PCR PURIFICATION KIT PROTOCOL

Purpose

This technique is used to remove small DNA fragments from non-specific amplification and also remove buffers and enzymes from a DNA sample.

KEYWORDS: DNA clean-up, PCR, silica-based column.

Total time: 10-15 minutes

Waiting time: 5 minutes

Resume

Bind DNA to the column → Wash & dry steps → Elution step → Dose the DNA

Materials

- Binding Buffer (*B2, Invitrogen*)
- Wash Buffer (*W1, Invitrogen*)
- Silica-based column (*PureLink Spin Column, Invitrogen*)
- DNA
- Binding Buffer HC (*B3, Invitrogen*)
- Elution Buffer

Protocol

1. In a tube, add 4 volumes (compared to the DNA volume to purify) of binding buffer (*B2, Invitrogen*) to remove **<100bp** fragments or binding buffer HC (*B3, Invitrogen*) to remove **<300 bp** fragments. MIX well.
2. Put a silica-based column (*PureLink Spin column, Invitrogen*) in a collection tube.
3. Load the sample into the column and centrifugate at 10 000g for 1 minute. Discard the flow-through.
4. **Wash step**. Add 650 µL of wash buffer (*W1, Invitrogen*) and centrifugate at 10 000g for 1 minute. Discard the flow-through.
5. Spin at max speed for 3 minutes in order to dry the column.

6. **Elution step**. Place the column in a clean tube. Add 50 µL of Elution Buffer and incubate at room temperature for 2 minutes. (*The elution can be done with H20 too).*

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Protocol

7. Keep the flow-through and store on ice. Dose the DNA.

8. Store at – 20°C.

Troubleshooting

Problem	Potential source of the problem	Solution(s)
Low DNA yield	PCR conditions are not optimized.	Check the amplicon on the gel to verify the PCR product prior to purification.
	Incorrect binding conditions.	For efficient DNA binding, always mix 1 volume of PCR (50-100µL) with 4 volumes of Binding Buffer. Be sure to add 100% isopropanol to the binding buffer before use.
	Ethanol was not added to the wash buffer.	Add 96-100% ethanol to the wash buffer befor use.
Primer dimers are present	Incorrect binding buffer used.	To efficiently remove primer dimers or short spurious PCR products (<300bp), use binding buffer HC (*B3, Invitrogen*). This buffer is specifically designed to remove <300bp DNA fragments, eliminating the need of a gel purification.
Downstream enzymatic reactions are inhibited	Ethanol is present in the purified DNA.	Traces of ethanol from the wash buffer can inhibit downstream enzymatic reactions. To remove the wash buffer, discard the wash buffer flow-through from de collection tube. Place the spin column into the collection tube and centrifugate the spin column at maximum speed for 2 – 3 minutes to completely dry the column.