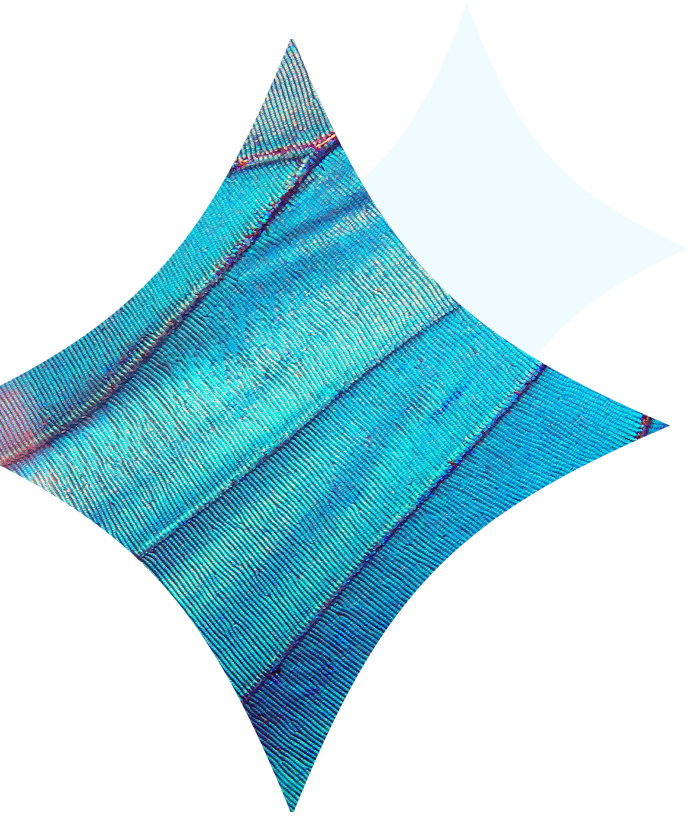
APPLICATION NOTE

Comparative analysis of size bias in PCR Multiplex amplification using DeCodi-Fi™ vs commercial HiFi polymerases



Goal

To evaluate the performance of DeCodi-Fi™ High-Fidelity Polymerase in multiplex PCR compared with four commercial HiFi polymerases, focusing on reducing size bias across eight DNA fragments from 100 bp to 10 kb, achieving balanced amplification of both short and long amplicons, and demonstrating the closest preservation of the original pool distribution through cosine dissimilarity analysis, thereby supporting its suitability for applications requiring uniform amplification such as multiplex long-range PCR and NGS library preparation.

Application Benefits

- DeCodi-Fi™ delivers more **uniform amplification** across fragment sizes (100 bp–10 kb) compared with commercial HiFi polymerases.
- DeCodi-Fi™ achieves **higher yields for long targets**, including the 10 kb fragment, without sacrificing performance on shorter fragments.
- DeCodi-Fi™ preserves the original pool distribution with the **lowest amplification bias**, ensuring reliable results for multiplex PCR, long-range PCR, and NGS library preparation.

This study was performed by Javier Gimpel and Jennifer Hsieh from Kura Biotech.
Please email sales@blikka.com for more information.
Formats available: DeCodi-Fi™ 2X All-in-One Mix, DeCodi-Fi™ High-Fidelity PCR kit.

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Background

PCR amplification is well known for its size bias, particularly when amplifying multiple targets of varying lengths in a single reaction. Shorter fragments are typically amplified more efficiently than longer ones, resulting in imbalanced representation, or, in some cases, complete dropout of larger targets [1-4]. To ensure accurate and comprehensive amplification, a solution that minimizes size bias is needed.

Objective

To evaluate and compare the **size bias profiles of DeCodi-Fi** and four commercial HiFi polymerase kits by analyzing their performance in **amplifying a multiplexed set of eight DNA fragments ranging from 100 bp to 10 kb**, using both mass- and molarity-normalized input pools. The goal is to determine which polymerase achieves the most uniform amplification across fragment sizes, thereby indicating the lowest amplification bias.

Methods

Amplification and tailing of individual targets:

Eight primer pairs were designed to target a range of selected fragment sizes on the lambda phage genome: 100 bp, 250 bp, 500 bp, 1 kb, 2 kb, 4 kb, 8 kb, and 10 kb. Each primer pair was tailed with universal ends, allowing amplification of the entire pool using a single pair of primers to control for primer bias. Individual target amplifications result in eight fragments, each extended by 67 bp to include the universal tails on both the 5′ and 3′ ends.

Pooling of targets:

Individual fragments were quantified using the BioTek Take 3 microvolume plate. The fragments were then pooled in two different ways. One pool was made based on equivalent mass, where each fragment was normalized to 10ng/ul, and then pooled in equal volumes, resulting in 1.25ng/ul of each fragment in the pool. A second pool

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was made based on equivalent molarity, where fragments were normalized to 1nM and then pooled in equal volumes, resulting in 0.125nM of each fragment in the pool.

Amplification of combined targets:

Master mixes for DeCodi-Fi and four other commercial HiFi competitors were prepared using 1 ng of input per reaction from the mass-equivalent pool, and 0.1 nM input from the molarity-equivalent pool. PCR conditions were identical for all kits: initial denaturation at 95 °C for 30 seconds, followed by 30 cycles of denaturation at 95 °C for 10 seconds, annealing at 68 °C for 30 seconds, and extension at 72 °C for 5 minutes, with a final extension at 72 °C for 5 minutes.

Gel electrophoresis and image analysis:

Amplification products were separated by gel electrophoresis using 0.7% agarose, visualized with GelRed, and documented with a Clinx gel documentation system. Relative intensity values were obtained from the gel image using IOCBIO Gel version 1.0.3.

Bias calculations:

In order to quantify the bias caused by amplification compared to the initial differences in quantity between the bands in each of the pools, we calculated the Cosine Dissimilarity value for each polymerase treatment against the unamplified pools. Cosine Dissimilarity measures the cosine of the angle between two intensity vectors and focuses on the similarity of the pattern of the intensities [5], i.e., how the amplification treatments changed the pattern of intensities of the bands as compared to the original relative intensities of the unamplified pools. A larger cosine dissimilarity value (closer to 1) indicates greater divergence from the unamplified pool, and thus greater size bias.

The formula to calculate cosine dissimilarity is as follows, where (E_i) is the mean intensity of the (i)-th measurement in the experimental group, and (C_i) is the mean intensity of the (i)-th measurement in the control group.

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$$\text{Cosine Similarity} = \frac{\sum_{i=1}^{8} E_i \cdot C_i}{\sqrt{\sum_{i=1}^{8} E_i^2} \cdot \sqrt{\sum_{i=1}^{8} C_i^2}}$$

$$\text{Cosine Dissimilarity} = 1 - \text{Cosine Similarity}$$

Results:

Amplification performance of DeCodi-Fi and Q5 was evaluated using a bioanalyzer. Results show that DeCodi-Fi produces stronger and more uniform peaks across all eight fragment sizes (100 bp–10 kb) compared to Q5. Notably, DeCodi-Fi yields a significantly stronger 10 kb peak, as well as enhanced amplification across most targets, indicating reduced size bias and more balanced performance.

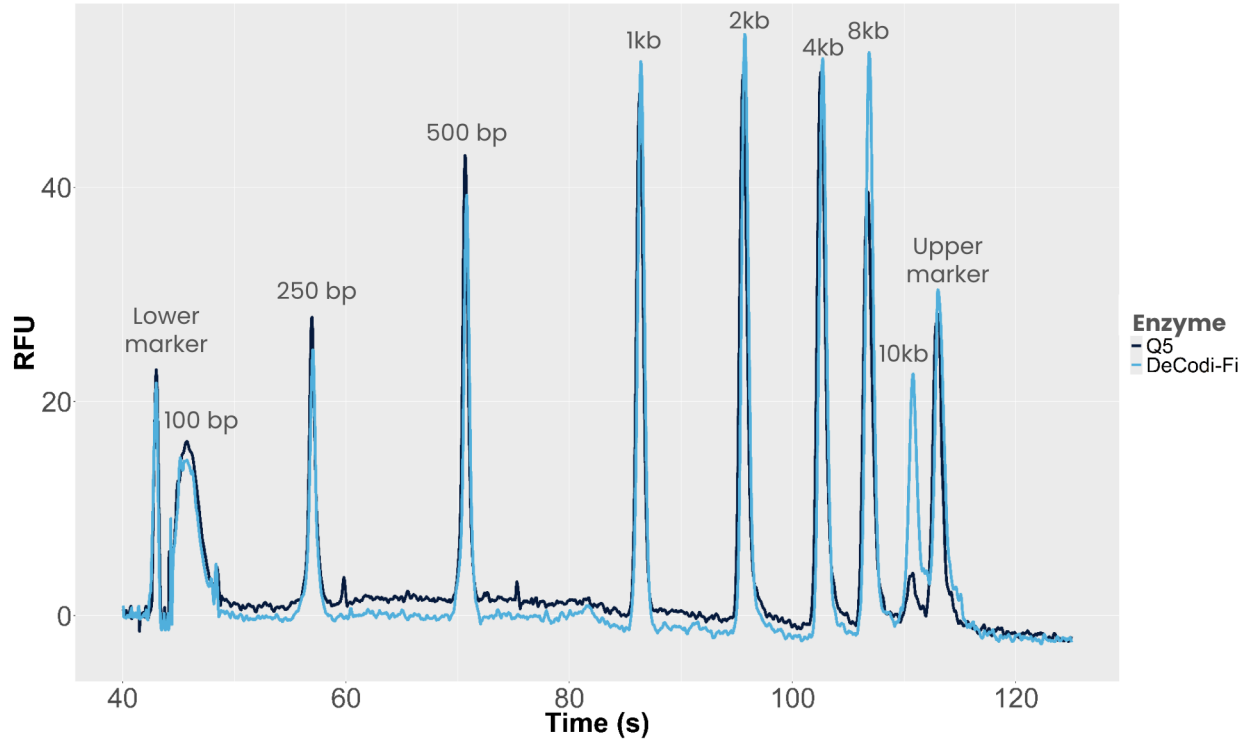

Figure 1. Amplification profiles of DeCodi-Fi and Q5 across a multiplexed 8-fragment pool (100 bp–10 kb), analyzed by bioanalyzer after 30 PCR cycles. Electropherogram traces represent fluorescence intensity (FU) as a function of migration time for DeCodi-Fi (light blue) and Q5(dark blue).

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To further benchmark DeCodi-Fi's performance, we compared it to additional commercial polymerases, Kapa HiFi, SuperFi II, and Phusion, using a multiplexed pool of 8 fragments and gel electrophoresis. Gel images showed that **DeCodi-Fi consistently amplified all targets across a wide size range (100 bp to 10 kb) with more balanced intensity**, both by equal mass and molarity inputs (Figures 1 & 2). In contrast, Kapa HiFi showed a high level of size bias and underperformed, particularly with larger fragments.

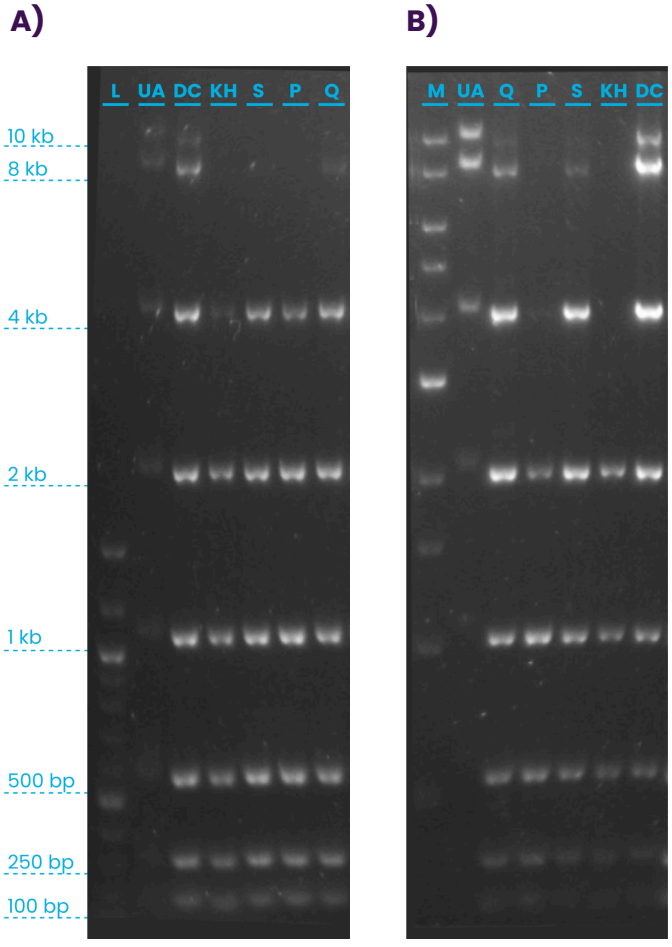

Figure 2. Amplification of 8 DNA fragments from (A) mass-normalized pools and (B) molarity-normalized pools.
Agarose gel images comparing the amplification of 8 DNA fragments using DeCodi-Fi and other polymerases, from pools normalized either by mass (A) or molarity (B) **L:**100 bp ladder (NEB), **M:**1kb ladder (NEB). **UA:** Unamplified pool (10 ng loaded in A, 5 nM in B). **DC:** DeCodi-Fi, **KH:**Kapa HiFi, **S:** SuperFi II, **P:** Phusion, **Q:**Q5.

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Quantitative band intensity analysis confirmed these trends (Figure 3). **DeCodi-Fi yielded the highest intensity** compared to all competitors for the 10 kb fragment while maintaining uniform amplification across other sizes. Other enzymes, especially Kapa HiFi, showed low amplification beyond 2 kb.

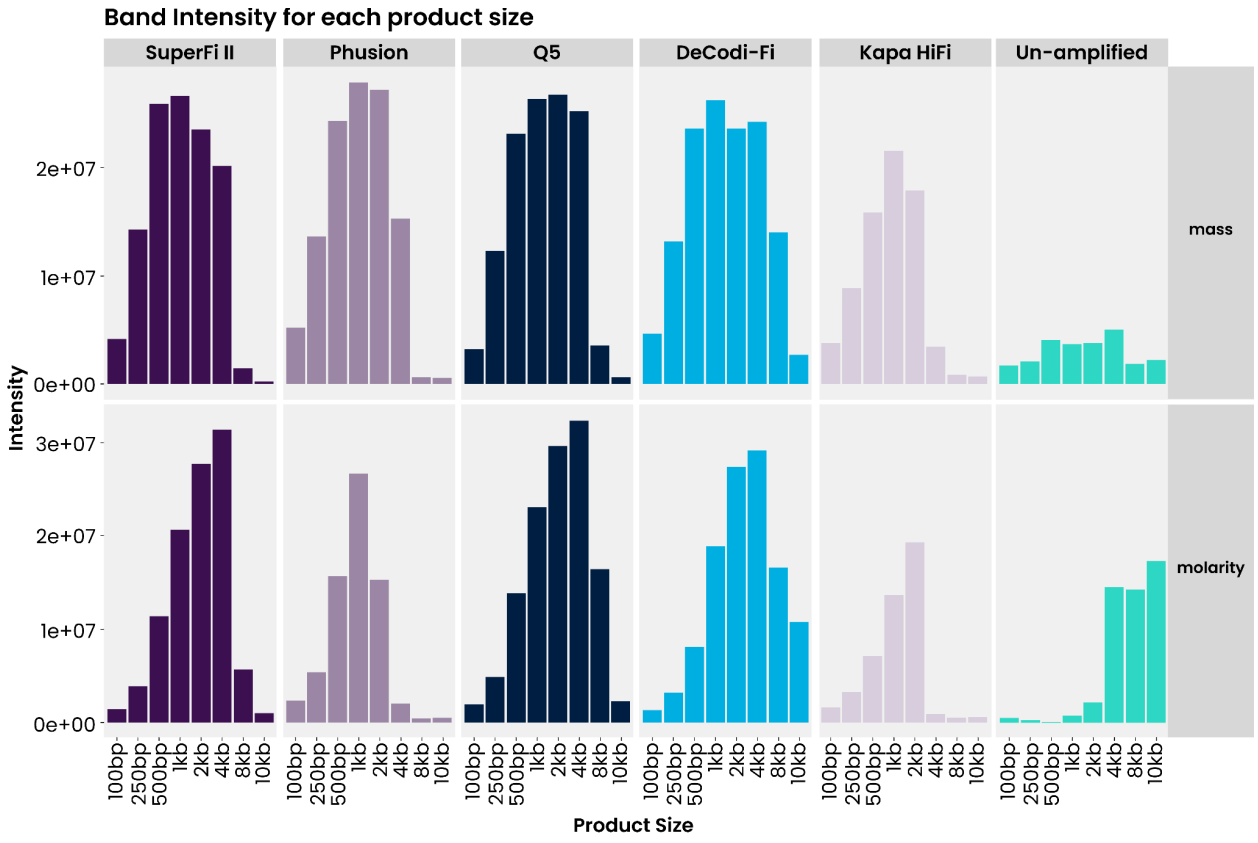

Figure 3: Band intensity for each fragment size, extracted from gel images. The top row presents results from pools normalized by equivalent mass, while the bottom row shows results from pools normalized by equivalent molarity. All polymerases displayed a positive bias toward mid-sized fragments (500 bp to 2 kb) and a negative bias against the shortest and longest fragments. However, DeCodi-Fi consistently outperformed the other kits, achieving the highest yield for the 10 kb fragment and comparable yields across all other sizes. In contrast, Kapa HiFi showed limited performance beyond 2 kb.

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Cosine dissimilarity analysis comparing amplified products to unamplified pools (Figure 4) further confirmed DeCodi-Fi's higher uniformity. Among all polymerases tested, DeCodi-Fi showed the lowest dissimilarity values for both mass- and molarity-normalized pools, indicating the least amplification bias and the best preservation of the original fragment distribution.

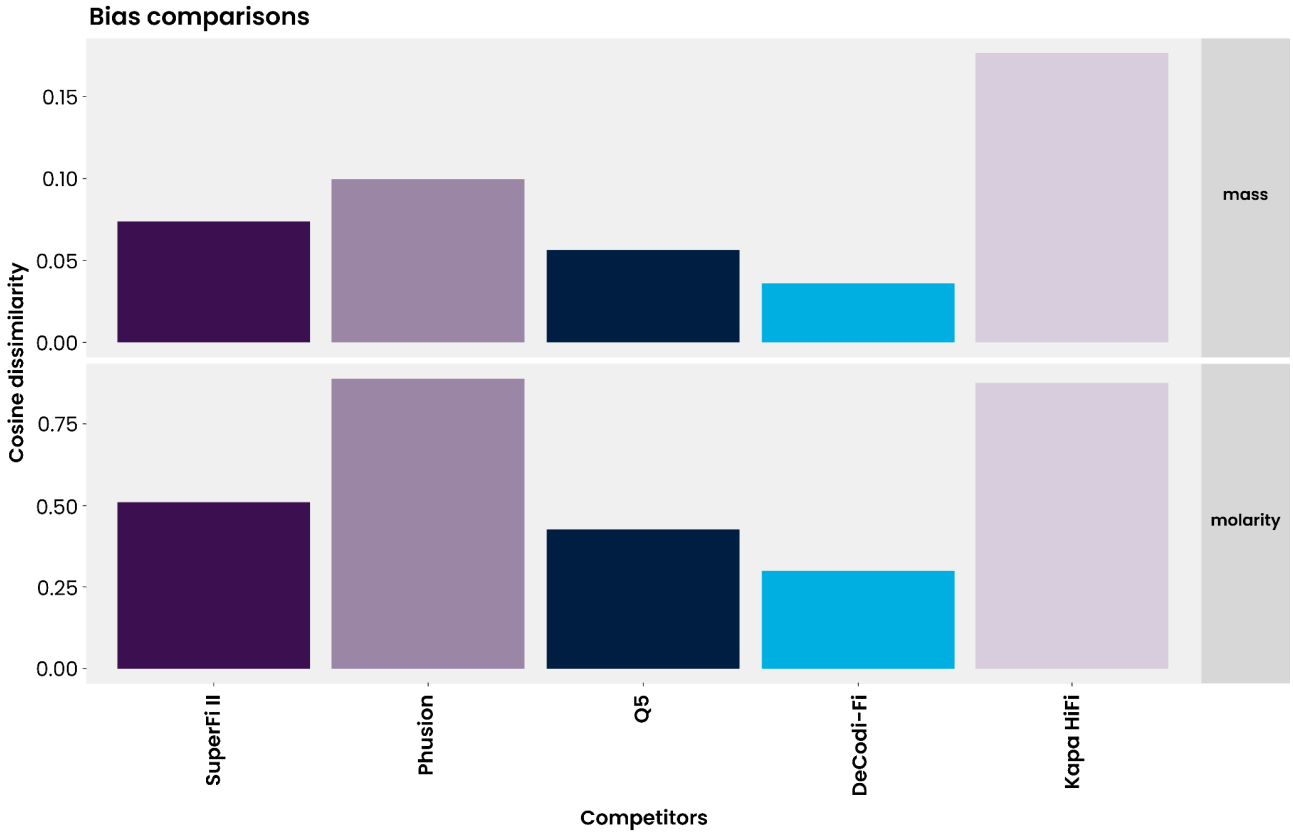

Figure 4: Cosine dissimilarity values comparing the magnitude of bias across polymerases, as compared to unamplified pools. Kapa HiFi produced higher cosine dissimilarity values, indicating greater divergence from the unamplified pools and has among the highest size bias, together with Phusion. In contrast, Decodi-Fi produced the lowest cosine dissimilarity values in the amplification of both types of pools, indicating the lowest size bias as compared to competitors.

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Conclusions

In conclusion, this comparative study demonstrated that while size bias is an inherent challenge in PCR amplification, DeCodi-Fi consistently outperformed the other commercial polymerase kits tested. DeCodi-Fi achieved the highest yields for the 10 kb fragment and equivalent or better yields for all other fragments, resulting in a more uniform and accurate amplification profile. The cosine dissimilarity analysis further confirmed DeCodi-Fi's higher performance, showing values most closely aligned with those of the unamplified pools. This makes DeCodi-Fi a robust and reliable choice for applications demanding unbiased amplification of a wide range of fragment sizes, particularly in long-range PCR and NGS library preparation.

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