

Supplementary Information for “Optimisation of a field-deployable portable qPCR workflow for the detection of *Aedes aegypti* in Kenya”

Sequence ID	Start	Alignment	End	Organism	Country
		1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23			
Query_5116049	(+) 1	A T T A C T A T G T T A T T A A C A G A C C G	23		
PQ424090.1	(+) 430		452	Aedes aegypti	Brazil: M...
PX556796.1	(+) 548		570	Aedes aegypti	Kenya
PX556803.1	(+) 543		565	Aedes aegypti	Kenya
PP124964.1	(+) 309		331	Aedes aegypti	Indones...
LC832630.1	(+) 575		597	Aedes aegypti	Saudi A...
OK285171.1	(+) 301		323	Aedes aegypti	Indonesia
OK570383.1	(+) 539		561	Aedes aegypti	
OM630610.1	(+) 529		551	Aedes aegypti	Central...
OL963810.1	(+) 404		426	Aedes aegypti	Kenya: K...
PX765404.1	(+) 379		401	Aedes aegypti	India: K...
OM865392.1	(+) 374		396	Aedes aegypti	
OK285157.1	(+) 301		323	Aedes aegypti	Indonesia
PP124982.1	(+) 309		331	Aedes aegypti	Indones...
LC832167.1	(+) 574		596	Aedes aegypti	Saudi A...
OK285147.1	(+) 301		323	Aedes aegypti	Indonesia
MZ230363.1	(+) 569		591	Aedes aegypti	Viet Na...
OQ283997.1	(+) 346		368	Aedes aegypti	Cambodia
PQ197330.1	(+) 2,094		2,116	Aedes aegypti	Banglad...
PX556808.1	(+) 543		565	Aedes aegypti	Kenya
OL680022.1	(+) 521		543	Aedes sp.	

Figure S1. Multiple sequence alignment of the probe-binding region showing complete conservation among *Ae. aegypti* reference sequences. The image corresponds to the first 20 hits. The query ID (Query_5116049) represents the probe sequence. The red boxes show reference sequences from Kenya.

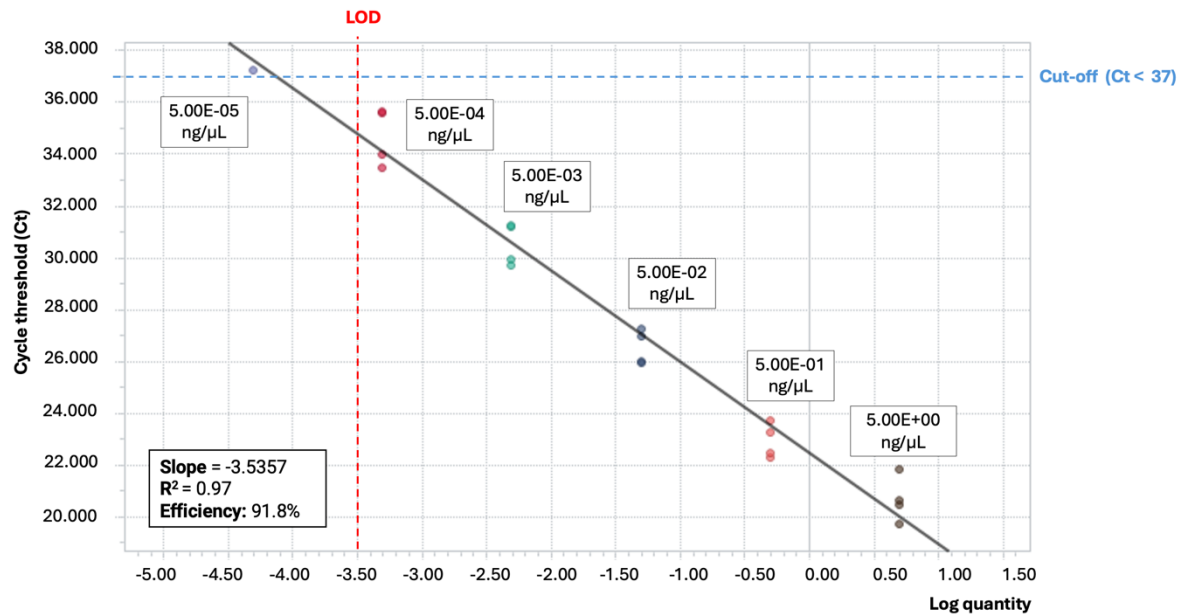


Figure S2. COI probe-based assay Ct values for a standard curve across a tenfold dilution series of gDNA for the detection of *Ae. aegypti*. Points represent four technical replicates per DNA concentration. The black solid line indicates the linear regression fit used to estimate amplification efficiency. The red dashed line indicates the LOD at 5×10^{-4} ng/μL, and the blue dashed line indicates the Ct cut-off at 37. Plot obtained from the Roche LightCycler 96 software.

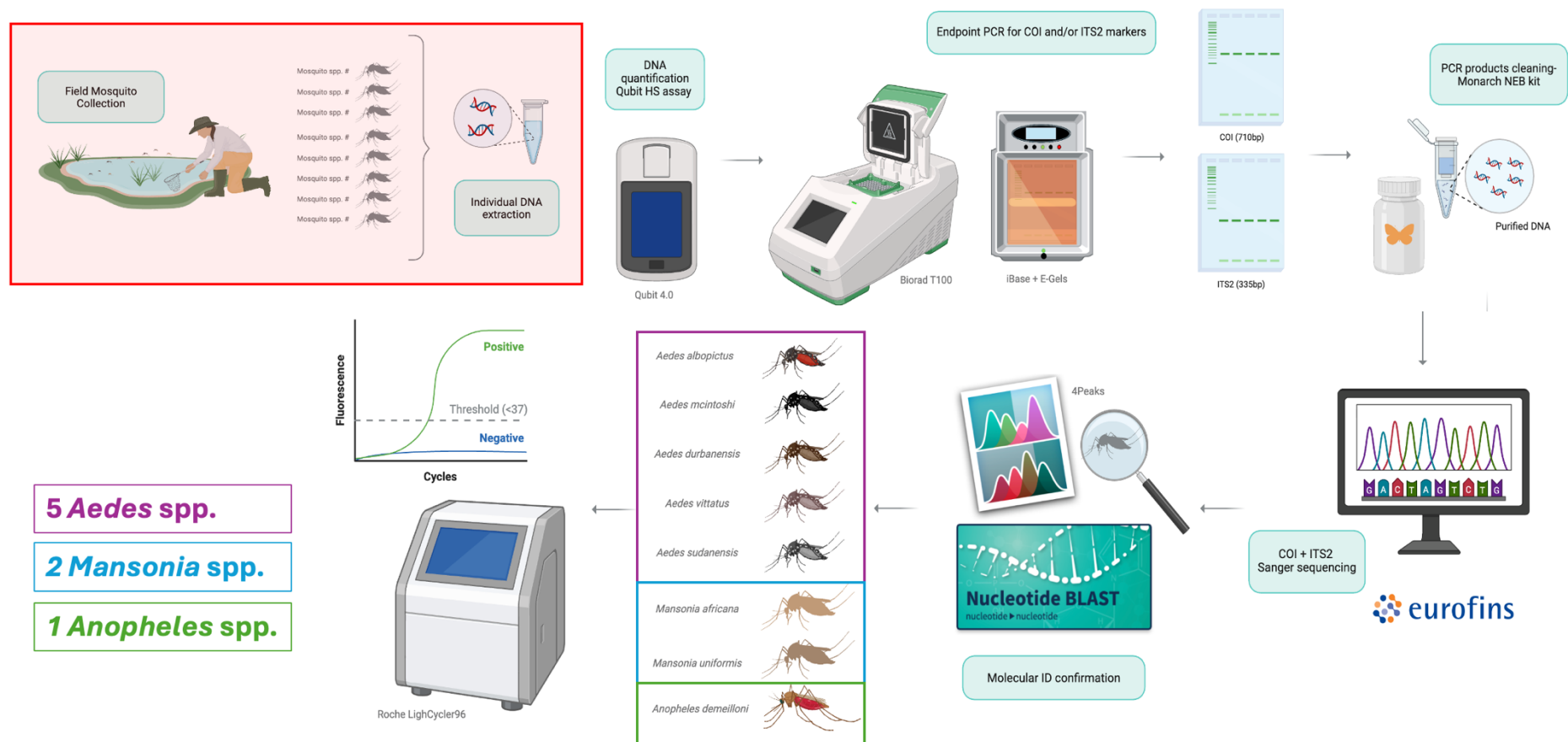


Figure S2. Diagram of the workflow for the molecular identification of donated samples for *in vitro* specificity assay. The red box highlights the sampling stage. Samples were collected in the field by others and donated for use in this study. Created in Biorender.com

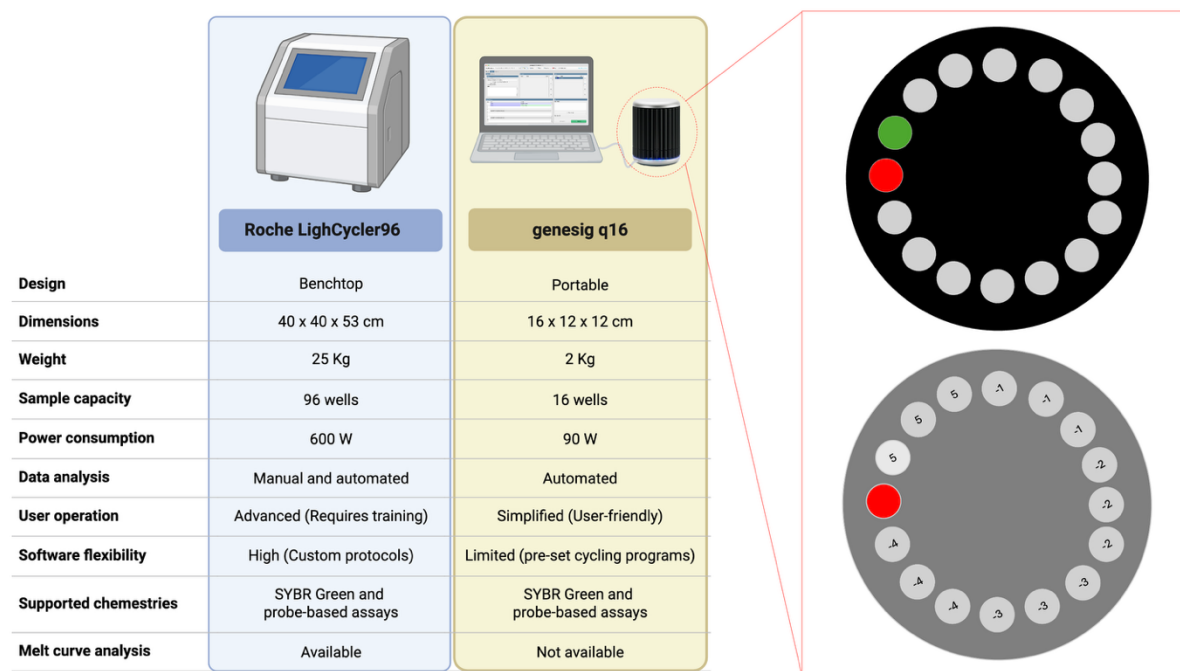


Figure S4. Technical and operational specifications of the conventional and portable qPCR platform. In the red box, examples of well arrangements for the genesig q16. **(Top)** Sample layout recommended by the manufacturer: the red well represents the negative control, the green well indicates the positive control (both wells predefined), and the grey wells are for testing 14 samples. **(Bottom)** arrangement employed to create the standard curve using only one control, the negative well in red. The positive control well was used for the first replicate at the highest DNA concentration, followed by the remaining concentrations. All concentrations were tested in triplicate. Created in Biorender.com.

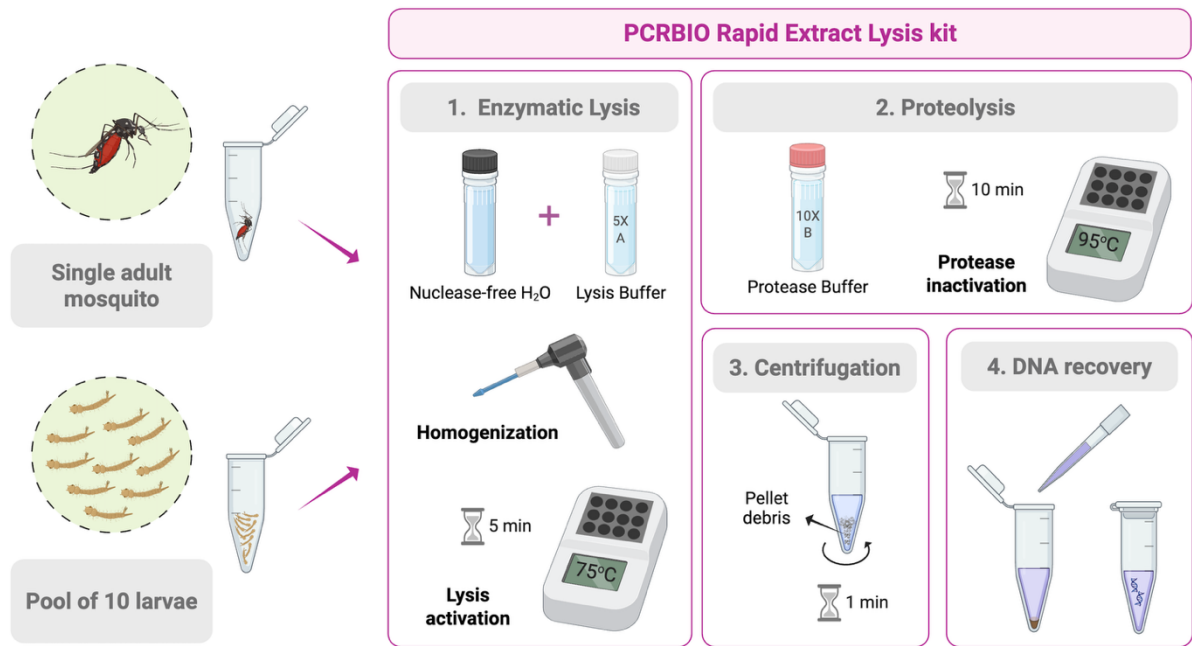


Figure S5. Methodological diagram of the rapid enzymatic DNA extraction process for field-collected samples.