RNA extraction from a mycobacterium under ultrahigh electric field intensity in a microfluidic device

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Supporting Information: Fig. S1-S5, Table S1 and Video S1

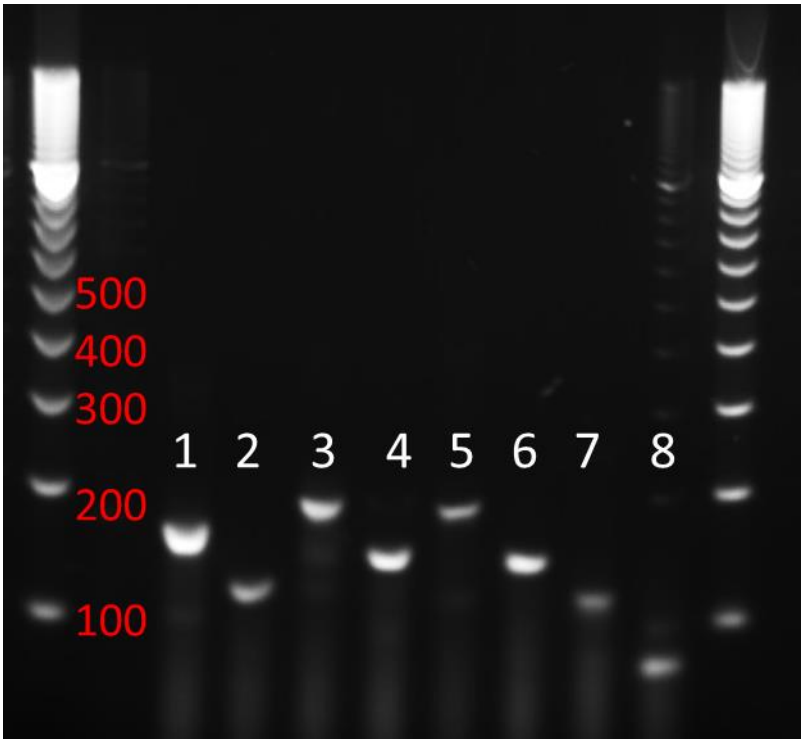

Fig. S1 RT-PCR product using the primers in **Table S1** and the primers containing added T7 promoter sequences. The RT-PCR products generated using primers containing T7 promoter sequences were 40 bp longer than the DNA templates generated using primers in **Table S1**. PCR products and 100 bp DNA ladder (Biorad) were loaded on 2% agarose gel. The PCR products containing T7 promoter sequences were in Lane 1 (SigA), 3 (ClpX), 5 (DnaE) and 7 (KatG). The PCR products without T7 promoter sequence (using primers in **Table S1**) were in Lane 2 (SigA), 4 (ClpX), 6 (DnaE) and 8 (KatG).

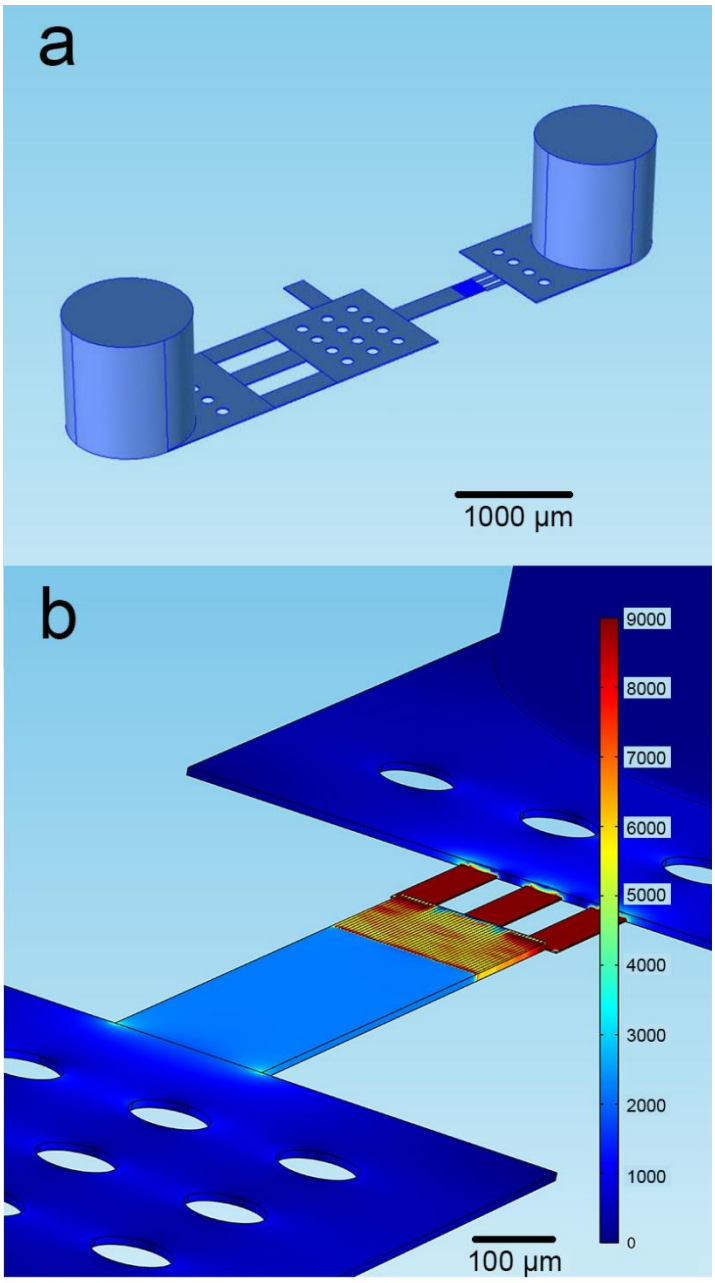

Fig. S2 COMSOL Multiphysics modelling of electric field intensity in the electrolysis channel with packed beads. (a) The overview of the modelled structure. (b) A zoom-in image of the electrolysis channel with 786 V DC voltage applied across the two reservoirs. The average field intensity in the space among beads was 6000 V/cm.

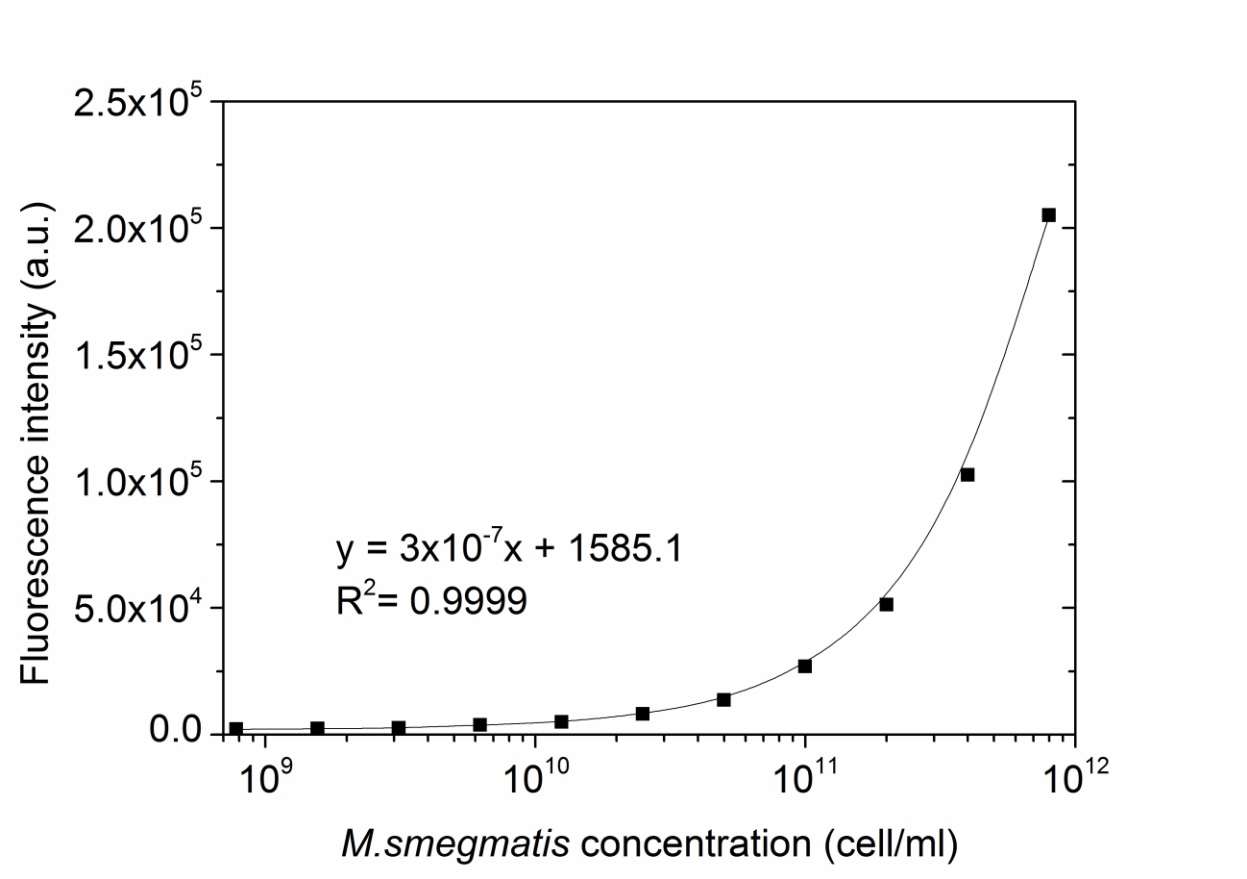

Fig. S3 A standard curve for quantifying mycobacterium concentrations in the electrolysis channel. GFP expressing *M.smegmatis* with different concentrations were loaded in a straight microfluidic channel with the same depth as the electrolysis channel. The fluorescence intensity was calculated based on z-stacking of 10 images.

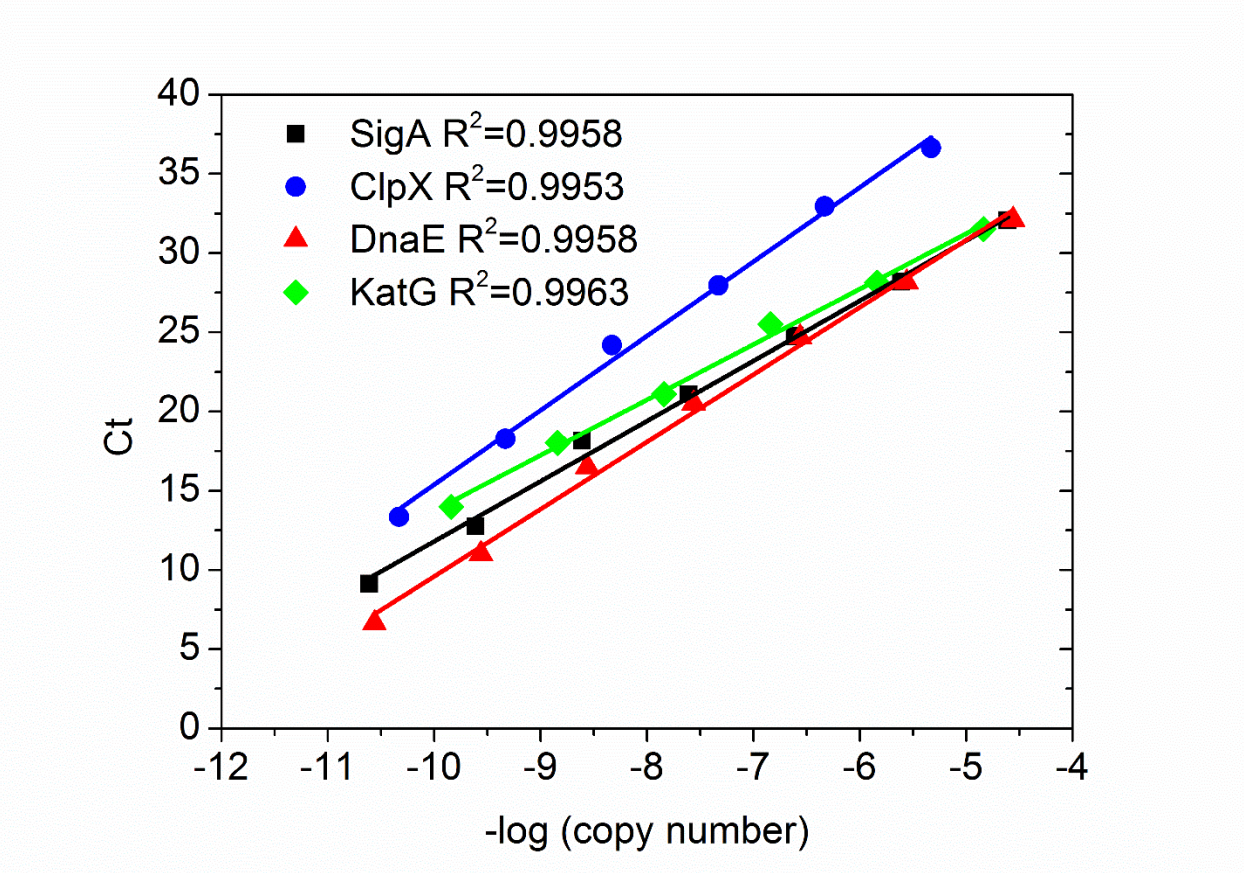

Fig. S4 The qPCR standard curves for all 4 mRNAs established using *in-vitro* synthesized RNA with known copy numbers (obtained by a Qubit 2.0 fluorometer).

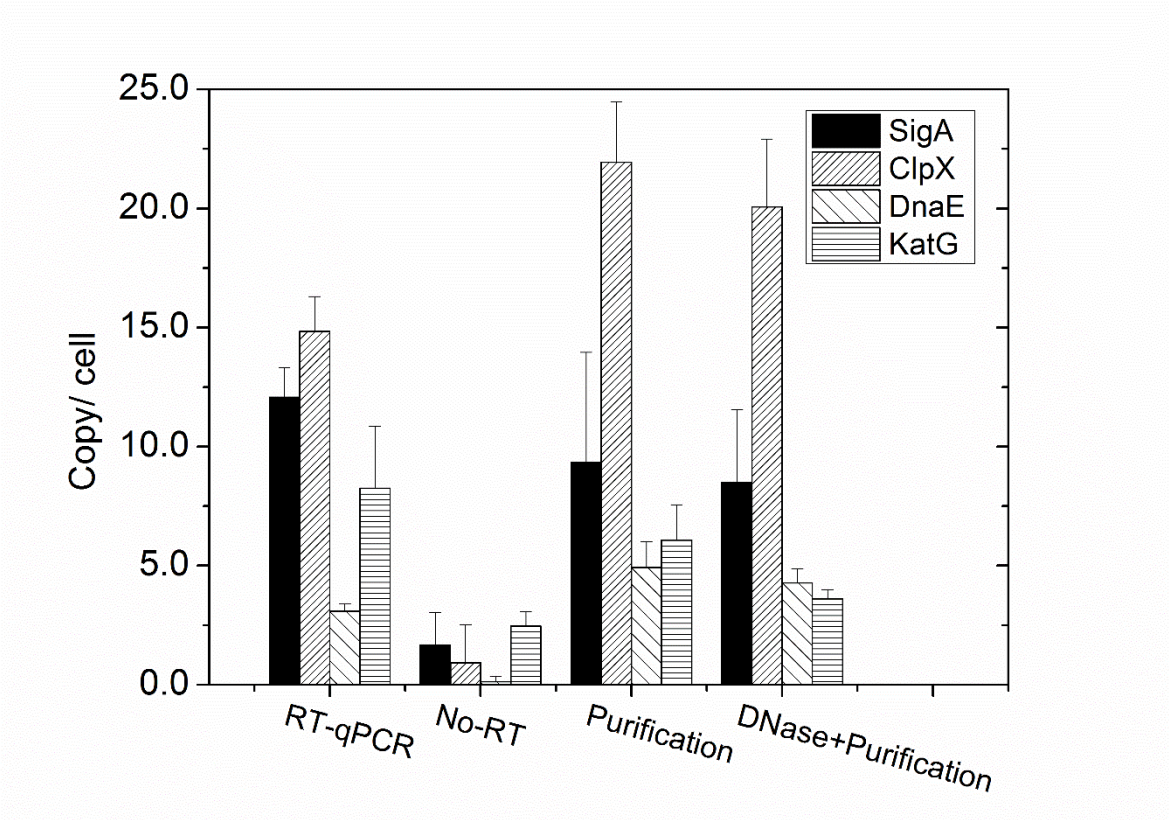

Fig. S5 The effect of DNA templates in the lysate on RT-qPCR results. All samples were extracted by applying 30 pulses of 6000 V/cm at 5 s duration from roughly 2 × 10^5 *M.smegmatis* cells (N=3). “No-RT” was performed without reverse transcription step. “Purification” was performed by purifying the sample using phenol/chloroform extraction, ethanol precipitation and resuspended in 20 µl water. “DNase+Purification” was performed by first treating the sample with 0.02 U/µl DNase I at 37 ℃ for 10 min, stopped by adding EDTA (at a concentration of 5 mM) and incubated at 75 ℃ for 10 min. The sample was then purified by phenol/chloroform extraction and ethanol precipitation. Based on comparisons between RT-qPCR and No-RT, we conclude that the fractions of RNA copy per cell values generated by DNA templates in the lysate are 14% for SigA, 6% for ClpX, 4% for DnaE and 30% for KatG.

Table S1 The primer sequences used in qRT-PCR.

mRNA	Forward primer sequences	Reverse primer sequences
SigA	GACTACACCAAGGGCTACAAG	TTGATCACCTCGACCATGTG
ClpX	TGACCAAGTCCAACATCCTG	CAACATCCTCACCGACGTAG
DnaE	CGTCTACCAAGAGCAGATCATG	TTCCTTGAAGCCCTTGTACTC
KatG	GACCGCGAATGACCTTGTGT	TGTCGGACTGGGCATAAACC

Video S1 On-chip electric lysis of GFP-expressing *M. smegmatis*. 30 electrical pulses (5 s each with an intensity of 6000 V/cm) were applied. The video was taken under fluorescence imaging at the rate of 1 image per 3.5 s and was played at 10 frames per second.