Staying on target: using nature’s error-correcting tools to make molecular diagnostics more robust

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Rapid, sensitive, and specific genetic amplification methods have become an indispensable tool for a wide array of molecular diagnostic applications such as disease detection, food safety testing, and environmental monitoring. Although the polymerase chain reaction (PCR) is the most well-known method, loop mediated isothermal amplification (LAMP) has recently emerged as a popular technique because of its sensitivity, specificity, and isothermal reaction condition that removes the need for thermal cycling. Unfortunately, LAMP’s powerful amplification mechanism makes it highly susceptible to carryover contamination, wherein amplified DNA products from previous LAMP reactions become templates for reamplification and lead to false positive results. Existing methods for preventing contamination are costly and failure-prone, and there is thus a pressing need for novel strategies to eliminate carryover contamination in LAMP reactions.

Toward this end, I report an enzyme-based method that effectively eliminates LAMP carryover contamination. Inspired by similar approaches used for PCR, my method eliminates carryover contamination by chemically tagging and selectively degrading LAMP amplicons between subsequent reactions. Specifically, the method incorporates deoxyuracil (dU) nucleosides into all LAMP reactions so that all amplified products are tagged with uracil, thus differentiating them chemically from natural DNA. All subsequent LAMP reactions are then treated with uracil-DNA-glycosylase (UDG), an enzyme that specifically degrades uracil-containing DNA, leaving only natural DNA intact for amplification and detection.

In this presentation, I show that my UDG-LAMP assay can effectively eliminate carryover contamination without compromising the performance of the LAMP reaction. As an example, I demonstrate detection of pathogen DNA in the presence of carryover contamination. I show that UDG-LAMP enables end-point detection of low concentrations of pathogen DNA while avoiding false positive results. I further apply UDG-LAMP to restore the fidelity of quantitative detection methods in the presence of contamination. These results demonstrate that UDG-LAMP vastly improves the utility and robustness of LAMP. This assay’s capacity to eliminate carryover contamination may be especially useful in environments where contamination prevention is not feasible including point-of-care diagnostics in low resource settings.