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VeriFi™ Library Amplification Mix

Product description:

VeriFi™ Library Amplification Mix is a superior proofreading polymerase mix with AptaLock™ hot start technology. This 2x ready mix is designed for library amplification in NGS workflows and GC-rich PCR applications where the ability to amplify difficult targets without bias is required.

VeriFi™ Library Amplification Mix contains the highly processive VeriFi™ Hot Start Polymerase, developed for robust and versatile high fidelity PCR. The mix contains buffer, dNTPs, $MgCl_2$, and enhancers and has been optimised to minimise GC-dependant bias during amplification. This unique mix composition enables improved library amplification, allowing for the aquistion of superior quality NGS datasets with a higher number of discrete reads than similar high fidelity mixes.

The mix is improved by PCRBIO's innovative AptaLock™ technology. This hot start mechanism uses a proprietary aptamer-like molecule that reversibly inhibits the enzyme at ambient temperatures. This unique hot start molecule prevents primer dimer formation and non-specific amplification to maximise the sensitivity and specificity of your PCR. This feature makes the enzyme highly suitable for difficult templates and enables reactions to be set up at room temperature.

VeriFi™ Library Amplification Mix can also be used to amplify difficult templates with very high or low GC content, when other proofreading enzymes fail, and can be used in multiplex PCR assays.

Component	50 x 50 µL rxns	250 x 50 µL rxns
2x VeriFi™ Library Amplification Mix	1 x 1.25 mL	5 x 1.25 mL

Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4 °C for 1 month.

Limitations of product use

The product may be used for in vitro research purposes only.

Technical support

Help and support is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting you can submit a technical enquiry online, or alternatively email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Bioanalyzer results or screen grabs of gel images
- Relevant sequencing data

Important considerations

2x VeriFi™ Library Amplification Mix: The 2x mix contains VeriFi™ Hot Start Polymerase, 6 mM $MgCl_2$, 2 mM dNTPs, enhancers and stabilisers. Adding more PCR enhancers or $MgCl_2$ to the reaction is not recommended. The mix composition has been optimised to maximise PCR success rates.

Primers: For NGS library amplification, primers targeting the ligated adapters (e.g., P5 and P7 for Illumina platforms) should be used at a concentration between 0.4 µM and 1 µM. For standard end-point PCR, primers should have a predicted melting temperature between 60 ˚C and 70 ˚C, using default Primer 3 settings (http://bioinfo.ut.ee/primer3/) and the final concentration in the reaction should be between 0.2 µM and 0.6 µM.

Denaturation: Denaturation should be performed at 95 ˚C. However, if the presence of high GC regions results in low yields, increasing the melting temperature to 98-100 ˚C can improve the amount of product.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 60 ˚C annealing temperature then increase in 2 ˚C increments if non-specific products are present.

Extension: Optimal extension is achieved at 72 ˚C. The optimal extension time is dependent on amplicon length and complexity of template. 30 seconds per kilobase (kb) is recommended for most applications. Two-step cycling protocols may also be used with combined annealing and extension at 68-72 ˚C.

Cycle number: Amplifying an NGS library excessively can lead to undesirable artifacts and cause amplification bias. The number of amplification cycles should be kept to the minimum necessary in order to obtain yields sufficient for downstream processes. Yields between 250 ng and 1000 ng are typically sufficient for most NGS applications. Depending on the DNA input, 5-15 cycles are generally enough. For non-NGS applications, 25-35 cycles are preferrable.

Reaction setup

1. Prepare a master mix based on the following table:

Reagent	25 µL reaction	50 µL reaction	Final concentration	Notes
2x VeriFi™ Library Amplification Mix	12.5 µL	25.0 µL	1x	
Forward primer (10 µM)	1.0 µL	2.0 µL	400 nM*	*See above for optimal primer concentration
Reverse primer (10 µM)	1.0 µL	2.0 µL	400 nM*	
Template DNA	<100 ng genomic DNA <5 ng less complex DNA	<200 ng genomic DNA <10 ng less complex DNA	variable	
PCR grade dH_2O	Up to 25 µL final volume	Up to 50 µL final volume		

2. Cycle using conditions based on the following tables:

3-step cyling:

Cycles	Temperature	Time	Notes
1	95 ˚C	1 min	Initial denaturation
5-15 (for NGS), 25-35 (standard)	95 ˚C 60 - 72 ˚C 72 ˚C	15 seconds 15 seconds 30 seconds/kb	Denaturation (see “Important considerations” above) Anneal Extension

2-step cyling:

Cycles	Temperature	Time	Notes
1	95 ˚C	1 min	Initial denaturation
5-15 (for NGS) 25-35 (standard)	95 ˚C 68 - 72 ˚C	15 seconds 30 seconds/kb	Denaturation (see "Important considerations” above) Extension