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Sequence Analysis of a Plasmid

Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

1 Works for me dx.doi.org/10.17504/protocols.io.43ngyme

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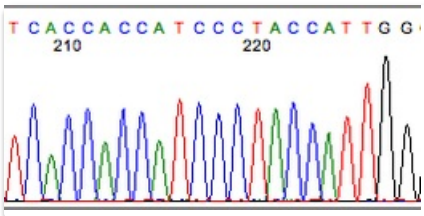
ABSTRACT

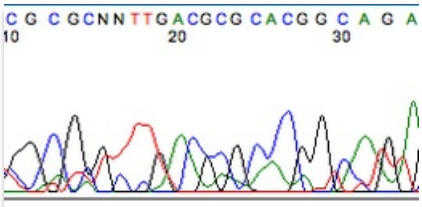
This protocol is for sequence analysis of a plasmid. To see the full abstract and additional resources, please visit https://www.addgene.org/protocols/sequence-analysis/.

Sample data

A good sequencing reaction will produce between 300-900 base pairs of useable sequence. You should receive your sequencing results as a trace file (.ab1) which graphically depicts the sequence as a series of colored peaks corresponding to one of the four nucleotide bases. This is an example of a trace file from a high-quality portion of a sequencing reaction:



Sequence near the beginning or end of a sequencing reaction is often unreliable. Although your sequencing results may indicate bases at specific locations, by looking at the trace file, you will see that these base calls are unreliable. This is an example of a trace file from a portion of a sequencing reaction with high background:



EXTERNAL LINK

https://www.addgene.org/protocols/sequence-analysis/

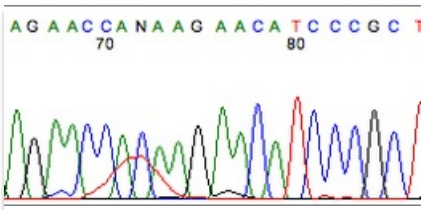
GUIDELINES

Tips and FAQs

- **My sequence doesn't match Addgene's sequencing result, what should I do?**
  Check your trace file first; the apparent mismatch/mutation may be the result of a mis-called peak in the trace file. If the mutation is not an artifact, please email help@addgene.org with the sequence, trace file, and primer used.

- **What program can I use to view my trace file?**
  There are many free programs available that can open .ab1 files, such as 4Peaks (Mac), SnapGene Viewer (Mac/PC), FinchTV (Mac/PC), Sequence Scanner (PC), and Chromas(PC).

- **How can I tell if a peak is mis-called?**
  Open the trace file and use the search feature in the program to locate the incorrect sequence. Look at the peaks in the area and make sure they are justifiable peaks. For instance, in the trace file below, you can see that just after base 70 there are multiple peaks in the same location. Looking at the trace file will give you more information than simply looking at the bases provided by your sequencing provider.



- **My sequence has "N"s in it- what does that mean?**
  "N" is the letter used to denote that the position could be any of the 4 bases. Check the trace file and see if you can manually call the correct base at the position. Sometimes an "N" is the result of an erroneous insertion by the sequencing reaction.