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THE EFFECT OF COUPLING EFFICIENCY ON THE YIELD AND PURITY OF OLIGONUCLEOTIDES SYNTHESIZED USING PHOSPHORAMIDITE METHOD

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ABSTRACT

Coupling efficiency is a way of measuring how efficiently the DNA synthesizer is adding new bases to the growing DNA chain. To measure coupling efficiency, dimethyl trityl (DMT) group is used, which is colorless when attached to DNA base, but gives a characteristic orange color once removed upon addition of the base to the growing chain of DNA. The intensity of this color can be measured by UV spectrometry and it is directly related to the number of DMT molecules released throughout the synthesis. The aim of this study was to determine the effect of coupling efficiency on the yield and purity of the synthesized oligonucleotides by using the DNA synthesizer Mermade 12. Brucella-specific primers Forward (5'CATGCGCTATGTCTGGTTTAC3') reverse (5'TAATAAGACTCGGCTTTGTGA3'), were arranged in the synthesis chamber with CPG standard columns C and A, respectively, with an estimated activator volume of 3.320 ml, trityl average size 10, trityl area threshold 20000. β-actin primers, forward (5'GGACTTCGAGCAAGAGATGG3') reverse (5'AGCACTGTGTTGGCGTACAG3'), were arranged in the synthesis chamber with CPG standards columns G and G, respectively and an estimated activator volume 3.230 ml, trityl average size 10, trityl area threshold 20,000. The purity and quantity of the primers was determined by Epoch microplate spectrophotometer. Brucella-specific forward and reverse primers showed average coupling efficiencies of 99.97 and 99.95%, with a purity (OD260/280) of 1.85 and 1.84 and yields of 2456ng/µl and 2440 ng/µl, respectively. The β-actin forward and reverse primers showed average coupling efficiencies of 68% and 42%, with purities of 1.14 and 1.12 and yields of 468 and 365 ng/µl, respectively. The coupling efficiency correlates with quantity and purity

of synthesized oligonucleotides. Hence, oligonucleotides with low coupling efficiency should not be used in downstream experiments.

KEYWORDS: Mermade 12, DNA synthesizer, Coupling efficiency, Trityl value.

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MATERIALS AND METHODS

Brucella species specific primer forward 5'CATGCGCTATGTCTGGTTTAC3' and reverse 5'TAATAAGACTCGGCTTTGTGA3' were arranged in the synthesis chamber of DNA synthesizer Mermade 12(Figure 1) with CPG standard columns C and A respectively with an estimated activator volume of 3.320ml, Trityl average size 10, Trityl area threshold 20000. B-actin primers forward 5'GGACTTCGAGCAAGAGATGG3' and reverse 5'AGCACTGTGTTGGCGTACAG3' were arranged in synthesis chamber (Figure 2) with CPG standard columns G and G respectively and an estimated activator volume 3.320ml, trityl average size 10, trityl area threshold 20,000. The purity and quantity of primers was determined by low volume epoch microplate spectrophotometer.

Figure: 1 Mermade 12.

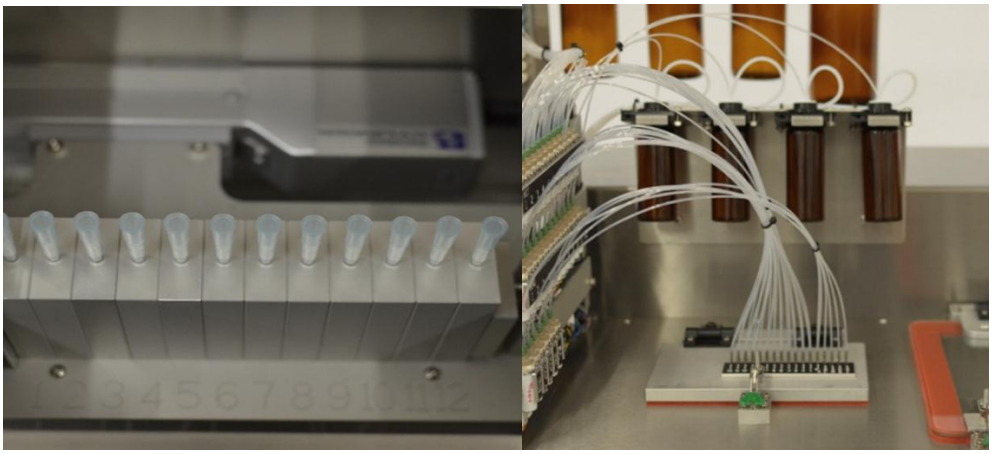


Figure: 2 Depicts the columns and valves for dispensing chemicals.

RESULTS

Brucella species specific forward and reverse primers showed average coupling efficiencies of 99.97% and 99.95% with purities(OD260/280) of 1.85 and 1.84 and yields of 2456ng/ul and 2440ng/ul respectively. The B-actin forward and reverse primers showed average coupling efficiencies of 68% and 42% with purities of 1.14 and 1.12 and yields of 468ng/ul and 365ng/ul respectively. Primers with low purity has shown poor online trityl graphs while monitoring the trityl graphs using Mermade 12 software. (Figure:3 and 4).

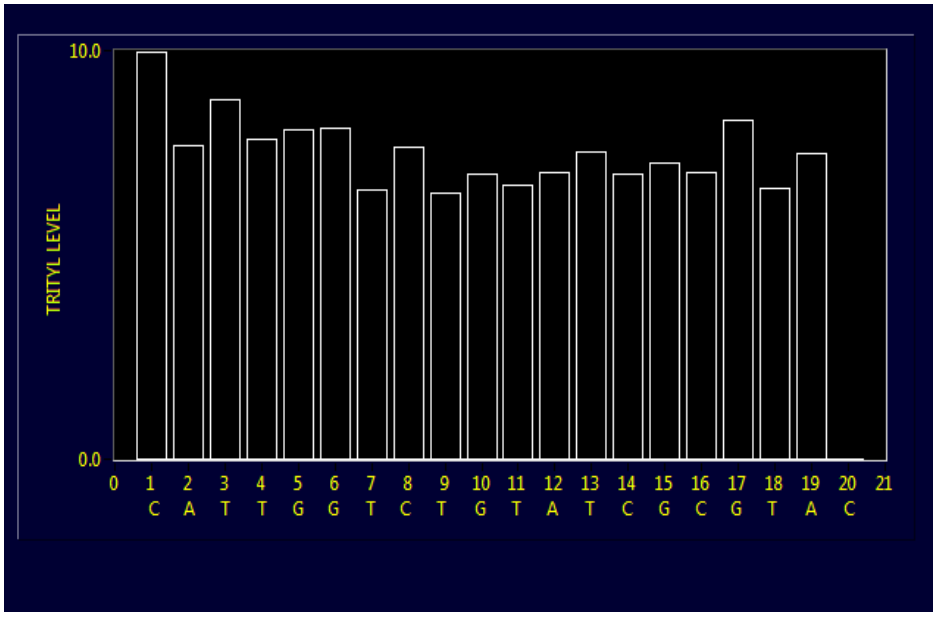


Figure: 3 Trityl graph of primer with high yield and purity.

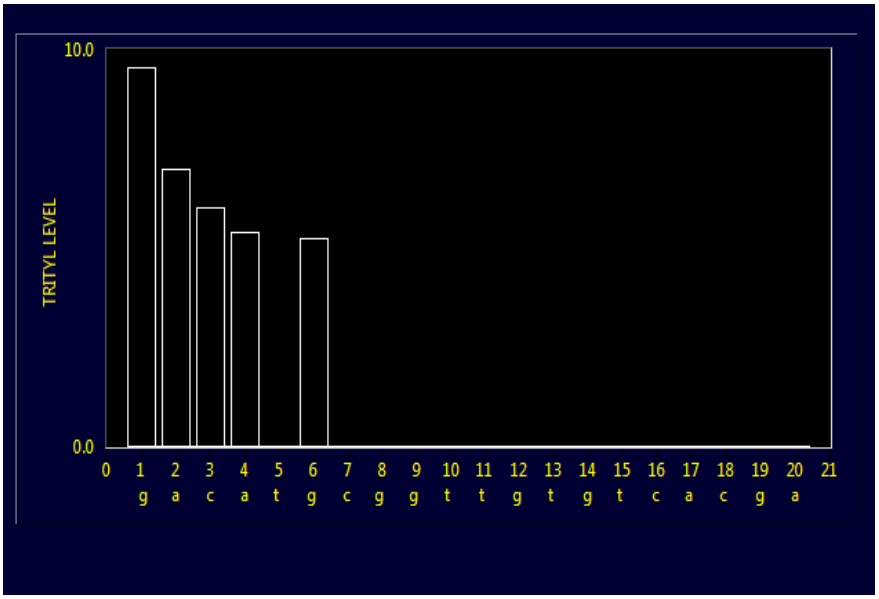
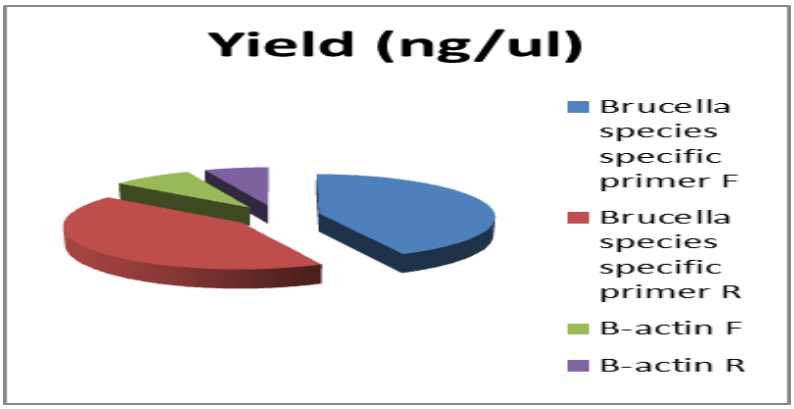


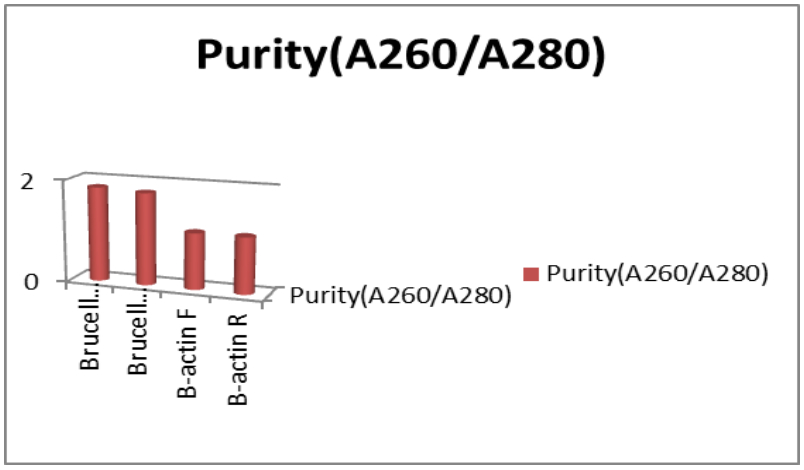
Figure 4: Trityl graph of primer with low yield and purity.

Primer	Yield (ng/ul)	Purity(A260/A280)
Brucella species specific primer F	2456	1.85
Brucella species specific primer R	2440	1.8
B-actin F	468	1.1
B-actin R	365	1.1

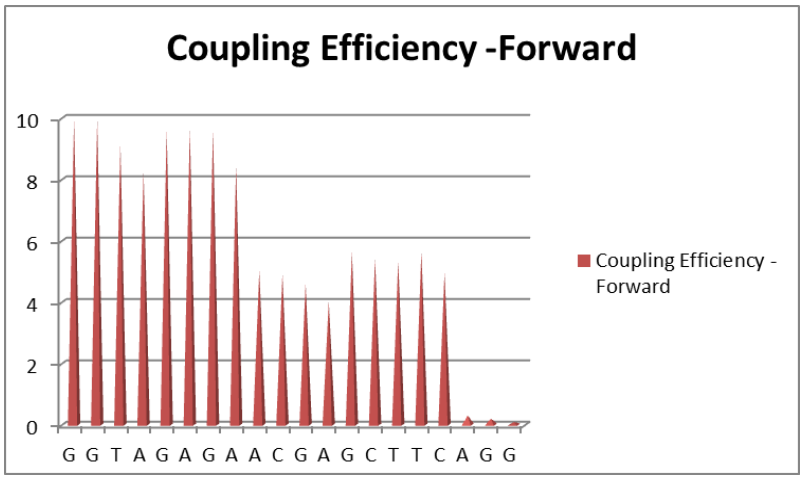
Values after quantitation in biotek spectrophotometer.



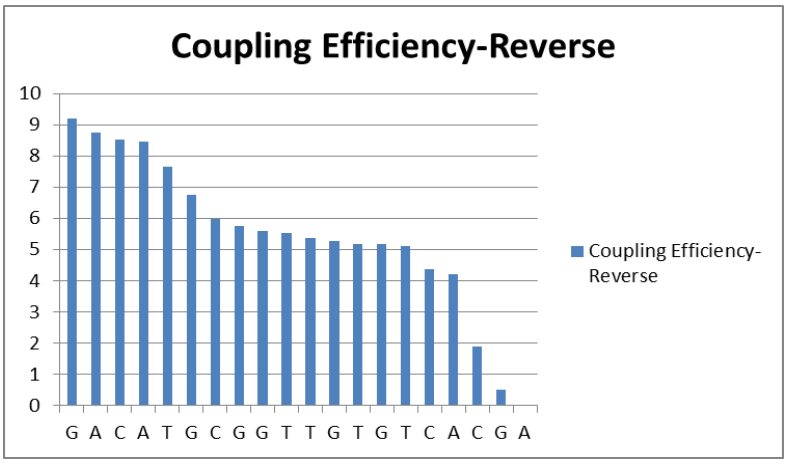
Primer Yield after synthesis using phosphoramidite chemistry.



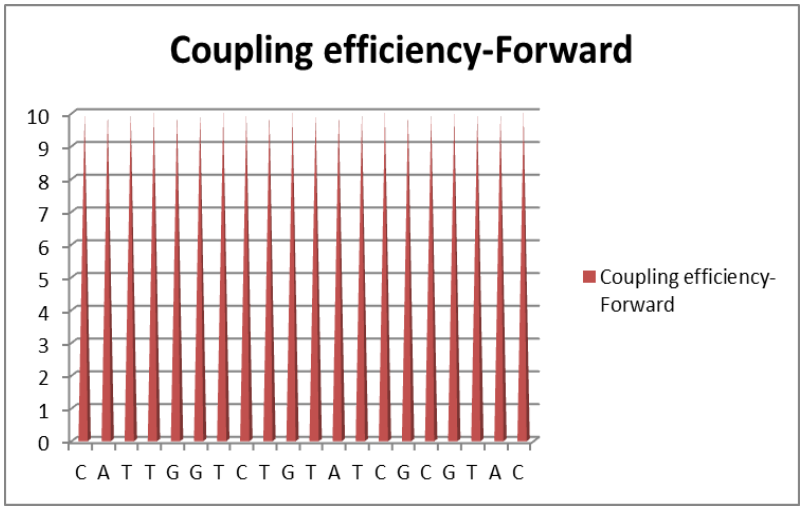
Purity of primers after synthesis based on their coupling efficiency.



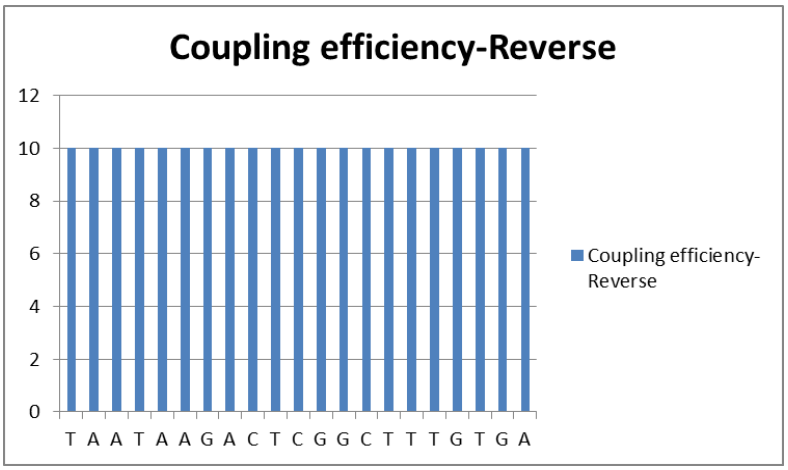
Graph showing the coupling efficiency of B-ACTIN forward primer.



Coupling efficiency of B-actin reverse primer using phosphoramidite synthesis.



Graph depicting the coupling efficiency of Brucella species specific forward primer.



Graph depicting the coupling efficiency of Brucella species specific reverse primer.

CONCLUSION

The coupling efficiency is correlated with the quantity and purity of synthesized oligonucleotides. Hence oligonucleotides with low coupling efficiency should not be used in downstream experiments.

ACKNOWLEDGEMENT

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