

SOP: SP044

Sequence Alignment by Vector NTI Contig Express

Materials and Reagents:

1. Dell Dimension XPS T450 Rm C210
2. Electronic sequence chromatograms from forward, reverse, and any internal primers (note 1)
3. Electronic sequence of gene of interest in recombinant plasmid map (see SOP SP043) or from Tuberculist at <http://genolist.pasteur.fr/TubercuList/>, or other sources (eg GenBank).
4. Folder containing all sequence files and serving as save site for Contig(s) to be generated by this protocol

Protocol:

1. ____ Create a folder (note 2) containing electronic text and chromatograms compiled for sequence of interest.
2. ____ For pMRLB plasmid sequences, route folder to Dell desktop → Contract Related folder → rec plasmid info folder → specific plasmid folder.
3. ____ For sequences not (yet) assigned TB Contract reagent status, route folder to personal domain on T drive or other server.
4. ____ Open Vector NTI 9 – design window opens.
5. ____ Pull down Assemble (in toolbar) → select Contig Express → Open New Assembly Project. Contig Express Project window opens.
6. ____ Pull down Project (in toolbar) → select Add Fragments → select ABI files for sequence of interest.
7. ____ Migrate to folder created for this sequencing project in step 1.
8. ____ Open "All Files". ABI chromatogram list will appear.
9. ____ Highlight and Open ABI chromatogram file(s) in contig project window.
10. ____ In Vector NTI design window, pull down File → Local Database. Exploring window opens.
11. ____ Select subset with recombinant plasmid map file.
12. ____ Highlight plasmid map file and drag to contig project window.
13. ____ Alternative (steps 14 - 23): Create Vector NTI sequence file from Tuberculist (or other sequence source.)
14. ____ Download Tuberculist DNA sequence ± upstream/downstream sequence.
15. ____ Open As Windows default.
16. ____ Save as seq file, eg. Rv1908c.seq to personal domain.
17. ____ In Vector NTI Exploring window, pull down DNA/RNA in top toolbar.
18. ____ Press Import → Molecule from Text File.
19. ____ Press FastA – OK.

20. ____ Navigate to file. Select file. Open.
21. ____ Insert in DNA/RNA Main or in desired subset. New File window appears.
22. ____ Fill in gene name in General tab window. eg Rv1908c.seq
23. ____ Click OK. This file is available to join the contig.
24. ____ Drag file from project subset window to Contig Project window.
25. ____ In Contig Express Project window, highlight sequences to be aligned using Shift Click.
26. ____ In toolbar, press Assemble Selected (icon with arrow over three bars). Contig 1 is created.
27. ____ Blue arrows by sequences indicates contig has been assembled.
28. ____ Grey arrow indicates no match.
29. ____ Select Contig 1 icon to display alignment.
30. ____ Left window shows alignment fragments. Click Contig 1 to add description, change name.
31. ____ Right window shows graphic of file overlaps as bars. Green line shows region of poor alignment.
32. ____ Lower window shows base by base alignment in text.
33. ____ Clicking file name highlights that file in both graphic and text view.
34. ____ Contig 1, the consensus sequence, is shown in lower panel.
35. ____ Dots indicate unreadable sequence.
36. ____ Red sequence indicates mismatch.
37. ____ Add viewable sequence chromatograms to resolve mismatches (steps 38 – 41).
38. ____ Click in text pane. Pull down View → Show All Chromatograms. Chromatogram(s) are displayed below the corresponding sequence.
39. ____ View rating for one nucleotide by holding cursor over peak. Trace values appear.
40. ____ If value is high for a base reported as an “n”, change it in the text line.
41. ____ Note true mismatches (lack of agreement in sequence from two primers).
42. ____ Submit plasmid for resequencing of region(s) of unresolved mismatch.
43. ____ Information in all panes (description, graphic, text) can be printed if desired.

Notes:

1. Sequence is acquired from vendors (eg. SeqWright, Macromolecular Resources) as text and electronic chromatograms. In this protocol, electronic chromatograms are required for alignment with recombinant plasmid map (created in SOP SP043 Recombinant Plasmid Map Design – Vector NTI) or with gene sequence from Tuberculist database or other sources.

2. Label the folder "Contig.construct name.date" eg: Contig, Rv.1908,pET23b. 2-23-05. Store the contig folder in personal domain on T drive (or other server) unless it is added to the data assembly for TB contract plasmids. See SOP RM006 pMRLB Plasmid Documentation and SOP RM007 Non-pMRLB Plasmid Documentation for detailed listing of documentation requirements.

References:

Vector NTI 9 Online Help