Application Note

VeriFi® Library Amplification Mix: A robust PCR mix for difficult samples

Pedro Quintas, PhD, Constantine Garagounis, PhD, & Matteo Beretta, PhD

Introduction

Challenging contexts for PCR

PCR inhibition can be a serious challenge for molecular biologists. While in most cases good practice during sample extraction and PCR setup can minimise or eliminate inhibition, there are many situations where PCR inhibitors remain an issue. This can be because certain sample types are rich in inhibitory compounds, for instance those containing blood, tissue, or cellular debris, or because sample preparation has not been optimally performed. Additionally, there is an increasing interest in conducting PCR on crude samples, which, by definition, will likely contain PCR-inhibiting compounds. Clinical diagnostics, forensic testing, and environmental monitoring require PCR reagents that can withstand such inhibitors to provide reliable results.

Having an inhibitor-tolerant polymerase is therefore beneficial in overcoming this challenge. In order to identify the best polymerase for use in such challenging PCRs, we tested a number of commercially available proofreading polymerases (VeriFi® Hot Start Mix, NEBNext Ultra II Q5 Master Mix, and Quantabio repliQa Hifi ToughMix) against our newest proofreading mix, VeriFi® Library Amplification Mix, in the presence of multiple common PCR inhibitors found in blood samples and DNA extraction solutions.

Blood inhibitor tolerance – a critical advantage

DNA PCR testing on blood or blood-derived samples is used in a multitude of diagnostic tests and research settings. However, a number of components found in crude blood are inhibitors of PCR and these can easily contaminate DNA extracted from blood samples, if extraction is not carried out perfectly. Key PCR inhibitors found in blood include haematin, heparin and haemoglobin. All three of these compounds can inhibit PCR in different ways. Haematin, a byproduct of haemoglobin, affects the activity of DNA polymerases and can lead to decreased PCR efficiency or complete reaction failure. Haemoglobin, while not as potent an inhibitor, can bind DNA and affect DNA polymerase thermostability, which reduces overall amplification yields. While heparin, which is often used as an anticoagulant in blood samples, can interfere both with DNA polymerase activity and with binding of primers to their DNA target, thereby hindering PCR reactions. As such, developing polymerases that are resistant to, or at least tolerant to these inhibitors is useful in a clinical setting and in research based on blood-sample testing.

Broad-spectrum inhibitor resistance

We also tested the robustness of these polymerases against SDS, phenol, and urea, all of which can often be present in improperly extracted DNA samples, or as laboratory contaminants. SDS, a detergent, can denature proteins, including polymerases, and is a common contaminant in DNA extracted from tissues or cell lines. Phenol, used in DNA extraction, can carry over into PCR reactions, inhibiting amplification by enzyme denaturation or intercalation into DNA. Urea, a chaotropic agent, can unfold proteins, including polymerases, and is often present in urine-derived samples. Resistance to these inhibitors is advantageous for amplifications from a wide variety of biological and environmental

samples, clinical specimens, and complex mixtures, where such extraction contaminants can be prevalent.

Inhibitor resistance in proofreading polymerases is essential for successful amplification from complex samples. VeriFi® Library Amplification Mix showcases high resistance to all of the tested inhibitors. VeriFi® Library Amplification Mix's tolerance to these substances ensures successful amplification from forensic samples, biopsy tissues, blood spots and samples contaminated with common laboratory chemicals.

Method

PCR amplification was performed using mouse genomic DNA (10 ng / 20 µL reaction) as template and primers for a 2700 bp region of the GAPDH gene (Fwd: TGAAAGACAAGAAACAGGGGAGC; Rev: TGCTGTGTCACTACCGAAGAAC). The concentration of each primer was 400 nM for VeriFi® Hot Start Mix and VeriFi® Library Amplification Mix, 500 nM for NEBNext Ultra II Q5 Master Mix, and 300 nM for repliQa HiFi ToughMix, in accordance with each manufacturer's instructions. Different concentrations of inhibitors were added to the reaction mixes, according to Table 1.

Inhibitor	Concentrations in reaction
Haematin	5, 2, 1, 0.2 µM
Haemoglobin	2, 1, 0.5, 0.1 mg/mL
Heparin	200, 100, 50, 10 µg/mL
Phenol	0.2, 0.1, 0.05, 0.01 mM
SDS	0.05, 0.02, 0.01, 0.005 %
Urea	0.5, 0.2, 0.1, 0.05 M

Table 1. Concentrations of the tested inhibitors.

Cycling conditions were according to the respective manuals, so that each polymerase was tested under its recommended conditions. Following the PCR, the amplicons were quantified on an Agilent TapeStation using D5000 tapes.

Results

Challenging proofreading polymerases with common blood sample inhibitors

We tested the proofreading polymerases with three common blood sample inhibitors in order to evaluate the usefulness of each mix in testing blood and clinical samples (Figure 1).

Haematin: At 0.2 µM, all mixes perform comparably well. At increased concentrations of 1 and 2 µM, VeriFi® Library Amplification Mix shows no reduction in performance, and remains the most efficient among the tested mixes. At 2 µM and 5 µM, VeriFi® Library Amplification Mix has the highest yield of all, suggesting that this mix has better performance under higher concentrations of haematin compared to all the other mixes (Figure 1, left).

Haemoglobin: VeriFi® Library Amplification Mix exhibits tolerance comparable to other mixes at lower concentrations (0.1 and 0.5 mg/mL). However,

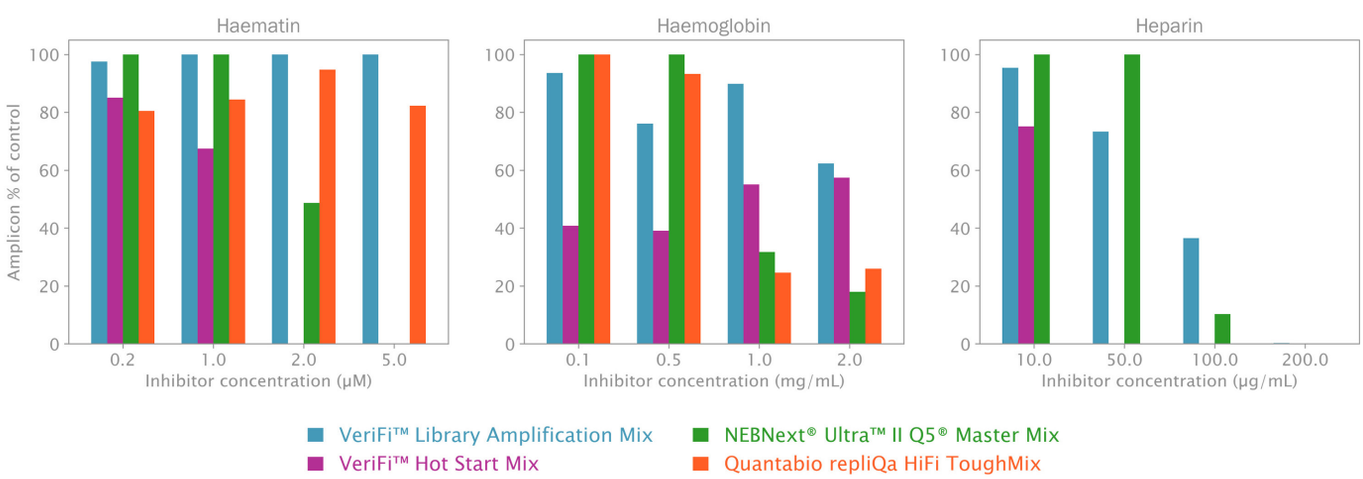

Figure 1. Amplification yield of proofreading polymerase mixes in the presence of common PCR inhibitors in blood samples.

PCRs were set up with the proofreading polymerase PCR mixes indicated in the figure above, as described in methods. Reactions were run in the presence of increasing amounts of the blood-derived PCR inhibitors indicated (X-axes). PCR yields were calculated for each mix and inhibitor concentration as a percentage of a positive control (uninhibited reaction) for each mix (Y-axes). VeriFi® Library Amplification Mix retains the highest yields at the highest inhibitor concentrations.

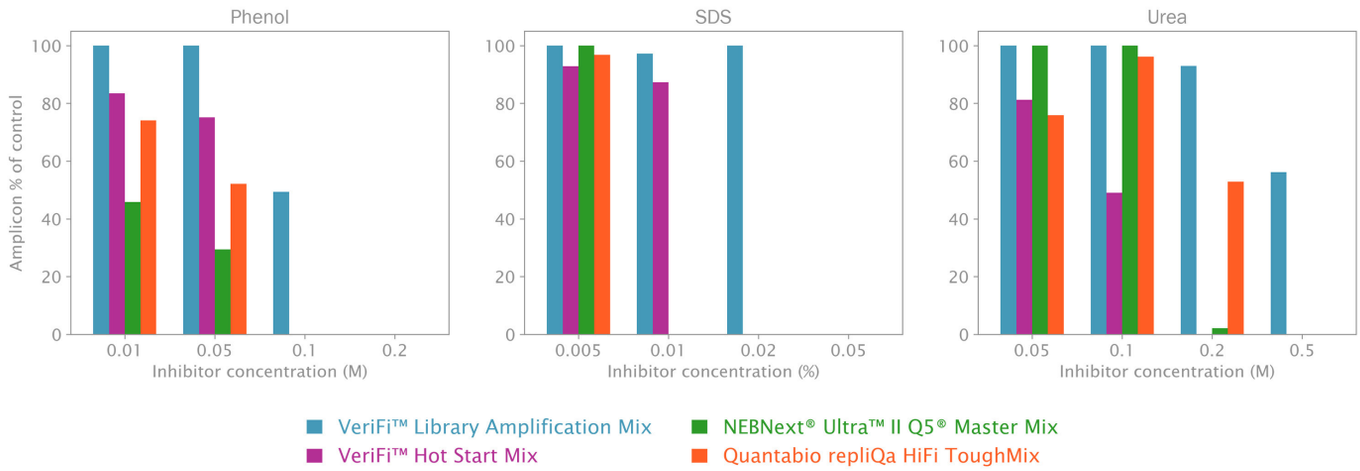

Figure 2. Amplification yield of proofreading polymerase mixes in the presence of common laboratory chemical PCR inhibitors.

PCRs were set up with the proofreading polymerase PCR mixes indicated in the figure above, as described in methods. Reactions were run in the presence of increasing amounts of the common chemical PCR inhibitors indicated (X-axes). PCR yields were calculated for each mix and inhibitor concentration as a percentage of a positive control (uninhibited reaction) for each mix (Y-axes). VeriFi® Library Amplification Mix retains the highest yields at the highest inhibitor concentrations.

for 1 mg/mL and 2 mg/mL haemoglobin, VeriFi® Library Amplification Mix again shows the least impact on amplification, maintaining higher levels than all other mixes (Figure 1, centre).

Heparin: Even at moderate heparin concentrations (10 and 50 µg/mL), VeriFi® Library Amplification Mix compares well with NEBNext Ultra II Q5 Master Mix (Green), which is the top performing mix at these lower inhibitor concentrations, suggesting minimal interference with polymerase activity. At 100 µg/mL heparin, VeriFi® Library Amplification Mix achieves better amplification rates than all other mixes, indicating greater resistance to heparin (Figure 1, right).

Given these results, we can conclude with confidence that VeriFi® Library Amplification Mix has a generally stronger tolerance to the blood-derived sample inhibitors haematin, haemoglobin, and heparin at the tested concentrations, compared to NEBNext Ultra II Q5 Master Mix, repliQa HiFi ToughMix, and VeriFi® Hot Start Mix.

Challenging proofreading polymerases with common extraction inhibitors

We further tested the proofreading polymerases with three common laboratory chemicals: phenol, SDS (sodium dodecyl sulfate) and urea. All three of these compounds are potent PCR inhibitors, and are often used in DNA extraction solutions. We chose these inhibitors in order to evaluate the reliability of each mix when using improperly extracted, or contaminated DNA samples in PCR workflows.

Phenol: At 0.01, 0.05, and 0.1 M phenol, VeriFi® Library Amplification Mix shows higher amplification yields compared to all other mixes. It is the only mix that retains any PCR yield at 100 mM phenol. These results indicate VeriFi® Library Amplification Mix has the best resistance to phenol (Figure 2, left).

SDS: At the lowest concentration of SDS (0.005%), VeriFi® Library Amplification Mix amplification efficiency is on par with other mixes. VeriFi® Library Amplification Mix was the only polymerase to retain activity at 0.02% SDS (Figure 2, centre). Thus, VeriFi® Library Amplification Mix displays strong resistance, suggesting it is suited for samples where residual SDS from lysis steps may be present.

Urea: VeriFi® Library Amplification Mix handles urea concentrations up to 0.2 M effectively, showing only a modest decrease in performance, compared to all of the other mixes. At the highest concentration (0.5 M), VeriFi® Library Amplification Mix's is the only mix to retain activity (Figure 2, right), indicating its reliability in PCR on urine samples or samples extracted with urea-containing solutions.

Taking these observations into account, we can say that the VeriFi® Library Amplification Mix generally displays a robust performance against Phenol, SDS, and urea. While it does experience some reduction in performance at higher concentrations

Inhibitor	Concentrations in reaction
Haematin	>5 µM
Haemoglobin	>2 mg/mL
Heparin	80 µg/mL
Phenol	100 mM
SDS	0.035%
Urea	0.5 M
Ethanol	6.5%
Guanidine	0.65%
Bilirubin	65 µg/mL
Humic Acid	> 2 µg/mL
Xylan	10 mg/mL

Table 2: Maximum inhibitor concentration at which VeriFi® Library Amplification Mix retains 50% yield of uninhibited reactions.

of these compounds, it is the only mix among those tested that retains activity, enabling it to maintain reasonable levels of amplification efficiency at higher concentrations of these inhibitors.

Tolerance to other inhibitors

While we primarily focused on the main inhibitors found in clinical or extracted samples, we also tested the impact of other common PCR inhibitors on VeriFi® Library Amplification Mix. In Table 2 we summarise the tested inhibitor concentrations at which VeriFi® Library Amplification Mix retains 50% PCR yield.

Conclusion

VeriFi® Library Amplification Mix has demonstrated superior performance in the presence of a wide array of PCR inhibitors. Its enhanced resistance to heparin, haematin, and haemoglobin makes it particularly suited for amplification from blood-derived samples, which is invaluable in clinical diagnostics and forensic applications. Its tolerance to SDS, phenol, and urea further extends its utility in PCR testing complex biological mixtures and poorly prepared DNA samples, where such contaminants can be common. Considering the robust inhibitor resistance profile, VeriFi® Library Amplification Mix stands out as a broad spectrum problem solver for difficult PCRs. It is the proofreading polymerase of choice for researchers facing the challenge of PCR amplification in the presence of diverse inhibitors, no matter what the downstream application is, from cloning to NGS library amplification.

Disclaimer & Product Use

This document has not been peer-reviewed.

NEBNext Ultra II Q5 Master Mix and repliQa HiFi ToughMix are trademarks or registered trademarks of New England Biolabs and Quantabio, respectively.

If you would like to discuss which products are best suited to your application, or need further technical advice on how to use VeriFi® Library Amplification Mix or VeriFi® Hot Start Mix, contact our team of experts at info@pcrbio.com.