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

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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 One*Taq* Hot Start Quick-Load 2X Master Mix is used, reactions can be set up at room temperature. If One*Taq* 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	
PCR Primer	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 (NEB #M0486) or One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer (NEB #M0488)*

Sterile H_2O

PCR primers

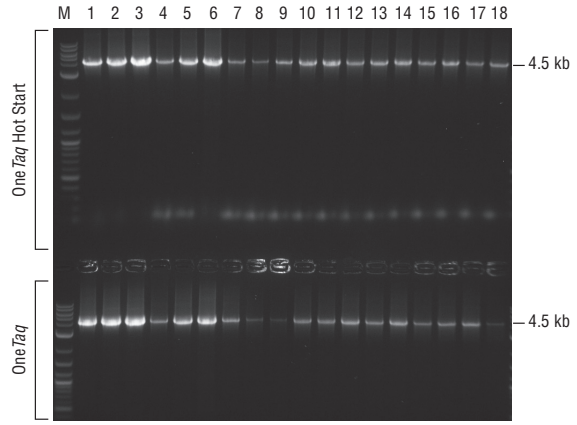
* For amplicons with a GC content over 65% GC, One*Taq* Quick-Load 2X Master Mix with GC Buffer or One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer may be used.

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.

FIGURE 1:
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.

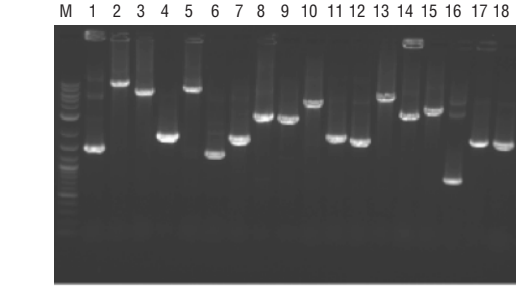


Reactions were set up according to the protocol and analyzed by agarose electrophoresis.
Marker M is the 1 kb DNA Ladder (NEB #N3232).

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.

FIGURE 2:
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.

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
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