Supplementary Information (SI) for Analytical Methods.

Designing a Locally Produced DENV-1 Nucleic Acid Diagnostic for Low Resource Regions with Endemic Disease

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Supplementary Information

Supplementary Section 1: Fluorescence Calibrations

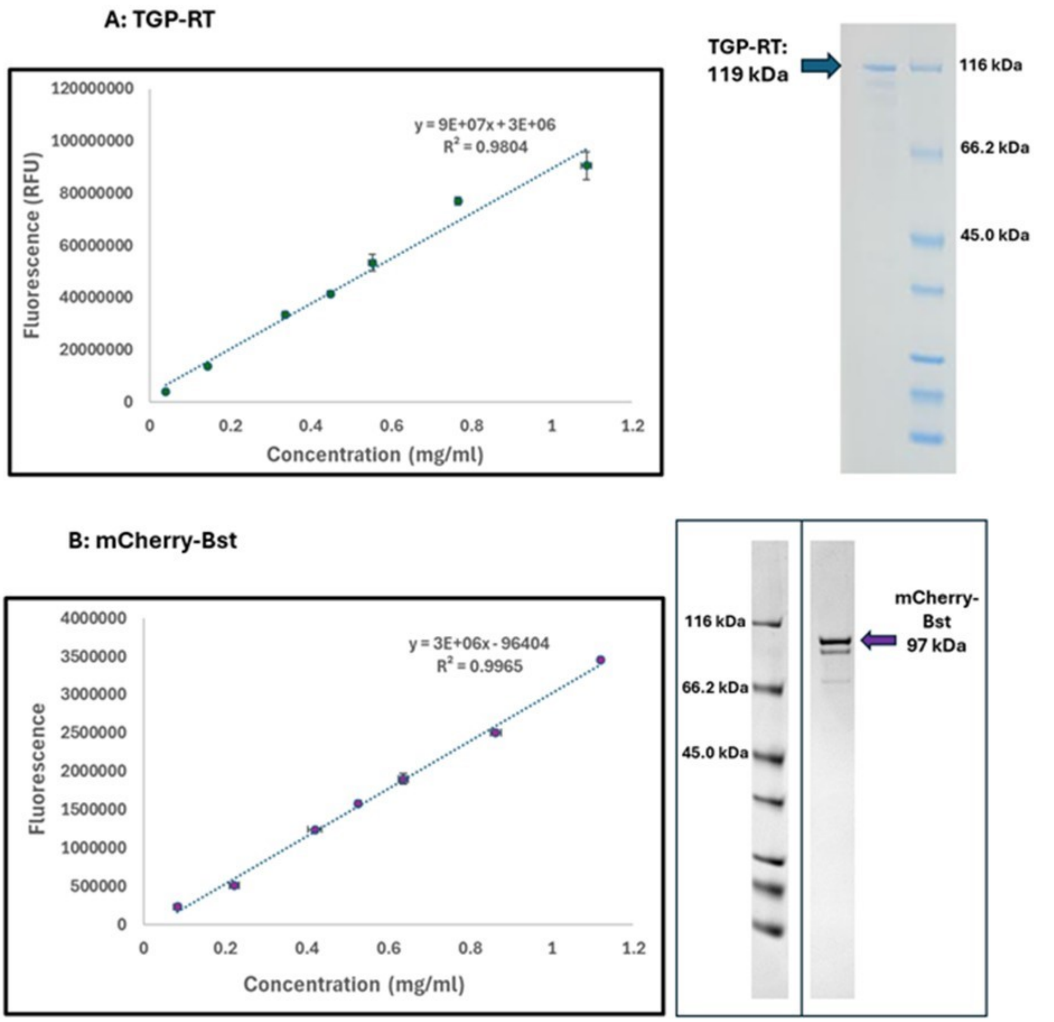


Figure S1: Fluorescence calibrations against concentration for TGP-RT (A) and mCherry-Bst (B), measured using the recombinant mCherry (mCherry-Bst) or TGP (TGP-RT) fluorophore, with SDS-PAGE displaying the His-purified enzyme utilised for calibration. Equations of linear fits are displayed with corresponding R value.

Nickel His-Bind Resin Purification of Fusion Constructs

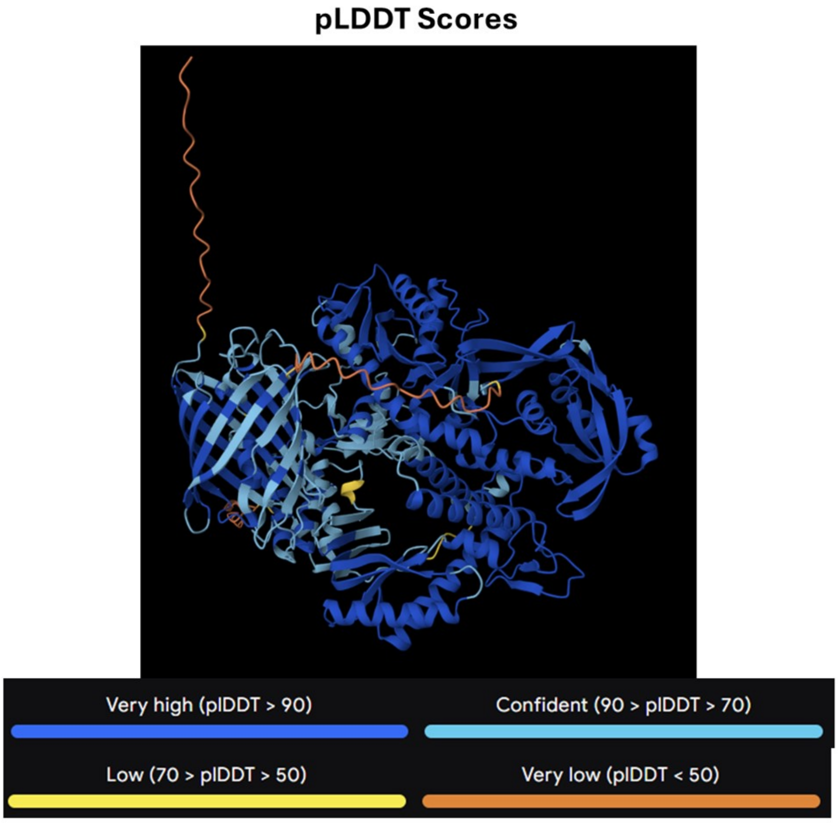
Thawed cells were lysed as per silica purification. Nickel His-Bind resin columns were prepared as per the manufacturer's guidelines. The column was washed using a 3X bed volume of bind buffer (20 mM sodium phosphate, 500 mM sodium chloride, 20 mM imidazole, pH 7.8), before lysate loading and incubation (30 mins). The column was washed with 10X bed volume of bind buffer and then a 6X bed volume of wash buffer (20 mM sodium phosphate, 500 mM sodium chloride, 50 mM imidazole, pH 7.8). Bound protein was eluted using a 6X bed volume of elute buffer (20 mM sodium phosphate, 500 mM sodium chloride, 250 mM imidazole, pH 7.8). Eluted protein was run through a CentriPure 25-Z25M gel filtration column, equilibrating and eluting with the appropriate storage buffer (mCherry-Bst or TGP-RT). Storage buffers for His-purified enzyme contained 25% glycerol. Protein was concentrated using an Amicon^{R} pro 50,000 MW column. Purified protein was stored at –20˚C.

Supplementary Section 2: CAB LAMP Primer Sequences

Table S1: CAB2, CAB3, and CAB5 LAMP primer set sequences.

Primer Set	Sequence (5' to 3')
CAB2	
F3	CATGATGGGAAAGAGAGAGA
B3	TGAGTATGTATCCAAGTTTGTG
FIP	TCTAAAAAGCGCGCTCCCAAAGTTCGGAAAGGCAAAAGG
BIP	GTTTGAAGCCCTTGGTTTCATGAGAGTCCTTCTCCTTCCAC
LF	CCACATGTACCATATTGCG
LB	ATCACTGGTTCAGCAGAGAG
CAB3	
F3	CAATATGGTACATGTGGTTGG
B3	GGCTGTGTCATCTGCATAC
FIP	ATTCTCTCTGCTGAACCAGTGATCCGCGCTTTTTAGAGTTTGA
BIP	TCACTCAGTGGAGTGGAAGGAATTTCCCCCTGGAATCTTTG
LF	TCATTCATGAAACCAAGGGC
LB	ACTTGGATACATACTCAGAGAC
CAB5	
F3	CATGATGGGAAAGAGAGAGAA
B3	AGTTTGTGGAGTCCTTCTC
FIP	GCTCCCAACCACATGTACCATAAAATTAGGAGAGTTCGGAAAGG
BIP	GCGCTTTTTAGAGTTTGAAGCCCCACTCCACTGAGTGAAT
LF	TTGCGCGACTTCCTTTTG
LB	GAAGATCACTGGTTCAGCAG

Supplementary Section 3: Alphafold Modelling of TGP-RT



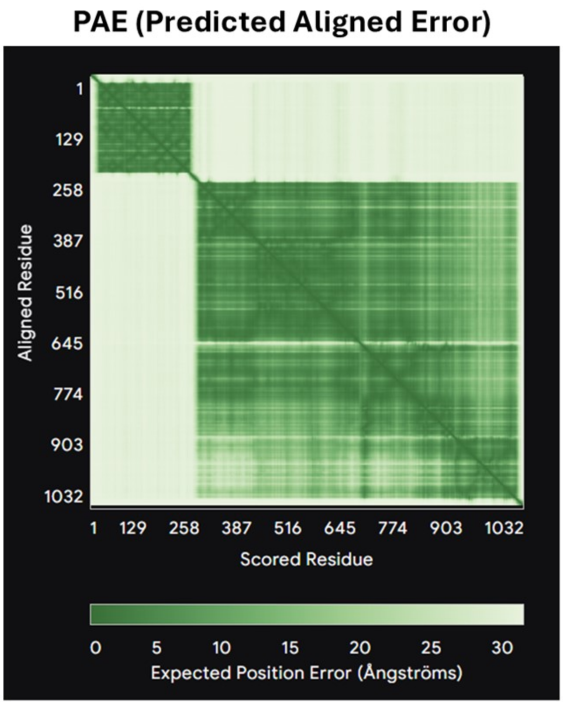


Figure S2: Alphafold Confidence scores for the annotated TGP-RT model presented in figure 3 (main text). Predicted local distance difference test (PLLDT) and Predicted Aligned Error (PAE) scores are displayed for the construct.

Supplementary Section 4: Surface Charge Modelling

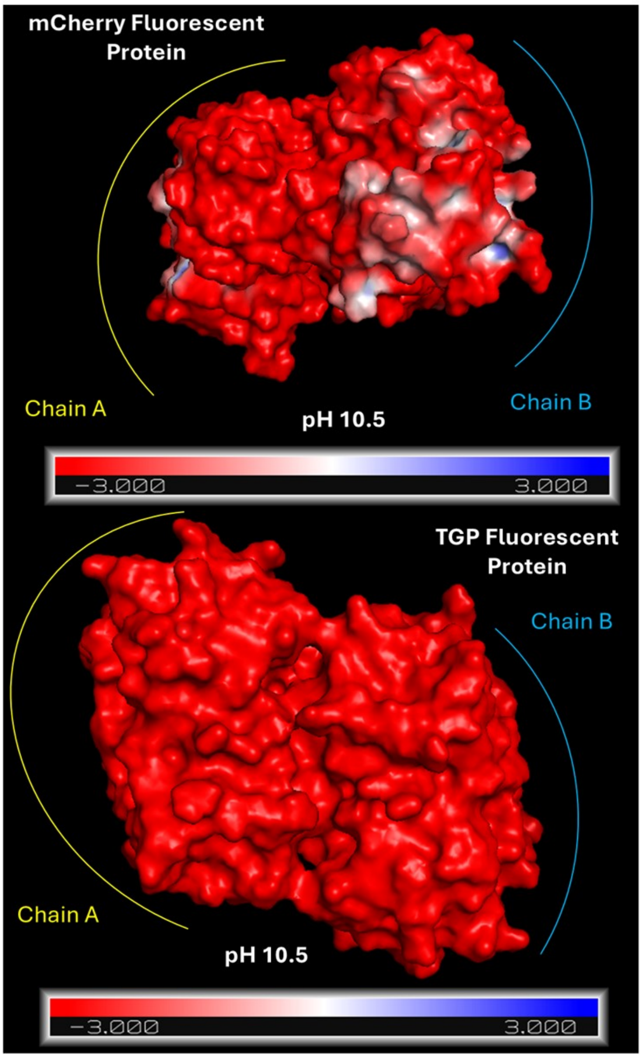


Figure S3: Surface charge models of mCherry (PDB: 4ZIN) and TGP (PDB: 8TJH) fluorescent proteins prepared with PDBQ2PR/APBS software and visualised using PyMol. Chains A/B represent repeat units present in crystal structures. Proteins were modelled at pH 10.5, analogous to pre-elution pH. Scale showing –3 to +3 kT/e.

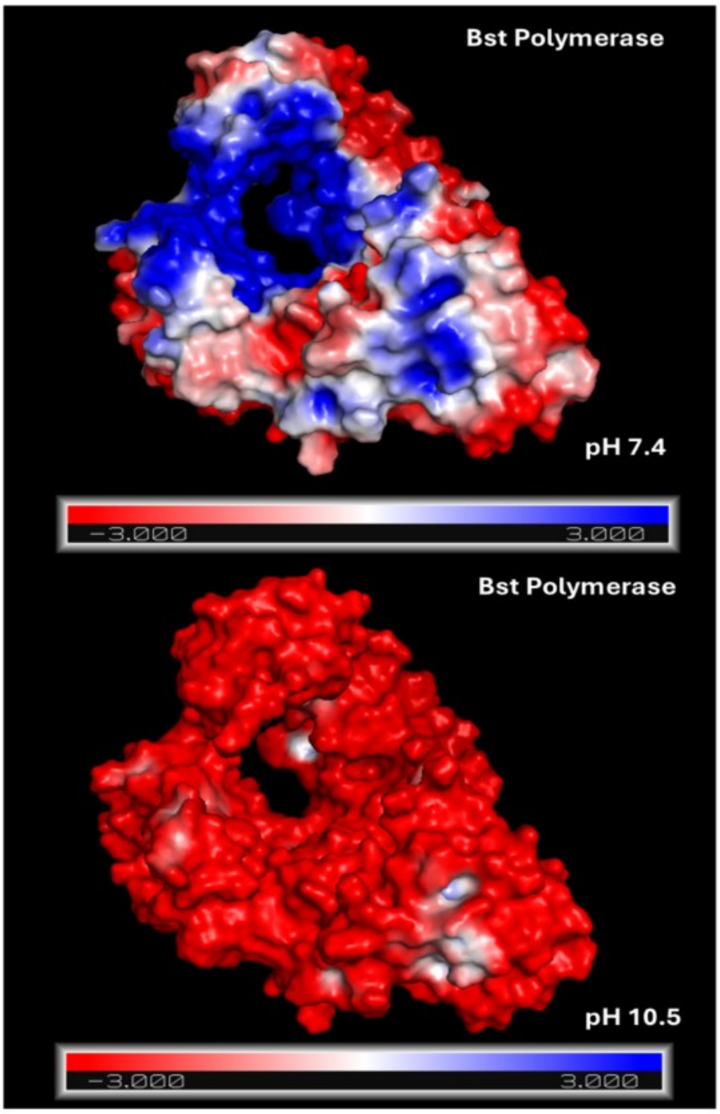


Figure S4: Surface charge models of Bst Polymerase (PDB: 6MU5) prepared with PDBQ2PR/APBS software and visualised using PyMol. The polymerase was modelled at both pH 7.4 and pH 10.5, analogous to pre-elution and post-elution pH. Scale showing –3 to +3 kT/e.

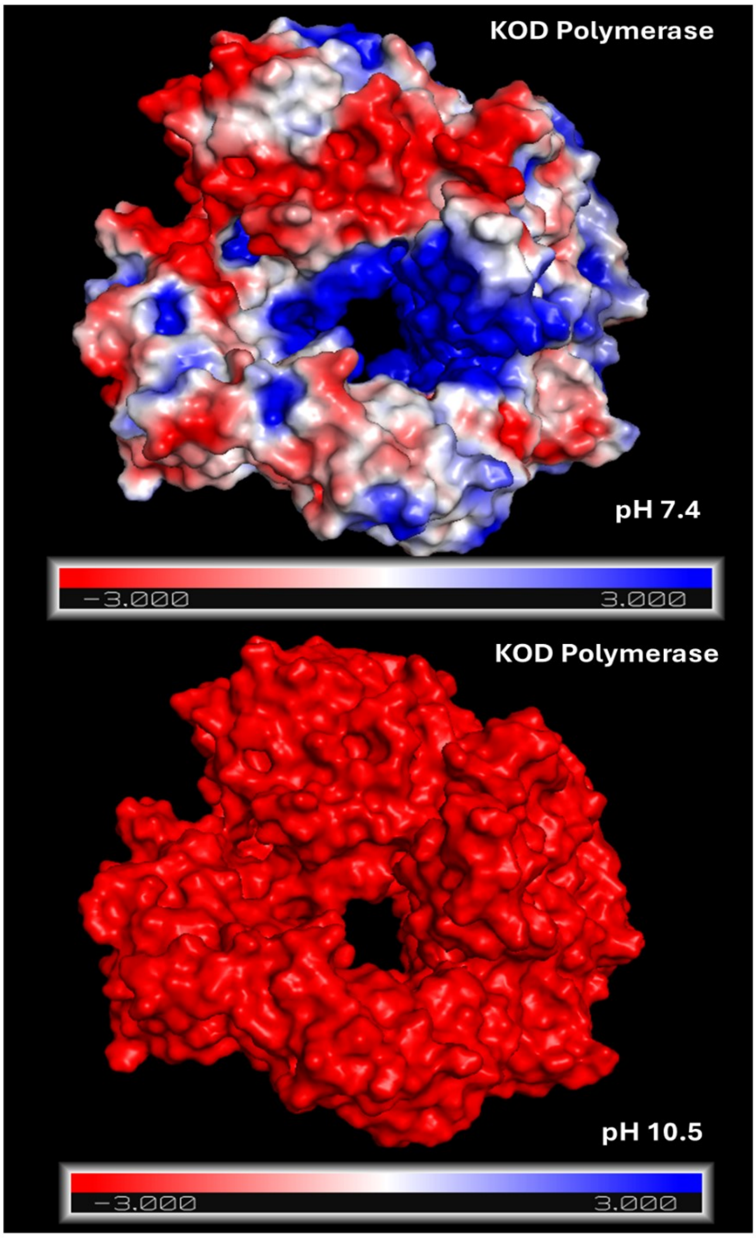


Figure S5: Surface charge models of KOD Polymerase (PDB: 4K8Z) prepared with PDBQ2PR/APBS software and visualised using PyMol. The polymerase was modelled at both pH 7.4 and pH 10.5, analogous to pre-elution and post-elution pH. Scale showing –3 to +3 kT/e.

Supplementary Section 5: Clustal Alignment of DENV S1 CAB3 Regions

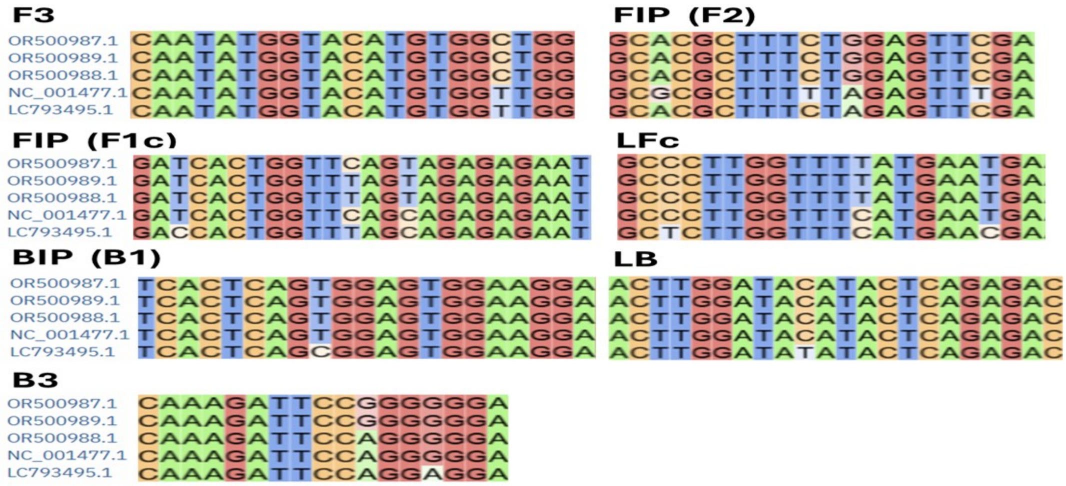


Figure S6: Clustal alignment of DENV serotype 1 variants with earlier reference NC_001477.1 from which the CAB3 primer set was designed, against sequences OR500987.1, OR500989.1, OR500988.1, and LC793495.1, for the CAB3 primer binding regions indicated.