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Abstract

Denaturation bubble-mediated strand exchange amplification (SEA) is a novel, rapid isothermal nucleic acid amplification that has been applied for point-of-care molecular diagnosis in food safety, meat adulteration, forest disease and animal disease. Nevertheless, the absence of specialized strategy for SEA primer design leads to long-time primer screening before SEA reaction execution, which largely increases time consumption when SEA is utilized for detecting new targets. In this work, we investigated the impact of the following primers' attributes on SEA efficiency, including Tm value, 3' end G/C content, self-complementary and 3' complementary. We demonstrated that optimal Tm value and reaction temperature were all 61° C, while the 3' terminal nucleotide should be G/C, as SEA reactions induced by primers possessing these attributes exhibited significantly lower threshold time (Tt) value. Moreover, self-complementary and 3' complementary of primers should be avoided. We also discussed the consideration priority order of these factors, which was self-complementary and 3' complementary, Tm value and 3' end G/C content in turn. Because the SEA primer design strategy is first presented, our work will greatly promote the application of SEA in point-of-care test.

1. Introduction

Denaturation bubble-mediated strand exchange amplification (SEA) is a novel isothermal nucleic acid amplification technology first presented in 2016, mediated by the invading of only one pair of primers with amplification products of 40–45 bp to the DNA breathing-induced variable-bubbles of dsDNA. Similar to other isothermal amplification technologies, SEA (designed as isothermal PCR) can realize exponential amplification via Bst 2.0 WarmStart DNA polymerase at a single temperature. Unlike traditional PCR, SEA requires no electrically powered thermal cycling equipment. Due to the advantages of simple, fast, low cost and sensitive, SEA has been applied for molecular detection of Escherichia coli, Listeria monocytogenes, Mycoplasma pneumoniae, Staphylococcus aureus, Bursaphelenchus xylophilus and meat adulteration. In our previous work, the limit of detection of SEA was 1.0 × 10^-13 M (approximately 6.0 × 10^5 copies of targets in 10 L reaction system). However, research on primer design in SEA was rarely reported, and the time-costing primer screening would restrict the application and popularity of this technology. Therefore, a primer design strategy for SEA is urgently needed.

2. Materials and methods (summary)

DNA primers were synthesized by Personal Biotechnology Co. (Shanghai, China). SEA detection kit was purchased from Navid Biotechnology Co. (Qingdao, China). Primers were designed using NUPACK, DNAMelt, NOVOPRO and NCBI BLAST web tools. Genomic DNA of M. pneumoniae, C. trachoma, S. domestica, B. cereus and S. aureus was extracted. SEA reaction was performed in a 10 L system comprised of 1 L template, 1.5 L each primer (10 M), 5 L 2× reaction mix, and 0.25× Eva Green. PCR products of 1 pM or 0.1 pM were used as templates. Reaction mixtures were incubated at settled temperature for 60 min, and SEA amplifications were monitored by CFX Connect Real-Time PCR System at 1-min intervals.

3. Results and discussion

3.1. Optimization of primer Tm value and reaction temperature

SEA is based on variable-bubble-mediated isothermal amplification. The reaction rate is affected by the balance of three factors: probability of bubble formation, efficiency of Bst polymerase and specific binding of primers to targets. We designed a series of M. pneumoniae specific primers (Mp1-Mp4) with varied Tm values (65° C, 63° C, 61° C, 59° C) for SEA reactions over a temperature range from 57° C to

65° C. The lowest Tt values of primers Mp1-Mp4 were 22 min, 15 min, 11 min, and 23 min, all reached at 61° C, demonstrating that 61° C was most beneficial for SEA reaction. Although bubble formation probability and Bst polymerase activity were high at 63° C or 65° C, these temperatures strongly influenced primer binding to target sites; too low a temperature (59° C) restricted DNA breathing and Bst polymerase activity. Therefore, SEA reached the highest efficiency at 61° C, which was also the optimal primer Tm value.

We also assessed the effect of Tm value difference within primer pairs. Primer pairs specific to C. trachoma (Ct1-Ct3) and S. domestica (Sd1-Sd3) with distinct Tm differences were employed. Primer pairs with smallest Tm value difference exhibited lowest Tt value, while those with largest difference showed the highest. This could be attributed to the fact that primers with similar Tm values possess close annealing temperatures, so amplification reactions on complementary strands occur at close rates, acquiring higher efficiency. Therefore, primers with similar Tm value are preferable for SEA.

3.2. Optimization of 3' G/C content

During SEA, the combination of primers to target sites is accomplished through invading of primer to the randomly deformed bubbles, rather than annealing following complete melting as in PCR. Hence primers must fast and stably combine to target sites during short DNA breathing intervals, especially at the 3' end. We compared the Tt value of M. pneumoniae (Mp3, Mp5, Mp6) and C. trachoma (Ct1, Ct4, Ct5) primer pairs with different G/C content in the 3' end last five nucleotides. Primers with higher combined 3' end G/C content exhibited lower Tt value. This is in contrast to PCR primers, which should generally avoid high C/G content at the 3' end. The 3' end of SEA primers should possess at least two G/C within the last five nucleotides, while the terminal nucleotide should be G/C.

3.3. Influence of self-complementary and 3' end complementary

Self-complementary and 3' end complementary of primers obstruct primer binding to target sites. We assessed their influence using primer pairs specific to C. trachoma (Ct1, Ct6, Ct2) and B. cereus (Bc1-Bc3) with different potential self-complementary and 3' complementary sites. The number of complementary sites was positively correlated with Tt value: primers with fewest total potential complementary sites displayed lowest Tt value. Notably, the influence of potential complementarity was more significant than Tm value and 3' end G/C content on amplification efficiency. In summary, self-complementary and 3' complementary should be avoided during SEA primer design, similar to PCR primers.

3.4. Priority of Tm value and 3' end G/C content

We identified the priority of Tm value and 3' end G/C content using two S. aureus primer pairs (Sa1 and Sa2). The amplification efficiency induced by Sa1 was significantly lower than Sa2 (higher Tt value), though the base pairs formed by 3'-terminal G/C were more stable than A/T. It could be concluded that Tm value and Tm value difference should take priority over 3' G/C content. The consideration priority order of these factors was: self-complementary and 3' complementary, Tm value and Tm value difference, and 3' G/C content.

4. Conclusion

We investigated the effect of primers' Tm value, 3' end G/C content, self-complementary and 3' complementary on SEA efficiency, and summarized a strategy for SEA primer optimization: optimal primers' Tm value is 61° C, with Tm value difference within a primer pair less than 1° C; primers should possess at least two G/C in the 3' end last five nucleotides (3'-terminal nucleotide should be G/C); and self-complementary and 3' complementary should be avoided. Avoiding self-complementary and 3' complementary should be considered first, followed by proper Tm value and 3' end G/C content. This primer design strategy would simplify SEA experiment design and promote the application of SEA in point-of-care testing.

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References

[1] C. Shi, F. Shang, M. Zhou, P. Zhang, Y. Wang, C. Ma, Triggered isothermal PCR by denaturation bubble-mediated strand exchange amplification, Chem. Commun. 52 (2016) 11551–11554.

[2] T. Notomi et al., Loop-mediated isothermal amplification of DNA, Nucleic Acids Res. 28 (2000) e63.

[3] J. Compton, Nucleic acid sequence-based amplification, Nature 350 (1991) 91-92.

[4] M. Vincent, Y. Xu, H. Kong, Helicase-dependent isothermal DNA amplification, EMBO Rep. 5 (2004) 795–800.

[5] I.A. Nanayakkara, I.M. White, Demonstration of a quantitative triplex LAMP assay for detection of MRSA, Analyst 144 (2019) 3878–3885.

[6] R. Liu et al., A simple isothermal nucleic acid amplification method for on-site identification for adulteration of pork source in mutton, Food Contr. 98 (2019) 297–302.

[7] M. Zhang et al., Rapid detection of foodborne pathogen Listeria monocytogenes by strand exchange amplification, Anal. Biochem. 545 (2018) 38–42.

[8] W. Shi et al., Rapid diagnosis of Mycoplasma pneumonia infection by denaturation bubble-mediated strand exchange amplification, Sci. Rep. 9 (2019) 896.

[9] C. Liu et al., Rapid and simple detection of viable foodborne pathogen Staphylococcus aureus, Front. Chem. 7 (2019) 124.

[10] D. Sint, L. Raso, M. Traugott, Advances in multiplex PCR: balancing primer efficiencies and improving detection success, Methods Ecol. Evol. 3 (2012) 898–905.