P28: Impact of different storage conditions of formamide in the quality of sequences

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Introduction: Obtaining high quality sequences is essential to proper reading and interpretation. A crucial step for this goal is the denaturation of sequencing products which is usually performed using a denaturing agent such as formamide. It has been described that the reduction of formamide quality may cause irregular cytosine and guanine peaks in an electropherogram.According to manufacturers recomendations, formamide should be stored at -20ºC. Under other conditions such as exposition to light and air and temperature changes for a certain period of time, quality may decrease.

Objectives: The purpose of this study was to understand which formamide storage-related variables can be responsible for decreasing sequence quality.

Materials and Methods: Three distinct PCR products were used: a wildtype product smaller than 200 bp; a wildtype product larger than 300bp; and a product with a point mutation. Formamide was stored under different conditions (exposure to light, air and different temperatures) during certain periods of time. The products were sequenced by Sanger method and subsequent electropherograms analysis was performed.The quality of sequences was scored by evaluating the degree of background noise and presence/absence of irregular cytosine and guanine peaks.

Results and Discussion: With the increase of exposure periods to non-ideal storage conditions we verified the presence of irregular cytosine and guanine peaks in some sequences, which result from a decrease of the quality of formamide. However, by evaluating the score of those cases, we concluded that the mentioned artifact does not affect significantly sequence interpretation. Therefore, we believe that formamide can remain in any of those conditions (including exposure to light and air) up to 1 month without significant impact in the sequence quality.

Conclusion: Formamide showed high stability when stored in non-ideal conditions, not compromising sequence interpretation in the studied periods of time.

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References

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