*Pfu*Turbo Cx Facilitates High Throughput Methylation Studies

Efficient proofreading and read-through of uracils are essential.

Customer Success Story

University of Otago, Dunedin School of Medicine, Department of Pathology, Dunedin, New Zealand

The Challenge:

- Harsh conditions used in bisulfite treatment can reduce the yield of input DNA available for PCR to less than 10%
- Extensive PCR amplification can then be required to compensate for the loss of DNA
- However, many DNA polymerases used in PCR "stall" in uracil-rich templates or do not have a proofreading function, resulting in insufficient amplification and introduction of mutations

The Solution:

The Agilent *Pfu*Turbo Cx HotStart DNA Polymerase efficiently reads through uracil in the template strand while simultaneously proofreading to prevent PCR-induced point mutations.

The end result is sufficient amplification of DNA heavily degraded by the bisulfite treatment process to enable sequencing of multiplexed templates on the Illumina HiSeq 2000 sequencer.

The laboratory of Professor Ian Morison at the University of Otago studies the epigenetics of hematological cancers and development. Epigenetic deregulation leading to inappropriate silencing of tumor suppressor genes has been widely implicated in the development of cancer [1].

In order to gain a full epigenetic understanding of cancer, a large sample population size is needed, necessitating high throughput bisulfite sequencing. Dr. Euan Rodger and Aniruddha Chatterjee of the Morison research group have helped fine tune a method for sequencing up to five multiplexed reduced representation bisulfite sequencing (RRBS) libraries on a single flow cell lane of the Illumina HiSeq 2000 [2]. However, to ensure maximum efficiency, every library amplification reaction must generate sufficient DNA to perform the sequencing reactions.

Using standard protocols with a competitive DNA polymerase did not amplify bisulfite-converted libraries at 20 or 30 cycles of PCR (Figure 1). This may have been due to stalling of the DNA polymerase caused by the high frequency of uracil. *Pfu*Turbo Cx efficiently reads through uracil in the template strand, and its enhanced proofreading activity prevents PCR-induced point mutations, resulting in sufficient high fidelity library DNA for sequencing (Figure 1).

It has been recommended in the literature [3] to use 1 µL DNA template and 0.5 µL *Pfu*Turbo Cx enzyme, to be added in 25 µL PCR master mix (lanes 2 and 2′ in Figure 1). However, the Morison research group has observed that increasing the DNA template and polymerase enzyme (3 µL DNA template and 1.2 µL *Pfu*Turbo Cx enzyme in 25 µL PCR master mix) yields better amplification results (lanes 1 and 1′ in Figure 1). Based on these results, they prepared a PCR master mix of 25 µL by adding 3 µL DNA template and 1.2 µL *Pfu*Turbo Cx enzyme to perform semi-quantitative PCR of their library samples. Figure 2 shows an example of three RRBS libraries successfully amplified by the modified protocol. Based on the gel results, 15 PCR cycles were used for library amplification for subsequent multiplex sequencing.

Conclusion

*Pfu*Turbo Cx HotStart DNA Polymerase overcomes the degradation of bisulfite-treated RRBS library template to generate sufficient amplified library DNA of high fidelity for sequencing when other DNA polymerases fail, thus enabling high throughput methylation studies using the technique developed in the laboratory of Professor Morison.

References

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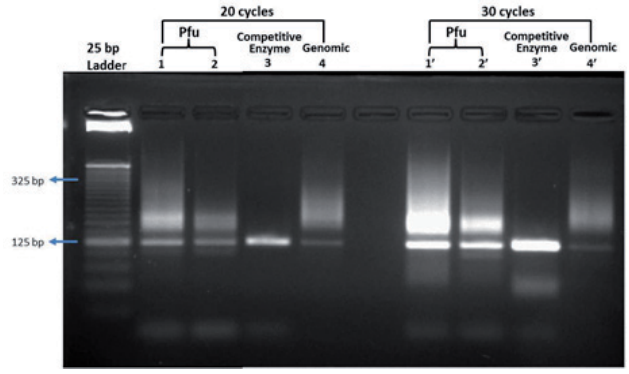

Figure 1. PCR amplification of an RRBS bisulfite library for 20 or 30 cycles using different amounts (1,2,1′2′) of either *Pfu*Turbo CX or a competitive DNA polymerase. 4 and 4′: amplification of a non-bisulfite converted library.

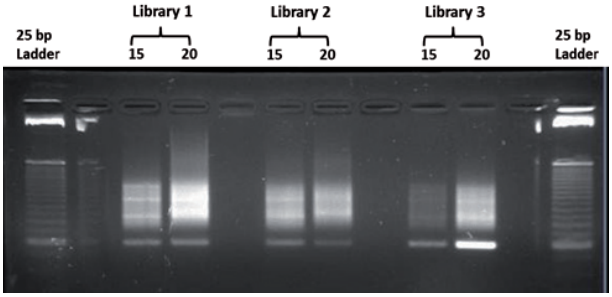

Figure 2. Three different RRBS libraries were amplified after bisulfite conversion with 15 and 20 PCR cycles using *Pfu*Turbo Cx HotStart DNA Polymerase as described in the text and electrophoresed in 3% Nusieve gel for 90 minutes.

U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

Europe
info_agilent@agilent.com

www.agilent.com/genomics/PfuTurboCx

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