200 units 1,000 units 5,000 units
One*Taq* Quick-Load DNA Polymerase	M0509S M0509L M0509X	100 units 500 units 2,500 units
One*Taq* 2X Master Mix with Standard Buffer	M0482S M0482L	100 rxns 500 rxns
One*Taq* Quick-Load 2X Master Mix with Standard Buffer	M0486S M0486L	100 rxns 500 rxns
One*Taq* Hot Start DNA Polymerase	M0481S M0481L M0481X	200 units 1,000 units 5,000 units
One*Taq* Hot Start 2X Master Mix with Standard Buffer	M0484S M0484L	100 rxns 500 rxns
One*Taq* Hot Start 2X Master Mix with GC Buffer	M0485S M0485L	100 rxns 500 rxns
One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer	M0488S M0488L	100 rxns 500 rxns
One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer	M0489S M0489L	100 rxns 500 rxns
One*Taq* RT-PCR Kit	E5310S	30 rxns
One*Taq* One-Step RT PCR Kit	E5315S	30 rxns

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