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LAMP-Seq enables sensitive, multiplexed COVID-19 diagnostics using molecular barcoding

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Frequent testing of large population groups combined with contact tracing and isolation measures will be crucial for containing Coronavirus Disease 2019 outbreaks. Here we present LAMP-Seq, a modified, highly scalable reverse transcription loop-mediated isothermal amplification (RT–LAMP) method. Unpurified biosamples are barcoded and amplified in a single heat step, and pooled products are analyzed en masse by sequencing. Using commercial reagents, LAMP-Seq has a limit of detection of ~2.2 molecules per μl at 95% confidence and near-perfect specificity for severe acute respiratory syndrome coronavirus 2 given its sequence readout. Clinical validation of an open-source protocol with 676 swab samples, 98 of which were deemed positive by standard RT–qPCR, demonstrated 100% sensitivity in individuals with cycle threshold values of up to 33 and a specificity of 99.7%, at a very low material cost. With a time-to-result of fewer than 24 h, low cost and little new infrastructure requirement, LAMP-Seq can be readily deployed for frequent testing as part of an integrated public health surveillance program.

As of February 2021, the global spread of a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in over 109 million confirmed cases, and approximately 2.4 million deaths have been attributed to Coronavirus Disease 2019 (COVID-19)[1]. Current containment strategies based on 'test–trace–isolate' face major issues: (1) many infected individuals do not show any symptoms and, therefore, remain untested[2]; (2) supply chain issues limit testing capacity; and (3) the successive (rather than parallel) testing of contact individuals causes a substantial lag in identifying infection chains, resulting in undetected spread due to delayed diagnosis. By contrast, repeated testing of large groups of individuals, regardless of symptoms or

contact status, is predicted to be an effective measure to decrease SARS-CoV-2 transmission[3–5]. Furthermore, this strategy helps to pinpoint outbreak areas and ongoing community transmission, thus enabling local interventions that maximize human health while minimizing the societal impact of restrictive isolation measures.

The current gold standard diagnostic test for detection of active SARS-CoV-2 infection is viral RNA extraction from a biospecimen followed by RT–qPCR to amplify and detect conserved regions of the SARS-CoV-2 genome. With increasing infection numbers, this has been complemented by antigen-based tests, which provide rapid results but have limited sensitivity[6], and sequencing-based approaches, which have increased throughput but still require

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RNA extraction and/or thermocycling devices[7–11]. Here we describe LAMP-Seq, an approach that combines RT–LAMP[12,13] with molecular barcoding to detect viral genomes in unpurified lysates at high throughput.

Results

Scalable deep sequencing–based SARS-CoV-2 detection. RT–LAMP uses six target-specific primers and a strand-displacing polymerase (Fig. 1a), and it has been shown to detect pathogens in unpurified samples at high sensitivity[14]. To establish a barcoded RT–LAMP reaction suitable for large-scale next-generation sequencing (NGS)-based detection, we designed a barcoded primer set based on a validated RT–LAMP amplicon[15] that matched 94.1% of 42,904 SARS-CoV-2 genomes available in the National Center of Biotechnology Information database (as of 11 March 2021; Supplementary Data 1). The barcodes (10-nt long, GC content of 30–70% and lacking homopolymer repeats of four or more nucleotides) were inserted into the forward inner primer (FIP) (denoted as 'LAMP barcodes' (LAMP-BCs)) (Fig. 1b). To reduce the risk of sample misattribution due to sequencing errors, we ensured a Levenshtein edit distance[16] between any barcode pair of three or more. Further scalability can be achieved by introducing a second level of barcodes at the PCR stage on pooled samples, using two standard PCR barcodes (PCR-BCs): i5 and i7 (Supplementary Data 1). The final structure of the sequencing library is shown in Fig. 1b.

We first determined the molecular sensitivity of barcoded RT–LAMP reactions using in vitro transcribed (IVT) template RNA and commercial RT–LAMP reagents (Methods). We included F3 and B3 primers with or without locked nucleic acid (LNA) modifications, which can increase binding affinity[17] (Fig. 1c; positions of LNA modifications are described in Methods). We obtained a limit of detection at 95% confidence (LoD-95) of about 18 RNA molecules per assay, using probit analysis (equivalent to 2.2 copies per μl (Fig. 1d)). This is similar to the assay sensitivity of the standard *E* gene RT–qPCR assay used for clinical diagnostics at the University Hospital Bonn (LoD-95: 18.4 molecules, using IVT RNA). Templating individually barcoded LAMP reactions that differ 100- or 10,000-fold in the amount of RNA template, combining them for PCR amplification and sequencing the products resulted in saturated read numbers (Fig. 1e). This indicates that RT–LAMP saturation effectively compresses the dynamic range from input viral loads to sequencing reads, enabling analysis of large numbers of samples spanning a wide range of viral loads in one sequencing run.

Next, we optimized the pooling of barcoded RT–LAMP reactions to (1) minimize the levels of barcode swapping and (2) ensure a sufficient number of individually validated barcodes. We observed moderate levels of barcode swapping when we pooled six barcoded RT–LAMP reactions, three of which were templated with IVT RNA before PCR and sequencing (Fig. 1f, left panel). We hypothesized that introducing barcoded primers into the PCR reaction led to amplification and re-barcoding of amplicons. In support of this, we eliminated detectable barcode swapping by diluting the pooled RT–LAMP reactions 10[6]-fold in the final PCR reaction (Fig. 1f, right panel). Finally, we pooled 480 barcoded FIPs and performed LAMP-Seq in quadruplicate. The barcode distribution in the products revealed that ~5% of barcode sequences performed poorly or even failed to engage in LAMP-Seq (Fig. 1g). The least efficient barcode primers displayed a marked enrichment for a GTCC motif or truncations thereof, especially toward the 3′ end of the barcode (Fig. 1g, inset). This is the reverse complement of the 3′ end of the FIP, suggesting that it was sequestering FIPs at their 3′ end by forming intramolecular structures. Thus, this homology should be avoided in barcode design, and we provide 192 experimentally validated FIP barcodes in Supplementary Data 1 (N-FIP barcode primers TIER-1). To enable scaling of LAMP-Seq, we additionally tested

an N_{10}-barcode library and identified about 10,000 functional barcodes. Of these, we in silico curated a set of 3,840 barcodes based on a minimum 3-edit distance, which are independent from Primer Set TIER 1 (Supplementary Data 1 (N-FIP barcode primers TIER-2)).

For LAMP-Seq to be safely deployed on patient samples, we confirmed that SARS-CoV-2 virus was inactivated in QuickExtract lysis buffer both after 30 min of incubation at 65 °C and after 10 min at 95 °C. Both incubations resulted in a >40,000-fold reduction in viral infectivity, whereas a 30-min incubation at 22 °C resulted in residual SARS-CoV-2 infectivity. The inactivation efficiency of the lysis buffer was further demonstrated by observing a >10[7]-fold reduction in viral infectivity of high dose of vesicular stomatitis virus (VSV) after incubation at 65°C for 20 min (Supplementary Table 1).

Clinical validation of LAMP-Seq using commercial reagents. We next tested 57 high-incidence human samples using LAMP-Seq side by side with a clinically approved diagnostic RT–qPCR pipeline. Upon informed consent, two oropharyngeal swab samples were collected from each individual using two separate cotton swabs. One randomly selected swab was analyzed using a validated clinical diagnostics pipeline comprising rehydration, robotic RNA purification and RT–qPCR using *E* gene-specific primers (which have been reported to be more sensitive than primers for other targets[18]; Fig. 2a, upper panel). The other swab was immediately inserted into a tube containing QuickExtract lysis buffer[19] (Fig. 2a, lower panel), and 8.3 μl of lysate was processed with LAMP-Seq in quadruplicate, using individual LAMP-BCs and PCR-BCs, with a multiplexed E1 or β-actin control in one of the replicates (Methods). Unfiltered LAMP-Seq data displayed the expected read structure, comprising primer sequences, viral genome sequences and matching barcodes (Fig. 2b). After sequencing, the median read count for four positive replicates was determined, and sample replicates were deemed positive if they showed at least 10% of that read number. Twenty-two of 25 individuals who were identified as positive for SARS-CoV-2 RNA by RT–qPCR were also identified as positive by LAMP-Seq (with two or more positive replicates). The three non-detected samples showed very low viral titers in the clinical pipeline (cycle threshold (Ct): 36.96–38.52). The remaining 32 individuals were identified as negative for viral RNA (with above-threshold number reads for β-actin; Fig. 2c). Together, our data using commercial LAMP reagents suggest that LAMP-Seq is a highly sensitive and specific SARS-CoV-2 testing approach.

Development of an open-source LAMP-Seq protocol. To further increase the potential for large-scale application of LAMP-Seq, we established an open-source version that allows for substantial cost reduction and independence from supply chains. We replaced all cost-driving items (lysis buffer and LAMP enzyme master mix) by self-produced buffers and enzymes (Methods) and reduced dNTP and primer concentrations. Notably, we found that a Bst polymerase large fragment from a *Geobacillus* strain sampled in Idaho[20] (Bst-LF-Idaho) alone is sufficient to maintain a high sensitivity of LAMP-Seq, without a reverse transcriptase but in the presence of LNA modifications in the F3/B3 primers (Supplementary Fig. 1). Applying this open-source protocol to different amounts of chemically inactivated SARS-CoV-2 particles (Methods) revealed slightly increased LoD-95 of 39 molecules per reaction or about 4.7 molecules per μl (Fig. 3a). We also confirmed high LAMP-Seq specificity, as we did not detect any of 15 other viral sequences (Fig. 3b). As cross-contamination of adjacent wells is a major concern in LAMP protocols, we performed a checkerboard experiment with 192 positive and 192 negative samples in duplicates (Fig. 3c). Using LAMP-BC Primer Set TIER 1, we observed a robust black–white pattern as expected. Sporadic sub-threshold reads (less than 10% of median positive reads) occurred in seven of 192 positions, and we also observed
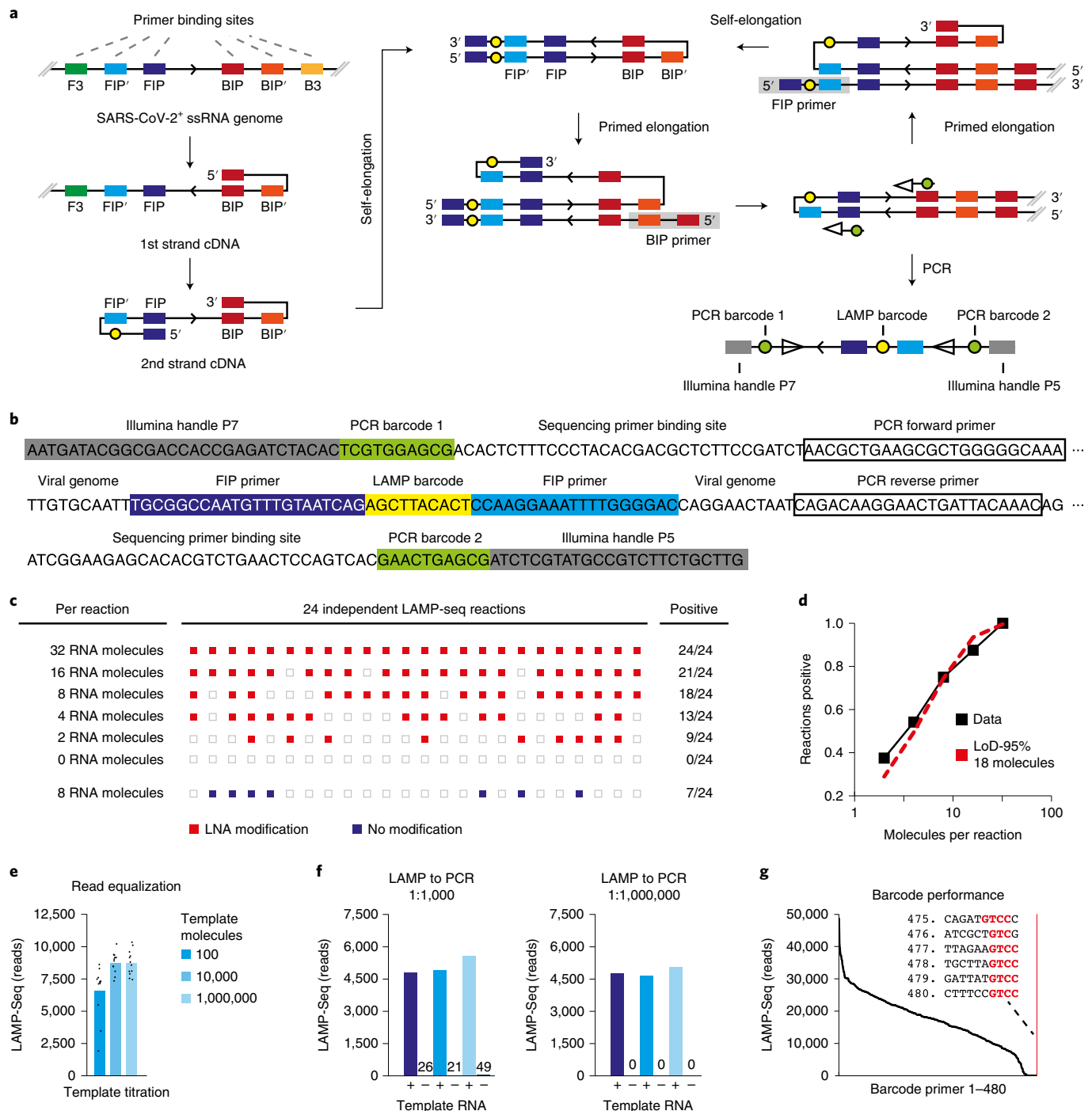
Fig. 1 | LAMP-Seq: a scalable deep sequencing–based approach for SARS-CoV-2 detection. a, Schematic of anticipated enzymatic reactions and reaction products. **b**, Annotated amplicon sequence for Illumina NGS of SARS-CoV-2 RT–LAMP products. **c**, Sensitivity assessment of LAMP-Seq using 24 reactions per condition, templated with the indicated numbers of RNA molecules per reaction. Filled squares indicate barcodes with read numbers >10% of median calculated from 24 positive samples. LNA modifications increase melting temperature from 53.7 °C to 60.1 °C for F3 and from 50.0 °C to 57.3 °C for B3. **d**, Estimation of the LoD-95 based on probit analysis of the fraction of positives among 24 replicates. **e**, LAMP-Seq reactions templated with 100, 10,000 or 100,000 RNA molecules. Reactions were PCR amplified and sequenced on an Illumina MiSeq sequencer. Absolute read counts per sample are shown from ten experimental replicates per condition. **f**, Quantitative assessment of barcode swapping in LAMP-Seq and dependence on pre-dilution of pooled RT–LAMP reactions before PCR (left panel, 1,000-fold; right panel, 1,000,000-fold). LAMP-Seq was performed as described in the Methods section, with the exception that synthetic RNA was used as the template instead of a swab sample, no Bst 3.0 or Tris buffer was added, and no LNA-modified primers were used. Numbers in the plot indicate read numbers for non-templated negative control reactions. **g**, Empirical performance assessment of 480 randomly chosen LAMP-Seq barcode primers. The barcoded FIPs were mixed at an equimolar concentration and used as a pool in four replicate LAMP-Seq reactions templated by RNA. Raw sequencing data were analyzed using LAMP-Seq Inspector v1.0 (http://manuscript.lamp-seq.org/Inspector.htm). Read counts are shown for barcodes in descending order. The six worst-performing barcode sequences are highlighted in red, and the respective sequences are provided in the inset. ssRNA, single-stranded RNA.
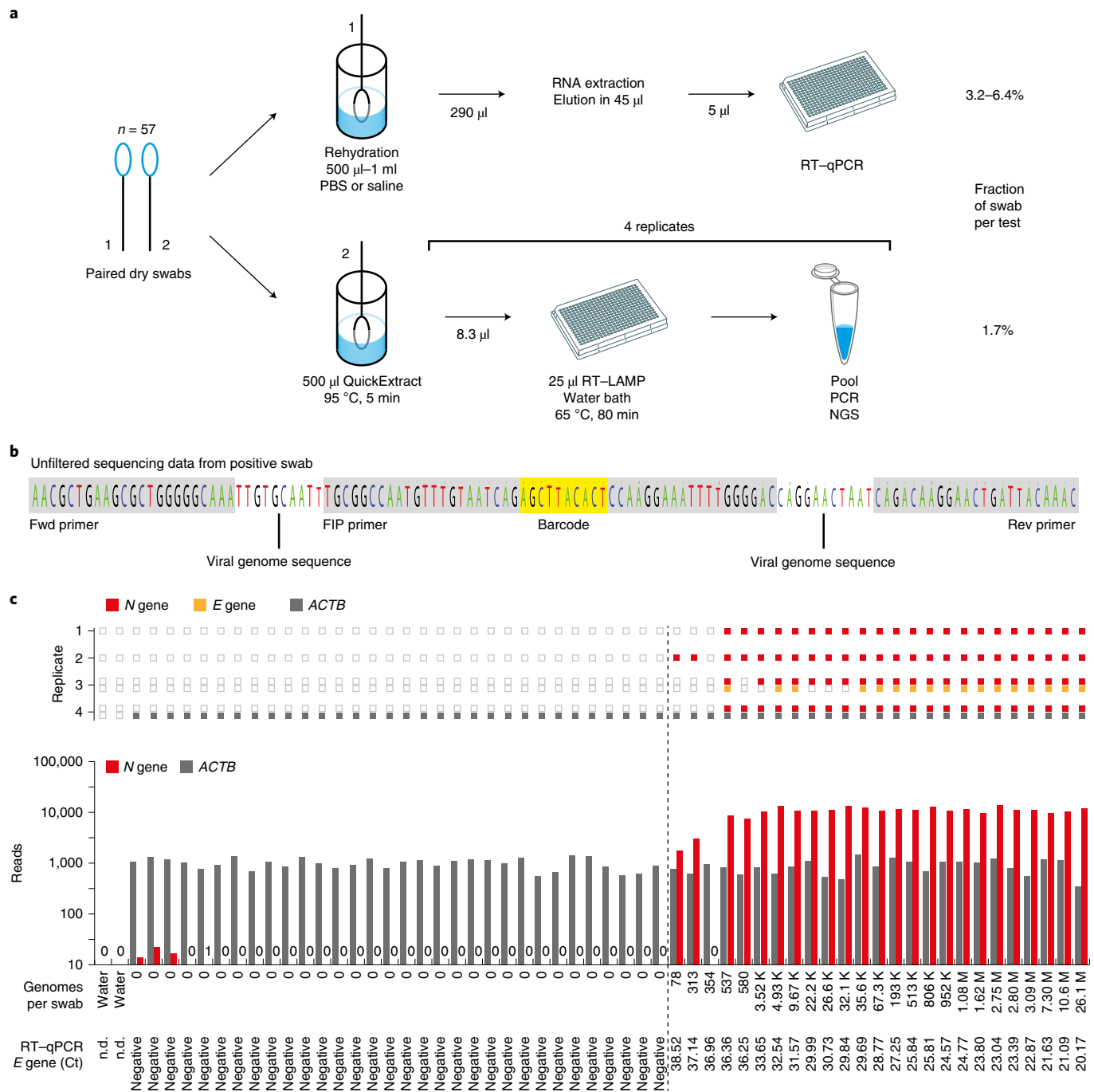
Fig. 2 | Clinical validation of LAMP-Seq. a, Outline of the protocol employed for validating LAMP-Seq (bottom workflow) against an established clinical RT–qPCR pipeline (top workflow). **b**, Sequencing data obtained from a SARS-CoV-2-positive swab sample using LAMP-Seq. Base frequencies are depicted by the size of each letter without applying any read filtering. **c**, Upper panel, positive LAMP-Seq replicates (threshold at 10% of median read number of four LAMP-Seq reactions with lowest corresponding Ct values) are indicated by filled squares. Replicates 1 and 2 detect just the *N* gene; replicate 3 is multiplex detection for *N* and *E* genes; replicate 4 is multiplex detection for *N* gene and *ACTB* (β-actin; endogenous control). Lower panel, cumulative read numbers obtained per sample, ordered by estimated viral genomes per swab as determined by RT–qPCR (*E* gene) on a paired swab, taking into account different rehydration volumes. The raw RT–qPCR Ct values are also shown. Of note, the *E* gene primer set did not bear LNA modifications and offers lower sensitivity, which could be useful for encoding log-scale quantitative information. n.d., not determined.

three false negatives. This latter effect, however, was not bar-code dependent, as the second well for those samples (including identical LAMP-BCs but different PCR-BCs) was positive in each case. With this checkerboard experiment, we showed that (1) cross-contamination of adjacent wells can be expected to be minimal, and (2) LAMP-Seq generates sporadic dropouts.

Both potential issues can be addressed by requiring two of four replicates to be positive. To run four replicates on each sample (100 µl of RT–LAMP) using open-source components, the cost, excluding equipment depreciation, labor and licenses, amounts to US$ 2.73 (as compared to US$ 12.69 or the commercial protocol) (Supplementary Fig. 2).
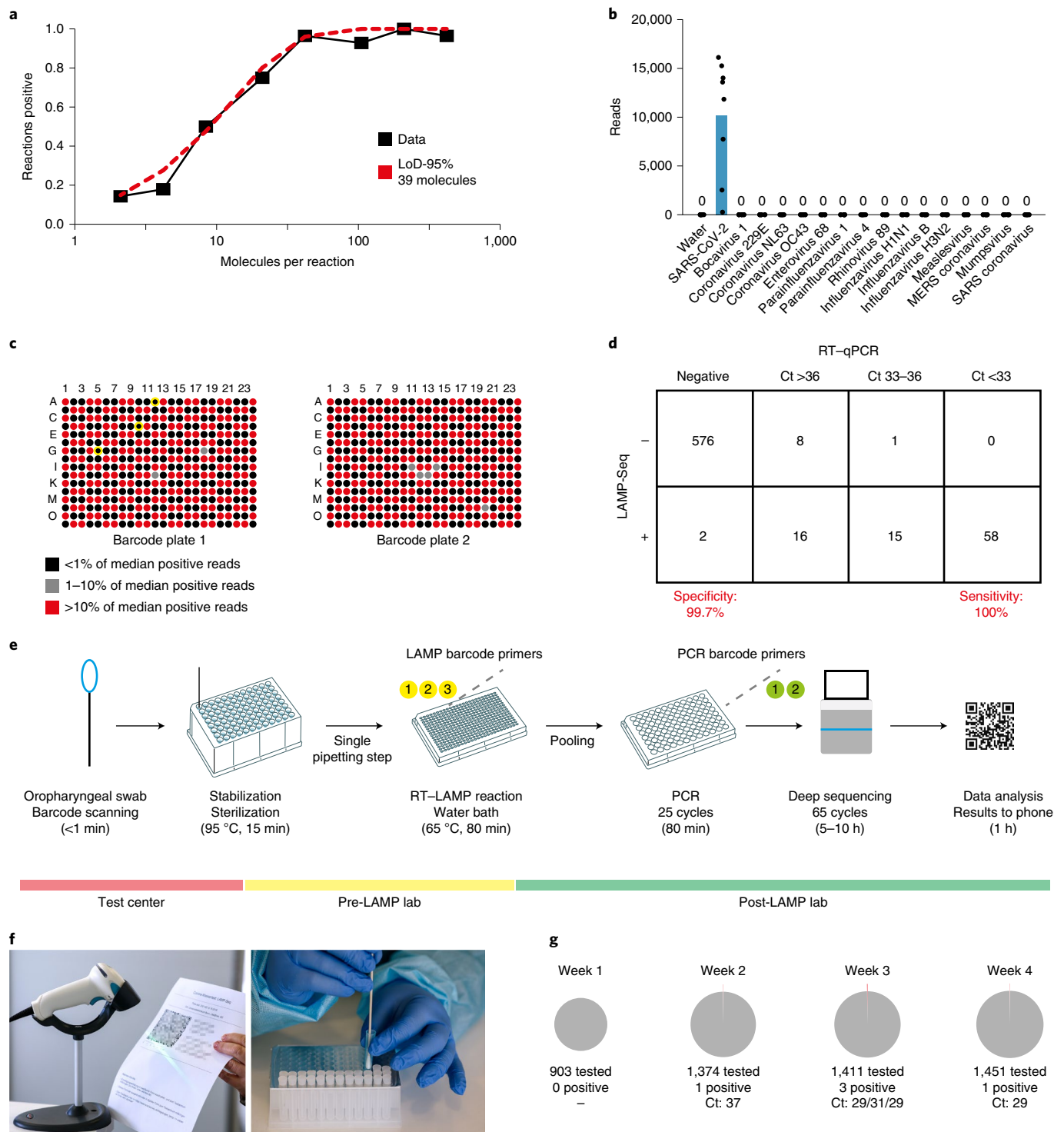
Fig. 3 | Validation of an open-source LAMP-Seq protocol. a, Estimation of the LoD-95 based on probit analysis of the fraction of positive replicates using a titration of chemically inactivated SARS-CoV-2 viral particles provided by the XPRIZE Foundation. **b**, Specificity determination using IVT RNA of a panel of human virus genomes. **c**, 2 × 384 LAMP-Seq reactions alternatingly inoculated with IVT RNA (500,000 molecules per reaction) in a checkerboard pattern to evaluate barcoding specificity and cross-contamination. Yellow circles indicate false-negative replicates. **d**, Summary of clinical validation study using open-source LAMP-Seq on 676 swab samples, performed on liquid-handling robots and analyzed in parallel by clinical RT–qPCR after RNA extraction from the same lysate. Samples annotated as Ct > 36 include late calls. **e**, Schematic outline of a proposed scalable testing procedure involving self-registration, semi-centralized barcoded RT–LAMP, pooling, sequencing and electronic results reporting. Colored bars indicate testing site (red), pre-LAMP (yellow) and post-LAMP (green) areas. **f**, Photographs showing the scanning process of individual QR codes generated during self-registration and 96-well lysis plates for immediate elution of virus particles from oropharyngeal swabs without cross-contamination of wells (plate is pre-sealed; swabs are inserted into wells using a disposable funnel; and then the well is covered with a silicone plug). **g**, Summary statistics from a 4-week-long pilot study of open-source LAMP-Seq for SARS-CoV-2 detection. In total, 5,139 asymptomatic volunteers were tested, of whom five were found to be positive in at least two replicates and subsequently confirmed by clinical RT–qPCR from the same lysate (Ct values shown below). The average time spent per person in our testing center was 2 min.

We validated open-source LAMP-Seq on 676 residual swab samples from clinical testing, with four replicates per sample and using automated liquid handling. One positive (IVT RNA) and one negative control was included on each 96-well plate. LAMP-Seq displayed a sensitivity of 100% (with two or more positive replicates) among positive samples with corresponding Ct values < 33 (Fig. 3d, right), in concordance with our LoD estimates. Among 578 samples that were negative by RT–qPCR, three were detected as positive by LAMP-Seq, one of which was subsequently confirmed to be a true positive using a more sensitive RT–qPCR protocol (Methods). Of 16 samples with Ct values between 33 and 36, 15 samples were identified as positive in LAMP-Seq, whereas 24 weakly positive samples (Ct above 36) were detected stochastically (Fig. 3d, middle).

Finally, we implemented LAMP-Seq in an end-to-end workflow (Fig. 3e) that was tested in a pilot study among medical and non-medical staff at the University Hospital Bonn (Application Note and Fig. 3f). Briefly, 96-well lysis plates were filled with open-source lysis buffer and controls, heat sealed with a pierceable foil and transported to the testing site. After self-registration, participants presented at the center. Supported by a visual and audio software, trained staff pierced one position on the plate with a disposable funnel device, took the oropharyngeal swab and then introduced it through the funnel into the well. After submerging for 10 s, the swab was removed together with the funnel, and the well was then closed with a silicone plug. After transport to the pre-LAMP lab, lysates were sterilized in a thermoblock at 95 °C, and 8.3 µl of lysed sample was stamped into a pre-made, barcoded RT–LAMP 384-well plate. This is the only mandatory liquid transfer step per sample. In the post-LAMP lab, plates were heated in a water bath to 65 °C and subsequently pooled using either multi-channel pipetting or centrifugation (Methods). After small-scale PCRs on individual pools, the library was loaded on a MiSeq device. In total, LAMP-Seq was performed within 12 h (2 h swab to lab; 4 h inactivation and LAMP-based library prep; 5 h sequencing; and 0.5 h analysis). For samples with at least two of four positive replicates, the sample was located on the original 96-well lysis plate and entered into clinical qPCR testing. Positive test results were reported to the participant and health authorities. Negative results were reported to each tested individual digitally, based on an individual QR barcode (Application Note). Within 4 weeks, we tested 5,139 samples and identified five positive samples, all of which were confirmed by qPCR from remaining lysate (Ct values: 29–37; Fig. 3g).

Discussion

We have shown here the feasibility of deploying LAMP-Seq for population testing. By employing sample-specific barcodes at the first step of the protocol after sample lysis, large-scale pooling of samples can be achieved during downstream processing. Additionally, early pooling in combination with an isothermal amplification step reduces supply chain issues and the requirements for technical infrastructure. Our protocol also circumvents the requirement for RNA extraction. Using LNA modifications and further protocol optimization, we achieved an assay sensitivity and target specificity approaching that of the current gold standard qPCR, at considerably lower cost. LAMP-Seq uses existing NGS infrastructure to deliver results at scale, in theory ranging from several hundreds to thousands of samples per day per sequencing facility, and it could be deployed in developing countries. LAMP-Seq allows multiplexing of several target sequences in a single reaction, which enables scalable differential diagnosis of a multitude of pathogens (for example, influenza) or, alternatively, the detection of specific virus variants within one reaction. Future developments will include determination of its compatibility with other types of human samples (for example, saliva[21]) and combination with a fast read-out at point of care[22] to help identify the most scalable solution for unsupervised at-home sample collection. For such a decentralized deployment scenario, it will be favorable to have tens of thousands of unique barcodes. To minimize expected costs for primer synthesis and validation, we simulated a compressed barcode space where each LAMP reaction gets more than one barcode and also described the possibility for additional barcodes in the backward inner primer (BIP) (Supplementary Notes and Supplementary Fig. 3). Finally, the inclusion of UDG/UTP in the LAMP-Seq protocol could be envisaged to help reduce amplicon-driven contamination[23,24] in situations where distinct pre-LAMP and post-LAMP areas are not available or sequencing of replicates is not possible. Once established, LAMP-Seq infrastructure could rapidly counter future waves of viral spread or novel pandemic outbreaks.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41587-021-00966-9.

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Methods

All clinical specimens either were collected upon informed consent under a human subjects protocol approved by the ethics committee of the Medical Faculty of the University Hospital Bonn (149/20 and 500/20) or were left-over specimens from viral diagnostic testing. No data on age, gender or ethnicity were collected.

LAMP-Seq testing for SARS-CoV-2 using commercial buffers and enzymes.

1. An inoculated cotton dry swab (nerbe plus, 09-819-5000) is inserted into 500 µl of QuickExtract (Lucigen, QE09050) supplemented with 2 ng µl⁻¹ of RNase-free plasmid DNA (pX330, Addgene, no. 42230) in a 15-ml Falcon tube or a 1.5-ml microcentrifuge tube, incubated for at least 10 min at room temperature and heated to 95 °C for 5 min.

2. Lysate (100 µl) is incubated with 35 mg of activated carbon and ion-exchange beads for 30 min, and 90 µl of the mixture is transferred to a fresh tube. The pH of the lysis buffer is adjusted by adding 2.25 µl of 1 N HCl.

3. A LAMP-Seq Master Mix for 110 reactions (including 10% overage) is prepared on ice, containing:
   a. 1,147 µl of LAMP Master Mix (New England Biolabs, E1700L)
   b. 516 µl of 1 M Tris-HCl pH 8.6
   c. 33.4 µl of C-BIP primer (CGCATTGGCATGGAAGTCACTTTGATG GCACCTGTGTAG; 100 µM, HPLC-purified, IDT)
   d. 4.2 µl of C-F3-LNA primer (A+AC+AC+AA+GC+TTTCGGCAG; 100 µM, HPLC-purified, IDT; + denotes LNA modification in subsequent position)
   e. 4.2 µl of C-B3-LNA primer (G+AA+AT+TT+GG+ATCTTTGT-CATCC; 100 µM, HPLC-purified, IDT; + denotes LNA modification in subsequent position)
   f. 8.3 µl of C-LF primer (TTCCTTGTCTGATTAGTTC; 100 µM, HPLC-purified, IDT)
   g. 8.3 µl of C-LB primer (ACCTTCGGGAACGTGGTT; 100 µM, HPLC-purified, IDT)
   h. 6.7 µl of ACTB-BIP primer (CTGAACCCCAAGGCCAACCGGCTG GGGTGTTGAAGGTC; 100 µM, IDT)
   i. 0.83 µl of ACTB-F3 primer (AGTACCCCATCGAGCACG; 100 µM, IDT)
   j. 0.83 µl of ACTB-B3 primer (AGCCTGGATAGCAACGTACA; 100 µM, IDT)
   k. 1.67 µl of ACTB-LF primer (TGTGGTGCCAGATTTTCTCCA; 100 µM, IDT)
   l. 1.67 µl of ACTB-LB primer (CGAGAAGATGACCCAGATCATGT; 100 µM, IDT)
   m. 2.3 µl of RNase-free plasmid DNA (pX330, 1 µg µl⁻¹, Addgene, no. 42230)
   n. 57.3 µl of Bst 3.0 (8,000 units per ml, New England Biolabs, M0374L)

4. 25-µl LAMP-Seq reactions containing the following components are assembled in a 384-well plate:
   a. 16.2 µl of LAMP-Seq Master Mix
   b. 0.5 µl of an aqueous solution of:
      i. barcoded C-FIP (TGCGGCCAATGTTTGTAATCAG NNNNNNNNNNCCAAGGAAATTTTGGGGAC, where Ns denote a barcode sequence; 60 µM, IDT)
      ii. barcoded ACTB-FIP (GAGCCACACGCAGCTCATTGTA NNNNNNNNNNNTCACCAACTGGGACGACA, where Ns denote a barcode sequence; 12 µM, IDT)
   c. 8.3 µl of swab lysate from Step 2

5. The RT–LAMP plate is thoroughly sealed using foil (BIOplastics, no. 157300), and the plate is submerged in a 65 °C water bath for 80 min, avoiding air bubbles under the plate.

6. Reactions are pooled on ice. If replicates of one sample have identical barcodes, a multi-channel pipette has to be used (0.25 pipette tips per sample). If each replicate on the 384-well LAMP plate has individual barcodes, pooling can be performed by brief top-down centrifugation into a disposable container (for example, pipette box lid).

7. The pool is diluted 1:100,000 in double distilled water (together with the subsequent dilution of 1 in 10 at the PCR stage; this results in a final dilution of the pool of 1 in 1,000,000).

8. For each pool, an 18-cycle 50-µl PCR reaction is performed:
   a. 25 µl of NEBNext 2× Master Mix (New England Biolabs)
   b. 0.25 µl of PCR-C-fwd primer (ACACTCTTTCCCTACACGACGCTC TTCCGATCTAACGCTAAGCGCTGGGGGCAAA; 100 µM, IDT)
   c. 0.25 µl of PCR-C-rev primer (TGACTGGAGTTCAGACGTGTGCTC TTCCGATCTGTTTGTAATCAGTTCCTTGTCTG; 100 µM, IDT)
   d. 5 µl of diluted RT–LAMP reaction
   e. 19.5 µl of water
   f. PCR cycle conditions: 20 s at 98 °C, 20 s at 65 °C and 30 s at 72 °C

9. For each pool, a secondary 18-cycle 50-µl PCR reaction is performed with:

   a. 25 µl of NEBNext 2× Master Mix (New England Biolabs)
   b. 0.25 µl of pool-specific fwd barcoding primer (AATGATACGGCGAC CACCGAGATCTACACNNNNNNNNNNNNACACTCTTTCCCTACAC GACGCT, where Ns denote a specific barcode sequence; 100 µM, IDT)
   c. 0.25 µl of pool-specific rev barcoding primer (CAAGCAGAAGACGG CATACGAGATNNNNNNNNNNNNGTGACTGGAGTTCAGACGTGT GCT, where Ns denote a specific barcode sequence; 100 µM, IDT)
   d. 5 µl of the previous PCR reaction
   e. 19.5 µl of water
   f. PCR cycle conditions: 20 s at 98 °C, 20 s at 65 °C and 30 s at 72 °C Of note, Steps 8 and 9 can also be combined into a single PCR reaction as described for the open-source protocol (Step 8).

10. The PCR products are pooled on ice, cut from a 2% agarose E-Gel, purified twice using a silica spin column (Qiagen), quantified using a NanoDrop photospectrometer (Thermo Fisher Scientific) and sequenced on an Illumina MiSeq or iSeq sequencer.

11. Using the LAMP-Seq Inspector software (http://manuscript.lamp-seq. org/Inspector.htm), barcodes co-occurring with the correct viral genome sequence, excluding sequence portions covered by primers, are determined. This analysis can also be performed using a 'kallisto | bustools' workflow[25].

LAMP-Seq testing for SARS-CoV-2 using open-source buffers and enzymes.

1. The following lysis buffer LSB is prepared and stored on ice:
   a. 300 mM Tris-HCl pH 8.5
   b. 2.7 units per ml of proteinase K (NEB, P8107S)
   c. 2.5 M betaine (Sigma-Aldrich, 61962-50G)

2. Dry swabs from routine clinical testing are inserted into a 96-well 1-ml deep-well plate (Eppendorf, 0030501217) containing 700 µl of LSB lysis buffer and, optionally, 175 µl of a dry volume of activated carbon and weak acid cation exchange beads, incubated for at least 10 s at room temperature and heated to 95 °C for up to 15 min.

3. A LAMP-Seq Master Mix for six 384-well plates (including overage) is prepared on ice, containing:
   a. 8 ml of isothermal reaction buffer (New England Biolabs, B0537S)
   b. 2.8 ml of dNTP mix 10 mM (NEB, N0447L)
   c. 17.92 ml of 1 M Tris-HCl pH 8.5
   d. 4.8 ml of MgSO₄ 100 mM (NEB, B1003S)
   e. 320 µl of C-BIP primer (CGCATTGGCATGGAAGTCACTTTGAT GGCACCTGTGTAG; 100 µM, IDT)
   f. 160 µl of C-F3-LNA primer (A+AC+AC+AA+GC+TTTCGGCAG; 100 µM, IDT; + stands for LNA modification in subsequent position)
   g. 160 µl of C-B3-LNA primer (G+AA+AT+TT+GG+ATCTTTGT-CATCC; 100 µM, IDT; + stands for LNA modification in subsequent position)
   h. 320 µl of C-LF primer (TTCCTTGTCTGATTAGTTC; 100 µM, IDT)
   i. 320 µl of C-LB primer (ACCTTCGGGAACGTGGTT; 100 µM, IDT)
   j. 80 µl of pUC19 plasmid DNA (1 µg µl⁻¹, New England Biolabs N3041L)
   k. 12.16 ml of water
   l. 3.2 ml of wild-type Bst-LF-Idaho polymerase (1 mg ml⁻¹).

4. 25-µl LAMP-Seq reactions containing the following components are assembled in a 384-well plate:

   a. 15.7 µl of LAMP-Seq Master Mix
   b. 1 µl of barcoded C-FIP (TGCGGCCAATGTTTGTAATCAG-NN NNNNNNNN-CCAAGGAAATTTTGGGGAC, where Ns denote a barcode sequence; 10 µM, IDT)
   c. 8.3 µl of swab lysate from Step 2.

Of note, we observed a similar sensitivity when scaling down to 12.5 µl per reaction in a 384-well plate.

1. Plates are submerged in a water bath at 65 °C for 80 min, protected by two nested Ziploc bags.
2. Reactions are pooled on ice. If replicates of one sample have identical barcodes, a multi-channel pipette has to be used (0.25 pipette tips per sample). If each replicate on the 384-well LAMP plate has individual barcodes, pooling can be performed by brief top-down centrifugation into a disposable container (for example, pipette box lid).
3. The pool is diluted 1:40,000 in double distilled water (together with the subsequent dilution of 1 in 10 at the PCR stage; this results in a final dilution of the pool of 1 in 400,000).
4. One-step PCR reactions are performed (25 cycles, Ta = 65 °C) containing:

   a. 12.5 µl of NEBNext 2× Master Mix (New England Biolabs)
   b. 2.5 µl of diluted LAMP pool
   c. 2.5 µl of primer mix, containing:

      i. 5 µM unique fwd primer (AATGATACGGCGACCACCGA GATCTACAC-NNNNNNNNNN-ACACTCTTTCCCTACAC

GACGCTCTTCCGATCTAACGCTGAAGCGCTGGGGGCAAA,
where Ns denote a barcode sequence; Ultramer, IDT)
   ii. 5 µM unique rev primer (CAAGCAGAAGACGGCATACGAG
   AT-NNNNNNNNNN-GTGACTGGAGTTCAGACGTGTGCTC
   TTCCGATCTGTTTGTAATCAGTTCCTTGTCTG, where Ns
   denote a barcode sequence; Ultramer, IDT)
      a. 7.5 µl of water
9. PCR products are pooled and purified using a Qiagen PCR spin purification column and eluted in 30 µl of water.
10. The library band at 258 bp is gauged against a 3× dilution series of a previously run reference library using a 2% Agarose EX E-Gel (Thermo Fisher Scientific).
11. A MiSeq Nano kit (Illumina, MS-103-1001) is loaded according to the manufacturer's protocol, with 10% PhiX spike-in (Illumina) and the following cycle numbers:

   a. 100 cycles read-1
   b. 8 cycles index-1
   c. 8 cycles index-2

Sequencing on the iSeq platform.

1. An N-100 random-index library is generated by NEBNext PCR (NEB) using template oligo iSeq-N100 (Supplementary Data 1; Ultramer, IDT), and primers iSeq-N100-fwd and iSeq-N100-rev (Supplementary Data 1; IDT). PCR products are purified using a silica-based PCR purification kit (Qiagen) and quantified using a NanoDrop photospectrometer (Thermo Fisher Scientific).
2. The library band at 258 bp is gauged against a 3× dilution series of a previously run reference library using a 2% Agarose EX E-Gel (Thermo Fisher Scientific).
3. An iSeq v2 reagent cartridge (20031371, Illumina) is thawed in a water bath at room temperature for 1 h; the flow cell is inserted; and the kit is kept at room temperature. The LAMP-Seq library and the N-100 random-index library are diluted to 2 nM each in water. Next, 1 µl of the combined library is mixed with 150 µl of Tris pH 8.5. Then, 20 µl of the final dilution is loaded into the reagent cartridge and sequenced according to the manufacturer's instruction using the following cycle numbers:

   a. 100 cycles read-1
   a. 8 cycles index-1
   a. 8 cycles index-2

Checkerboard validation of 96 LAMP-Seq barcodes.

1. A 384-well LAMP-Seq plate is prepared using a Beckman Coulter FXP pipetting robot, following the open-source LAMP-Seq protocol. Wells contain all reaction components except template (total: 16.7 µl per well), and 96 barcoded primers are tested per plate, as each primer is spotted to four adjacent wells.
2. A dilution of IVT-generated template RNA with 60,200 molecules per µl in water is created.
3. In the first row of a 96-well PCR plate, 300 µl of template RNA dilution is spotted, alternating with water.
4. Using a 12-channel pipette, 8.3 µl of template or water (amounting to 500,000 or 0 molecules per LAMP reaction) are spotted to quadrants 1 and 4 of the LAMP-Seq plate and mixed four times without performing a blow-out.
5. The template plate is turned 180°, and 8.3 µl of template or water is spotted to quadrants 2 and 3 of the LAMP-Seq plate and mixed four times without performing a blow-out.
6. The LAMP-Seq plate is heated to 65 °C for 80 min and pooled, diluted, amplified and sequenced on a MiSeq platform (Illumina) as described in the above open-source protocol.

Clinical RT–qPCR pipeline.

Swabs were rehydrated in 600 µl ml^{-1} of PBS, saline or LAMP-Seq lysis buffer. Viral RNA was extracted using the chemagic Prime viral DNA/RNA 300 kit (PerkinElmer) on a chemagic Prime 8 system (PerkinElmer). The viral sample (150–290 µl) was mixed with 10 µl of the internal control sample and 300 µl of lysis buffer. Extraction was performed according to the manufacturer's protocol, and viral RNA was eluted in 45 µl of elution buffer for subsequent analysis. Detection of viral RNA using one-step real-time RT–PCR was performed according to Corman et al.[18] with the iTaq Universal Probes One-Step Kit (Bio-Rad), using 5 µl of eluate per reaction and primers and probes against the *E* gene (E_Sarbeco_F1: ACAGGTACGTTAATAGTTAATAGCGT, E_Sarbeco_R2: ATATTGCAGCAGTACGCACACA and E_Sarbeco_P1: FAM-ACACTAGCCAT CCTTACTGCGCTTCG–BBQ; TIB MolBiol). Spike-in RNA of the bacteriophage MS2 served as an internal control and was detected using the Luna Universal Probe One-Step RT–qPCR Kit (New England Biolabs) using 2 µl of eluate and corresponding primers and probes (MS2_F: TGCTCGCGGATACCCG, MS2_R: AACTTGCGTTCTCGAGCGAT and MS2_P: YAK-ACCTCGGGTTTCC

GTCTTGCTCGT–BBQ; TIB MolBiol). The reactions for the *E* gene and internal control were performed using dual detection of FAM and YAK/VIC in a LightCycler 480 (Roche).

Clinical RT–qPCR protocol 2 (high sensitivity). Next, 20 µl of extracted viral RNA (see clinical RT–qPCR pipeline) was analyzed using the New Coronavirus Nucleic Acid Detection Kit (PerkinElmer), according to the manufacturer's protocol, in a total sample volume of 30 µl. Samples were analyzed using QuantStudio (Thermo Fisher Scientific; comparative Ct method of three fluorophores: internal control: VIC; *N* gene: FAM; and ORF1b: ROX).

Viruses and cells. The SARS-CoV-2 strain MUC-IMB-1 was isolated and kindly supplied by Rosina Ehmann and Gerhard Dobler (Bundeswehr Institute of Microbiology). The virus was propagated and titrated on Vera E6 cells (ATCC CRL-1586). All work with SARS-CoV-2 was conducted in a Biosafety Level (BSL)-3 facility in accordance with the biosafety guidelines of the Israel Institute for Biological Research (IIBR). VSV serotype Indiana, kindly provided by Eran Bacharach (Tel-Aviv University), was propagated and titrated on Vero cells (ATCC CCL-81). All work with VSV was conducted in a BSL-2 facility in accordance with the biosafety guidelines of the IIBR.

Lysis buffer inactivation assay. QuickExtract DNA extraction solution (Lucigen) was tested in accordance with the manufacturer's suggested buffer-to-sample ratio. Universal transfer medium (UTM; Copan) aliquots were inoculated with either 5×10^6 plaque-forming units (PFU) per ml of SARS-CoV-2 or 2×10^9 PFU per ml of VSV and incubated at 22 °C, 65 °C or 95 °C for 10–30 min. Positive and negative control samples included UTM inoculated with viable virus without lysis buffer and UTM inoculated with lysis buffer without virus, respectively. The LoD was defined as the first serial dilution of the negative control that did not cause a cytopathic effect (CPE) by itself (represented in log scale). Briefly, Vero E6 cells (for SARS-CoV-2) or Vero cells (for VSV) were cultured in DMEM supplemented with 10% FBS, MEM non-essential amino acids, 2 mM L-glutamine, 100 U ml^{-1} of penicillin, 0.1 mg ml^{-1} streptomycin and 12.5 U ml^{-1} of nystatin (Biological Industries). Monolayers (2.5×10^5 cells per well in 24-well plates) were washed once with MEM Eagle medium without FBS and infected with 200 µl of ten-fold serial dilutions of the samples. After 1 h of incubation, the wells were overlaid with 1 ml of MEM medium containing 2% FCS, MEM non-essential amino acids, 2 mM L-glutamine, 100 U ml^{-1} of penicillin, 0.1 mg ml^{-1} of streptomycin, 12.5 U ml^{-1} of nystatin and 0.15% sodium bicarbonate (Biological Industries). The cells were then incubated at 37 °C/5% CO_2 for 5 d (SARS-CoV-2) or 1 d (VSV). CPE was determined by counter-staining with crystal violet solution.

Bst-LF-Idaho polymerase protein expression and purification. Bst polymerase large fragment from a *Geobacillus* strain sampled in Idaho[20] was cloned into a pET vector with an N-terminal His6-3C-tag (the full plasmid sequence is provided in Supplementary Data 1). Recombinant protein was expressed in *Escherichia coli* BL21 Rosetta (DE3) cells in TB autoinduction media supplemented with 17 mM KH_2PO_4, 72 mM K_2HPO_4, 1.5% lactose, 0.05% glucose and 2 mM $MgSO_4$ at 18 °C overnight. Cells were harvested by centrifugation and resuspended in lysis buffer (50 mM Tris/HCl pH 8.0, 1 M NaCl, 20 mM imidazol and 10% glycerol) followed by sonication. The lysate was cleared in a Beckman Coulter Avanti JNX-26 centrifuge with a JA-25.50 rotor (20,000 r.p.m. for 30 min at 4 °C) and applied to a HisTrap FF column (GE Healthcare). After washing with 10 column volumes of lysis buffer, protein was eluted in elution buffer (50 mM Tris/HCl pH 8.0, 0.5 M NaCl, 200 mM imidazol and 10% glycerol). Fractions of the main peak were pooled and diluted 1:10 with IEX loading buffer (20 mM Tris/HCl, 100 mM NaCl and 10% glycerol), and the affinity tag was removed using 1:100 3C protease overnight at 4 °C. Protein was loaded onto a reverse HisTrap FF column coupled to a HiTrapQ HP column (GE Healthcare). After loading, the HisTrap column was removed, and protein was eluted from the HiTrapQ column with 25% IEX elution buffer (20 mM Tris/HCl, 1 M NaCl and 10% glycerol). Fractions of the main peak were pooled and diluted 1:5 with heparin loading buffer (20 mM Tris/HCl, 100 mM NaCl and 10% glycerol). Sample was loaded onto a HiPrep heparin FF column (GE Healthcare) and eluted using 40% heparin elution buffer (20 mM Tris/HCl, 1 M NaCl and 10% glycerol). Fractions of the main peak were concentrated using Amicon filters (Millipore) and applied to size exclusion chromatography using a Superdex 200 Prep Grade column (GE Healthcare) equilibrated with SEC buffer (25 mM Tris/HCl pH 8.0 and 250 mM KCl). Fractions of the main peak were pooled, concentrated to 1 mg ml^{-1} using Amicon filters and stored in 1-ml aliquots in storage buffer (10 mM Tris/HCl pH 7.5, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol) at −20 °C.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Genomic sequences of SARS-CoV-2 are available at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/sars-cov-2/). Read statistics and read counts are provided in Supplementary Data 2. Example

LAMP-Seq data are available at the Sequence Read Archive (accession number PRJNA729981).

Plasmid availability

The expression plasmid pET-Bst-LF-Idaho is available from http://www.addgene.org/ (no. 170469).

Code availability

The LAMP-Seq Inspector tool for processing raw LAMP-Seq data is available at http://manuscript.lamp-seq. org/Inspector.htm. Python scripts (v3.6.0) for designing the error-correcting barcodes are available at https://github.com/feldman4/dna-barcodes. Jupyter Notebooks for numerical simulations and MATLAB scripts (R2020a Update 4) for figure generation are available at https://github.com/ dbli2000/SARS-CoV2- Bloom-Filter.

References

25. Booeshaghi, A. S. et al. Reliable and accurate diagnostics from highly multiplexed sequencing assays. *Sci. Rep.* **10**, 21759 (2020).

Author contributions

K.U.L.: investigation, methodology, project administration and writing—original draft. R.M.S.: investigation, resources and project administration. D.L.: investigation and formal analysis. M. Jacobs: investigation. R.H.: investigation and methodology. K.B.: resources, software and project administration. J.L.: investigation. M. Słabicki: investigation. A.B.-S.: investigation. O.I.: investigation. S.W.: investigation. T.E.: investigation. N.P.: investigation. W.R.: investigation. G.W.: resources. D.F.: investigation. B.L.: methodology. N.I.: methodology. L.M.H.: methodology. E.B.: methodology. I.H.K.: resources. M. Schmitz: resources. A.W.: investigation. M.D.: resources. E.S.: resources. M. Jentzsch: project administration. J.D.B.: investigation. J.S.: investigation. J.R.: investigation. B.C.: investigation. M.G.: methodology and resources. M.H.: supervision. R.M.: supervision and writing. M.M.N.: supervision and funding acquisition. P.H.: investigation and methodology. M.E.: supervision. A.R.: supervision and funding acquisition. F.Z.: supervision and funding acquisition. J.L.S.-B.: conceptualization, project administration, software, supervision, funding acquisition, investigation and writing—original draft.

Competing interests

K.U.L., D.L., F.Z. and J.S.-B. are inventors on patent applications filed by the Broad Institute and others related to this work with the specific aim of ensuring that this technology can be made rapidly available for research and deployment. F.Z. is a co-founder of Editas Medicine, Beam Therapeutics, Pairwise Plants, Arbor Biotechnologies and Sherlock Biosciences. A.R. is a founder and equity holder of Celsius Therapeutics, an equity holder in Immunitas Therapeutics and, until 31 August 2020, was a scientific advisory board member of Syros Pharmaceuticals, Neogene Therapeutics, Asimov and Thermo Fisher Scientific. Since 1 August 2020, A.R. has been an employee of Genentech, a member of the Roche Group. P.H. and M.M.N. are scientific advisory board members of HMG Systems Bioengineering and receive salaries from Life & Brain. M.M.N. served on scientific advisory boards for the Lundbeck Foundation and Robert-Bosch-Stiftung, was reimbursed for travel expenses by Shire and holds shares in Life & Brain. J.D.B. is a co-founder of Coral Genomics and a scientific advisory board member of Alix Ventures.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41587-021-00966-9.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Self-registration of probands was enabled by a pilot software that was GDPR-compliant. This software cannot be made available as it might qualify as a medical device, which falls under regulation procedures in some European countries.
Data analysis	The LAMP-Seq Inspector tool for processing raw LAMP-Seq data is available at: http://manuscript.lamp-seq.org/Inspector.htm. Python scripts (v3.6.0) for designing the error-correcting barcodes are available at: https://github.com/feldman4/dna-barcodes. Jupyter Notebooks for numerical simulations and MATLAB scripts (R2020a Update 4) for figure generation are available at: https://github.com/dbli2000/SARS-CoV2-Bloom-Filter.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

SARS-CoV-2 genomic sequences are available at NCBI (https://www.ncbi.nlm.nih.gov/sars-cov-2/). Read statistics and read counts are provided in Supplementary Data 2. Example LAMP-Seq data are available at SRA (accession number PRJNA729981).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For each investigation involving human research participants, the sample size was determined by the maximum number of available research participants or clinical samples.
Data exclusions	Clinical samples (panel 3D) were left-over specimen from routine clinical diagnostics. Individual samples were excluded if they did not meet quality criteria (i.e., failure of internal extraction control). For each analyzed sample, all replicates were included in data analysis.
Replication	The number of replicates are indicated for each Figure panel. No contradictory results were obtained.
Randomization	In our study, no assignment to groups took place. Therefore, no randomization was required.
Blinding	Test results obtained from LAMP-Seq (positive / negative) and from qPCR (Ct-values) were generated by the data analysis algorithm (LAMP-Seq, see Methods) and the LightCycler software, respectively. Therefore, no blinding was required. Clinical samples were pseudonymized.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	ATCC CRL-1586 (VERO-E6), ATCC CCL-81 (VERO) were purchased from ATCC.
Authentication	For the two cell lines used in the study, no approved authentication protocol exist. Therefore we purchased the cell lines from an official supplier (ATCC), confirmed their origin (monkey) using NGS, and confirmed that they follow published characteristics as described by ATCC, including shape, growth rate and sensitivity to viral infections.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	No population-specific characteristics were assessed at any point.
Recruitment	Recruitment of human research participants was either performed upon direct contact after individuals tested positive at the University Hospital Bonn, or were invited to participate in the UKB pilot study through public announcements. These specimens were collected upon informed consent under a human subjects protocol approved by the ethics committee of the

Medical Faculty of the University Hospital Bonn, Germany (149/20 and 500/20). No data on age, gender or ethnicity were collected. Additional viral samples were obtained from diagnostic laboratories as left-over specimen. Clinical samples were enriched for a previous positive qPCR result, thus increasing the prevalence of positives in our clinical cohorts. No self-selection took place.

Ethics oversight   All studies involving human research participants have been approved by the Ethics Committee of the Medical Faculty of the University Hospital Bonn, under accession numbers 149/20 and 500/20.

Note that full information on the approval of the study protocol must also be provided in the manuscript.