



Package contents

Catalog No.

A38539050

Size

50 rxns

Kit contents

A38539250 250 rxns



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. 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	5–10 μL	1–2 μM ^[2]
Adapter-ligated DNA	15–20 μL	varies

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

^[2] 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.

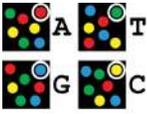
Purchaser notification

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The example PCR procedure below shows appropriate volumes for a single 50- μ L 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	<p>a. Add the following components to each PCR tube.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>Volume</th> <th>Final conc.</th> </tr> </thead> <tbody> <tr> <td>2X Library Amplification Master Mix^[1]</td> <td>25 μL</td> <td>1X</td> </tr> <tr> <td>10 μM Primer Mix</td> <td>5–10 μL</td> <td>1–2 μM^[2]</td> </tr> <tr> <td>Adapter-ligated DNA</td> <td>15–20 μL</td> <td>varies</td> </tr> <tr> <td>Total volume:</td> <td>50 μL</td> <td>—</td> </tr> </tbody> </table> <p>¹ Provides MgCl₂ at a final concentration of 2 mM in the reaction. ² See “Optimization strategies”, page 1, for more information.</p> <p>b. Mix and then briefly centrifuge the components.</p>	Component	Volume	Final conc.	2X Library Amplification Master Mix ^[1]	25 μ L	1X	10 μ M Primer Mix	5–10 μ L	1–2 μ M ^[2]	Adapter-ligated DNA	15–20 μ L	varies	Total volume:	50 μ L	—													
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3 	Determine the required number of PCR cycles	<p>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.</p> <table border="1"> <thead> <tr> <th>Input DNA^[1]</th> <th>PCR cycle number</th> </tr> </thead> <tbody> <tr> <td>250 ng</td> <td>1–2</td> </tr> <tr> <td>100 ng</td> <td>2–4</td> </tr> <tr> <td>50 ng</td> <td>4–7</td> </tr> <tr> <td>25 ng</td> <td>5–8</td> </tr> <tr> <td>10 ng</td> <td>6–9</td> </tr> <tr> <td>5 ng</td> <td>7–10</td> </tr> <tr> <td>1 ng</td> <td>10–13</td> </tr> <tr> <td>0.1 ng</td> <td>13–15</td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th>Input RNA^[1]</th> <th>PCR cycle number</th> </tr> </thead> <tbody> <tr> <td>25–50 ng</td> <td>8–10</td> </tr> <tr> <td>10–25 ng</td> <td>9–11</td> </tr> <tr> <td>5–9 ng</td> <td>10–12</td> </tr> <tr> <td>1–4 ng</td> <td>12–14</td> </tr> </tbody> </table> <p>^[1] The DNA input amount to Library Prep kit.</p>	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	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
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5  Analyze by sequencing		<p>After amplification, use your library immediately for post-PCR cleanup and sequencing, or store it at –20°C.</p>																											

¹ See “Determine the number of PCR cycles”, page 2, for the required number of PCR cycles.

² Annealing temperature of 60°C is recommended for standard Illumina™ adapter/primer pairs. Optimization of annealing temperature may be required for different adapters and primers. Note that the annealing temperature for the 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.

Package contents

Catalog
A385390
A385392

Storage conditions

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Related products

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Kit contents

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Reagents provided are sufficient for 50 or 250 amplification reactions of 50 µL each. Note that you will need to supply the appropriate primer mix to perform the library amplification reactions.

Component	Cat. No. A38539050 (50 rxns)	Cat. No. A38539250 (250 rxns)
2X Library Amplification Master Mix	1.25 mL	5 × 1.25 mL

- Invitrogen™ Collibri™ Library Amplification Master Mix (2X) is a ready-to-use solution designed for the amplification of

Component	50-µL rxn	Final conc.
2X Library Amplification Master Mix ^[1]	25 µL	1X

Important guidelines

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- Thaw frozen reagents on ice before use, and keep them on ice until ready to use.
- Mix reagents thoroughly before each use.
- Carefully mix and centrifuge all tubes before opening to ensure homogeneity and to improve recovery. Prepare a master mix for the appropriate number of samples to be amplified plus at least one extra to account for volume loss from pipetting.
- When using Collibri™ Library Amplification Master Mix, it is not necessary to perform the PCR set-up on ice.
- Avoid cross-contamination by using aerosol-resistant barrier tips and by analyzing PCR products in a separate area from PCR assembly.
- The Platinum™ SuperFi™ DNA polymerase cannot read dUTP, dITP, or their derivatives in the template strand. Therefore, we do not recommend the use of these analogues or primers that contain them.
- The Collibri™ Library Amplification Master Mix is compatible with carboxylated beads that may be carried over from library prep protocols, e.g. Agencourt™ AMPure™ XP (Beckman Coulter, Inc.).
- Use SPRI Beads or PCR purification columns for post-PCR clean-up.

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.

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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.

The example PCR procedure below shows appropriate volumes for a single 50- μ L 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.

Optimization strategies

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1

Notes about reaction components

2X Collibri™ Library Amplification Master Mix: The master mix contains all necessary reaction components. Only template library DNA and primers need to be added, thus reducing the number of pipetting steps during PCR reaction setup. The master mix provides 2 mM MgCl₂ and 200 μ M of each dNTP in the final reaction mix.

The Collibri™ Library Amplification Master Mix is supplemented with an inert blue dye. This provides a visual aid when pipetting and decreases the risk of pipetting errors during reaction set-up. The dye does not interfere with PCR performance and are compatible with cleanup, quantification, and sequencing protocols.

Primers: The recommended final primer concentration is 1 μ M, but this can be varied in the range of 0.2–2 μ M, if needed. Increase the primer concentration up to 2 μ M when the required amplification yield is >500 ng or when the library DNA input to PCR reaction is \geq 50 ng.

Template: To ensure greatest success for library amplification, use high quality, purified DNA templates. Libraries prepared from difficult samples, such as FFPE DNA, may require 1–3 additional PCR cycles.

2

Notes about cycling parameters

Make sure that the heated lid temperature is set several degrees above 98°C to avoid sample condensation. The lid can be pre-heated before putting the samples in the thermocycler.

Initial denaturation: 30-second initial denaturation at 98°C is sufficient for most libraries.

Denaturation: Keep the denaturation time as short as possible. For most libraries, 15 seconds at 98°C is recommended, but the time can be varied from 5 to 30 seconds depending on library length and complexity.

Annealing: Annealing temperature of 60°C is recommended for standard Illumina™ adapter/primer pairs. Optimization of annealing temperature may be required for different adapters and primers. As a starting point, use the T_m calculator available on our website (www.thermofisher.com/tmcalculator) to calculate the recommended annealing temperature. If necessary, use a temperature gradient to further optimize and empirically determine the ideal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR).

Extension: Extension time of 30 seconds is sufficient for most libraries with an average insert size up to 800 bp. Libraries with longer insert sizes might need optimization, where the recommended extension time is 15–30 seconds per 1 kb.

3

of PCR cycles

25 ng	5–8
10 ng	6–9
5 ng	7–10
1 ng	10–13
0.1 ng	13–15

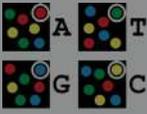
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12–14

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

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5 	Analyze by sequencing	<p>Optimization of annealing temperature for SuperFi™ DNA Polymerase is available using the T_m calculator on our website at www.thermofisher.com.</p> <p>Store it at -20°C.</p>						

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