SimpliFi HS Mix

Amplifying ALL of your DNAfor more accurate NGS results

SimpliFi HS Mix is a high-fidelity polymerase mix using aptamer-based hot-start technology, highly suited to amplification of DNA from crude samples, for fast, inexpensive target enrichment, NGS library amplification and cloning applications.

- **Robust:** optimized enzyme/buffer mix promotes reliable amplification of a broad range of targets, including complex DNA extracted from human, animal and plant samples
- **Specific:** the aptamer-based hot-start remains completely inactive during PCR set-up and after amplification, to prevent non-specific products
- **Optimized:** high yields with minimal optimization, regardless of a template's GC content, reducing time to results and eliminating the cost of unnecessary repeats
- **Enhanced accuracy:** higher than 130x Taq fidelity, reducing errors for next generation sequencing (NGS) library amplification.

SimpliFi HS Mix is a combination of the latest advances in buffer chemistry and PCR enhancers and stabilizers, together with an aptamer-mediated hot-start polymerase, dNTPs and $MgCl_2$. It has been designed for highly reproducible, accurate assay results in the presence of inhibitors. The advanced buffer chemistry and enhancers has been developed for fast PCR and is designed for superior sensitivity and specificity, making SimpliFi HS Mix perfect for NGS library amplification.

APPLICATIONS

- Gene expression
- Viral and bacterial detection
- Robust PCR
- High-specificity PCR
- High-fidelity PCR
- GC/AT-rich PCR

FIDELITY

SimpliFi HS Mix is a high-fidelity, high-efficiency hot-start PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The fidelity of the SimpliFi HS Mix was determined using a method designed by Lee D. *et al.* (2016)[1] and compared to commercially available high-fidelity master mixes (Fig. 1). The results are demonstrating the exceptional fidelity of SimpliFi HS Mix, offering higher level of confidence for preserving DNA sequence accuracy during the preparation of DNA for next-generation sequencing.

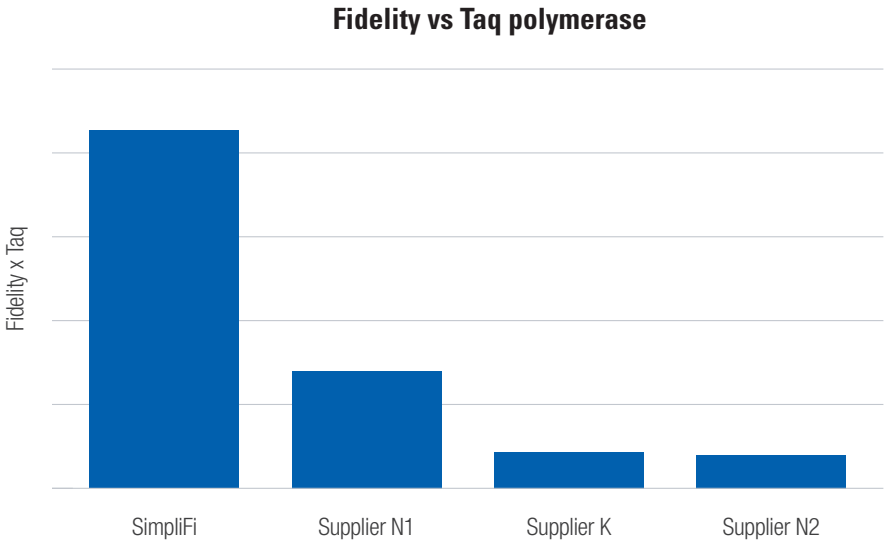

Fig 1. Fidelity comparison across commercially available high-fidelity polymerases.
Purified plasmid DNA was extended using SimpliFi, the complementary strands were synthesized using a standard high-fidelity polymerase, in both cases using primers containing a partial Illumina adapter, a random product barcode and a condition barcode. Primers complementary to the partial Illumina adapter are used to PCR amplify the complementary strands, forming the sequencing library. After next-generation sequencing, reads are grouped according to condition barcode and product barcode. Sequences are aligned to the correct sequence and errors are called. Errors are only kept if they are present in all copies, otherwise they are discarded as sequencing error. Exactly the same method was used to determine the error rate for supplier N1, Supplier K and Supplier N2 and the fidelity values were normalized to Taq polymerase fidelity.

HIGH YIELD AND SENSITIVITY

SimpliFi HS Mix is supplied as an optimized 2x master mix that only requires the addition of template and primers, thereby reducing the number of pipetting steps during PCR set-up, for improved speed, throughput, sensitivity (Fig. 2) and reproducibility. The optimized mix also makes SimpliFi HS Mix useful for crude samples such as 2% whole blood (Fig. 3).

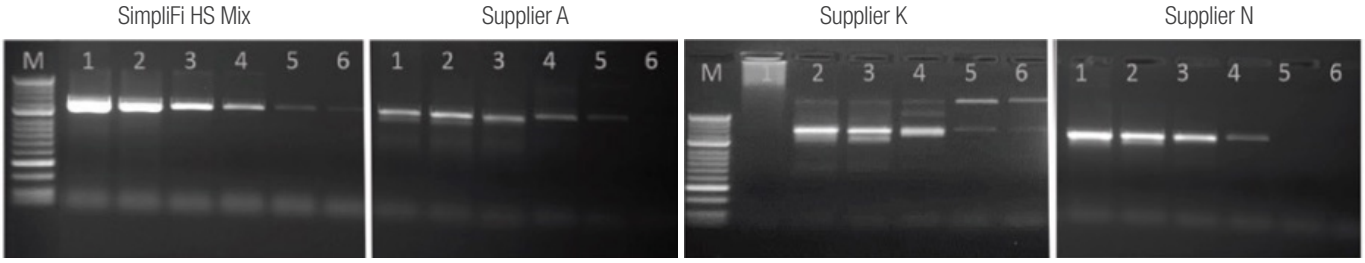

Fig. 2. High sensitivity and yield
SimpliFi HS Mix was compared with high-fidelity mixes from other suppliers for the amplification of a 1.2 kb fragment of the F8 (Coagulation Factor VIII) gene. A five-fold serial dilution of human genomic DNA was used as a template and all reactions were set up according to the manufacturers' recommendations. Marker is HyperLadder™ 50 bp, (BIO-33054). SimpliFi HS Mix delivers higher yield and sensitivity as compared with the competing products.

MULTIPLEXING

Optimizing the buffer and using an aptamer hot-start has also made SimpliFi HS Mix ideal for amplifying multiplex targets (Fig. 3), so that it can be used for high-throughput applications with limited starting material, such as genotyping and forensic analysis.

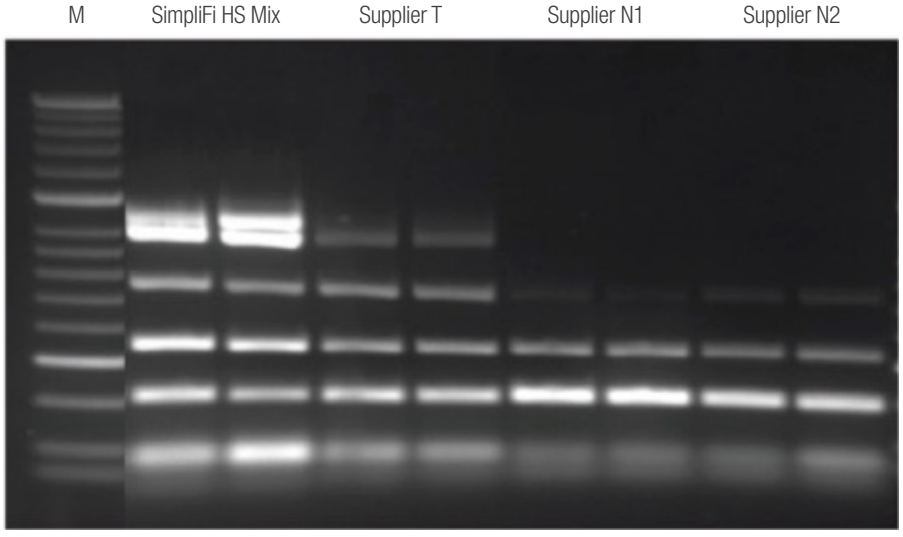

Fig. 3. Multiplexing with inhibitor-rich samples
Multiplexed PCR amplification was performed using 6 targets (961 bp, 892 bp, 793 bp, 548 bp, 332 bp and 196 bp) directly from 20% whole blood in EDTA, set up according to the manufacturers' recommended protocols. Marker is HyperLadder 50 bp, (BIO-33054). SimpliFi HS Mix is able to outperform other suppliers in the presence of 20% whole blood in EDTA in a multiplexed PCR reaction.

OPTIMIZED FOR LOW GC BIAS

PCR amplification can be susceptible to bias resulting from genomes that contain unusually high or low GC content, SimpliFi HS Mix has been developed to address this through careful selection of high-quality polymerase and optimization of the buffer (Fig. 4), making it ideal for NGS library preparation (Fig. 5).

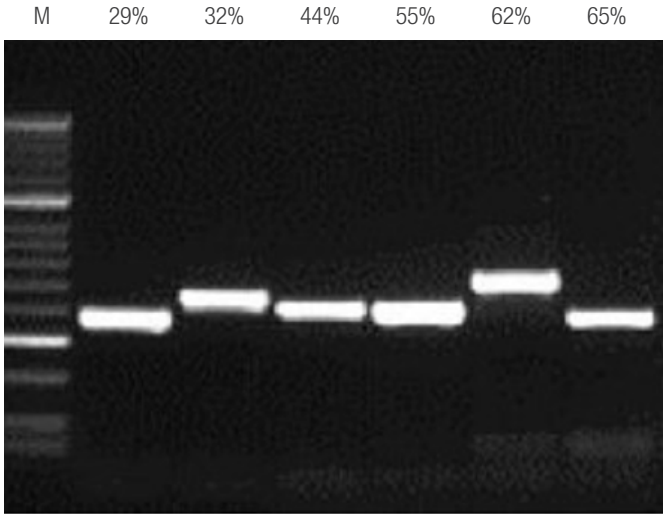

Fig. 4. Amplification of DNA containing different GC-content
SimpliFi HS Mix was used for the amplification of 6 targets: RBB8 (161 bp), RBB8 (450 bp), IL 10 (417 bp), Mpex (418 bp), EGFR (525 bp) and EGFR (380 bp) (29%, 32%, 44%, 55%, 62% and 65% GC rich respectively) from human genomic DNA. Marker is HyperLadder 50 bp, (BIO-33054). The results illustrate that SimpliFi HS Mix can be used for efficient amplification with DNA containing different GC content making SimpliFi HS Mix suitability for applications requiring low GC-bias.

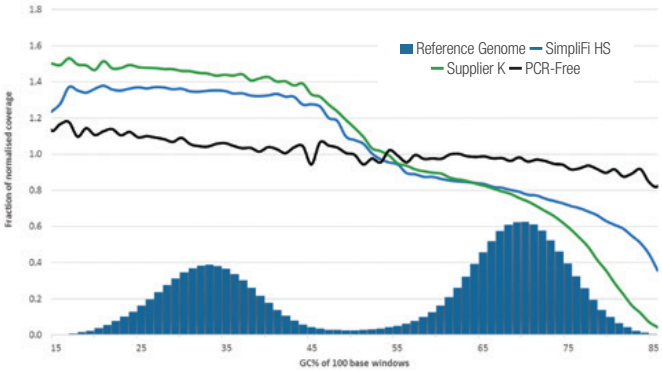

Fig. 5. GC bias with both high and low GC content
Libraries were prepared from 100 ng (amplified libraries) or 1 µg (PCR-free library) of genomic DNA from *S. aureus* (32% GC) and *R. spheroides* (69% GC), mixed equimolarly. End-repair and ligation steps were carried out using JetSeq™ ER and Ligation Kit (BIO-68026) and for all the samples. Library amplification consisted of 10 PCR cycles using SimpliFi HS Mix and a mix from supplier K, following the manufacturer's recommendations. GC bias plots were generated, with %GC content of 100 bp windows on the X axis. Normalized coverage is indicated for the High- SimpliFi HS Mix (blue line) and supplier K (green line) and can be compared to a PCR-free reference library (black line), which has the horizontal bias distribution closer to 1 (representing unbiased coverage). The results illustrate SimpliFi HS Mix has a lower bias across the entire range of GC content, compared to the PCR-amplified library from supplier K, especially for GC percentages higher than 65%.

Ordering Information

Product	Size	Cat. #
SimpliFi HS Mix (2x)	100 Reactions	BIO-25060
SimpliFi HS Mix (2x)	500 Reactions	BIO-25061

For related products such as library preparation kits, quantification kits and clean-up and selection beads visit **www.bioline.com**

[1] Lee D.F., *et al.* (2016) Mapping DNA polymerase errors by single-molecule sequencing. *Nucleic Acids Res.* 44(13): e118; PMID: 27185891

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