Control Reaction Protocol for PreCR Repair Mix

Overview

PCR conditions will vary depending on the PCR polymerase and PCR buffer. The control reaction was optimized for *Taq* DNA polymerase with ThermoPol buffer (NEB#M0267). If using another polymerase or buffer it may be necessary to optimize the reaction conditions. The forward and reverse control PCR primers, the L1 Primer Mix, come pre-mixed at a concentration of 20 µM of each. It is important to run a negative control at the same time as the repair reaction. For the negative control add 1 µL of H2O instead of the 1 µL of PreCR Repair Mix at the appropriate time. This is the unrepaired DNA reaction for comparison.

The following protocol is for a 50 µL PCR reaction.

Protocol

1. At room temperature, combine 38 µl H_2O, 5 µl ThermoPol Buffer, 0.5 µl 100X NAD^+, 0.5 µl of 10 mM dNTPs, and 3 µl of the supplied UV damaged Lambda DNA.
2. Add 1 µl PreCR Repair Mix and mix by gently pippeting up and down 3 times.
3. Incubate the repair reaction for 15 minutes at 37°C.
4. Place the reaction on ice.
5. Directly to the repair reaction, add 1 µl of the control primers, 0.5 µl of 10 mM dNTPs and 0.5 µl of *Taq* DNA polymerase (5 units/µl, not supplied). Mix gently.
6. Place into a thermocycler running the following program once the block temperature reaches > 90°C. Thermocycler program; 2 min at 95°C for 1 cycle, then 10 sec at 95°C, 30 sec at 65°C, 1 min at 72°C for 25 cycles and finally a 4°C hold. A PCR product of 1 kb should be seen.

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.