Jan 18, 2019

Sanger Sequencing

DOI
dx.doi.org/10.17504/protocols.io.pxddpi6

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Protocol Citation: Lívia Sacchetto, Izabela M Rezende 2019. Sanger Sequencing. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.pxddpi6**

Protocol status: Working
We use this protocol and it's working

Created: May 05, 2018

Last Modified: January 18, 2019

Protocol Integer ID: 11973

Abstract

For sequence by dideoxy-method using the ABI3130 platform (Applied Biosystems, USA) and specific primers.

Materials

MATERIALS

- Microcentrifuge Tubes
- Filter Tips
- Nuclease-Free Water, 1000ml **Promega Catalog #**P1199
- Primer
- Micropipettors
- Sequencing plate
- PCR purified template
- Sequencing buffer
- Sanger sequencer
- adhesive plate seal

1 In a 0.2 mL microtube, place the items below:

	PCR product	20 to 30 ng of DNA per 100 bp
	Primer Forward or Reverse	10 pmol
	BigDye™ Terminator v3.1 5X Sequencing Buffer (Applied Biosystems)	2 µL
	Nuclease-free water	to a final volume total of 10,0 µL

2 Pipette each reaction from the tube into the corresponding well of the sequencing plate (10 µL).

3 Seal the plate; centrifuge briefly to collect the contents of the wells at the bottom. Protect from extended light exposure or elevated temperatures before cycling.

4 Use Sanger sequencing with the Applied Biosystems® 3730 DNA Analyser, according to the manufacturer's instructions.