Sequential Reaction Protocol for PreCR Repair Mix

Overview

This procedure is recommended if optimal PreCR DNA repair is desired and a PCR buffer other than ThermoPol buffer is used for the PCR reaction.

The following protocol is recommended for a 50 µl PCR reaction:

Protocol

1. At room temperature, combine 1X ThermoPol Buffer, 100 µM dNTPs, 1X NAD^+ ,50-500 ng of the damaged template DNA and H_2O to 49 µl.
2. Add 1 µl of the PreCR Repair Mix, and mix gently.
3. Incubate the repair reaction for 15-20 minutes at 37°C.
4. Place the reactions on ice.
5. The DNA repaired by the PreCR Repair Mix can now be used as a template for the subsequent PCR reaction in the desired buffer. Testing various amounts of the repaired template DNA in a 50 µl reaction is recommended, with 5 µl as a starting point.

   Notes: It is common for PCR inhibitors to co-purify with DNA from degraded samples. One method to overcome this is to use albumin to bind the inhibitor and prevent it from interfering with PCR. If PCR inhibitors are suspected to be present in your samples we recommend that albumin be added to both the repair and PCR reactions to a final concentration of 1 mg/ml. If possible, it is best to perform both the repair and PCR reactions with and without albumin.