WATCHMAKER GENOMICS

# STELLARSCRIPT REVERSE TRANSCRIPTASE PORTFOLIO

## PRECISION ENZYMES TO FUEL CUTTING-EDGE APPLICATIONS

The **StellarScript® Reverse Transcriptase Portfolio** features three M-MLV variants with reduced RNase H activity and enhanced processivity, activity, thermostability, and inhibitor tolerance in comparison to wild-type enzyme.

## HIGHLIGHTS

- **Enhanced thermostability** improves cDNA product specificity and improves reverse transcription through GC-rich regions
- **Increased activity** generates higher cDNA yields of longer lengths for high-sensitivity performance with degraded RNA and **inputs as low as 10 pg**
- **Improved inhibitor tolerance** ensures compatibility with a broad range of sample sources and applications

## SPECIFICATIONS

- Concentration: 200 U/µL
- Purity (SDS-PAGE): >99%
- dsDNA exonuclease: <1% released[1]
- ssDNA exonuclease: <1% released[1]
- DNA contamination (*E. coli*, mammalian, library): <10 copies[1]
- Phosphatase assay: <1% release[1]
- Endonuclease: Not detectable[1]
- Nonspecific RNase: Not detectable[1]

## APPLICATIONS

- First strand cDNA synthesis
- RT-qPCR/qRT-PCR
- RT-PCR
- Primer extension
- RACE
- RNA sequencing
- Single cell RNA sequencing[2]

[1]As assessed using 400 U per assay

[2]StellarScript and StellarScript HT+ Reverse Transcriptases only

For research use only. Not for use in diagnostic procedures.

## SELECT THE IDEAL REVERSE TRANSCRIPTASE FOR YOUR APPLICATION

A tailored suite of reverse transcriptase variants with a wide range of enhanced properties fuel multiple applications including:

- **StellarScript** for template switching and robust performance
- **StellarScript HT** for increased thermostability and inhibitor tolerance
- **StellarScript HT+** for template switching and the highest thermostability and inhibitor tolerance

**Table 1. Overview of StellarScript portfolio enzyme properties and relevant applications**

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

"+" signs indicate strength of a property or fit of an application for a specific enzyme.

## IMPROVE SPECIFICITY WITH INCREASED THERMOSTABILITY

The high degree of secondary structure in many RNA species—such as high-GC transcripts—can make the generation of full-length cDNA difficult and decrease overall assay sensitivity. Increased reverse transcription temperatures can relax secondary structure, but this approach requires a highly thermostable reverse transcriptase. The StellarScript suite contains variants with a wide range of optimal reverse transcription temperatures to increase cDNA product specificity.

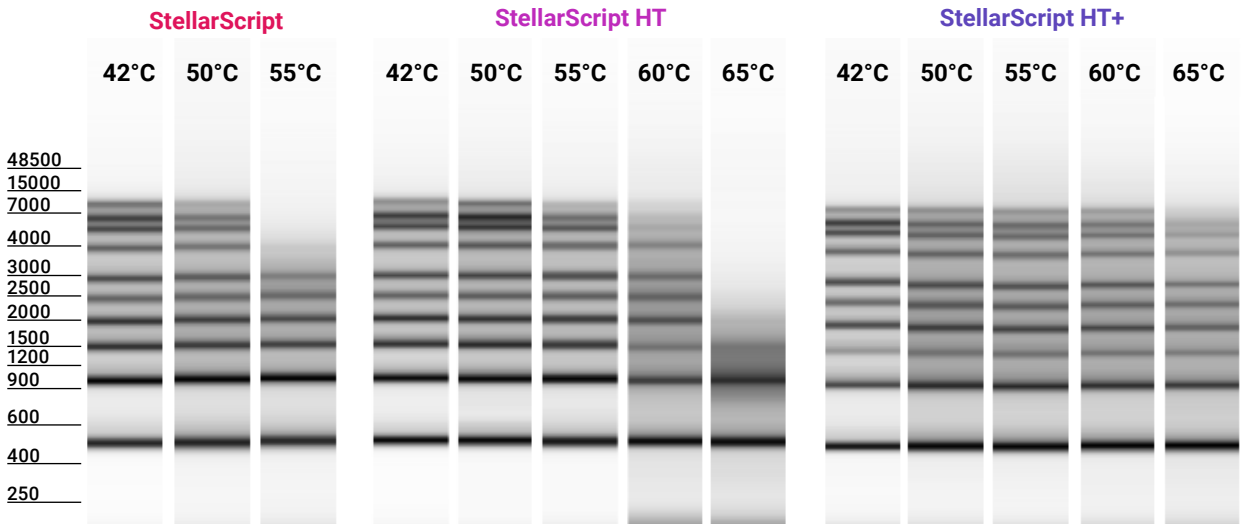



**FIGURE 1. Overcome RNA secondary structure.** StellarScript, StellarScript HT, and StellarScript HT+ were run in oligo-dT-primed first strand synthesis for 30 min using a 0.5 to 9 kb RNA ladder as indicated. Reverse transcriptase thermostability increases from StellarScript to HT and HT+.

## SAFEGUARD PERFORMANCE WITH INHIBITOR TOLERANCE

Inhibitors can dampen reverse transcription efficiency and impact sensitivity in applications such as RT-qPCR. Improved robustness in the presence of common inhibitors safeguards performance and ensures compatibility across a wide range of sample types and sources. StellarScript HT+ delivers the highest level of inhibitor tolerance across the StellarScript portfolio.

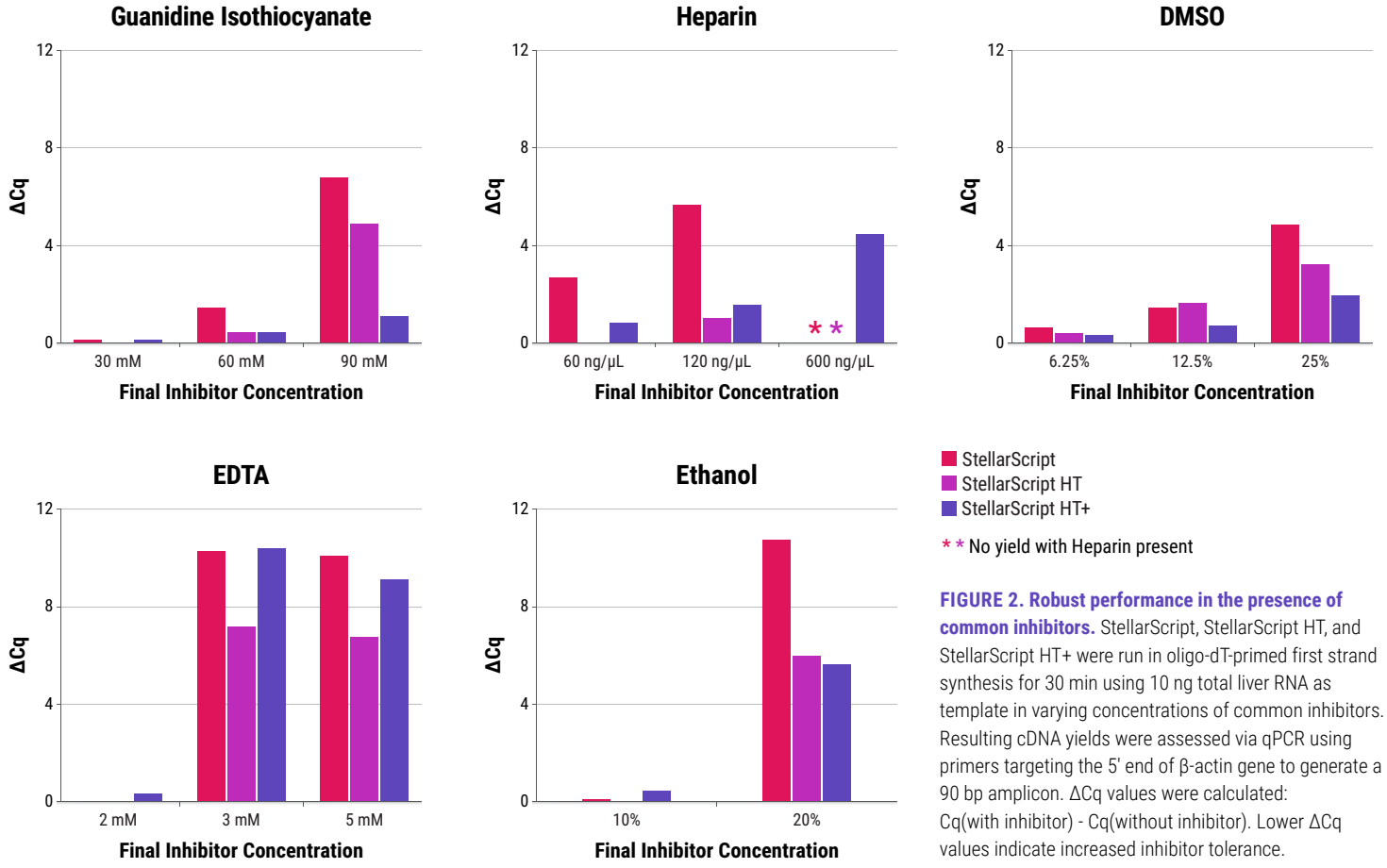



**FIGURE 2. Robust performance in the presence of common inhibitors.** StellarScript, StellarScript HT, and StellarScript HT+ were run in oligo-dT-primed first strand synthesis for 30 min using 10 ng total liver RNA as template in varying concentrations of common inhibitors. Resulting cDNA yields were assessed via qPCR using primers targeting the 5' end of β-actin gene to generate a 90 bp amplicon. ΔCq values were calculated: Cq(with inhibitor) - Cq(without inhibitor). Lower ΔCq values indicate increased inhibitor tolerance.

## ACHIEVE SENSITIVE PERFORMANCE WITH FFPE

Formalin-fixed paraffin-embedded (FFPE) RNA is difficult to process due to template damage and the presence of residual crosslinks. The StellarScript suite delivers high-quality performance with this challenging sample type, even with low template amounts.

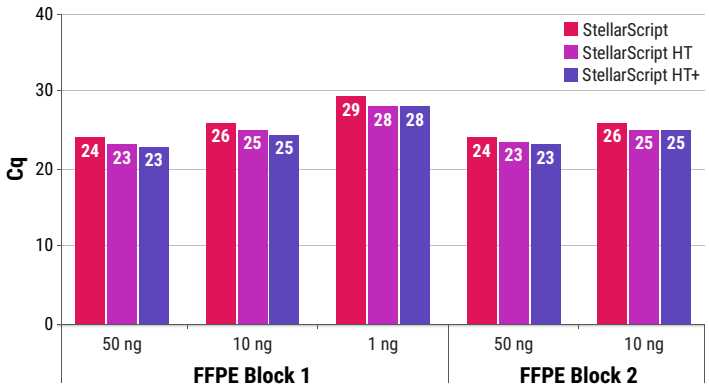



**FIGURE 3. Reliable performance with FFPE.** StellarScript, StellarScript HT, and StellarScript HT+ were run in oligo-dT and randomly primed first strand synthesis at 50°C for 30 min using total RNA inputs from two independent FFPE blocks as indicated. Resulting cDNA yields were assessed via qPCR using primers targeting the 5' end of β-actin gene to generate a 90 bp amplicon. All enzymes show similar sensitivity.

## STELLARSCRIPT FOR ROBUST PERFORMANCE

StellarScript provides enhanced thermostability in comparison to wild-type M-MLV reverse transcriptase and performs equivalently or better in comparison to SuperScript™ II with respect to both cDNA yield and lengths generated at elevated temperatures.

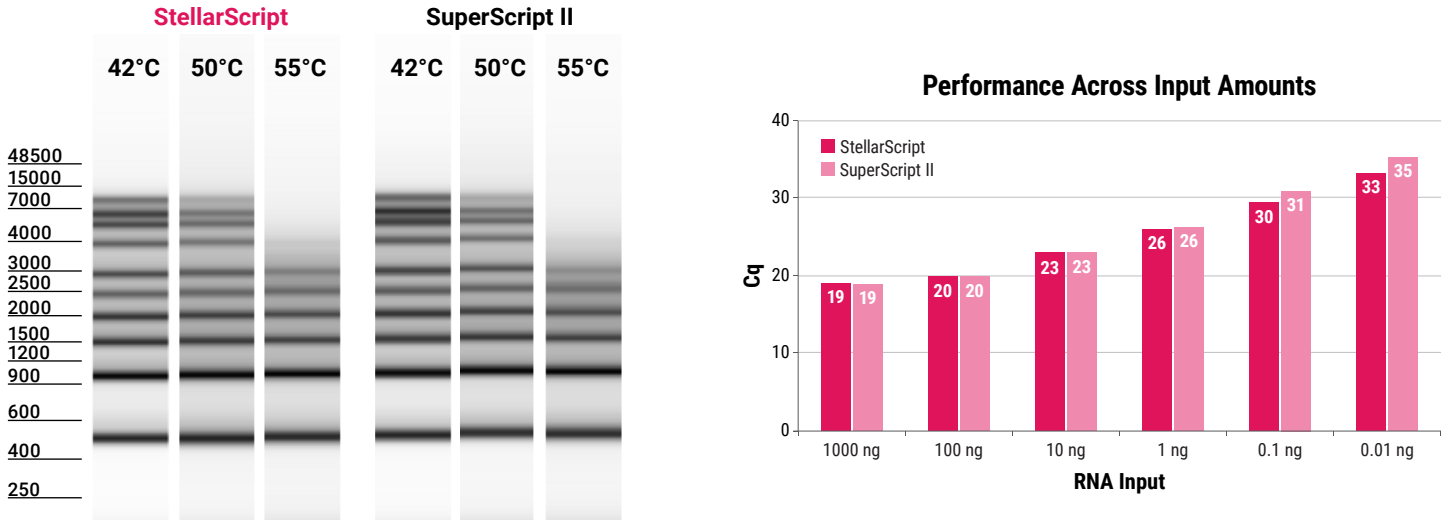



**FIGURE 4. StellarScript provides equivalent performance to SuperScript II.** **(Left)** Watchmaker's StellarScript and ThermoFisher Scientific's SuperScript II were run in oligo-dT primed first strand synthesis at 42°C, 50°C, or 55°C for 30 min using a 0.5 to 9 kb RNA ladder as template. Both enzymes have robust processivity at 42°C that decreases with increased temperature. **(Right)** StellarScript and SuperScript II were run in an oligo-dT and randomly primed first strand synthesis at 42°C for 40 min using 1000 ng to 0.01 ng total liver RNA. Resulting cDNA yields were assessed via qPCR using primers targeting the 5' end of β-actin gene to generate a 90 bp amplicon. StellarScript showed equivalency with inputs of 1 ng or more and showed slight outperformance with lower input amounts.

## STELLARSCRIPT HT FOR IMPROVED THERMOSTABILITY

StellarScript HT generates higher cDNA yields and performs equivalently with respect to processivity and cDNA yield generation relative to SuperScript III at elevated temperatures.

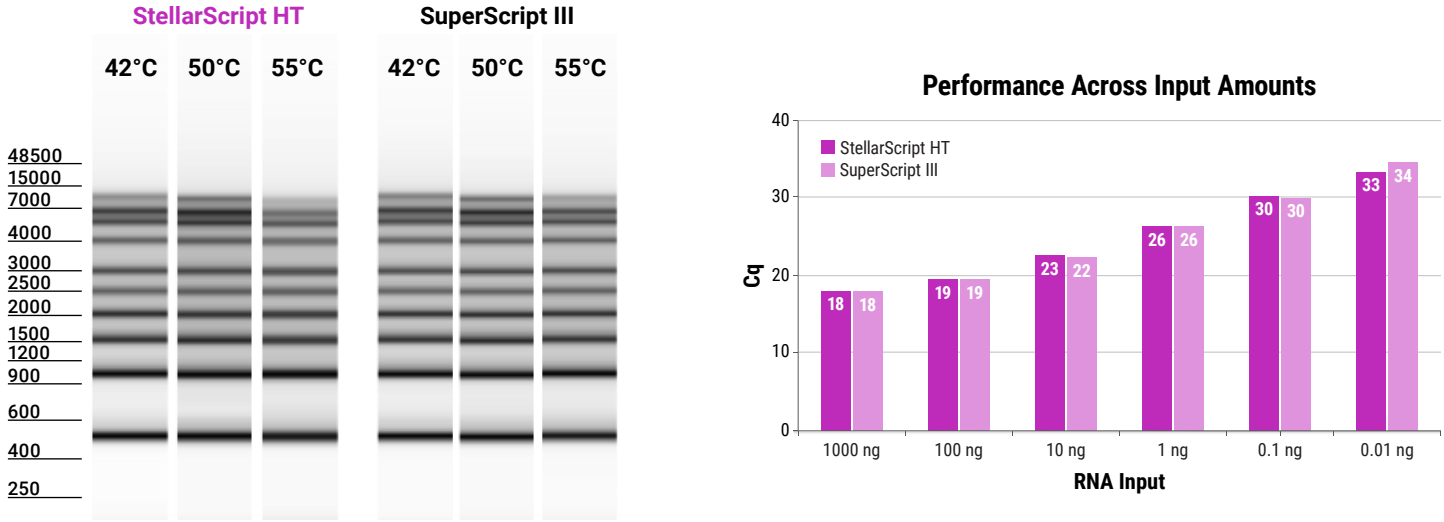



**FIGURE 5. StellarScript HT provides equivalent performance to SuperScript III.** **(Left)** Watchmaker's StellarScript HT and ThermoFisher Scientific's SuperScript III were run in oligo-dT-primed first strand synthesis at 42°C, 50°C, or 55°C for 30 min using a 0.5 to 9 kb RNA ladder as template. Both enzymes have robust processivity across the temperatures assessed. **(Right)** StellarScript HT and SuperScript III were run in an oligo-dT and randomly primed first strand synthesis at 50°C for 40 min using 1000 ng to 0.01 ng total liver RNA as template. Resulting cDNA yields were assessed via qPCR using primers targeting the 5' end of β-actin gene to generate a 90 bp amplicon. StellarScript HT showed overall equivalency to SuperScript III.

## STELLARSCRIPT HT+ FOR MAXIMUM THERMOSTABILITY

Increased thermostability enables reverse transcription at elevated temperatures to overcome RNA template secondary structure and generate cDNAs from difficult targets, such as viral RNA. It further improves specificity by minimizing nonspecific primer binding.

StellarScript HT+ is the most thermostable enzyme in the portfolio, generating higher cDNA yields and better maintaining its folded protein structure at elevated temperatures relative to SuperScript IV and Maxima H Minus.

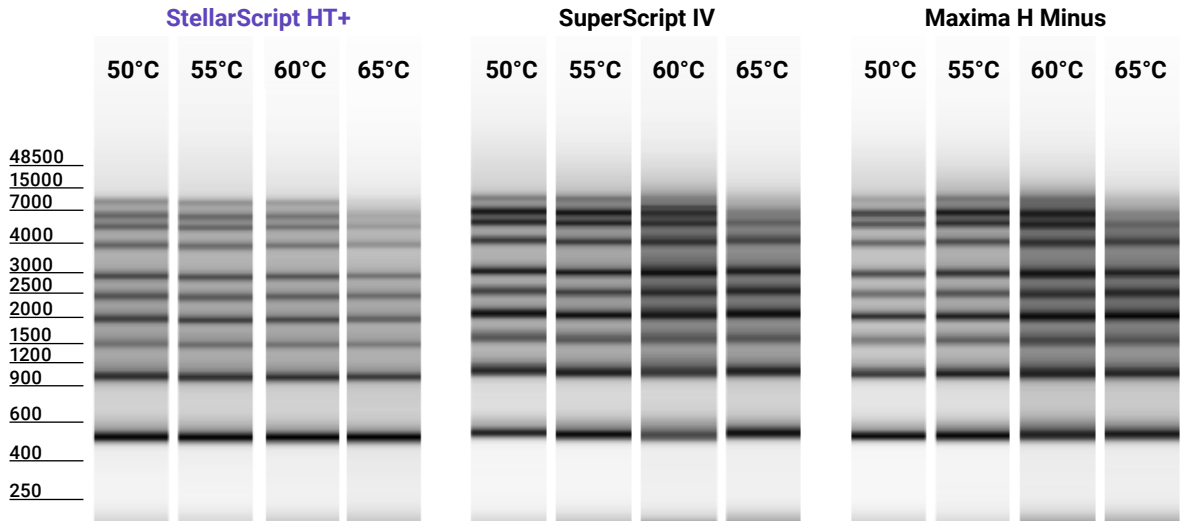
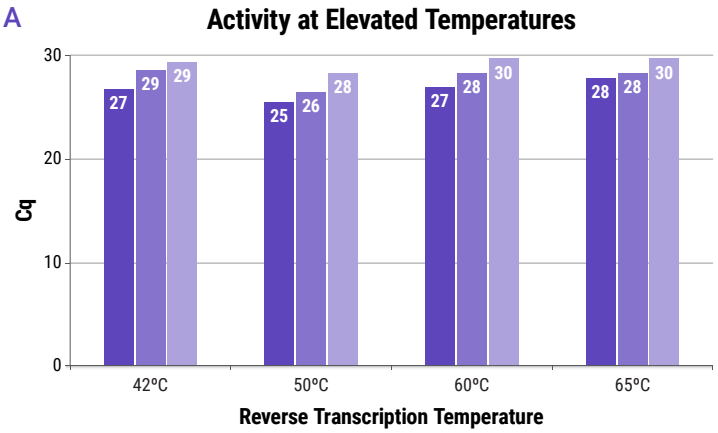



**FIGURE 6. StellarScript HT+ provides equivalent processivity at elevated temperatures.** Watchmaker's StellarScript HT+ and ThermoFisher Scientific's SuperScript IV and Maxima H Minus were run in an oligo-dT-primed first strand synthesis at 50°C, 55°C, 60°C, or 65°C for 30 min using a 0.5 to 9 kb RNA ladder as template. All enzymes have robust processivity up to 60°C.



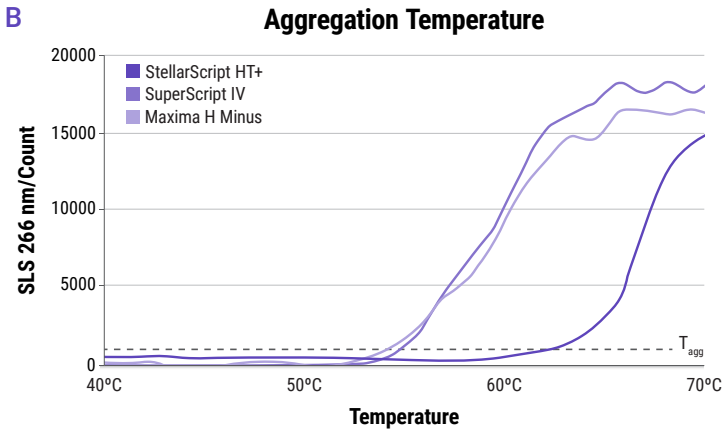
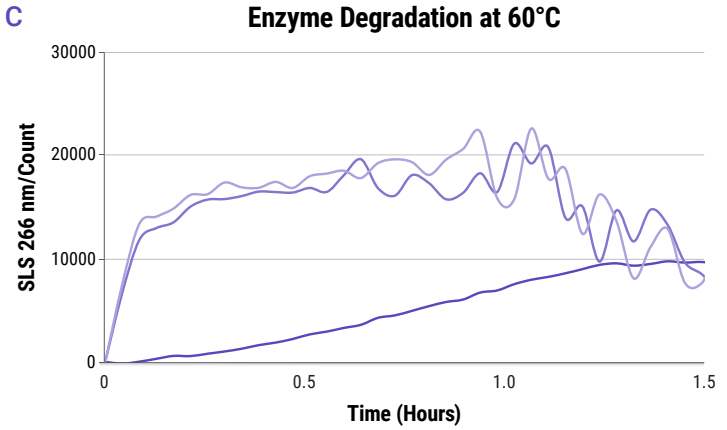
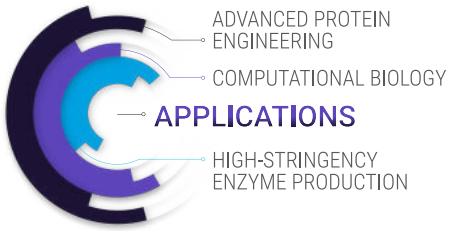




**FIGURE 7. StellarScript HT+ delivers superior thermostability. (A)** Watchmaker's StellarScript HT+ and ThermoFisher Scientific's SuperScript IV and Maxima H Minus were run in an oligo-dT-primed first strand synthesis was employed for 25 min using 10 ng total liver RNA, as indicated. Resulting cDNA mass 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 at any evaluated temperature, indicating improved efficiency at elevated temperatures. **(B)** Enzymes were further assessed via static light scattering under increasing temperature to determine their respective aggregation temperatures, at which point protein unfolding begins and **(C)** during a time course study at 60°C to measure their stability in reverse transcription reaction conditions. The enhanced thermostability of StellarScript HT+ is demonstrated by its increased aggregation temperature and improved stability over time at 60°C.



## OUR TECHNOLOGY: THE NEW FRONTIER OF PROTEIN ENGINEERING

Industry-leading assays require best-in-class reagents. Watchmaker's focus on application-driven development and deep domain expertise in advanced enzyme engineering, computational biology, and high-stringency protein production enables us to address unmet needs with respect to performance, scalability, and consistency of supply. Our purpose-designed enzymes deliver outstanding performance from prototype to large volume production.




## ADVANTAGES OF PARTNERING WITH WATCHMAKER

- High-stringency enzyme manufacturing ensures quality performance across scales and lots
- Custom formats available to support lyophilization applications
- Expedited custom labeling and kitting formats from bulk to finished goods
- Application-relevant kit-based lot testing
- ISO13485-compliant Quality Management System
- Flexible terms designed with both start-up and large organizational needs in mind

Contact sales@watchmakergenomics.com
or visit watchmakergenomics.com/stellarscript to learn more.

| PRODUCT | 10 kU[1] | 40 kU[1] | 200 kU[1] |
|---|---|---|---|
| StellarScript Multi Reverse Transcriptase Sample Pack (200 U/µL)<br>*incl. 10X RT Reaction Buffer, 10 mM dNTP Mix, 100 mM DTT, and 50 µL each of StellarScript RT, StellarScript HT RT, and StellarScript HT+ RT* | 7K0083-50UL | – | – |
| StellarScript Reverse Transcriptase Kit (200 U/µL)<br>*incl. 10X RT Reaction Buffer, 10 mM dNTP Mix, 100 mM DTT* | 7K0080-50UL | 7K0080-200UL | 7K0080-1ML |
| StellarScript HT Reverse Transcriptase Kit (200 U/µL)<br>*incl. 10X RT Reaction Buffer, 10 mM dNTP Mix, 100 mM DTT* | 7K0070-50UL | 7K0070-200UL | 7K0070-1ML |
| StellarScript HT+ Reverse Transcriptase Kit (200 U/µL)<br>*incl. 10X RT Reaction Buffer, 10 mM dNTP Mix, 100 mM DTT* | 7K0081-50UL | 7K0081-200UL | 7K0081-1ML |

[1]One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble material in 10 min at 37°C using poly(A)/oligo (dT) as a substrate.



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