ARIA

DNA purification: Cleanup Protocol

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DNA purification: Cleanup

Introduction

The DNA purification cleanup Kit allows to remove residual DNA from previous reactions. In this page, we expose how we have done it in our laboratory.

Materials

- Monarch® PCR & DNA Cleanup Kit
- DNA sample: from PCR amplification

Procedure

Before Starting

1. The input amount of DNA to be **purified** should not exceed the binding capacity of the column (5 µg).
2. **Centrifugation** should be carried out at **16,000 x g** in a standard laboratory microcentrifuge at room temperature.
3. For the 50-prep kit, add 14 ml of isopropanol to the **DNA Cleanup Binding Buffer.**
4. For the 50-prep kit, add 20 ml of ethanol to the Monarch **DNA Wash Buffer.**
5. Always keep all buffer bottles **tightly closed** when not in use.

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6. **Dilute** sample with DNA Cleanup Binding Buffer according to the table below. Mix well by pipetting up and down or flicking the tube. Do not vortex. A starting sample volume of 20–100 µl is recommended. For smaller samples, TE can be used to adjust the volume.

DNA sample dilution

	A	B	C
1	SAMPLE TYPE	RATIO OF BINDING BUFFER: SAMPLE	EXAMPLE
2	dsDNA > 2 kb (plasmids, gDNA)	2:1	200 µl:100 µl
3	dsDNA < 2 kb (some amplicons, fragments)	5:1	500 µl:100 µl
4	ssDNA > 200 nt**	7:1	700 µl:100 µl

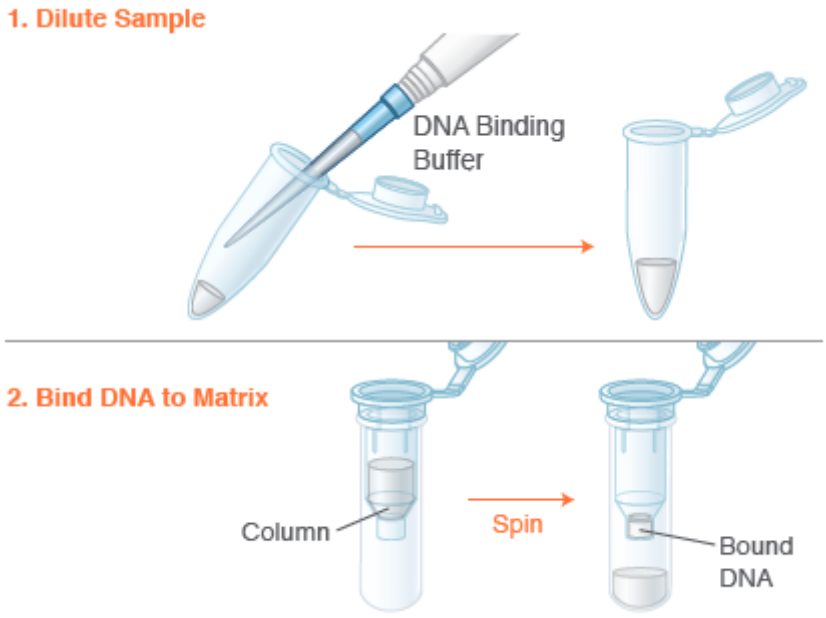
7. **Insert** column into collection tube and load sample onto column and close the cap. Spin for 1 minute, then discard flow-through.

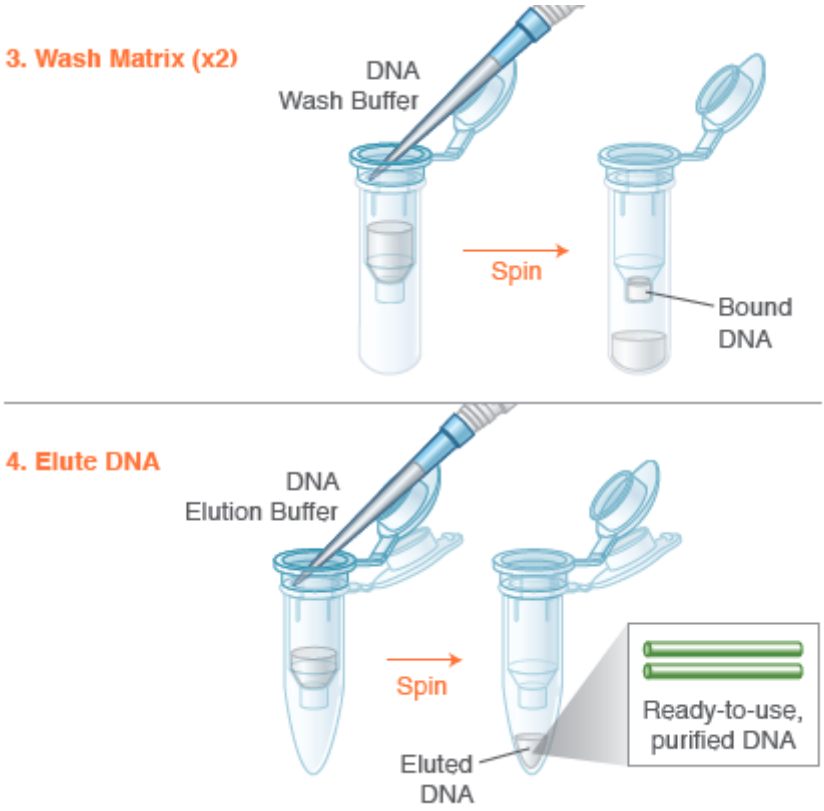
8. **Re-insert** column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional.

9. **Repeat** wash (Step 11).

10. **Transfer** column to a clean 1.5 ml microfuge tube. Whilst, use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.

11. **Add** ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA [1].





Bibliography

1. New England Biolabs. (n.d.). Protocol for DNA cleanup and concentration using the monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). Retrieved October 17, 2021, from Neb.com website: https://international.neb.com/protocols/2015/11/23/monarch-pcr-and-dna-cleanup-kit-protocol