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.

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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.

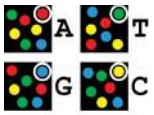
Steps	Action	Procedure details
4 	**Incubate reactions in a thermal cycler**	

PCR cycles	Step	Temperature	Time
1	Initial denaturation	98°C	30 seconds
1–15[1]	Denature	98°C	15 seconds
	Anneal[2]	60°C	30 seconds
	Extend	72°C	30 seconds
1	Final extension	72°C	1 minute
		4°C	hold

[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.

5 	**Analyze by sequencing**	After amplification, use your library immediately for post-PCR cleanup and sequencing, or store it at –20°C.

Package contents Catalog
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A385402

Storage conditions Store all
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Related products Go to th

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

Kit contents

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Reagents provided are sufficient for 50 or 250 amplification reactions of 50 µL each.

Component	Cat. No. A38540050 (50 rxns)	Cat. No. A38540250 (250 rxns)
2X Library Amplification Master Mix	1.25 mL	5 × 1.25 mL
10 µM Primer Mix	500 µL	5 × 500 µL

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ease (proofreading)

	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.
- We recommend an annealing temperature of 60°C 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 the Platinum™ SuperFi™ DNA Polymerase is typically higher than for other DNA polymerases. As a starting point, use the T_m calculator on our website at **www.thermofisher.com/tmcalculator** to calculate the recommended annealing temperature. If necessary, optimize by testing at increments of 1–2°C until optimal results are achieved.
- 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.

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.

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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.

Optimization strategies

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Notes about reaction components

2X 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 $MgCl_2$ and 200 µM of each dNTP in the final reaction mix.

The Collibri™ Library Amplification Master Mix is supplemented with an inert blue dye. Mixing with the yellow dye contained in the Primer Mix turns the final PCR solution green. This provides a visual aid when pipetting and decreases the risk of pipetting errors during reaction set-up. The dyes do not interfere with PCR performance and are compatible with cleanup, quantification, and sequencing protocols.

Primers: The 10 µM Primer Mix targets the P5 and P7 regions of Illumina™ adapters. The primer mix is supplemented with an inert yellow dye to provide visual aid when pippetting. The recommended final primer concentration is 1 µM, but this can be varied in the range of 0.2–2 µM, if needed. Increase the primer concentration up to 2 µM when the required amplification yield is >500 ng or when the library DNA input to PCR reaction is ≥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.

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: 60°C is the optimal annealing temperature for the primer mix supplied with the kit. For other primer sets, use the T_m calculator available on our website (**www.thermofisher.com/tmcalculator**) as a starting point 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.

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Determine the required number of PCR cycles

250 ng	1–2
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[1] The DNA input amount to Library Prep kit.

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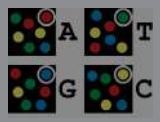
Steps	Action	Procedure details
4	 **Incubate reactions in a thermal cycle**	n of annealing temperature may be atinum™ SuperFi™ DNA Polymerase is e the T_m calculator on our website at
5	 **Analyze by sequencing**	r store it at –20°C.

Troubleshooting

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Observation	Recommended action
No product at all or low yield.	▪ Make sure that there are no pipetting errors. ▪ Do not use primers or template that contain dUTP or dITP. ▪ Template DNA may be damaged prior to library preparation, causing reduced conversion rate. Use carefully purified template. ▪ Increase the number of cycles. ▪ Decrease annealing temperature (only if custom primers are used). ▪ Check the purity and concentration of the primers. ▪ Check primer design.
Overamplification–High molecular weight smears	▪ Reduce the total number of cycles. ▪ Reduce the library DNA input amount to the PCR reaction. ▪ Increase the concentration of primers. ▪ Decrease extension time. ▪ Increase annealing temperature or try 2-step protocol. ▪ Optimize denaturation time and temperature.