Spotlight

Performance of Herculase II Fusion DNA Polymerase in NGS

DNA library preparation methods for next-generation sequencing (NGS) often require PCR amplification. GC and length biases can be introduced into NGS libraries by PCR amplification, which can be detrimental to high-throughput sequencing methods and reporting.

A study by Jesse Dabney and Matthias Meyer highlights the performance of ten different DNA polymerases, including Agilent Herculase II Fusion and Pfu Turbo Cx HotStart polymerases.[1] It was performed to determine whether use of alternate polymerases can impact GC-content and DNA fragment length biases.[1]

The DNA polymerases were used to amplify DNA libraries from human B-lymphocytes across 15, 25, and 40 PCR cycles. The libraries were then sequenced on an Illumina GAII sequencing platform. The median length of the original input DNA was 185 bp.[1]

Herculase II Fusion DNA Polymerase outperforms nine competitor polymerase buffer systems in GC-content and length distributions of a single human sequencing library

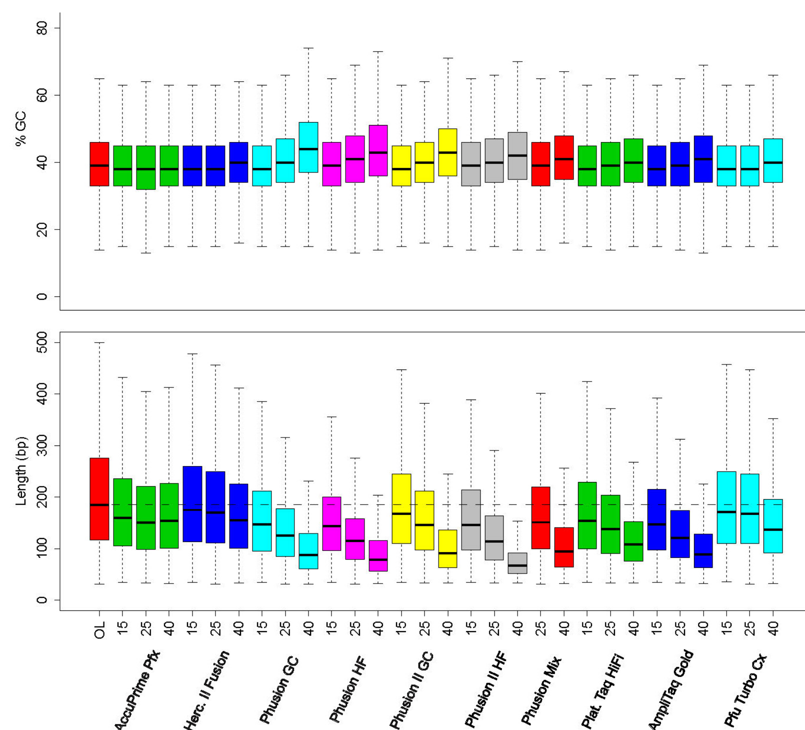

Figure 1. GC-content and length distributions of a single sequencing library amplified with 10 different polymerase-buffer systems. Distributions are in ascending cycle number order (15, 25 and 40) and color coded by polymerase. Boxes (inter quartile range, IQR) represent the middle 50% of the data. Median values are denoted by black lines through the IQR, and whiskers extend to 1.5 IQR. The original, unamplified library is depicted in the left-most red column. The dotted line across the length plot represents the median value of the original library. Distributions for Phusion Mix 15 cycles were excluded due to insufficient sequence retrieval.*

Description	Part number
Herculase II Fusion DNA Polymerase, 40 rxn	600675
Herculase II Fusion DNA Polymerase with dNTP, 200 rxn	600677
Herculase II Fusion DNA Polymerase with dNTP, 400 rxn	600679
Pfu Turbo Cx Hotstart DNA Polymerase, 100U	600410
Pfu Turbo Cx Hotstart DNA Polymerase, 500U	600412
Pfu Turbo Cx Hotstart DNA Polymerase, 1000U	600414

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The group observed the following results:

Post-15 PCR Cycles:

The GC-content for all libraries was maintained within 1% of the original input of 39%. All polymerases reduced the length of DNA fragments. While other commercial polymerases reduced the length of DNA fragments by an average of 38 to 41 bp, Herculase II Fusion polymerase showed the least fragment length reduction, an average decrease of only 10 bp.[1] Performing nearly as well, Pfu Turbo Cx HotStart polymerase showed an average length decrease of 14 bp.[1]

Post-25 PCR Cycles

The GC-content of all libraries was maintained within 1 to 2% of the original input of 39%. Similar to the data taken after 15 PCR cycles, all polymerases reduced the length of DNA fragments. While the competitor polymerases reduced the length of DNA fragments by an average of approximately 70 bp, Herculase II Fusion polymerase continued to perform the best, showing an average decrease of only 14 bp.[1] Pfu Turbo Cx HotStart polymerase showed an average length decrease of 16 bp.[1]

Post-40 PCR Cycles

Product performance among the 10 polymerases diverged from previous data trends, prompting the researchers to ensure that performance was not the result of PCR plateau effects. This was ruled out by performing higher resolution PCR assays with an increased number of timepoints per cycle.[1] After 40 cycles, the GC-content of all libraries was within approximately 5% of the original input. As observed before, all polymerases reduced the length of DNA fragments. While some of the polymerases reduced DNA fragment length by up to 116 bp on average, Herculase II Fusion polymerase only showed an average length decrease of approximately 30 bp[1]. Pfu Turbo Cx HotStart polymerase showed an average length decrease of 47 bp.[1]

The higher resolution assay incorporating data from additional timepoints during PCR was performed using the best and worst performing polymerases of the 10 that were tested. Overall, DNA libraries showed no correlation between increased GC-bias and cycle number when using Herculase II Fusion polymerase. Among all polymerases tested, Herculase II Fusion polymerase came out ahead, showing the weakest correlation between fragment length bias and cycle number ($r = -0.66$, $P = 0.02$, Pearson).[1]

In contrast, libraries amplified using competitor polymerases showed a clear correlation between increased GC-bias and cycle number ($r = 0.97$, $P = 9.15 \times 10^{-7}$, Pearson). Additionally, libraries amplified using the same polymerases displayed a strong correlation between decreasing DNA fragment length and cycle number ($r = -0.96$, $P = 1.62 \times 10^{-6}$, Pearson).[1]

Conclusion

DNA fragment length and GC-biases introduced by PCR amplification can be considerably minimized by using fit-for-purpose polymerases: Herculase II Fusion or Pfu Turbo Cx HotStart polymerases.

1. Dabney, J.; Meyer, M. Length and GC-Biases during Sequencing Library Amplification: A Comparison of Various Polymerase-Buffer Systems with Ancient and Modern DNA Sequencing Libraries. *Biotechniques* **2012**, *52* (2), 87–94. https://doi.org/10.2144/000113809.

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