

INTRODUCTION



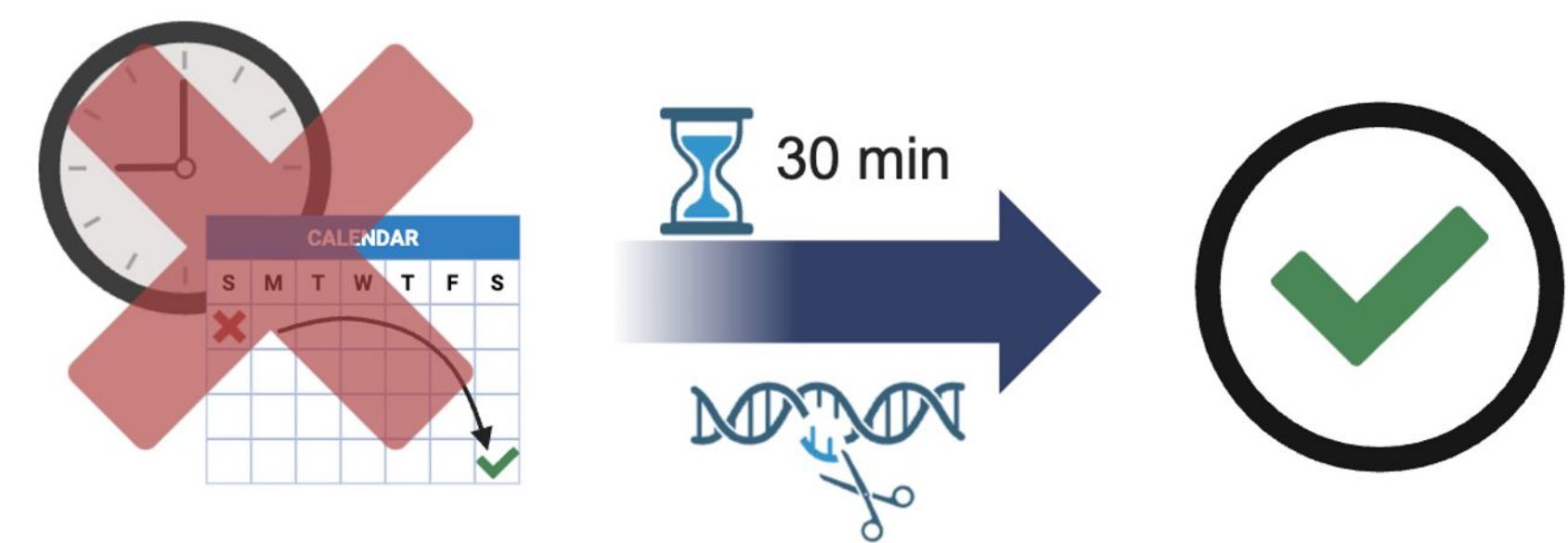
Tinea capitis and onychomycosis are common pediatric fungal infections that remain diagnostic and therapeutic challenges. Tinea capitis rates have increased over the past decade, disproportionately affecting Black children in the U.S., while onychomycosis is more frequent in children with immune-altering conditions such as Down syndrome.



Antifungal therapy requires weeks of treatment and carries risks of side effects, resistance, and undertreatment.¹ Confirmatory testing is recommended before antifungal use, yet current diagnostic methods are slow, expensive, and often inaccessible.²⁻³ As a result, empiric treatment is common, contributing to both overtreatment and delayed care.

GOAL

The goal of this project is to develop a rapid, low-cost point-of-care diagnostic to enable same-day detection of fungal infections and improve diagnostic accessibility in dermatologic care.

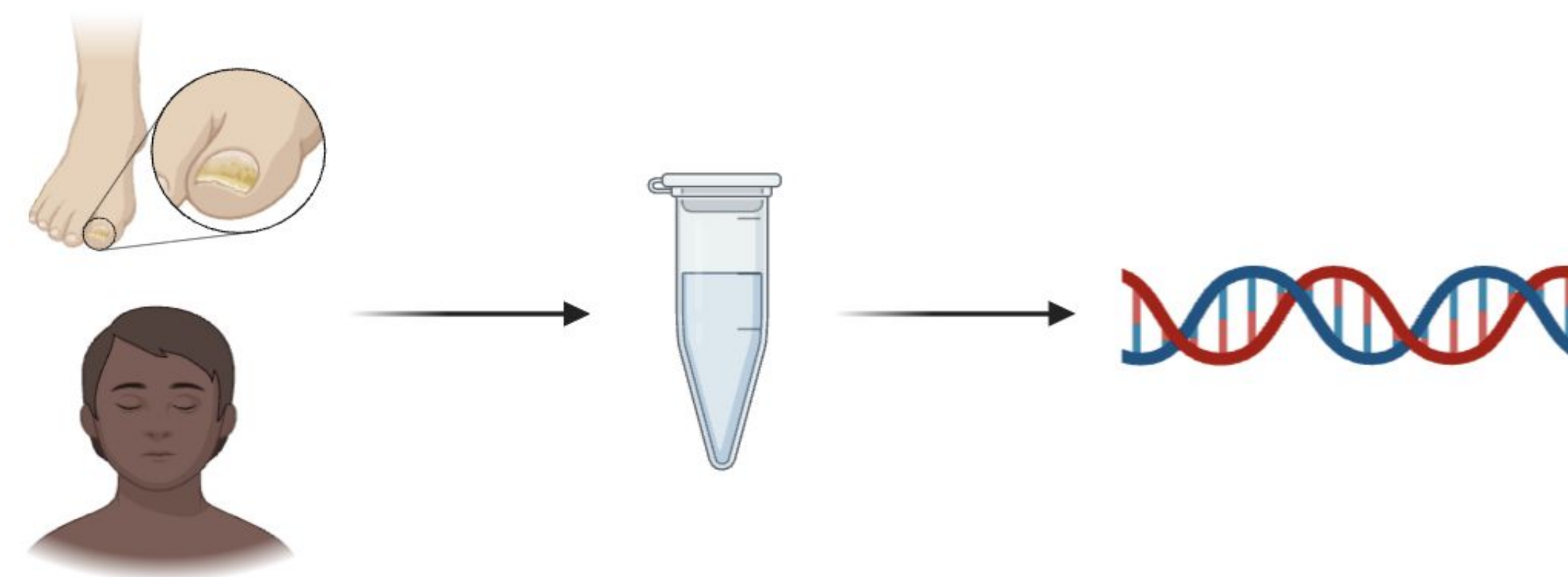


FUTURE DIRECTIONS

- Future work will focus on developing and validating a CRISPR-Cas12a guide for *T. indotineae*, an emerging terbinafine-resistant dermatophyte species that is genetically similar to other *Trichophyton* strains, making highly specific guide design challenging.
- Nanopore sequencing will be incorporated to confirm on-target LAMP amplification, identify sequence variation among closely related species, and ensure assay specificity. Integrating Nanopore data will also allow iterative refinement of primer and guide regions, improving the assay's accuracy and adaptability for future clinical use.

METHODS

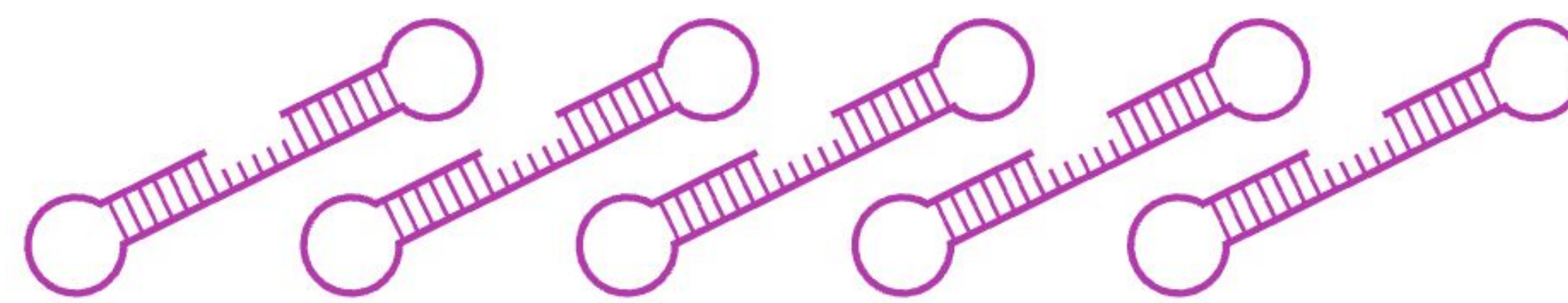
Step 1: Sample collection and DNA preparation.



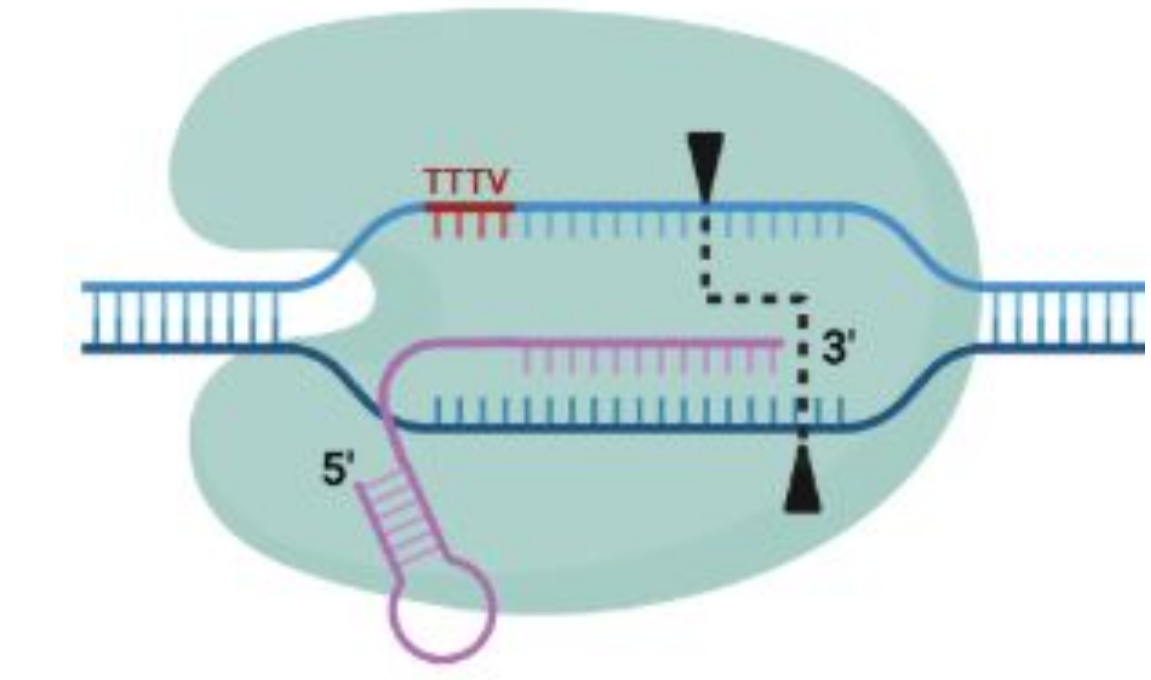
Step 2: Conserved **TOPII** gene regions are identified as genus-specific targets for *Microsporum*, *Trichophyton*, and *Epidermophyton*.



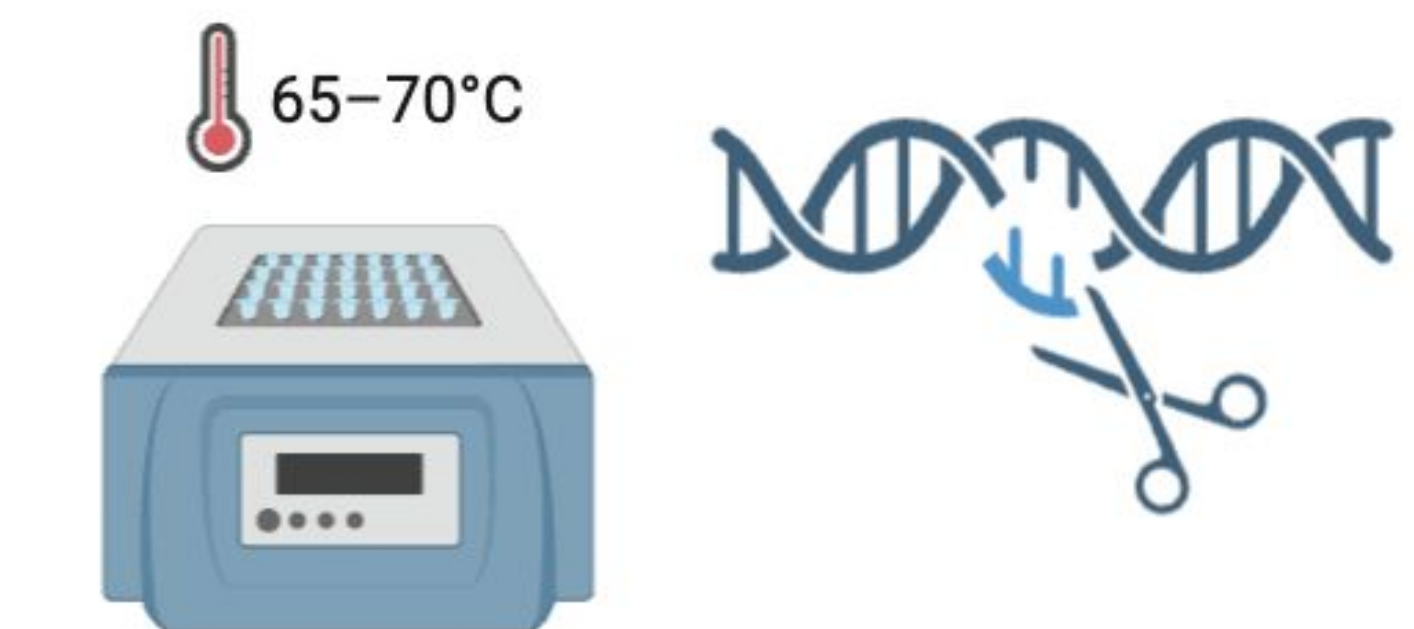
Step 3: LAMP primers are designed to amplify the selected gene region under isothermal conditions.



Step 4: A **Cas12a** guide RNA is designed within the amplified region to enable precise detection.



Step 5: LAMP amplification for ~20 minutes is followed by CRISPR-Cas12a detection (~10 minutes), which produces a visible fluorescent signal.



Step 6: Assay performance is being optimized and validated with additional isolates and negative controls.



PROGRESS TO DATE

- Genus-specific LAMP primers and CRISPR-Cas12a guides designed for *Microsporum* and *Epidermophyton*.
- CRISPR-Cas12a guides successfully detected *Trichophyton* species after LAMP amplification.



Figure A: Colorimetric LAMP amplification of *Trichophyton rubrum* DNA showing positive reactions (yellow) within 20–25 minutes, with no cross-reactivity to other dermatophytes and non-dermatophyte controls.

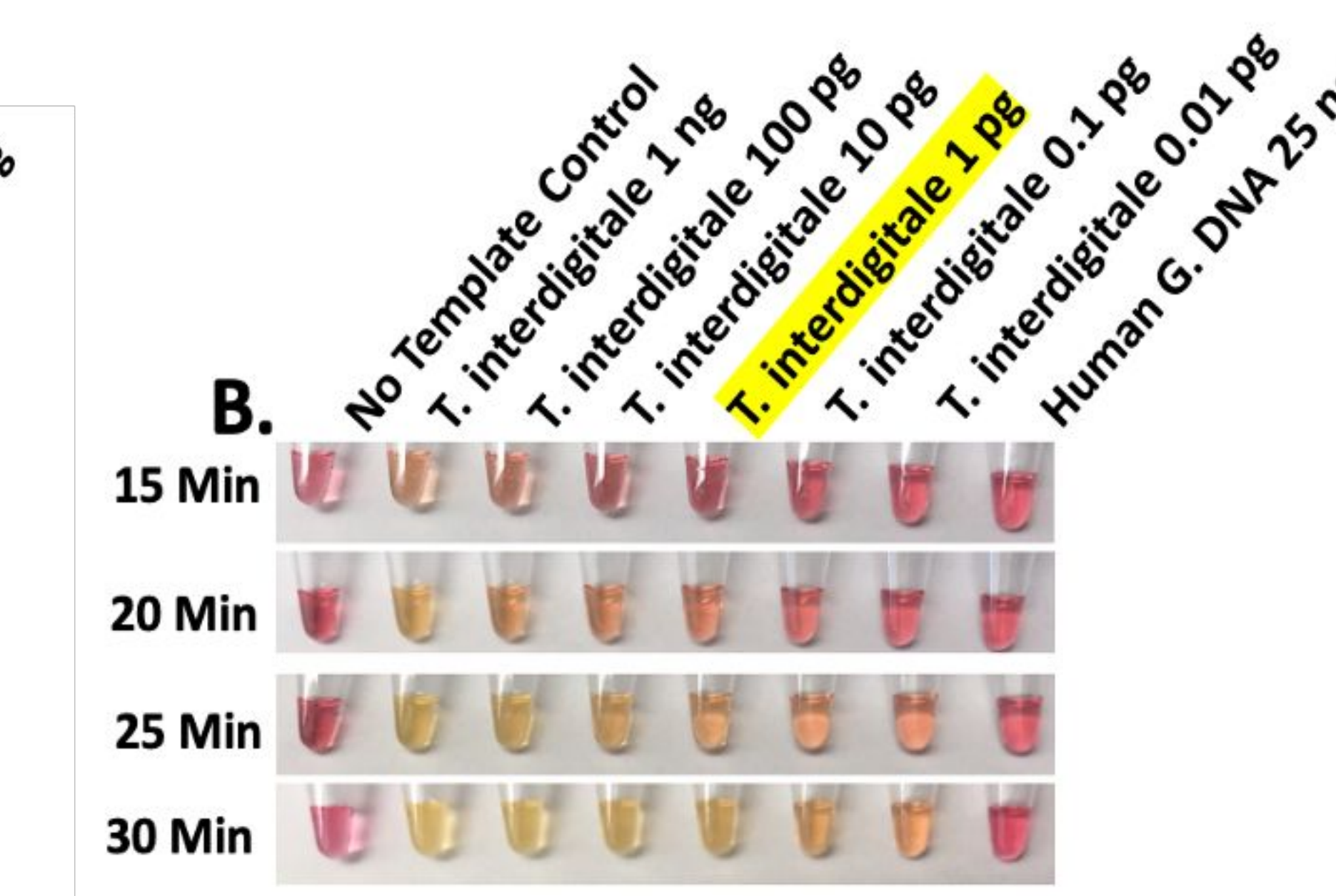


Figure B: Colorimetric LAMP amplification of *T. interdigitale* plasmid construct demonstrating specific amplification of the target sequence without cross-reactivity to human genomic DNA.

ACKNOWLEDGEMENTS

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