Guidelines for Preventing Contamination of PCR

Protocol

This protocol is for the Guidelines for Preventing Contamination of PCR

During PCR more than 10 million copies of a template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are the following:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up mixtures for PCR in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and set up PCR.
- Use certified reagents, including high quality water (e.g., Water, nuclease-free).
- Always perform No-Template-Control (NTC) reactions to check for the absence of contamination.

For detailed instructions for the set-up of a PCR laboratory and its maintenance, refer to PCR Methods and Applications, 3, 2, S1-S14, 1993.

PCR frequently is contaminated by amplicons from previous PCR held in the same room. One of the most popular and efficient methods for prevention of carryover contamination is a use of uracil DNA glycosylase* (UDG) (1). A part or all of the dTTP in the PCR reaction is substituted by dUTP and therefore all PCR products generated in your working environment contain dUTP. Prior to each PCR, short incubation with UDG eliminates such contaminating amplicons carried over from the previous PCR. Incorporation of dUTP does not affect the intensity of ethidium bromide staining or the electrophoretic mobility of the PCR product, therefore the reactions can be analyzed by standard agarose gel electrophoresis.

Taq DNA polymerase and all other non-proofreading polymerases will incorporate dUTP into a PCR product, but proofreading polymerases or enzyme mixes containing such proofreading polymerases (e.g, DreamTaq™ DNA Polymerase, High Fidelity Enzyme Mix or the Long PCR Enzyme Mix), do not incorporate dUTP or may incorporate with much less efficiency.

* Use of such enzyme in certain territories may be covered by patents and may require a license.

Reference

1. Longo, M.C., et al., Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions, *Gene 93*, 125-8, 1990.

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