The use of engineered reverse transcriptase and *Taq* DNA polymerase variants for improved activity and inhibitor tolerance in POC MDx applications

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Introduction

Point of care (POC) molecular diagnostics (MDx) enable same-day diagnosis, revolutionizing patient care and disease surveillance. The requirement for DNA purification is a limitation for POC devices because it increases both the cost and time to get from sample to answer. In order to enable the use of crude lysates or skip extraction altogether, we engineered new reverse transcriptase (RT) and *Taq* DNA polymerase (*Taq* DNAP) enzymes with higher inhibitor tolerance and polymerization speed, and evaluated them with custom inhibitor-tolerant buffers.

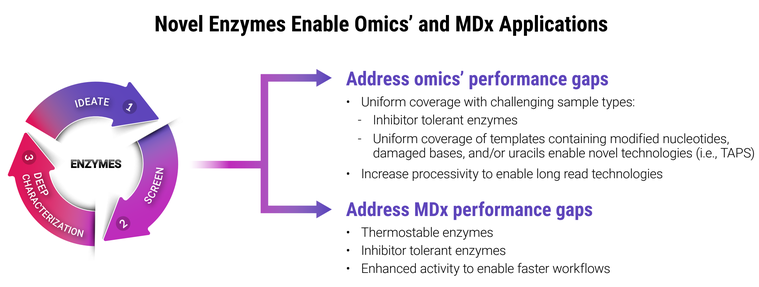

Figure 1. Overcoming performance gaps. Our enzyme engineering platforms are well suited to identify novel enzyme variants with properties desirable for genomics and MDx applications.

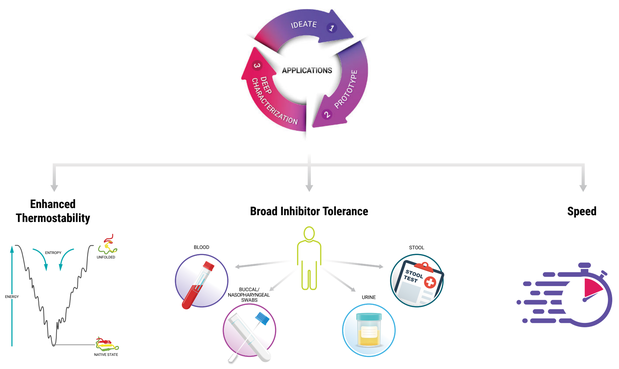

Figure 2. Purpose built reverse transcriptases and *Taq* DNA polymerases. To address current pain points in the molecular diagnostic field, we aimed to identify mutations in reverse transcriptases and *Taq* DNAP that would confer enhanced thermostability, inhibitor tolerance, and/or speed.

Engineered Polymerases

Watchmaker has previously detailed our engineered StellarScript® Reverse Transcriptases, purposefully designed for greater thermostability and inhibitor tolerance. In the work presented in this poster, we focused solely on StellarScript HT+ as the RT with the greatest ability to amplify targets in the presence of inhibitors.

Reverse Transcriptases

	Optimal Temp.	Thermal Stability	Inhibitor Tolerance	Template Switching	RT-qPCR	RT-PCR	RNA-seq	scRNA-seq	5' RACE	3' RACE
StellarScript	42°C	+	+	+	+	+	+	+	+	+
StellarScript HT	42 – 50°C	++	++	-	++	++	+	-	-	+
StellarScript HT+	42 – 65°C	+++	+++	+	+++	+++	+	+	+	+

Table 1. Overview of StellarScript portfolio enzyme properties and relevant applications. "+" signs indicate strength of a property or fit of an application for a specific enzyme.

Following onto this work, Watchmaker has also engineered the newly introduced StellarTaq™ DNA Polymerase. This engineered polymerase retains all the standard functions of a *Taq* polymerase, but significantly increases the speed of polymerization while improving inhibitor tolerance to the point of similar or higher performance compared to currently existing solutions available to laboratories focused on molecular diagnostic applications (see Figures 3 and 4).

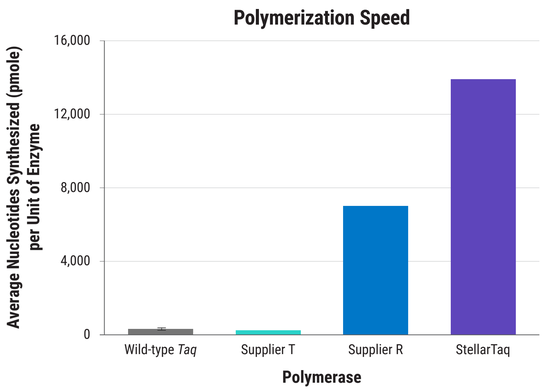

Figure 3. Relative polymerization speed of StellarTaq. StellarTaq DNAP, Wild-type *Taq* DNAP and competitor engineered *Taq* DNAPs were evaluated for polymerization speed by allowing extension of a pre-primed, ssDNA template at 72°C. Relative polymerization speed was measured over time by monitoring the fluorescence of an intercalating dye. StellarTaq had the fastest polymerization speed which enables faster workflows and ultra-fast PCR.

Engineered Polymerases (cont.)

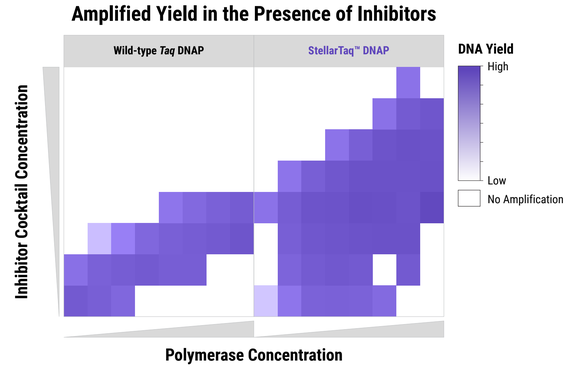

Figure 4. Amplification in the presence of inhibitors. StellarTaq DNAP and Wild-type *Taq* DNAP were evaluated in a probe-based qPCR assay using 0.35 ng of human genomic DNA (~100 copies) in the presence of increasing inhibitor cocktail and polymerase concentrations. StellarTaq maintains high DNA yield across a broad range of inhibitor and enzyme concentrations highlighting StellarTaq's broad utility with various sample types and applications.

Biological Sample Inhibitor Testing

StellarTaq and StellarScript HT+ were engineered by directed evolution, rational design and *in silico* modeling to achieve increased activity and inhibitor tolerance. These engineered variants have been characterized by using either a qPCR or one-step RT-qPCR assay to evaluate performance in the presence of key inhibitors. To start, StellarTaq was compared against Wild-Type *Taq* DNAP and two other available *Taq*-based engineered polymerases for the ability to amplify a known target in the presence of three biological samples types: urine, saliva, and blood.

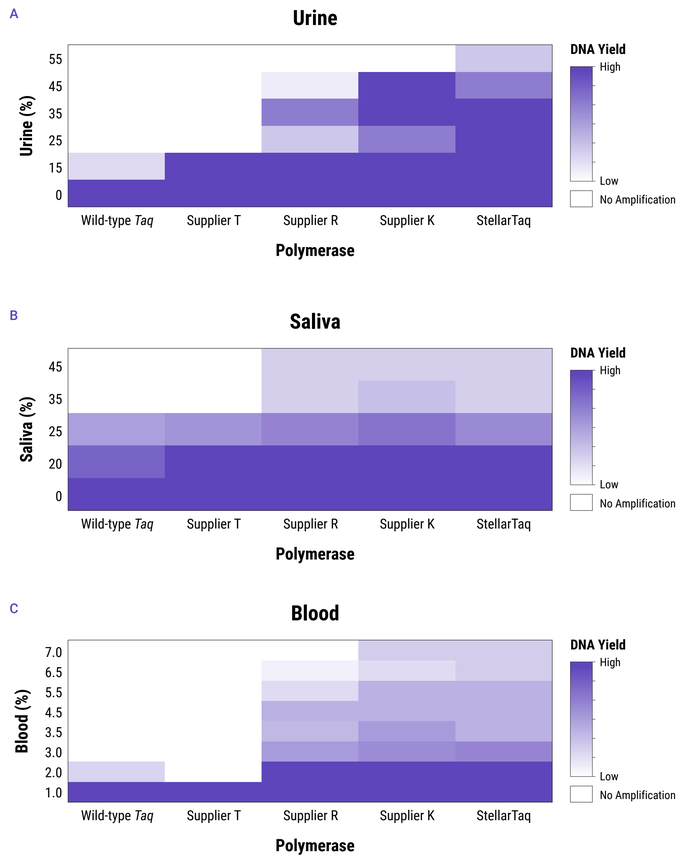

Figure 5. Robust amplification across biological sample types. StellarTaq DNAP, Wild-type *Taq* DNAP, and competitor-engineered *Taq* DNAPs were evaluated in probe-based qPCR. A 131 bp region of the SARS-CoV-2 ORF1ab gene was amplified from 20,000 copies of target DNA in the presence of increasing **(A)** Urine **(B)** Saliva and **(C)** Blood. ΔCq values were calculated: Cq (with inhibitor) - Cq (without inhibitor) and are displayed as a heat map where a lower ΔCq indicates increased DNA yield and inhibitor tolerance. Across all crude sample types, StellarTaq had the highest inhibitor tolerance enabling accurate qPCR results on crude sample types.

RT-qPCR Sensitivity

Reverse transcriptase is a known inhibitor of *Taq* DNAPs, thus the amount of RT added must be limited. This can affect the ability to generate sufficient amounts of cDNA template for sensitive downstream qPCR amplification. The solution to this issue is to engineer a *Taq* DNAP that is more resistant to the presence of an RT in the one-step RT-qPCR assay.

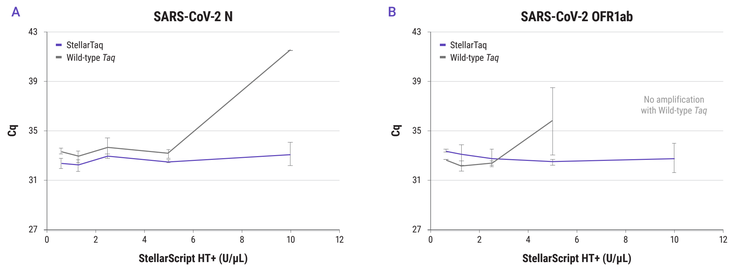

Figure 6. Achieve high sensitivity in RT-qPCR with StellarTaq DNAP and StellarScript HT+. A one-step multiplex RT-qPCR assay was carried out amplifying **(A)** a 67 bp region of SARS-COV2 N and **(B)** 131 bp region of SARS-COV2 ORF1ab from 10 copies of target RNA. To mimic a biological sample, 1 ng of universal human reference RNA was added. Yield impacts of increasing [StellarScript HT+] was assessed using StellarTaq DNAP (purple) vs Wild-type *Taq* DNAP (grey). StellarTaq has increased yield and sensitivity at higher concentrations of StellarScript HT+. This allows users to add higher concentrations of reverse transcriptase to their reactions for better yield and sensitivity specifically when working with challenging or crude sample types.

To take advantage of the enhanced ability of StellarTaq to tolerate higher RT concentrations, StellarScript HT+ has been engineered for improved robustness in the presence of common inhibitors safeguards performance and ensures compatibility across a wide range of sample types.

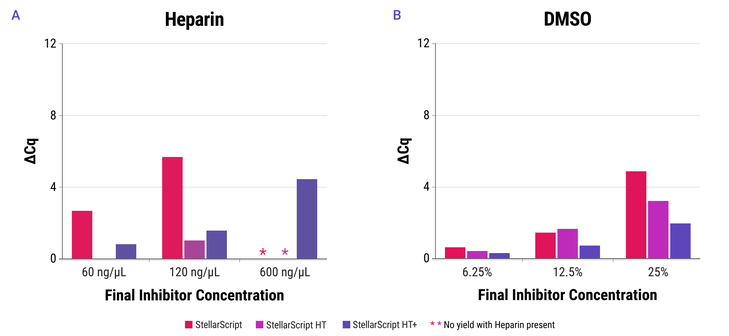

Figure 7. Robust performance in the presence of common inhibitors. StellarScript, StellarScript HT, and StellarScript HT+ were run in oligo-dT-primed first strand synthesis using 10 ng of total liver RNA as template in varying concentrations of inhibitors: **(A)** Heparin **(B)** DMSO. Resulting cDNA yields were assessed via qPCR using primers targeting the 5' end of β-actin gene to generate a 90 bp amplicon. Cq values were calculated: ΔCq (with inhibitor) - Cq (without inhibitor). Lower ΔCq values indicate increased inhibitor tolerance. StellarScript HT+ had the highest inhibitor tolerance.

StellarScript HT+ has demonstrated high thermostability, generating higher cDNA yields and better maintaining its folded protein structure at elevated temperatures relative to commonly used RTs.

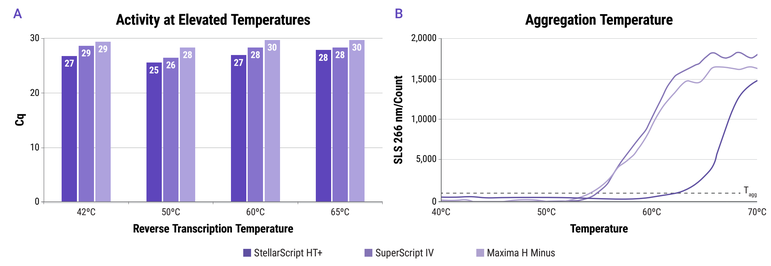

Figure 8. StellarScript HT+ delivers superior thermostability. (A) Watchmaker's StellarScript HT+ and ThermoFisher Scientific's SuperScript IV and Maxima H Minus were run in an oligo-dT-primed first strand synthesis with 10 ng of total liver RNA. Resulting cDNA was assessed via qPCR using primers targeting the 5' end of β-actin gene to generate a 90 bp amplicon. StellarScript HT+ produced higher yields (indicated by lower Cq values) than SuperScript IV and Maxima H Minus across all temperatures tested, indicating improved efficiency at elevated temperatures. **(B)** The thermostability of the enzymes was further assessed via static light scattering under increasing temperatures to determine their respective aggregation temperatures. StellarScript HT+ exhibited the highest thermostability which often translates to improved storage stability and supply chain efficiency.

Conclusions

- The Watchmaker Genomics' engineering platform has enabled the development of *Taq* polymerase and reverse transcriptase enzymes that are capable of more robust amplification, speed, and inhibitor tolerance for molecular diagnostic applications.
- These enzymes are available in either glycerol-free or high-concentration versions that make them ideal for use in lyophilized assay formats, increasing shelf life and reducing cold-chain storage concerns.

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