AMPLIQON
PCR ENZYMES & REAGENTS

Taq Master Mix RED and TEMPase Master Mix BLUE can be used to produce PCR products for sequencing

Conclusion

PCR products, generated with either Taq Master Mix RED or TEMPase Master Mix BLUE, can directly be used for sequencing after treatment with spin columns or ExoSAP-IT. If ExoSAP-IT is used, the PCR products should be diluted prior to sequencing. In a test, we used an 8 times dilution.

Introduction

Taq Master Mix RED is a convenient and reliable product featuring direct gel loading. It contains a red tracking dye that does not interfere with PCR.

TEMPase Master Mix BLUE is the hot start version of Taq Master Mix RED. It offers the same excellent performance, direct gel loading and complete inactivation at room temperature. It contains a blue tracking dye that does not interfere with PCR.

However, the tracking dyes might interfere with some downstream methods using fluorescence.

Non-radioactive sequencing methods use fluorescent dyes to track the nucleotides of a sequence. Samples for sequencing have to be treated to remove excess primers and dNTPs. Methods for sample treatment before sequencing are e. g. spin column purification, gel purification or enzyme based methods like ExoSAP-IT.

Previously we have shown that PCR product purification using spin columns remove all of the red and the blue tracking dyes and subsequent sequencing was successful.

In this project we investigated, whether treatment with ExoSAP-IT is sufficient to enable good sequencing results downstream of using Taq Master Mix RED or TEMPase Master Mix BLUE for PCR. ExoSAP-IT is an enzyme based method. Excess primers and dNTPs are degraded by Exonuclease I and Shrimp Alkaline Phosphatase. There is no buffer change or removal of red or blue dyes etc. associated with this method.

Experimental setup

- A PCR was run using Taq 2x Master Mix RED, TEMPase 2x Master Mix A BLUE, and as a positive control Taq 2x Master Mix.
- DNA quality was checked on an agarose gel and the amount of DNA/µl was estimated.
- PCR products were treated with ExoSAP-IT.
- 10 ng/µl and 5 ng/µl dilutions of ExoSAP-IT treated PCR products were sent for sequencing.

Colors of the samples ready for sequencing:

A PCR was run and products were treated with ExoSAP-IT and further diluted to a DNA concentration of approx. 10 ng/µl and 5 ng/µl. The total dilution of the PRC products was 8x and 16x. After dilution the color of TEMPase Master Mix BLUE was barely visible (figure 1 to the right) and the color of Taq Master Mix RED was weakly visible (figure 1 to the left).

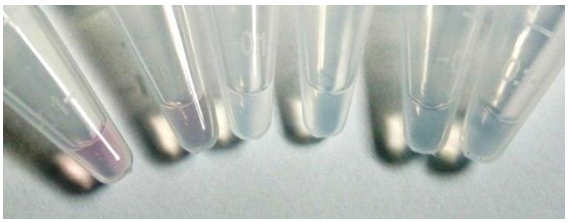

Figure 1: Colors of the samples ready for sequencing. From left to right: Taq Master Mix RED, 8 x diluted, Taq Master Mix RED, 16 x diluted, Taq Master Mix, 8 x diluted, Taq Master Mix, 16 x diluted, TEMPase Master Mix BLUE, 8 x diluted and TEMPase Master Mix BLUE, 16 x diluted.

Sequencing results

Figure 2 shows electropherograms of each of the three tested Master Mixes at the 8x dilution. The electropherograms of all three sequencing reactions look similar, indicating that Taq Master Mix RED and TEMPase Master Mix Blue, when diluted 8 times, can be used for sequencing without problems.

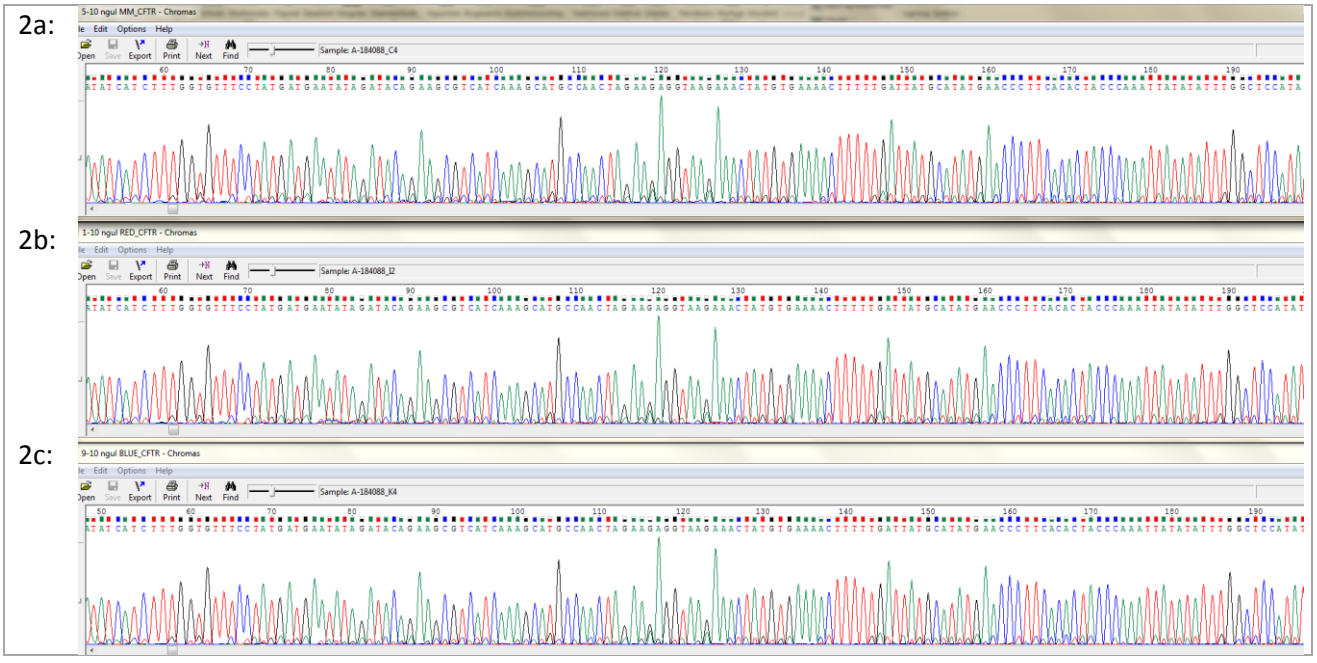

Figure 2. Sequencing electropherograms.

a: Taq Master Mix without loading dye, b: Taq Master Mix RED, c: TEMPase Master Mix Blue