APPLICATION NOTE

Resistance of High-Fidelity DNA polymerases to the presence of magnetic beads

Summary

Considering the wide use of "on bead" PCR for several molecular approaches with important impact in biotechnology and biomedicine, the selection of a suitable DNA polymerase with high levels of resistance to the inhibitory effect of magnetic beads is key for the success of any experimental plan that requires this type of matrices. In this report, we show the remarkable performance of DeCodi-Fi™ 2X All-in-One mix to both Sera-Mag™ Carboxylate-Modified Magnetic Beads and Sera-Mag™ Streptavidin-Coated Magnetic Beads in comparison to commonly used high-fidelity DNA polymerases. Inhibition resistance is evident while amplifying Illumina genomic libraries, but also with challenging PCR fragments of up to 15 kb(Kilobases). Overall DeCodi-Fi™ shows up as an excellent and affordable option with a wide range of resistance to magnetic beads in different PCR scenarios.

Introduction

Magnetic beads are widely used in different molecular biology applications, not only in classic DNA and protein purification approaches but also in a variety of downstream techniques, including standard polymerase chain reaction (PCR), emulsion PCR (Siu RHP., et al. 2021); digital droplet PCR (ddPCR)(Gu Z., et al. 2022); microfluidic PCR (Hilton JP., et al. 2012; Delley CL., et al. 2021); DNA library preparation and enrichment (Lyander A., et al. 2024), next generation sequencing (NGS) (McEvoy CR., et al. 2020); genotyping (Zhu J., et al. 2014); cell-free protein synthesis (Gan R., et al. 2008; Lee KH., et al. 2012), among others. Results are particularly relevant when low DNA input and/or low DNA quality are involved (Cravero K., et al. 2018; Lyander A., et al. 2024).

For those reasons, the choice of a DNA polymerase with high-fidelity and also a wide range of resistance to magnetic beads of different natures is critical for all bead-based PCR approaches that need this type of material. In this report, we show a comparative study of inhibition resistance of 4 high-fidelity DNA polymerases available in the market against two types of paramagnetic beads. The results show that DeCodi-Fi™ 2X All-in-One mix is highly resistant while showing the best performance among all the compared enzymes for the amplification of a long amplicon. Furthermore, DeCodi-Fi™ shows robust and consistent amplification of an Illumina fragmented gDNA library in a wide range of magnetic bead loads.

Results

High-fidelity DNA polymerase performance for the amplification of 15 kb lambda (λ) genome in the presence of paramagnetic beads.

The first comparison was performed using Sera-Mag™ Carboxylate-Modified Magnetic Beads in a gradient from 25 µg to 500 µg of beads. DeCodi-Fii™ 2X All-in-One shows the best performance among all the high-fidelity enzymes evaluated, with a resistance range between 25 µg to 300 µg of Carboxylate-Modified Magnetic Beads, with no evident yield compromise between 25 µg to 200 µg. Followed by Kapa HiFi Hot Start ReadyMix(Roche) with a resistance range between 25 to 100 µg of beads. Platinium SuperFi II (Thermofisher) and Q5 Hot Start High Fidelity(NEB) show the highest bead sensitivity. SuperFi II shows a good performance in control without beads, but the efficiency is notoriously affected at 25 µg and no product is observed with ≥50 µg of beads. On the other hand, Q5 showed the lowest yield among the 4 evaluated enzymes in the absence of magnetic beads, and no evident signal of PCR product at ≥25 µg of magnetic beads (Figure 1).

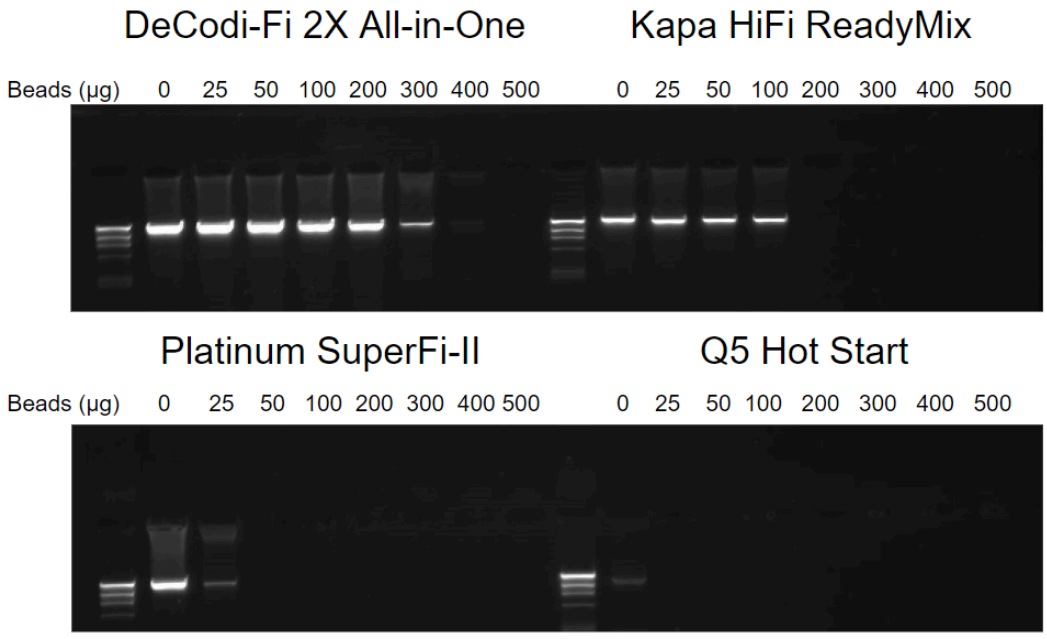

Figure 1: Effect of increasing amounts of Sera-Mag™ Carboxylate-Modified Magnetic Beads on PCR performance of high-fidelity enzymes.
15 kb DNA amplification was performed using 2 ng of λ genomic DNA as a template in a final volume reaction of 50 µL in the presence of increasing amounts of Sera-Mag™ Carboxylate-Modified Magnetic Beads. The gradient considers a range including 25-50-100-150-200-300-400 and 500 µg of magnetic beads. 1 µL of PCR reactions using DeCodi-Fii™ 2X All-in-One, Kapa or SuperFi II, were diluted and loaded in the gel. 10 µL of PCR reactions using Q5 were loaded in the gel. The gel shows one representative replicates from three independent PCR reactions.

Due to the fact that the enzymes with the best performance were DeCodi-Fi™ and Kapa ReadyMix, both were selected for resistance evaluation to Sera-Mag™ Streptavidin-Coated Magnetic Beads in the same gradient conditions. The results show that there is more sensitivity to this type of magnetic bead versus the carboxylate version for both enzymes while amplifying the same 15 kb product. DeCodi-Fi™ 2X All-in-One is resistant to 25 µg of beads with no effect on yield and a more variable performance from ≥50 µg but with partial resistance up to 200 µg of streptavidin magnetic beads with just 1 µL of PCR reaction volume loaded in the gel. Kapa ReadyMix shows no amplicon at ≥25 µg for Streptavidin beads, even with 5 µL loaded in the gel (Figure 2).

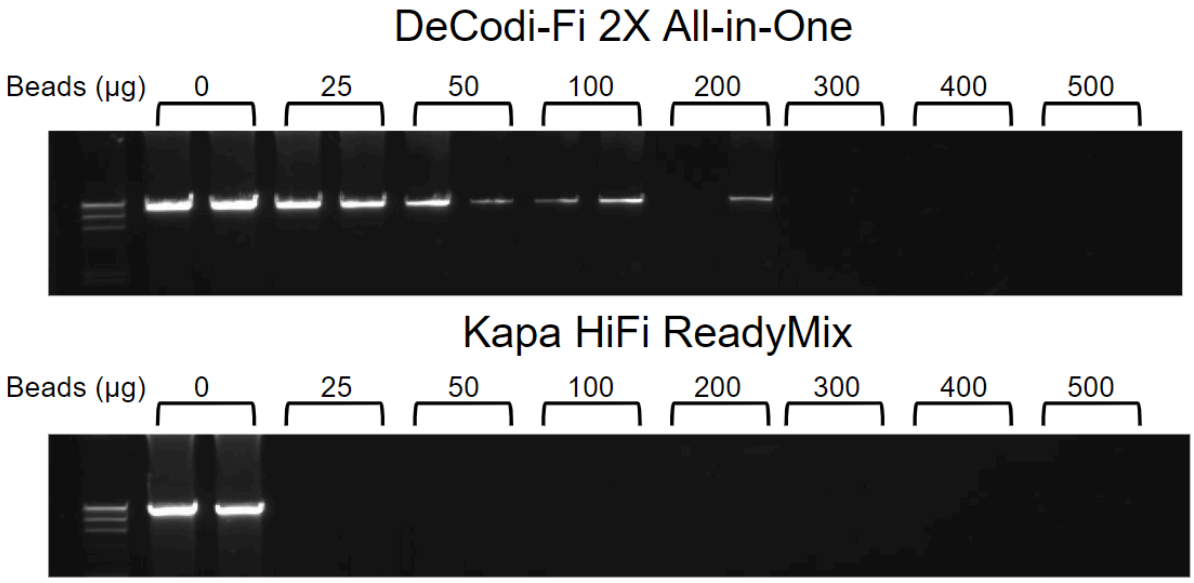

Figure 2: Effect of increasing amounts of Sera-Mag™ Streptavidin-Coated Magnetic Beads on PCR performance of high-fidelity enzymes.
15 kb DNA amplification was performed using 2 ng of λ genomic DNA as a template in a final volume reaction of 50 µL in the presence of increasing amounts of Sera-Mag™ Streptavidin-coated Magnetic Beads. 1 µL of PCR reactions using DeCodi-Fi™ 2X All-in-One were diluted and loaded in the gel. 5 µL of PCR reactions using Kapa ReadyMix were diluted and loaded. The gel shows two replicates from each condition.

DeCodi-Fi™ 2X All-in-One mix performance in genomic library amplification

It is important to note that the remarkable performance of DeCodi-Fi™ 2X All-in-One in comparison to other market options was evidenced in a challenging 15 kb long PCR. However, most applications deal with shorter DNA amplicons. A widely used example of this is DNA library amplification for downstream Illumina Next-generation Sequencing. To evaluate the performance of DeCodi-Fi™ 2X All-in-One in that

context, an *Escherichia coli* gDNA library was generated and amplified under specific cycling conditions (Table 4) in the presence of increasing amounts of Sera-Mag™ Carboxylate-Modified Magnetic Beads or 500 µg of Sera-Mag™ Streptavidin-Coated Magnetic Beads. The results show a consistent and high-yield amplification of the libraries in all conditions using DeCodi-Fi™ 2X All-in-One when compared to the no-bead control (Figure 3). This also shows that Decodi-Fi™ can tolerate even greater amounts of beads for shorter fragment amplification (~250 bp in this case) compared to the previous 15 kb.

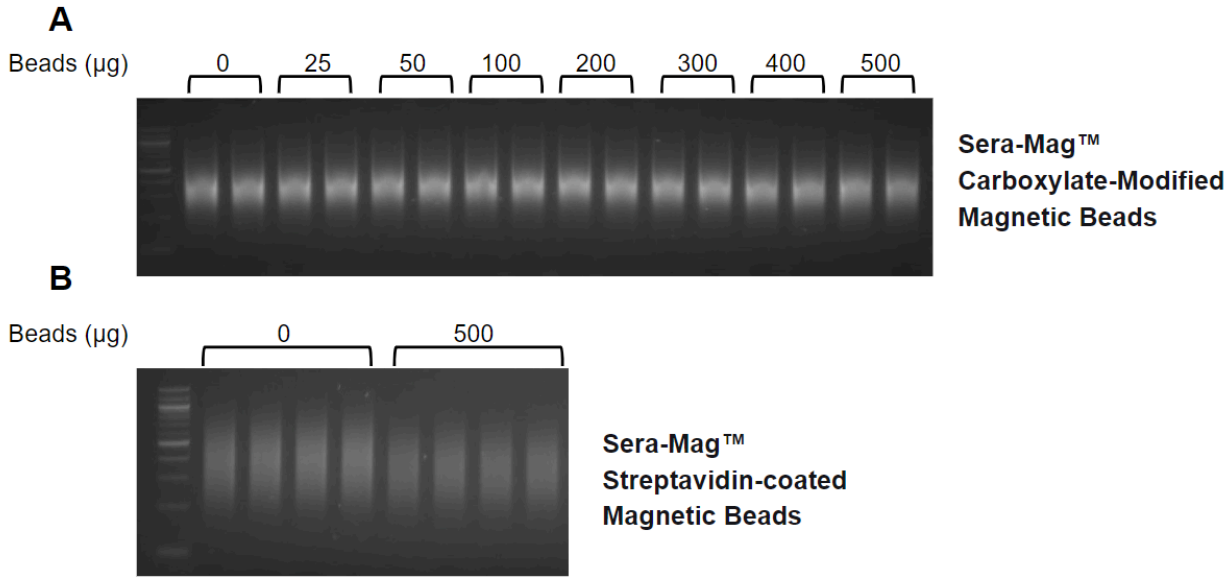

Figure 3: DeCodi-Fi™ 2X All-in-One amplification of Illumina fragmented gDNA libraries in the presence of Sera-Mag™ Carboxylate-Modified Magnetic Beads and Sera-Mag™ Streptavidin-Coated Magnetic Beads.

E. coli library amplification by DeCodi-Fi™ 2X All-in-One was performed using 2 ng of DNA template in a final reaction volume of 50 µL. **A)** PCR reaction in the presence of increasing amounts of Sera-Mag™ Carboxylate-Modified Magnetic Beads, results are shown in duplicate. **B)** PCR reaction in the absence and presence of 500 µg of Sera-Mag™ Streptavidin-coated Magnetic Beads, results are shown in four replicates for each condition.

Conclusion

This application note demonstrates the remarkable resistance of DeCodi-Fi™ to the inhibitory effects of magnetic beads within the PCR reaction, particularly towards Carboxylate and Streptavidin Beads. The results highlight DeCodi-Fi's superior performance compared to other high-fidelity DNA polymerases available in the market, especially in amplifying challenging PCR fragments up to 15 kb even in the presence of beads. DeCodi-Fi™ consistently exhibited robust amplification across a wide range of bead concentrations, proving to be an excellent choice for various PCR applications that involve magnetic beads.

Materials and Methods

Magnetic Beads concentration gradient: With the aim of evaluating the effect of paramagnetic beads presence in the PCR performance of the different high-fidelity enzymes, two types of paramagnetic beads were used. Sera-Mag™ Carboxylate-Modified Magnetic Beads and Sera-Mag™ Streptavidin-Coated Magnetic Beads, both from Cytiva. Beads were properly washed, diluted, and stored. Increasing amounts of each type of bead were used, in a gradient from 25 to 500 µg/ 50 µL reaction (considering 25-50-100-150-200-300-400 and 500 µg). Before the addition of PCR mixes a specific amount of magnetic beads was added in each 0.2 mL PCR tube in a magnetic rack and the magnetic beads storage buffer was removed and washed with nuclease-free water two times.

Long 15 Kb λ genome PCR:

DeCodi-Fi™ 2X All-in-One mix, Kapa readymix, Superfi II, and Q5 high-fidelity enzymes were used to amplify 2 ng of λ DNA template (NEB cat. No.N3011S) in a 50 µL total volume reaction, using a 25 cycles PCR protocol in a Veriti™ Dx 96-well Thermal Cycler, following the cycling conditions specified in table 1. The primer sequence used for λ genome amplification, including primer forward and reverse is specified in table 2.

Table1. 15 kb λ PCR cycling conditions.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	3 minutes	1
Denaturation	95°C	30 seconds	25
Annealing	60°C/62°C*	15 seconds	
Extension	72°C	7 minutes	
Final extension	72°C	5 minutes	1

* 60°C was used for SuperFi II, 62°C was used for DeCodi-Fi 2X All-in-One mix, Kapa ReadyMix and Q5 DNA polymerases.

Cycling conditions in terms of annealing and extension time were selected considering each provider's specifications.

Table 2. Primer sequence and genome position for 15 kb λ amplification.

Primer name	Primer sequence	Genome Position	Amplicon length (bp)
15 kb λ forward	5`-C*CGCACCATTACGTCTGCAAAC*A*T*C-3`	15807	15682
15 kb λ reverse	5`-G*CGTGAAGGCCTGCATTATGTC*G*T-3`	31489	

*phosphorothioate-modified nucleotides.

PCR mastermix for DeCodi-Fi™ 2X All-in-One mix was made following the final PCR mix composition specified in Table 3.

Table 3. PCR mix composition for 15 kb λ amplification.

Component	Volume (50 µL reaction)	Final composition
DeCodi-Fi 2X All-in-One mix*	25 µL	1X
λ Template (1 ng/µL)	2 µL	2 ng
15 kb λ primer mix forward+reverse (5 µM each)	2 µL	0.2 µM
Nuclease-Free Water	21 µL	

* provides dNTPs mix and Mg2+ (at 2 mM final concentration).

For Kapa readymix, SuperFi II, and Q5 high-fidelity enzymes, master mixes were made following each provider's specifications.

1µL of PCR product using DeCodi-Fi™ 2X All-in-One mix and variable volumes (based on the difference in the yield obtained) from PCR product using competitors enzymes were loaded in a 0.7% agarose gel and a suitable volume of lambda *Hind*III

digest DNA ladder (NEB Cat. No N3012) was also loaded for size comparison. Gel electrophoresis was run for 40-50 minutes and agarose gels were visualized in a CLiNiX UV transilluminator imaging system.

Escherichia coli (*E. coli*) genomic library amplification

An *E. Coli* genomic DNA (gDNA) library with final fragment size of approximately 250 bp was generated using IDT xGen™ DNA Library Prep EZ with 500 ng of gDNA as starting material, following the provider specifications. 2 ng of purified library was used as template in a 50 µL total volume reaction, using a 14 cycles PCR protocol in a Veriti™ Dx 96-well Thermal Cycler, following the cycling conditions specified in table 4. The forward and reverse primers used for library amplification (i5 and i7 primers, respectively) were synthesized using the sequences developed by Quail and collaborators (Quail MA., et al. 2024).

Table 4. *E. coli* gDNA library PCR cycling conditions.

Step	Temperature	Time	Cycles
Initial denaturation	98°C	2 minutes	1
Denaturation	98°C	20 seconds	14
Annealing	60°C	15 seconds	
Extension	72°C	1 minute	
Final extension	72°C	2 minutes	1

PCR mastermix for DeCodi-Fi™ 2X All-in-One mix was made following the final PCR mix composition specified in Table 5.

Table 5. PCR mix composition for *E. coli* gDNA library amplification.

Component	Volume (50 µL reaction)	Final composition
DeCodi-Fi™ 2X All-in-One mix	25 µL	1X
E.coli library (1 ng/µL)	2 µL	2 ng
i5/i7 primer mix forward+reverse (5 µM each)	2 µL	0.2 µM
Nuclease-Free Water	21 µL	

3 µL or 1 µL of PCR product using DeCodi-Fi™ 2X All-in-One mix were mixed with nuclease-free water and DNA loading dye and loaded in a 2.0 % agarose gel, for libraries amplified in Sera-Mag™ Carboxylate-Modified Magnetic Beads or Sera-Mag™ Streptavidin-Coated Magnetic Beads, respectively. A suitable volume of

100 bp DNA ladder (NEB Cat. N3231S) was also loaded for size comparison. Gel electrophoresis was run for 40 minutes and agarose gels were visualized in a CLiNiX UV transilluminator imaging system.

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