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