Supplementary Information

Reagents & Materials

Recombinant *Leptotrichia wadei* Cas13 (LwaCas13a) was kindly provided by the Zhang Lab at the Broad Institute of MIT and Havard (Cambridge, MA USA). TrueGuide™ Synthetic sgRNA (CRISPR486687_SGM) with MSTN as gene target, Lipofectamine RNAiMAX transfection reagent, Novex™ 8 % TBE Gels, GeneRuler Ultra Low Range DNA Ladder (ready-to-use), Novex™ Hi-Density TBE Sample Buffer (5x), Novex™ TBE Running Buffer (5x), SYBR™ Safe DNA Gel Stain and RNase Away surface decontaminant were purchased from Thermo Fisher Scientific (Waltham, MA USA). Forward and reverse primer along with the crRNA were custom synthesised by GenScript (Piscataway, NJ USA). Analyte extraction was achieved with the miRNeasy Serum/Plasma Advanced Kit from Qiagen (Hilden, Germany). Isopropanol was acquired from Biosolve BV (Valkenswaard, Netherlands) while ethanol, 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethane-1-sulfonic acid (HEPES) powder, glycerol, dithiothreitol (DTT), Tris-hydrochlorid, sodium chloride, magnesium chloride, pooled human serum and recombinant Cas9 protein from *Streptococcus pyogenes* were procured from Merck (Darmstadt, Germany). Isothermal nucleic acid amplification was performed utilising the TwistAmp Basic kit from TwistDx (Maidenhead, United Kingdom) and M-MuLV Reverse Transcriptase, Murine RNase Inhibitor, Ribonucleotide Solution Mix as well as T7 RNA Polymerase were purchased from New England BioLabs (Frankfurt am Main, Germany). For nucleic acid detection, the RNaseAlert substrate from Integrated DNA Technologies (Coralville, IA USA) was used as reporter molecule.

Experimental Procedure

As the experimental procedure includes the generation of large quantities of amplified products, preventive measures were taken to avoid sample contamination. Hence, separate pre- and post-amplification work areas were set up and wiped down with RNase Away before each experiment. Sample extraction was conducted employing the Qiagen miRNeasy Serum/Plasma Advanced Kit using a sample input volume of 100 µL serum, adjusting the manufacturer's instructions to that effect. Subsequently, 1 µL of the obtained extract was forwarded to isothermal amplification of the target nucleic acid sequence via RT-RPA. Since the utilised TwistAmp Basic kit is designed for DNA amplification, the master mix was accommodated by the addition of reverse transcriptase and RNase inhibitor and scaled up according to the number of reactions needed (Table 1). Furthermore, the incubation temperature was set to 42 °C but apart from that the amplification procedure was conducted according to the manufactures' instructions. For PAGE screening of the target amplicon, 1 µL the obtained RT-RPA sample was diluted with 7 µL water and mixed with 2 µL Hi-Density buffer before directly loading it onto a TBE gel (Novex TBE Gel, 8 %) along with 2 µL GeneRuler Ultra Low Range DNA Ladder. After the gel electrophoresis was conducted at a constant voltage of 200 V for approximately 30 min, the gels were washed with water and subsequently stained with SYBR Safe (after 1:10000 dilution in running buffer) for 30 min at room temperature in the dark. Band visualisation was achieved via UV excitation utilising the Gel DocTM XR+ device from Bio Rad (Hercules, CA USA). Following this, the master mix required for SHERLOCK nucleic acid detection was prepared according to the number of reactions (Table 2) adding an excess volume of 15% to compensate for pipetting errors. After resuspension of the lyophilised RNaseAlert substrate with 2 µL 10X RNaseAlert Buffer, 18 µL SHERLOCK master mix and 1 µL RT-RPA reaction sample was added and the mixture was incubated at 37 °C for 30 min while protected from light exposure. The fluorescence FAM reporter signal (Em 520 nm) induced by collateral activity after target recognition was finally detected via the UV transilluminator of the Gel DocTM XR+ device.

Table 1: RT-RPA master mix for one reaction

Component	Volume [µL]
Forward primer 10µM	2.4
Reverse primer 10 µM	2.4
Rehydration Buffer (TwistAmp)	29.5
10X M-MuLV buffer	5
M-MuLV RT (200 U/µL)	1
Murine RNase Inhibitor (40 U/µL)	1.25
UltraPure water	4.95

Table 2: SHERLOCK master mix for one reaction

Component	Volume [µL]
UltraPure water	11.3
HEPES (pH 6.8, 1 M)	0.4
MgCl2 (1 M)	0.2
rNTP solution mix (25 mM each)	0.8
LwaCas13a in SB* (63.3 µg/mL)	2
Murine RNase Inhibitor (40 U/µL)	1
T7 RNA polymerase (5 U/µL)	0.5
crRNA (10 ng/µL)	1

*SB = LwaCas13a protein storage buffer after Kellner et al.[1]

In Vitro Study

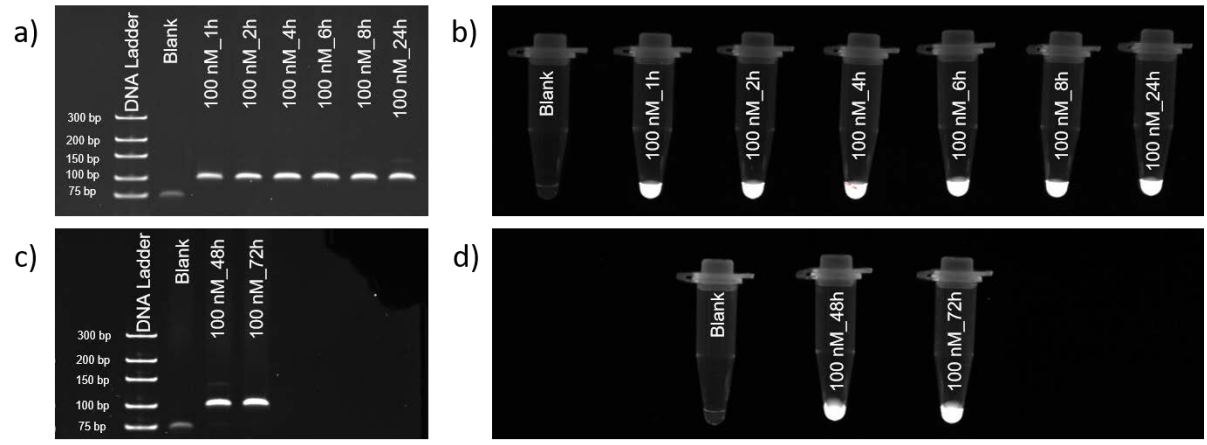
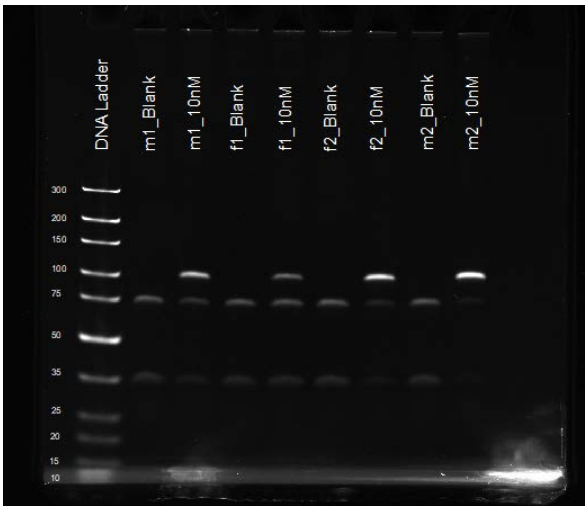


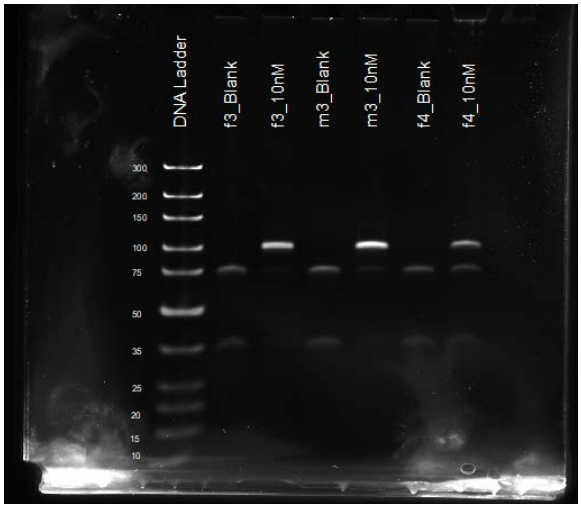
Figure S1. a) PAGE screening of in vitro samples after 1, 2, 4, 6, 8 and 24 h incubation time of RNP complex in pooled serum (comprising 100 nM sgRNA) b) SHERLOCK confirmation of in vitro samples after 1, 2, 4, 6, 8 and 24 h incubation time of RNP complex in pooled serum (comprising 100 nM sgRNA) c) PAGE screening of in vitro samples after 48 and 72 h incubation time of RNP complex in pooled serum (comprising 100 nM sgRNA) d) SHERLOCK confirmation of in vitro samples after 48 and 72 h incubation time of RNP complex in pooled serum (comprising 100 nM sgRNA)

References

1. Kellner, M. J.; Koob, J. G.; Gootenberg, J. S.; Abudayyeh, O. O.; Zhang, F., SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nat Protoc* **2019,** *14* (10), 2986-3012.

Raw images of all PAGE gels reported in the paper





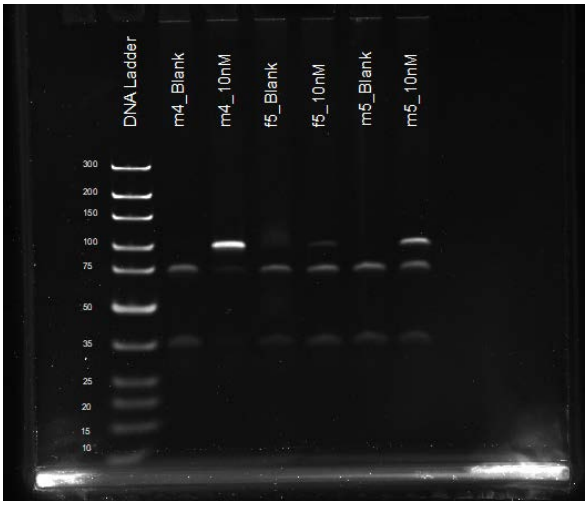
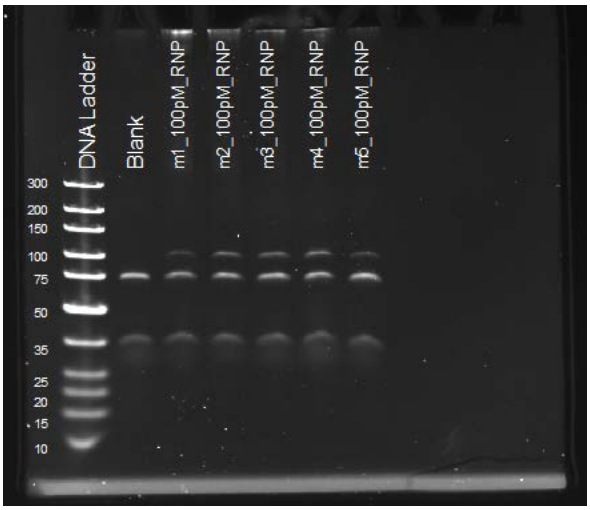


Figure S2. Specificity and sensitivity (sgRNA) of RT-RPA reaction via PAGE with 10 individual serum samples (5 male & 5 female) [Figure 4b]



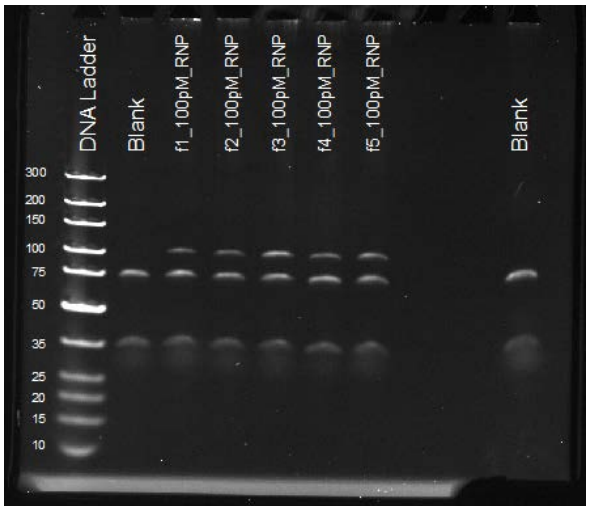


Figure S3. Sensitivity (RNP) of RT-RPA reaction via PAGE with 10 individual serum samples [Figure 4g]

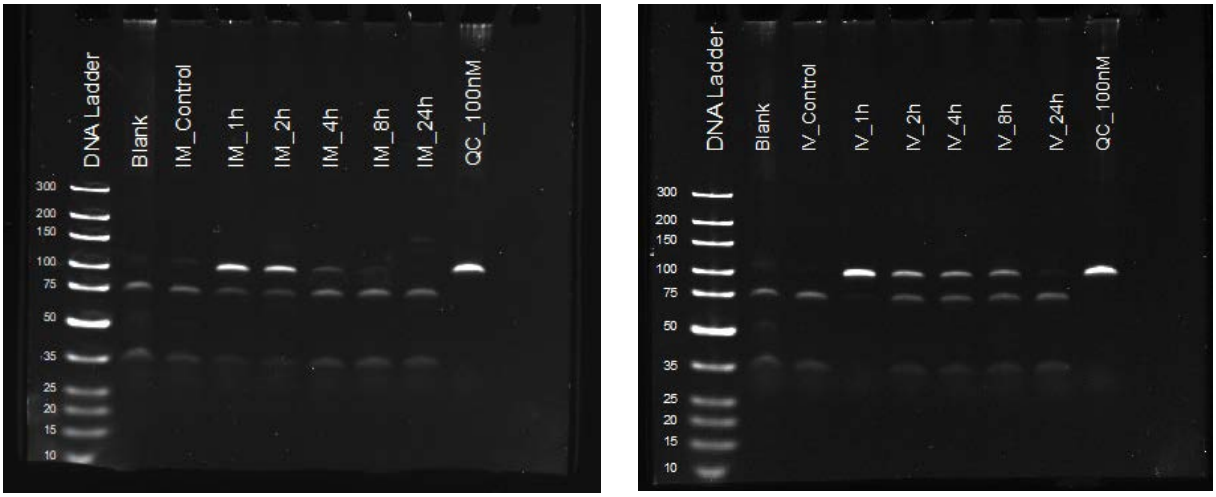


Figure S4. PAGE results of in vivo study after intramuscular injection and intravenous injection [Figure 6a & b]

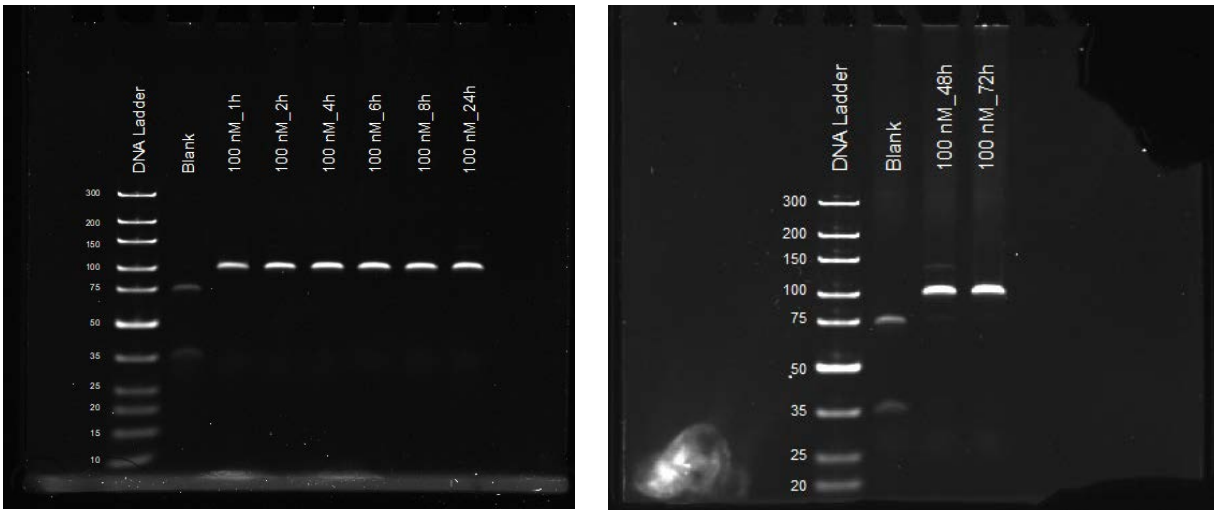


Figure S5. PAGE screening of in vitro samples after 1, 2, 4, 6, 8, 24, 48 and 72 h incubation time of RNP complex in pooled serum (comprising 100 nM sgRNA) [Figure S1a & c]

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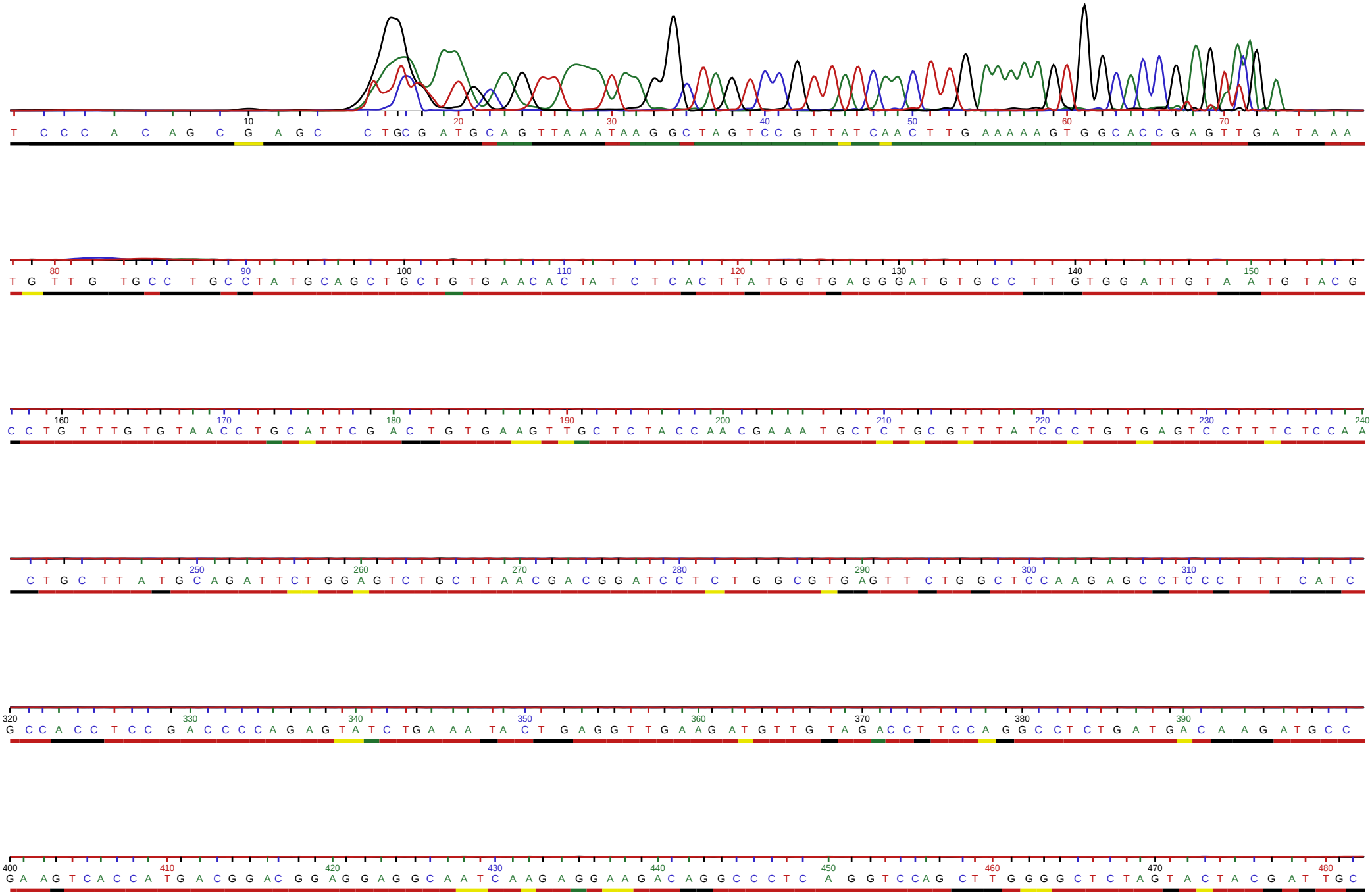

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