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16S_PCR_Sequence_Analysis

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Protocol status: Working

We use this protocol and it's working

Created: December 08, 2022

Last Modified: December 20, 2022

Protocol Integer ID: 73758

Keywords: Genotyping, consensus 16s rrna gene sequence, rrna gene sequence, consensus 16, reverse sequence, gene

Abstract

Protocol to generate a consensus 16S rRNA gene sequence from forward and reverse sequences.

Materials

Software

Serial Cloner

NAME

Franck Perez

DEVELOPER

http://serialbasics.free.fr/Serial_Cloner.html

SOURCE LINK

Software

FinchTV

NAME

macOS

OS

Geospiza Research Team

DEVELOPER

<https://digitalworldbiology.com/FinchTV>

SOURCE LINK

Troubleshooting



Adding U515 sequence feature

- 1 Open Serial Cloner.

Software

Serial Cloner

NAME

Franck Perez

DEVELOPER

http://serialbasics.free.fr/Serial_Cloner.html

SOURCE LINK

- 2 Go to "Features" → "Manage Features".
- 3 Add new collection. Name "16S rRNA Gene Conserved Regions".
- 4 Add new feature. Name "U515". Add sequence "GTGCCAGCAGCCGCGGTAA".
- 5 Check "Scan For This Feature" box. Click Ok.

Generating consensus 16S rNA gene sequence

- 6 Upload 16S rRNA gene forward and reverse sequences for the sample.
- 7 Go to "Function" → "Align Two Sequences".
- 8 Check "anti-parallel" box for reverse sequence.
- 9 Click "Local Align".



- 10 Click "Build Consensus". New dialog box will appear.
- 11 Select "Unsaved Consensus" window then click "Features" → "Scan Sequence". "U515" feature should be highlighted in consensus sequence.
- 12 Click "Features" tab in the "Unsaved Consensus" window.
- 13 The nucleotide positions of the "U515" marker will be given. Select the "from" box in top right corner of window and type in the nucleotide position that is -385 nt from the beginning of the "U515" marker. Select the "to" box in top right corner of window and type in the nucleotide position that is +216 nt from the end of the "U515" marker. A 620 bp consensus sequence should be generated.
- 14 To check for quality of consensus sequence select "Align Two Sequences" window and look for differences in base calls between forward and reverse sequences within the consensus sequence. Sometimes there will be a mismatch or gap between forward and reverse sequences. To assist in making the right base call at a certain nucleotide position use FinchTV to view chromatograms and determine the right call using the higher-quality base call.

Software

| | |
|---|-------------|
| FinchTV | NAME |
| macOS | OS |
| Geospiza Research Team | DEVELOPER |
| https://digitalworldbiology.com/FinchTV | SOURCE LINK |

- 15 Save consensus 16S rRNA gene sequence.
- 16 Annotate consensus 16S rRNA gene sequence by searching a database (e.g. NCBI) or using a 16S rRNA gene classifier (e.g. RDP classifier).