Invitrogen™ Collibri™ Library Amplification Master Mix

invitrogen

USER GUIDE Pub. No. MAN0017909 Rev. B

Package contents

Catalog No.	Size
A38539050	50 rxns
A38539250	250 rxns

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. 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

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 / 5–10 µL / 1–2 µM[2] 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] See **"Optimization strategies"**, page 1, for more information. **b.** Mix and then briefly centrifuge the components.
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.

Steps	Action	Procedure details

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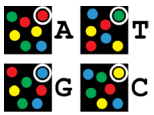
Incubate reactions in a thermal cycler

PCR cycles	Step	Temperature	Time
1	Initial denaturation	98°C	30 seconds
1–15[1]	Denature	98°C	15 seconds
	Anneal[2]	varies	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] Annealing temperature of 60ºC 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.

5 

Analyze by sequencing

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