LEO™ MASTERMIX (2X)

FOR HIGH GC RICH TEMPLATES & ROUTINE PCR

PRODUCT DETAILS

Mighty Leo Mastermix is 2X concentrated solution for PCR reaction which contains dNTPs, Taq DNA Polymerase, Mg+2 and other critical reaction components. It doesn't contain primers and DNA template. Leo Mastermix is designed for routine PCR and also for amplification of high GC templates. The pre loaded green dye makes it easier for users to load directly on the agarose gel post PCR.

FEATURES

- Extremely powerful taq based premix, specially optimized for GC rich templates.
- 5X better yield than any standard Taq DNA polymerase.
- Amplification upto 5KB is tested.
- Comes with pre-loaded green dye which separates into blue (Equivalent to ~ 3-5kb DNA fragment) and yellow (Equivalent to ~25bp DNA fragment) on 1% agarose gel.
- **Super thermostable:** Mastermix is made with proprietary formulation buffer which makes shipping possible at Room Temperature. The mastermix is tested for its massive amplification, even after incubating at 45℃ for at least 7 days !

CATALOG INFORMATION

- R8120 - 4 mL Pack
- R8121 - 16 mL Pack
- R8120X - BULK

www.dxbidt.com
Email: info@dxbidt.com
Ph: +91-7349708807

LEO™ MASTERMIX (2X)

TECHNICAL DATA

STUDY 1 DETAILS: EFFICEIENCY

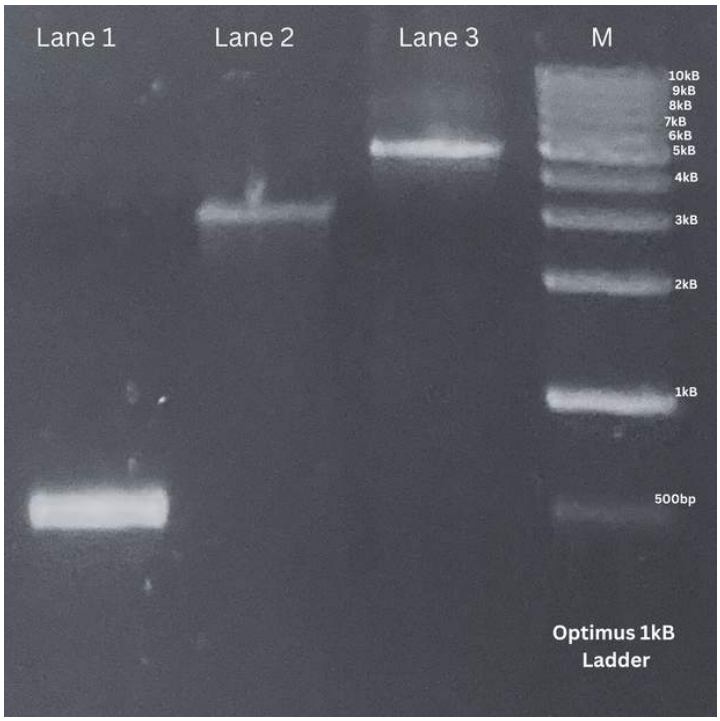
1ng of Lambda (λ) phage DNA was taken for amplification. 500bp , 3kb and 5kb fragments were amplified using LEO Mastermix. Average GC content: 57%.

PCR Conditions

Lambda template (1µL), forward & reverse primers (1µL each), LEO Mastermix (25µL) and make upto 50µL using Nuclease Free Water.

Cycling conditions and Gel

95℃ (2min), 25 Cycles [95℃-30s; 58℃-30s; 72℃-1min/kb], 72℃- 5to 10 minutes. 5µL of sample was loaded directly in 1% Agarose Gel which was run just for 15 minutes.



Leo Mastermix can easily amplify upto 5kB.

Lane 1: 500bp fragment
Lane 2: 3kb fragment
Lane 3: 5kB Fragment
Lane M: Optimus™ 1kb marker

LEO™ MASTERMIX (2X)

TECHNICAL DATA

STUDY 2 DETAILS: HUMIC ACID TOLERANCE

Leo™ mastermix contains unique buffer and reaction mix which can tolerate humic acid/fulvic acid which may be present in your DNA template samples. Humic/fulvic acid are PCR inhibitors and severely impact Taq DNA polymerase performance.

Study Details

Humic acid/Fulvic acid salt (70:15) was dissolved in nuclease free water and stock was prepared. To a PCR reaction volume of 50µL, concentration of humic acid/fulvic acid salt was tested at 3.5ng/µL, 7ng/µL, 10ng/µL, & 20ng/µL. A competitor product (Ampliqon) was also tested for comparison.

PCR Conditions

Sample: Lambda DNA (100pg), target 1KB fragment, forward/reverse primers 1µL each

Cycling conditions and Gel

95℃ (2min), 25 Cycles [95℃-30s; 58℃-30s; 72℃-1min/kb], 72℃- 2 minutes.
10 µL of sample was loaded directly in 1% Agarose Gel which was run just for 30 minutes.

Lane 1 : Marker
Lane 2: Leo, Without inhibitor
Lane 3: Leo + 3.5ng/µL Humic
Lane 4: Leo + 7ng/µL Humic
Lane 5: Leo + 10ng/µL Humic
Lane 6: Leo + 20ng/µL Humic

Lane 7 : Marker
Lane 8: Ampliqon, Without inhibitor
Lane 9: Ampliqon + 3.5ng/µL Humic
Lane 10: Ampliqon + 7ng/µL Humic
Lane 11: Ampliqon + 10ng/µL Humic
Lane 12: Ampliqon + 20ng/µL Humic

RESULTS:

Even in the presence of 10ng/µL humic acid LEO Mastermix work well (**1kb fragment**). At 20ng/µL humic amplification is fully inhibited. But for ampliqon red mastermix, ampification is fully inhibited at 3.5ng/µL humic. This test clearly demonstrates tolerance of LEO mastermix with respect to PCR inhibitors.

LEO™ MASTERMIX (2X)

TECHNICAL DATA

STUDY 3 DETAILS: COLONY PCR

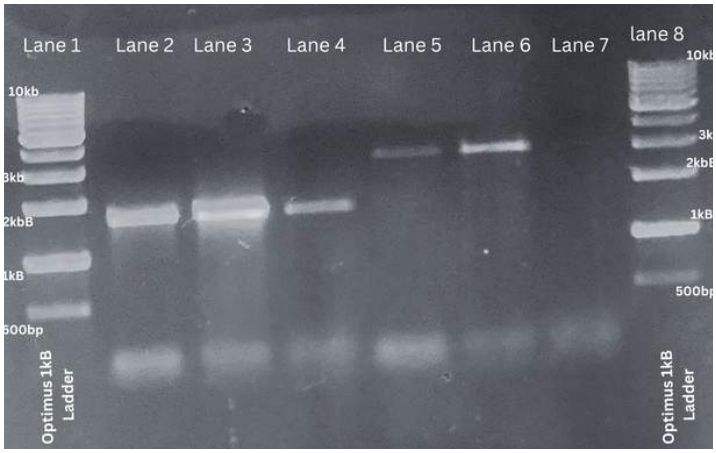
Leo Mastermix has extreme capabilities to detect positive colonies. To demonstrate this, two plasmids were chosen.
Plasmid 1: 6.7 KB total length. Gene fragment: 1.6KB
Plasmid 2: 8KB total length. Gene fragment: 2.9KB

PCR Conditions

One single colony was picked using 10µL tip and suspended in 100µL of nuclease free water. From this suspension, 1µL, 3µL and 1µL(diluted 10 times) were taken for colony PCR.
Forward & Reverse(10µM): 1µL each
Leo Mastermix: 25µL
Final Volume: 50µL

Cycling conditions and Gel

Plasmid 1: 95℃ (2min), 25 Cycles [95℃-30s; 58℃-30s; 72℃-1.5min], 72℃- 2 minutes.
Plasmid 2: 95℃ (2min), 25 Cycles [95℃-30s; 58℃-30s; 72℃-3min], 72℃- 5 minutes.
10 µL of sample was loaded directly in 1% Agarose Gel which was run just for 30 minutes.



Clear band was visible at 1.6kb and 2.9kb.
(Lane 1: Optimus™ 1kb marker), (Lane 2: 1µL colony, plasmid 1), (Lane 3: 3µL colony, plasmid 1), (Lane 4: 1µL,10X diluted colony, plasmid 1), (Lane 5: 1µL colony, plasmid 2), (Lane 6: 3µL colony, plasmid 2, (Lane 7: 1µL, 10X diluted colony, plasmid 2), (Lane 8:Optimus™ 1kb marker)

Result:

- LEO™ Mastermix has more tolerance to no.of colonies. Hence, 3µL neat sample gave good amplification.
- When sample is diluted 10 times, band was still visible for 1.6kB fragment (lane 4). But no band was observed for 2.9kb (lane 7). This information gives good insight to which dilution to follow when users work with various gene sizes.

LEO™ MASTERMIX (2X)

TECHNICAL DATA

STUDY 4 DETAILS: FREEZE THAW TOLERANCE

One of the most critical feature for any mastermix is its ability to withstand 'Freeze-Thaw' cycles. Leo™ mastermix out-performs this test. It's astonishingly stable for at least 50 freeze thaw cycles tested.

Freeze thaw test

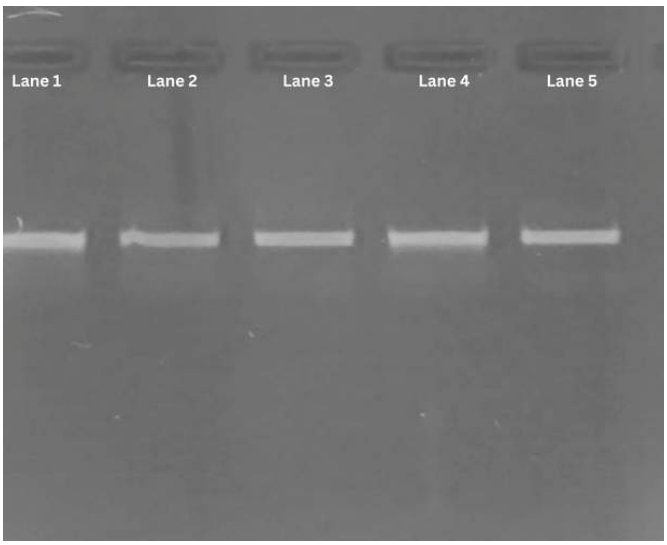
Leo™ mastermix was taken out from -20℃ freezer and thawed in hand for 3-4 minutes. After this, samples were vortexed and spun down for 10 mins. It was then kept on ice for 30 mins (for PCR activities),later were stored in -20℃ for 90 mins. Amplification was tested for every 15, 30, 40 and 50 cycles.

PCR Conditions

Sample: Lambda DNA (1ng), target 300bp, Forward/Reverse 1µL each

Cycling conditions and Gel

95℃ (2min), 25 Cycles [95℃-30s; 58℃-30s; 72℃-30s], 72℃- 2 minutes.
10 µL of sample was loaded directly in 1% Agarose Gel which was run just for 30 minutes.



Good amplification was observed even after 50 Cycles.
(Lane 1: Leo, Stored at -20℃, Cycle 0), (Lane 2: 15th Cycle), (Lane 3: 30th Cycle), (Lane 4: 40th Cycle), (Lane 5: 50th Cycle).

Result:

- Even after 50 cycles, the band intensity was comparable to the sample stored at -20℃. Leo is stable for at least 50 freeze thaw cycles.

LEO™ MASTERMIX (2X)

TECHNICAL DATA

STUDY 5 DETAILS: ROOM TEMPERATURE STABILITY

At dx/dt, we believe in providing high quality mastermixes at an affordable price. This would not be possible with cutting col-chain costs. Hence, we designed an unique buffer which keeps Leo™ mastermix and few other mastermixes stable at room temperature. At least for a week !

Study details

Leo Mastermix was kept in hot air oven set at 45℃ (Really!). It was stored for 8 days.

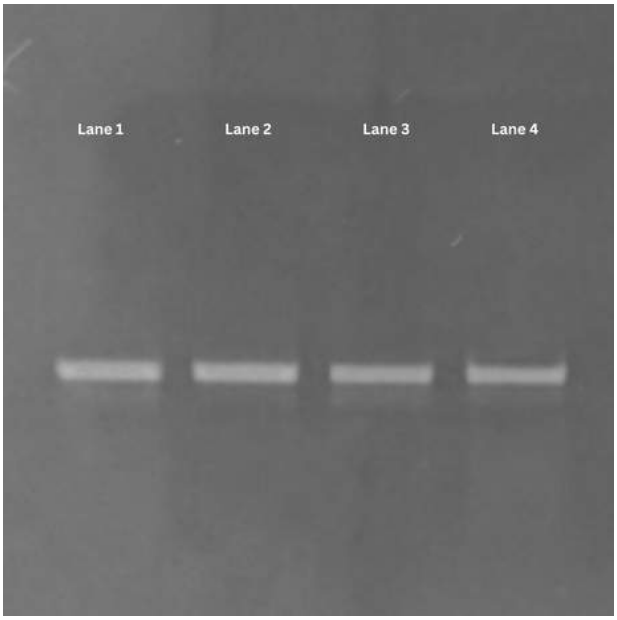
PCR Conditions

Sample: Lambda DNA (1ng), target 300bp fragment, Forward/Reverse 1µL each

Cycling conditions and Gel

95℃ (2min), 25 Cycles [95℃-30s; 58℃-30s; 72℃-30s], 72℃- 2 minutes.

10 µL of sample was loaded directly in 1% Agarose Gel which was run just for 20- 30 minutes.



Good amplification was observed even after 8 Days.

(Lane 1: Leo, Stored at -20℃, Day 0), (Lane 2:Leo stored at 45℃, Day 3), (Lane 3: Lane 2:Leo stored at 45℃, Day 6), (Lane 4: Leo stored at 45℃, Day 8).

Result:

- Leo is stable even after 8 days, when stored at 45℃.