



## Sanger Sequencing

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### ABSTRACT

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

### PROTOCOL STATUS

#### Working

We use this protocol in our group and it is working

### MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Microcentrifuge Tubes		
Filter Tips		
Nuclease-Free Water, 1000ml	P1199	Promega
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.



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