Invitrogen™ Collibri™ Library Amplification Master Mix with Primer Mix

invitrogen

USER GUIDE Pub. No. MAN0017582 Rev. B

Package contents

Catalog No.	Size
A38540050	50 rxns (with primer mix)
A38540250	250 rxns (with primer mix)

Kit contents

Storage conditions

Store all contents at –20°C until the expiration date or at 4°C for up to 1 month. No negative effect on master mix performance has been observed for up to 30 freeze/thaw cycles.

Related products

Go to thermofisher.com/collibri to view related products.

Product description

- Invitrogen™ Collibri™ Library Amplification Master Mix (2X) is a ready-to-use solution designed for the amplification of next generation sequencing (NGS) libraries compatible with Illumina™ sequencing platforms. The master mix includes the Platinum™ SuperFi™ DNA Polymerase in combination with a proprietary reaction buffer that contains all the necessary components for efficient and uniform library amplification regardless of GC content, helping improve coverage across GC- and AT-rich sequences and other complex regions.
- Platinum™ SuperFi™ DNA Polymerase has both 5′ to 3′ polymerase and 3′ to 5′ exonuclease (proofreading) activities, but lacks the 5′ to 3′ exonuclease activity. Exceptionally strong proofreading activity ensures amplification of NGS libraries with supreme sequence accuracy.
- The Collibri™ Library Amplification Master Mix is supplemented with an inert blue dye. The master mix is supplied with a 10X Primer Mix that targets the P5 and P7 regions of Illumina™ adapters and contains a yellow dye. Mixing both components in a PCR reaction turns final solution green. This provides a visual aid when pipetting and decreases the risk of pipetting errors during reaction setup.
- Platinum™ hot-start technology inhibits DNA polymerase activity at ambient temperatures, allowing room temperature reaction setup and storage of pre-assembled PCR reactions for up to 24 hours prior to the PCR. Enzyme activity is restored after the initial denaturation step.

Important guidelines

Click here for important library amplification guidelines.

Online resources

Visit our product page for additional information and protocols. For support, visit thermofisher.com/support.

Master mix characteristics

Concentration:	2X
Enzyme:	Platinum™ SuperFi™ DNA Polymerase
Activities:	5′ to 3′ polymerase, 3′ to 5′ exonuclease (proofreading)
Hot-start:	Antibody
Fidelity vs. *Taq*:	>300X

PCR setup

Component	50-µL rxn	Final conc.
2X Library Amplification Master Mix[1]	25 µL	1X
10 µM Primer Mix[2]	5–10 µL	1–2 µM
Adapter-ligated DNA	15–20 µL	varies

[1] Provides $MgCl_2$ at a final concentration of 2 mM in the reaction.

[2] Use the Primer Mix supplied with the master mix or use another suitable library amplification primer mix. The recommended final concentration of each primer in the amplification reaction is 1–2 µM (see **"Optimization strategies"** for more information).

PCR protocol

See page 2 to prepare and run your PCR.

Optimization strategies

Click here for guidelines to optimize your library amplification.

Troubleshooting

Click here for guidelines to troubleshoot your library amplification.

Purchaser notification

Click here for Limited warranty, Disclaimer, and Licensing information.

Print Options

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The example PCR procedure below shows appropriate volumes for a single **50-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense appropriate volumes into each 0.2–0.5-mL PCR tube prior to adding template DNA and primers.

Steps	Action	Procedure details
1 	**Thaw reagents**	Thaw, mix, and briefly centrifuge each component before use. Avoid generating bubbles when mixing the Master Mix.
2 	**Prepare PCR reaction mix**	**a.** Add the following components to each PCR tube.

Component	Volume	Final conc.
2X Library Amplification Master Mix[1]	25 µL	1X
10 µM Primer Mix[2]	5–10 µL	1–2 µM
Adapter-ligated DNA	15–20 µL	varies
Total volume:	50 µL	—

[1] Provides $MgCl_2$ at a final concentration of 2 mM in the reaction.

[2] Use the Primer Mix supplied with the master mix or use another suitable library amplification primer mix. The recommended final concentration of each primer in the amplification reaction is 1–2 µM (see "**Optimization strategies**", page 1, for more information).

b. Mix and then briefly centrifuge the components.

Note: Collibri™ Library Amplification Master Mix is supplemented with a blue dye and the Primer Mix contains a yellow dye. Mixing both components in a PCR reaction turns the solution green. This provides a visual aid when pipetting and decreases the risk of pipetting errors during reaction setup.

3 

Determine the required number of PCR cycles

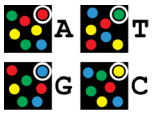
Note: The number of PCR cycles recommended here are optimized for NGS libraries to acquire at least 200 fmol of library DNA. The actual number of PCR cycles may differ depending on the library prep reagents, protocol, and input DNA quality. Add additional cycles to amplify libraries prepared from FFPE DNA or other challenging samples.

Input DNA[1]	PCR cycle number
250 ng	1–2
100 ng	2–4
50 ng	4–7
25 ng	5–8
10 ng	6–9
5 ng	7–10
1 ng	10–13
0.1 ng	13–15

[1] The DNA input amount to Library Prep kit.

Input RNA[1]	PCR cycle number
25–50 ng	8–10
10–25 ng	9–11
5–9 ng	10–12
1–4 ng	12–14

[1] The rRNA-depleted or poly(A)-enriched RNA input amount to Library Prep kit.

<table>
<tr><th>Steps</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>4  </td><td>Incubate reactions in a thermal cycler</td><td>
<table>
<tr><th>PCR cycles</th><th>Step</th><th>Temperature</th><th>Time</th></tr>
<tr><td>1</td><td>Initial denaturation</td><td>98°C</td><td>30 seconds</td></tr>
<tr><td rowspan="3">1–15[1]</td><td>Denature</td><td>98°C</td><td>15 seconds</td></tr>
<tr><td>Anneal[2]</td><td>60°C</td><td>30 seconds</td></tr>
<tr><td>Extend</td><td>72°C</td><td>30 seconds</td></tr>
<tr><td rowspan="2">1</td><td rowspan="2">Final extension</td><td>72°C</td><td>1 minute</td></tr>
<tr><td>4°C</td><td>hold</td></tr>
</table>
[1] See “Determine the number of PCR cycles”, page 2, for the required number of PCR cycles.
[2] 60°C annealing temperature is recommended for the Primer Mix supplied with the kit. Optimization of annealing temperature may be required for different user-supplied adapters and primers. Note that the annealing temperature for Platinum™ SuperFi™ DNA Polymerase is typically higher than for other DNA polymerases. To determine the optimal annealing conditions, use the T_m calculator on our website at thermofisher.com/tmcalculator as a starting point.
Note: See “Optimization strategies”, page 1, for guidelines to optimize cycling conditions.
</td></tr>
<tr><td>5  </td><td>Analyze by sequencing</td><td>After amplification, use your library immediately for post-PCR cleanup and sequencing, or store it at –20°C.</td></tr>
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