Normalization of Samples and Amplicons in Multiplex PCR-

Rapid species identification of spices and medicinal plants by Nanopore Sequencing

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Abstract

Optimized multiplex PCR amplification of DNA barcoding primers is essential for converting tedious Sanger based sequencing to robust and automated NGS based Sequencing. In addition, the normalisation of PCR product yield between samples and targets is required for successful conversion of sanger sequencing to NGS.

Spices and medicinal plants industry suffers from low cost substitutions and adulterations of herbs with closely related species. DNA barcoding offers accurate means to identify the plant species of interest as well as adultrants. Majority of DNA barcoding work is currently carried out by tedious Sanger sequencing of rbcL, matK, rpoB, trnH-psbA, and ITS gene markers. In this study, we have optimized a multiplex PCR method for simultaneous and uniform amplification for multiple plant DNA barcode targets in a single PCR reaction. Further, Nanopore DNA sequencing was performed using rapid barcode kit and MinION Mk1B.

Results from DNA barcoding of spice samples like Lantana, Four o'clock, Chilli, Ginger, Black pepper, Mustard and Indian medicinal herbs like Ashwagandha, Holy basil will be described. The same methodology can be applied for fungal, animal and insect species identification

OBJECTIVE

- To optimise the PCR primer concentration and reaction conditions to get near uniform yields of amplicons
- To obtain the uniform # of nanopore reads for multiple samples with varying concentrations
- To develop Nanopore sequencing based DNA barcoding to identify spices and medicinal plants

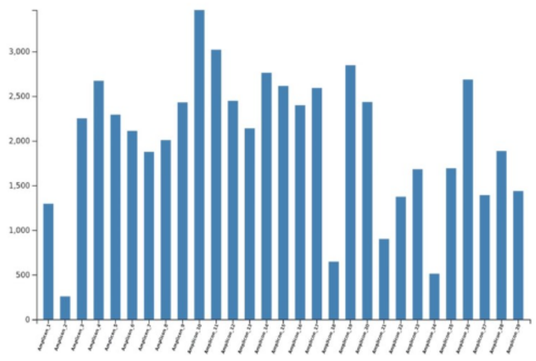
PROBLEM

Need for inter amplicon and inter sample normalization in Multiplex PCR based Nanopore Sequencing

Sample vs Coverage

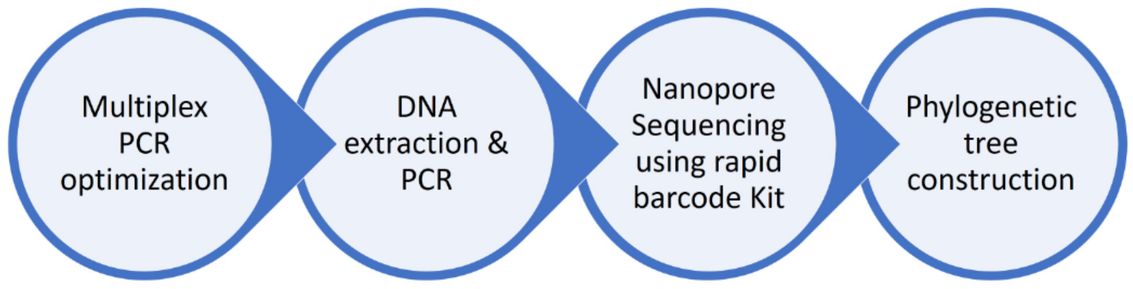
Sample Name	X Coverage	Genome Coverage (%)
Sample_1	4978	100
Sample_2	1949	100
Sample_3	1497	88
Sample_4	1313	88
Sample_5	4420	97
Sample_6	1566	100
Sample_7	3667	100
Sample_8	831	77

Amplicon distribution of sample_ 3



METHODS

Work Flow of DNA barcoding by Nanopore Sequencing



RESULTS

Multiplex PCR Typical results

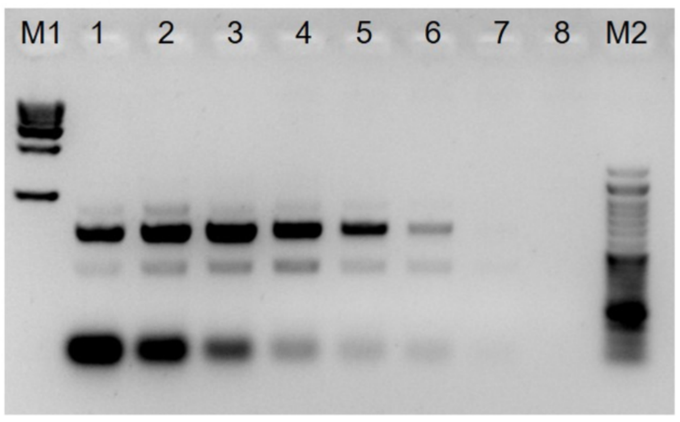


Figure 1: Agarose gel electrophoresis of multiplex PCR products. Lane M1: 1 kb DNA Ladder, Lane 1 -8: 1:2 serially diluted primer mix 16.6, 8.3, 4.1, 2.0, 1.0, 0.52, 0.26, and 0.13 µM respectively of MatK, rbcLa & rpoB targets. Lane M2: 100bp DNA Ladder A.

Multiplex PCR results after primer QTNorm

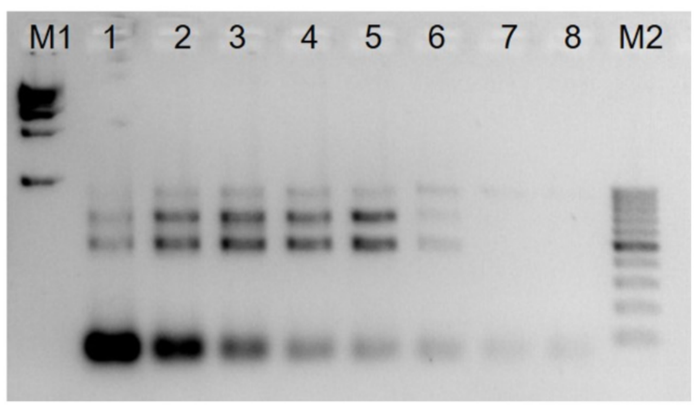


Figure 2: Agarose gel electrophoresis of multiplex PCR products. Lane M1: 1 kb DNA Ladder, Lane 1 -8: 1:2 serially diluted QTNorm primer mix of MatK, rbcLa & rpoB targets. Lane M2: 100bp DNA Ladder B.

CONCLUSION

DNA barcoding using the above optimised multiplex PCR coupled with Nanopore sequencing is quick , precise, cost effective and more suitable for large number of samples in spices and medicinal plants authentication.

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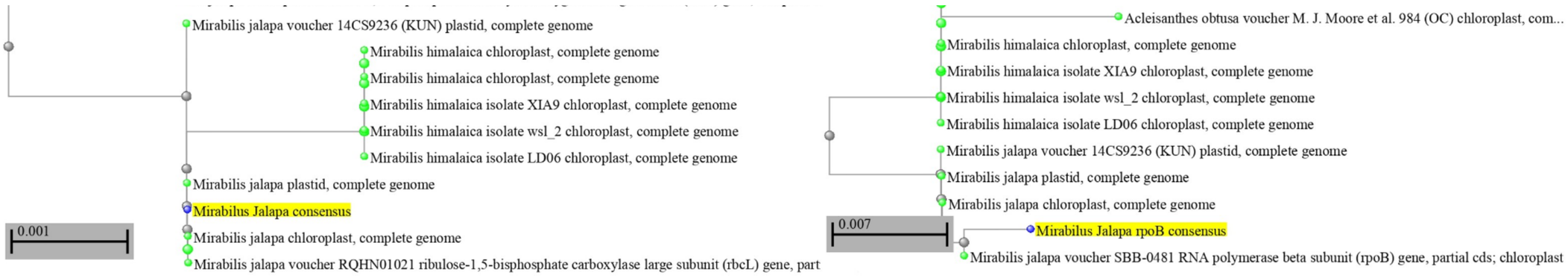

Figure 3: Phylogenetic Analysis results from obtained DNA Sequences from Nanopore Sequencing