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IN DEVELOPMENT

Vector Linearization

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COMMENTS 0

ABSTRACT

Protocol for the linearization of a previously obtained vector.

PROTOCOL CITATION

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GUIDELINES

Add phosphatase to remove phosphate, which remains from the end of linear vectors, thus preventing cells from relinearizing the vectors.

Equipment

1

Equipment

NanoDrop™ One UV-Vis Spectrophotometer	NAME
spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU
https://www.thermofisher.com/order/catalog/product/ND-ONE-W	LINK
Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm	SPECIFICATIONS

Measure DNA Concentration of the Plasmid

2 Use 1.0 µL to 1.5 µL of the elution buffer (used to elute the DNA) as blank.

3 Measure blank once to confirm the accuracy.

4 To measure DNA sample, add 1-1.5 µL (equivalent to blank)

Calculate the amount of Plasmid to be used

5 If 1 µg is 1000 ng , this value should be divided by the amount of DNA measured.

5.1 If the required amount is 3 µg and the measured sample was 450 ng/µL , the proper calculation should be: 3000/450 = ~6.67 = 7.00 µL

3h

Vector Linearization

6 3 µg Plasmid DNA

7 1 µL Enzyme A

8 1 µL Enzyme B

9 2 µL 10X Buffer

10 Remaining H2O 20 µL (up to)

3h

11 Incubate the reaction for 03:00:00 at 37 °C , then place it On ice .

11.1 Place it On ice and add 0.5 µL **phosphatase** to the linearized vector.