SNIPER: Engineered Specificity Unlocks Multi-Kilobase Isothermal Amplification for High-Throughput Sequencing

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Modern nucleic acid sequencing enables rapid and cost-effective genomic analysis, but for low-input samples, amplification via polymerase chain reaction (PCR) remains a critical bottleneck. Isothermal amplification (IA) offers a more scalable alternative by eliminating thermocycling, yet existing IA methods fail to generate amplicons beyond several hundred base pairs, limiting IA's integration into sequencing. The basis behind this limitation remains unclear. Here we show that longer targets experience increased base exposure during amplification, increasing mispriming and producing shorter off-targets that outcompete intended amplicons. To overcome this, we replaced conventional specificity controls with a single-stranded DNA-binding protein (SSB) that restored sufficient specificity to enable long-target amplification. However, yields remained low because primer invasion mediated solely by SSB depended on DNA breathing, restricting amplification to template ends where breathing was more frequent. We thus developed SNIPER. SNIPER uses end-complementary primers to exploit end breathing and SSB to suppress off-target priming, allowing only complementary primers to displace SSB and initiate extension. SNIPER achieved long-target amplification (>5.8 kb) and multiplex amplification of 4 multi-kilobase targets with sequencing adapter incorporation, while maintaining PCR-level sensitivity (1 copy/uL). SNIPER's practical utility was demonstrated in a multiplexed three-exon panel for non-small cell lung cancer screening and a one-pot RNA amplification workflow. Our findings identify specificity loss as a central barrier to long-target IA and establish engineering specificity as a path towards accessible high-throughput sequencing across laboratory, clinical and field settings.

Awards Won:
Society Alumni Special Award: Society for Science Alumni Special Award