Capturing sequence diversity in metagenomes with comprehensive and scalable probe design

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Metagenomic sequencing has the potential to transform microbial detection and characterization, but new tools are needed to improve its sensitivity. Here we present CATCH, a computational method to enhance nucleic acid capture for enrichment of diverse microbial taxa. CATCH designs optimal probe sets, with a specified number of oligonucleotides, that achieve full coverage of, and scale well with, known sequence diversity. We focus on applying CATCH to capture viral genomes in complex metagenomic samples. We design, synthesize, and validate multiple probe sets, including one that targets the whole genomes of the 356 viral species known to infect humans. Capture with these probe sets enriches unique viral content on average 18-fold, allowing us to assemble genomes that could not be recovered without enrichment, and accurately preserves within-sample diversity. We also use these probe sets to recover genomes from the 2018 Lassa fever outbreak in Nigeria and to improve detection of uncharacterized viral infections in human and mosquito samples. The results demonstrate that CATCH enables more sensitive and cost-effective metagenomic sequencing.

Sequencing of patient samples has transformed the detection and characterization of important human viral pathogens1 and has provided crucial insights into their evolution and epidemiology2–5. Unbiased metagenomic sequencing is particularly useful for identifying and obtaining the genome sequences of emerging or diverse species because it allows accurate detection of both new and known species and variants1. However, extremely low viral titers (as seen in the recent Zika virus outbreak6) or high levels of host material8 can limit its practical utility: a low ratio of viral to host material makes genome assembly difficult or prohibitively expensive. To fully realize the potential of metagenomic sequencing, new tools are needed that improve its sensitivity while preserving its comprehensive, unbiased scope.

Previous studies have used targeted amplification9–10 or enrichment via capture of viral nucleic acid using oligonucleotide
to designing probe sets to capture viral diversity. We used CATCH to design a probe set that targets all viral species reported to infect humans (V_all), which could be used to achieve more sensitive metagenomic sequencing of viruses from human samples. V_all encompasses 356 species (86 genera, 31 families), and we designed it using genomes available from NCBI GenBank (Supplementary Table 1). We constrained the number of probes to 350,000, significantly fewer than the number used in studies with comparable goals18,19, reducing the cost of synthesizing probes that target diversity across hundreds of viral species. The design output by CATCH contained 349,998 probes (Fig. 1c). This design represents comprehensive coverage of the input sequence diversity under conservative choices of parameters, values, for example, tolerating few mismatches between probe and target sequences (Fig. 1d). To compare the performance of V_all against probe sets with lower complexity, we separately designed three focused probe sets for commonly co-circulating viral infections: measles and mumps viruses (V้ม<sub>M</sub>, 6,219 probes), Zika and chikungunya viruses (V้ม<sub>CH</sub>, 6,171 probes), and a panel of 23 species (16 genera, 12 families) circulating in West Africa (V_all<sub>W</sub>, 44,995 probes) (Supplementary Fig. 3 and Supplementary Table 1).

We synthesized V_all<sub>W</sub> as 75-nucleotide (nt) biotinylated single-stranded DNA (ssDNA) and the focused probe sets (V้ม<sub>M</sub>, V้ม<sub>CH</sub>, V_all<sub>W</sub>) as 100-nt biotinylated ssRNA. The ssDNA probes in V_all<sub>W</sub> are more stable and therefore more suitable for use in lower-resource settings than ssRNA probes. We expect the ssRNA probes to be more sensitive than ssDNA probes in enriching target cDNA owing to their longer length and the stronger bonds formed between RNA and DNA14, making the focused probe sets a useful benchmark for the performance of V_all<sub>W</sub>.

**Enrichment of viral genomes upon capture with V_all**. To evaluate the enrichment efficiency of V_all<sub>W</sub>, we prepared sequencing libraries from 30 patient and environmental samples containing at least one of eight different viruses: dengue virus (DENV), GB virus C (GBV-C), hepatitis C virus (HCV), HIV-1, influenza A virus (IAV), Lassa virus (LASV), mumps virus (MuV), and Zika virus (ZIKV) (Supplementary Table 2). These eight viruses together reflect a range of typical viral titers in biological samples, including ones that have extremely low levels, such as ZIKV<sup>15</sup>. The samples encompass a range of source materials: plasma, serum, buccal swabs, urine,
avian swabs, and mosquito pools. We performed capture on these libraries and sequenced them both before and after capture. To compare enrichment of viral content across sequencing runs, we downsampled raw read data from each sample to the same number of reads (200,000) before further analysis. Downsampling to correct for differences in sequencing depth, rather than the more common use of a normalized count such as reads per million, is useful for two reasons. First, it allows us to compare our ability to assemble genomes (for example, due to capture) in samples that were sequenced to different depths. Second, downsampling helps to correct for differences in sequencing depth in the presence of a high frequency of PCR duplicate reads (Methods), as observed in captured libraries. We removed duplicate reads during analyses so that we could measure enrichment of viral information (that is, unique viral content) rather than measure an artifactual enrichment arising from PCR amplification.

We first assessed enrichment of viral content by examining the change in per-base read depth resulting from capture with \( V_{\text{ALL}} \). Overall, we observed a median increase in unique viral reads across all samples of 18x (first and third quartiles: \( Q_1 = 4.6, Q_3 = 29.6 \)) (Supplementary Table 3). Capture increased depth across the length of each viral genome, with no apparent preference in enrichment for regions over this length (Fig. 2a-b and Supplementary Fig. 4). Moreover, capture successfully enriched viral content in each of the six sample types we tested. The increase in coverage depth varied between samples, likely in part because the samples differed in their starting concentration, and, as expected, we saw lower enrichment in samples with higher abundance of virus before capture (Supplementary Fig. 5).

Next, we analyzed how capture improved our ability to assemble viral genomes. For samples that had incomplete genome assemblies (<90%) before capture, we found that application of \( V_{\text{ALL}} \) allowed us to assemble a greater fraction of the genome in all cases (Fig. 2c). Importantly, of the 14 samples from which we were unable to assemble any contig before capture, we were able to assemble 11 at least partial genomes (>50%) using \( V_{\text{ALL}} \), of which 4 were complete genomes (>90%). Many of the viruses we tested, such as HCV and HIV-1, are known to have high within-species diversity, yet the
enrichment of their unique content was consistent with that of less diverse species (Supplementary Table 3).

We also explored the impact of capture on the complete metagenomic diversity within each sample. Metagenomic sequencing generates reads from the host genome as well as background contaminants, and capture should reduce the abundance of these taxa. Following capture with VALL, the fraction of sequence classified as human decreased in patient samples while viral species with a wide range of pre-capture abundances were strongly enriched (Fig. 2d). Moreover, we observed a reduction in the overall number of species detected after capture (Supplementary Fig. 6a), suggesting that capture indeed reduces non-targeted taxa. Lastly, analysis of these metagenomic data identified a number of other enriched viral species present in these samples (Supplementary Table 4). For example, one HIV-1 sample showed strong evidence of HCV co-infection, an observation consistent with clinical PCR testing.

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**Fig. 2** Improvement in genome coverage and assembly, and shift in metagenomic distribution after capture. **a**, Distribution of the enrichment in read depth, across viral genomes, provided by capture with VALL on 30 patient and environmental samples with known viral infections. Each curve represents one of the 31 viral genomes sequenced here (one sample contained two known viruses). At each position across a genome, the post-capture read depth is divided by the pre-capture depth, and the plotted curve is the empirical cumulative distribution of the log of these fold-change values. A curve that rises fully to the right of the black vertical line illustrates enrichment throughout the entirety of a genome; the more vertical a curve, the more uniform the enrichment. Read depth across viral genomes DENV-SM3 (purple) and DENV-SM5 (green) is shown in more detail in **b**. **b**, Read depth throughout the DENV genome in two samples. DENV-SM3 (left) has few informative reads before capture and does not produce a genome assembly, but does following capture. DENV-SM5 (right) does yield a genome assembly before capture, and depth increases following capture. **c**, Percent of each viral genome unambiguously assembled in the 30 samples, which had eight known viral infections across them. Shown before capture (orange), after capture with VWAFR (light blue), and after capture with VALL (dark blue). Red bars below samples indicate ones in which we could not assemble any contig before capture but in which, following capture, we were able to assemble at least a partial genome (>50%). **d**, Left, number of reads detected for each species across the 30 samples with known viral infections, before and after capture with VALL. Reads in each sample were downsampled to 200,000 reads. Each point represents one species detected in one sample. For each sample, the virus previously detected in the sample by another assay is colored. Homo sapiens matches in samples from humans are shown in black. Right, abundance of each detected species before capture and fold change upon capture with VALL for these samples. Abundance was calculated by dividing pre-capture read counts for each species by counts in pooled water controls. Coloring of human and viral species is as in the left panel.
In addition to measuring enrichment on patient and environmental samples, we sought to evaluate the sensitivity of VALL on samples with known quantities of viral and background material. To do so, we performed capture with VALL on serial dilutions of Ebola virus (EBOV)—ranging from 10^6 copies down to a single copy—in known background amounts of human RNA. At a depth of 200,000 reads, use of VALL allowed us to reliably detect viral content (that is, observe viral reads in two technical replicates) down to 100 copies in 30 ng of background and 1,000 copies in 300 ng (Fig. 3a and Supplementary Table 5), each of which was at least an order of magnitude lower than without capture, and similarly lowered the input at which we could assemble genomes (Supplementary Fig. 7a).

Although we chose a single sequencing depth so that we could compare pre- and post-capture results, higher sequencing depths provide more viral material and thus more sensitivity in detection (Supplementary Fig. 7b,c).

Comparison of VALL to focused probe sets. To test whether the performance of the highly complex 356-virus VALL probe set matches that of focused ssRNA probe sets, we first compared it to the 23-virus VWAFR probe set. We evaluated the six viral species we tested from the patient and environmental samples that were present in both the VALL and VWAFR probe sets, and we found that performance was concordant between them: VWAFR provided almost the same number of unique viral reads as VALL (1.01 times as many; Q_1 = 0.93, Q_3 = 1.34) (Supplementary Table 3).
could unambiguously assemble was also similar between the probe sets (Fig. 2c), as was the read depth (Supplementary Figs. 4 and 8a,b). Following capture with V_{WAFR}, human material and the overall number of detected species both decreased, as with V_{ALL}, although these changes were more pronounced with V_{WAFR} (Supplementary Fig. 6a,b and Supplementary Table 4).

We next compared the V_{ALL} probe set to the two-virus probe sets V_{SM} and V_{ZC}. We found that enrichment for MuV and ZIKV samples was slightly higher using the two-virus probe sets than with V_{ALL} (2.26 times more unique viral reads; Q_i = 1.69, Q = 3.36) (Supplementary Figs. 4 and 8c,d, and Supplementary Table 3). The additional gain of these probe sets might be useful in some applications but was considerably less than the 18× increase provided by V_{ALL} against a pre-capture sample. Overall, our results suggest that neither the complexity of the V_{ALL} probe set nor its use of shorter ssDNA probes prevent it from efficiently enriching viral content.

Enrichment of targets with divergence from design. We then evaluated how well our V_{ALL} and V_{WAFR} probe sets capture sequence that is divergent from the sequences used in their design. To do this, we tested whether the probe sets, whose designs included human IAV, successfully enrich the genome of the nonhuman, avian subtype H4N4 (IAV-SM5). H4N4 was not included in the designs, making it a useful test case for this relationship. Moreover, the IAV genome has eight RNA segments that differ considerably in their genetic diversity; segment 4 (hemagglutinin, H) and segment 6 (neuraminidase, N), which are used to define the subtypes, exhibit the most diversity.

The segments of the H4N4 genome displayed different levels of enrichment following capture (Supplementary Fig. 9). To investigate whether these differences are related to sequence divergence from the probes, we compared the identity between probes and sequence in the H4N4 genome to the observed enrichment of that sequence (Fig. 3b). We saw the least enrichment in segment 6 (N), which had the least identity between probe sequence and the H4N4 sequence, as we did not include any sequences of the N4 subtypes in the probe designs. Interestingly, V_{ALL} did show limited positive enrichment of segment 6, as well as of segment 4 (H); these enrichments were lower than those of the less divergent segments. But this was not the case for segment 4 when using V_{WAFR}, suggesting a greater target affinity of V_{WAFR} capture when there is some degree of divergence between probes and target sequence (Fig. 3b), potentially due to this probe set's longer, ssRNA probes. For both probe sets, we observed no clear inter-segment differences in enrichment across the remaining segments, whose sequences have high identity with probe sequences (Fig. 3b and Supplementary Fig. 9). These results show that the probe sets can capture sequence that differs markedly from what they were designed to target, but nonetheless that sequence similarity with probes influences enrichment efficiency.

Quantifying within-sample diversity after capture. Given that many viruses co-circulate within geographic regions, we assessed whether capture accurately preserves within-sample viral species complexity. We first evaluated capture on mock co-infections containing 2, 4, 6, or 8 viruses. Using both V_{ALL} and V_{WAFR}, we observed an increase in overall viral content while preserving the relative frequencies of each virus present in the sample (Fig. 3c and Supplementary Table 4).

Because viruses often have extensive within-host viral nucleotide variation that can inform studies of transmission and within-host virus evolution^{33,34}, we examined the impact of capture on estimating within-host variant frequencies. We used three DENV samples that yielded high read depth (Supplementary Table 3). Using both V_{ALL} and V_{WAFR}, we found that the frequencies of all within-host variants were consistent with pre-capture levels (Fig. 3d and Supplementary Table 6; concordance correlation coefficient of 0.996 for V_{ALL} and 0.997 for V_{WAFR}). These estimates were consistent for both low- and high-frequency variants. Because capture preserves frequencies so well, it should enable measurement of within-host diversity that is both sensitive and cost-effective.

Rescuing Lassa virus genomes in patient samples from Nigeria. To demonstrate the application of V_{ALL} in the case of an outbreak, we applied it to samples of clinically confirmed (by qRT–PCR) Lassa fever cases from Nigeria. In 2018, Nigeria experienced a sharp increase in cases of Lassa fever, a severe hemorrhagic disease caused by LASV, leading the World Health Organization and the Nigeria Centre for Disease Control to declare it an outbreak^{35}. Previous genome sequencing of LASV has revealed its extensive genetic diversity, with distinct lineages circulating in different parts of the endemic region^{36}, and ongoing sequencing can enable rapid identification of changes in this genetic landscape.

We selected 23 samples, spanning five states in Nigeria, that yielded either no portion of a LASV genome or only partial genomes with unbiased metagenomic sequencing even at a reasonably high sequencing depth (>4.5 million reads)^{37} and performed capture on these using V_{ALL}. At equivalent pre- and post-capture sequencing depths (200,000 reads), use of V_{ALL} improved our ability to detect and assemble LASV. Capture considerably increased the amount of unique LASV material detected in all 23 samples (in 4 samples, by more than 100×), and in 7 samples it enabled detection when there were no LASV reads pre-capture (Supplementary Fig. 10a and Supplementary Table 7). This in turn improved genome assembly. Whereas pre-capture we could not assemble any portion of a genome in 22 samples (in the remaining sample, 2% of a genome could be assembled) at this depth, following use of V_{ALL} we could assemble a partial genome in 22 of the 23 samples (Fig. 4a and Supplementary Fig. 10b); most were small portions of a genome, although in 7 samples we assembled >50% of a genome. Assembly results with V_{ALL} were comparable without downsampling (Supplementary Fig. 10c), likely because we saturated unique content with V_{ALL} even at low sequencing depths (Supplementary Fig. 7b,c). These results illustrate how V_{ALL} can be used to improve viral detection and genome assembly in an outbreak, especially at the low sequencing depths that may be desired or required in these settings.

Identifying viruses in uncharacterized samples using capture. We next applied our V_{ALL} probe set to pools of human plasma and mosquito samples with uncharacterized infections. We tested five pools of human plasma from a total of 25 individuals with suspected LASV or EBOV infection from Sierra Leone, as well as five pools of human plasma from a total of 25 individuals with acute fevers of unknown cause from Nigeria and five pools of Culex tarsalis and Culex pipiens mosquitoes from the United States (see Methods for details). Using V_{ALL}, we detected eight viral species, each present in one or more pools: two species in the pools from Sierra Leone, two species in the pools from Nigeria, and four species in the mosquito pools (Fig. 4b and Supplementary Fig. 6c). We found consistent results with V_{WAFR} for the species that were included in its design (Supplementary Fig. 6d and Supplementary Table 4). To confirm the presence of these viruses, we assembled their genomes and evaluated read depth (Supplementary Fig. 11 and Supplementary Table 8). We also sequenced pre-capture samples and found substantial enrichment by capture (Fig. 4c and Supplementary Fig. 6c,d). Quantifying abundance and enrichment together provides a valuable way to discriminate viral species from other taxa (Fig. 4c), thereby helping to uncover which pathogens are present in samples with unknown infections.

Looking more closely at the identified viral species, all pools from Sierra Leone contained LASV or EBOV, as expected (Fig. 4b). The five plasma pools from Nigeria showed little evidence for pathogenic viral infections; however, one pool did contain
hepatitis B virus (HBV). Additionally, three pools contained GBV-C, consistent with expected frequencies for this region\textsuperscript{20,37}. In mosquitoes, four pools contained West Nile virus (WNV), a common mosquito-borne infection, consistent with PCR testing. In addition, three pools contained Culex flavivirus, which has been shown to co-circulate with WNV and co-infect Culex mosquitoes in the United States\textsuperscript{38}. These findings demonstrate the utility of capturing sequences in improving virus identification without a priori knowledge of sample content.

**Discussion**

CATCH condenses highly diverse target sequence data into a small number of oligonucleotides, enabling more efficient and sensitive sequencing that is only biased by the extent of known diversity. We show that capture with probe sets designed by CATCH improves viral genome detection and recovery while accurately preserving sample complexity. These probe sets have also helped us to assemble genomes of low-titer viruses in other patient samples: \textit{V_{ZK}}, for suspected ZIKV cases\textsuperscript{6} and \textit{V_{ALL}} for improving rapid detection of Powassan virus in a clinical case\textsuperscript{39}.

The probe sets we have designed with CATCH, and more broadly with comprehensive probe designs, improve the accessibility of metagenomic sequencing in resource-limited settings through smaller-capacity platforms. For example, in West Africa we are using the \textit{V_{ALL}} probe set to characterize LASV and other viruses in patients with undiagnosed fevers by sequencing on a MiSeq (Illumina). This could also be applied on other small machines such as the iSeq (Illumina) or MinION (Oxford Nanopore)\textsuperscript{40}. Further, the increase in viral content enables more samples to be pooled and sequenced on a single run, increasing sample throughput and decreasing per-sample cost relative to unbiased sequencing (Supplementary Table 9). Lastly, researchers can use CATCH to quickly design focused probe sets, providing flexibility when it is not necessary to target an exhaustive list of viruses, such as in outbreak response or for targeting pathogens associated with specific clinical syndromes.

Despite the potential of capture, there are challenges and practical considerations that are present with the use of any probe set. Notably, as capture requires additional cycles of amplification, computational analyses should account for duplicate reads due to amplification; the inclusion of unique molecular identifiers\textsuperscript{41,42} could improve determination of unique fragments. Also, quantifying the sensitivity and specificity of capture with comprehensive probe sets is challenging—as it is for metagenomic sequencing more broadly—owing to the need to obtain viral genomes for the hundreds of targeted species and the risk of false positives from components of sequencing and classification that are unrelated to capture (for example, contamination in sample processing or read misclassifications). Targeted amplicon approaches may be faster and more sensitive\textsuperscript{7} for sequencing ultra-low-titer samples, but the suitability of these approaches is limited by genome size, sequence heterogeneity, and the need for prior knowledge of the target species\textsuperscript{43,44}. Similarly, for molecular diagnostics of particular pathogens, many commonly used assays such as qRT–PCR and rapid antigen tests are likely to be faster and less expensive than metagenomic sequencing. Capture does increase the preparation cost and time per sample as compared to unbiased metagenomic sequencing, but this is offset by
reduced sequencing costs through increased sample pooling and/or lower-depth sequencing’ (Supplementary Table 9).

CATCH is a versatile approach that could also be used to design oligonucleotide sequences for capturing non-viral microbial genomes or for uses other than whole-genome enrichment. Capture-based approaches have successfully been used to enrich whole genomes of eukaryotic parasites such as *Plasmodium*45 and *Babesia*, as well as bacteria46. Because designs from CATCH scale well with the growing knowledge of genomic diversity22,21, it is particularly well suited for designing probes to target any microbes that have a high degree of divergence. This includes many bacteria, which, like viruses, have high variation even within species24. Beyond microbes, CATCH could benefit studies in other areas that use capture-based approaches, such as the detection of previously characterized fetal and tumor DNA from cell-free material49,50, in which known targets of interest may represent a small fraction of all material and for which it may be useful to rapidly design new probe sets for enrichment as novel targets are discovered. Moreover, CATCH can identify conserved regions or regions suitable for differential identification, which can help in the design of PCR primers and CRISPR–Cas13 crRNAs for nucleic acid diagnostics.

CATCH is, to our knowledge, the first approach to systematically design probe sets for whole-genome capture of highly diverse target sequences that span many species, making it a valuable extension to the existing toolkit for effective viral detection and surveillance with enrichment and other targeted approaches. We anticipate that CATCH, together with these approaches, will help provide a more complete understanding of microbial genetic diversity.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability, and associated accession codes are available at https://doi.org/10.1038/s41587-018-0006-x.

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Author contributions
H.C.M., D.J.P., A. G nirke, P.C.S., and C.B.M. initiated the study of improved design and application of comprehensive probe sets. H.C.M. conceived of CATCH and implemented it with advice from D.J.P., A. G nirke, and C.B.M. K.J.S. and C.B.M. conceived of experimental design for evaluating probe sets. C.B.M., J.Q., A.G.-Y., and K.J.S. developed enrichment protocols with help from A. Goldfarb. K.J.S., A.G.-Y., J.Q., and P.B. prepared samples, performed enrichment, and sequenced samples. A.P., S.W., A.C., A.E.L., and K.G.B. helped with sample preparation and enrichment. D.C.T., B.C., S.H., G.B.-L., Y.R.V., L.M.F., A.L.T., K.F.G., L.A.P., A.B., E.H., D.S.K., T.M.A., J.A.R., S.S., F.A.B., T.M.I.S., S.I., S.F.M., I.L., L.G., and I.B. collected and shared samples with known viral content. E.S.-L. and L.H. shared viral seed stocks. G.E. shared uncharacterized mosquito pools. I.O., P.E., O.A.F., A. Goba, D.S.G., and C.T.H. collected human plasma samples from Nigeria and Sierra Leone. H.C.M. and K.J.S. formulated and performed data analyses with help from D.K.Y. H.C.M., K.J.S., and C.B.M. wrote the manuscript with input from other authors.

Competing interests
H.C.M., D.J.P., A. G nirke, P.C.S. and C.B.M. are co-inventors on a patent application filed by the Broad Institute related to work in this manuscript (US 15/756546).

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Methods

Probe design using CATCH. Designing a probe set given a single choice of parameters. We first describe how CATCH determines a probe set that covers input sequences under some selection of parameters. That is, the input is a collection of (unaligned) sequences \(d\) and parameters \(\theta\) describing hybridization, and the goal is to compute a set of probes \(s(d, \theta)\). For example, \(d\) commonly encompasses the strain diversity of one or more species and \(\theta\) includes the number of mismatches that we should tolerate when determining whether a probe hybridizes to a sequence.

CATCH produces a set of candidate probes from the input sequences in \(d\) by stepping along them according to a specified stride (Fig. 1a). Optionally, CATCH uses locality-sensitive hashing (LSH) to reduce the number of candidate probes, which is particularly useful when the input is a large number of highly similar sequences. CATCH supports two LSH families: one under Hamming distance and another using the MinHash technique, which has been used in metagenomic applications. It detects near-duplicate candidate probes by performing approximate near-neighbor search using a specified family and distance threshold. CATCH constructs hash tables containing the candidate probes and then queries each (in descending order of multiplicity) to find and collapse near-duplicates. Because LSH reduces the space of candidate probes, it may remove candidate probes that would otherwise be selected in the steps described below, thereby increasing the size of the output probe set. Use of LSH to reduce the number of candidate probes is optional in our implementation of CATCH; we did not use it to produce the probe sets in this work. The approach of detecting near-duplicates among probes (and subsequently mapping them onto sequences, described below) bears some similarity to the use of P clouds for clustering related oligonucleotides to identify diverse repetitive regions in the human genome.

CATCH then maps each candidate probe \(p\) back to the target sequences with a seed-and-extend-like approach, in the process deciding whether \(p\) maps to a range \(r\) in a target sequence according to the function \(f_{SS}(p, r, \theta)\), which effectively specifies whether \(p\) will capture the subsequence at \(r\). Further, CATCH assumes that, because \(p\) captures an entire fragment and not just the subsequence to which it binds, \(p\) ‘covers’ both \(r\) and some number of bases (given in \(\theta\)) on each side of \(r\); we term this a ‘cover extension’. This yields a collection of bases in the target sequences that are covered by each \(p\), namely \((p, \{[s, e]\} \in \text{covered by } p)\) for all \(s \in d\) and \(e \in d\) for all candidate probes \(p\).

Next, CATCH seeks to find the smallest set of candidate probes that covers all sequences in \(d\). The problem is NP-hard. To determine \(s(d, \theta)\), an approximation of the smallest such set of candidate probes, CATCH treats the problem as an instance of the set cover problem. Similar approaches have been used in related problems in uncovering patterns in DNA sequence. Notably, these include PCR primer selection, string barcoding of pathogens, and other applications in microbial metagenomics; although these are not aimed at whole-genome enrichment for sequencing many taxa.

CATCH computes \(s(d, \theta)\) using the canonical greedy solution to the set cover problem, which likely provides close to the best achievable approximation. In this approximation-preserving reduction, each candidate probe \(p\) is treated as a set whose elements represent the bases in the target sequences covered by \(p\). The universe of elements is then all the bases across all the target sequences—that is, what it seeks to cover. To implement the algorithm efficiently, CATCH operates on sets of intervals rather than base positions and applies other techniques to improve performance for this problem.

Extensions to probe design. This framework for designing probes offers considerable flexibility. Supplementary Note 1 describes the default \(f_{SS}\) in CATCH and how it can be customized; how CATCH allows for differential identification, blacklisting of taxa, selected by CATCH for each probe to minimize probe overlap as described in Supplementary Note 1. Furthermore, in these three probe sets we included the reverse complement of each designed 140-nt oligonucleotide in the synthesis.

Analysis of probe set scaling with parameter values and input size. For all evaluations of how probe counts grew with respect to an independent variable (Fig. 1b and Supplementary Figs. 1c and 2), Supplementary Note 2 describes input data and how we used CATCH.
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Construction of sequencing libraries. We first removed contaminating DNA by pool (one mosquito pool contained six), resulting in 15 final pools (5 mosquito, with suspected Lassa and Ebola virus infections from Sierra Leone—we created acute non-Lassa virus fevers from Nigeria, and human plasma from 25 individuals 26 mosquito pools from the United States, human plasma from 25 individuals with duplicate. For samples where the microbial content was uncharacterized—

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Lassa outbreak, we extracted RNA using the Qiagen QIAamp viral mini kit, except in cases where samples were provided for secondary use as extracted RNA directly from the plasma. To enable comparison between pre- and post-capture results, we downsampled all raw reads to 200,000 reads using SAMtools6. We performed all analyses on downsampled datasets unless otherwise stated. We chose this number as 90% of all samples sequenced on the MiSeq (among the 50 patient and environmental samples used for validation) were sequenced to a depth of at least 200,000 reads. For those few low-coverage samples for which we did not obtain >200,000 reads, we performed all analyses using all available reads unless otherwise noted (Supplementary Table 3). Downsampling normalizes sequencing depth across runs and allows us to more readily evaluate the effectiveness of capture on genome assembly (that is, the fraction of the genome that can assemble) than an approach such as comparing viral reads per million. It also allows us to more readily compare unique content (see below). A statistic like unique viral reads per unique million reads can be distorted by sequencing depth in the presence of a high fraction of viral PCR duplicate reads: sequencing to a lower depth can inflate the value of this statistic as compared to sequencing to a higher depth.

We used viral-ngs to assemble the genomes of all viruses previously detected in these samples or identified by metagenomic analyses, including the LAV genomes from the 2018 Lassa fever outbreak in Nigeria and the EBOV genomes from the dilution series. For each virus, we taxonomically filtered reads against many available sequence databases for that virus (Supplementary Tables 2 and 3). We assembled each genome to scaffolding quality (Illuna) using the default settings or 300,000 assembled contigs (Supplementary Tables 5, 6 and 7). We set the parameters 'assembly_min_length_fraction_of_reference' and 'assembly_min_unambig' to 0.01 for all assemblies. We took the fraction of the genome assembled to be the number of base calls we could make in the assembly divided by the length of the reference genome used for scaffolding. To calculate per-base read depth, we aligned depthed reads from viral-ngs to the same reference genome that we used for scaffolding. We did this alignment with BWA4 through the 'align_and_plot_coverage' function of viral-ngs with the following parameters: -m 50000 --excludeDuplicates --aligner_options "-k 12 -B -O 3 --minScoreToFilter 60". We counted the number of aligned reads (unique viral reads) using SAMtools6 with its default settings view -F 1024. We calculated enriched viral content by comparing the number of aligned reads before and after capture. viral-ngs removes PCR duplicate reads with Picard based on alignments, allowing us to measure unique content. We excluded samples where one or more conditions had fewer than 100,000 raw reads for reasons of comparability. Excluded samples are highlighted in red in Supplementary Table 3.

To assess how the amount of viral content detected increases with sequencing depth (Supplementary Fig. 7bc), we used data from the Ebola dilution series on 10° and 10¹ copies. At these input amounts, both technical replicates, with and without capture and in both 30 ng and 300 ng of background, yielded at least 2 million sequencing reads. For each combination of input copies, background amount, and technical replicate, we ran 5 technical replicates and 3 biological replicates. We aligned all raw reads to n = {1, 10, 100, 1,000, 10,000, 100,000, 200,000, 300,000, ..., 1,900,000, 2,000,000} reads. For each n, we performed this downsampling five times. We depleted samples with viral-ngs, aligned depleted reads to the EBOV reference genome (Supplementary Table 5), and counted the number aligned, as described above. We plotted the number of aligned reads for each subsampling amount in

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Supplementary Fig. 7b, where shaded regions are 95% pointwise confidence bands calculated across the five downsampling replicates.

To analyze the relationship between probe–target identity and enrichment (Fig. 3b), we used an influenza A virus sample of avian subtype H4N4 (IAY-SM5). We assembled a genome of this sample both pre- and following capture with VALL to verify concordance; we used the VALL sequence for further analysis here because it was more complete. We aligned depleted reads to this genome as described above (with BWA using the align_and_plot_coverage function of viral-ngs and the following parameters: -n 50000 --excludeDuplicates --aligner-options -k 12 -B 2-0.3 --minScoreToFilter 60). For a window in the genome, we calculated the fold change in depth to be the fold change of the mean depth post-capture against the mean depth prep-capture within the window. Here we used windows of length 150 nt, sliding with a stride of 25 nt. We aligned all probe sequences in VALL and VstAIR designs to this genome using BWA-MEM with the following options: -M -k 8 -A 1 -B 1 -O 2 -E 1 -L 2 -T 20; these sensitive parameters should account for most possible hybridizations and include a low soft-clipping penalty to allow us to model a portion of a probe hybridizing to a target while the remainder hangs off. We counted the number of bases that matched between a probe and target sequence using each alignment’s MD tag (this does not count mismatches), and defined enrichment as the fold change between a probe and target sequence to be this number of matching bases divided by the probe length. We defined the identity between probes and a window of the target genome as follows: we considered all mapped probe sequences that had at least half their alignment within the window and took the mean of the top 25% of identity values between these probes and the target sequence. In Fig. 3b, we plot a point for each window. We did this separately with the probes in VALL and VstAIR designs.

Within-sample variant calling. For our comparison of within-sample variant frequencies with and without capture (Fig. 3d and Supplementary Table 6), we used three dengue virus samples (DENV-S1, DENV-S2, and DENV-S5). We selected these because they have relatively high depth of coverage, in both pre- and post-capture genomes (Supplementary Table 3); the high depth in pre-capture genomes was necessary for the comparison. We did not subsample reads before this comparison, to maximize coverage for detection of rare variants. For each of the three samples, we pooled data from three sequencing replicates of the same pre-capture library before downstream analysis. For each of these samples, we performed two capture replicates on the same pre-capture library (two replicates with VstAIR and two with VstAIR) and sequenced, and estimated, and plotted frequencies separately on these replicates.

After assembling genomes, we used V-Phaser 2.0, available through viral-ngs, to call with sample variants from mapped reads. We set the minimum number of reads required on each strand (vphaser_min_reads, each) to 2 and ignored indels. When counting reads with each allele and estimating variant frequencies, we excluded PCR duplicate reads through viral-ngs. In Fig. 3d, we show the frequencies for a variant if it was present at ≥1% frequency in any of the replicates (that is, either the pre-capture pool or any of the replicates from capture with VALL or VstAIR). The plot positions combined across the three samples that we analyzed.

We estimated the concordance correlation coefficient (ρc) between pre- and post-capture frequencies over points in which each was a pair of pre- and post-capture frequencies in a replicate. Because we had pooled pre-capture data, each pre-capture frequency for a variant was paired with multiple post-capture frequencies for that variant.

Metagenomic analyses. We used kraken v0.10.672 in viral-ngs to analyze the metagenomic content of our pre- and post-capture libraries. First, we built a database that included the default kraken ‘full’ database (containing all bacterial and viral whole genomes from RefSeq as of October 2015). Additionally, we included the whole human genome (hg38), genomes from PlasmoDB74, sequences of the probe designs (with 20-nt adaptors where applicable) developed here are available at https://github.com/broadinstitute/catch (see Supplementary Table 1 for links to specific versions used). Sequences of the probe designs (with 20-nt adaptors where applicable) developed here are available at https://github.com/broadinstitute/catch/tree/cf500c6/probe-designs. Sequencing data from this study, as well as viral genomes generated as part of this work, have been deposited in NCBI databases under BioProject accession PRJNA431306 (PRJNA436552 for the 2018 Lassa virus genomes).

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| ☑ | Clearly defined error bars |

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Software and code

Policy information about availability of computer code

| Data collection | We downloaded sequences from NCBI GenBank, using the Entrez module in Biopython (v1.66), and other resources. Details are given in the Methods section. See Supplementary Table 1 for links to downloaded accessions. |
| Data analysis | We used the publicly available software package viral-ngs v1.17.0 (http://viral-ngs.readthedocs.io/en/latest/) to analyze all sequencing data including demultiplexing of sequencing runs, viral genome assembly, taxonomic read classification, alignment to viral genomes, and detection of intra-host variants. Additional custom scripts specific to our compute environment were used for generating plots and parsing output. |

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Sequences used as input for probe design (Supplementary Table 1) are available in the repository at <https://github.com/broadinstitute/catch>. Sequences of the probe designs are available at <https://github.com/broadinstitute/catch/tree/cf500x6/probe-designs>. Viral genomes sequenced as part of this study will be deposited in NCBI GenBank (Clark et al. 2016) prior to publication under BioProject accession PRJNA431306 (PRJNA436552 for the 2018 Lassa virus genomes).

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Life sciences

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Life sciences study design

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

| Sample size | We performed experimental testing of CATCH using a total of 68 patient and environmental samples (30 samples for validation, 23 samples for 2018 Lassa virus sequencing, and 15 pools of samples with uncharacterized contents). Predetermining sample size is not applicable here as no statistical tests were performed. |
| Data exclusions | No data were excluded from the analyses, except for samples with too few raw sequencing reads (explained in Methods and highlighted in Supplementary Table 3). These limits were determined prior to data analysis based on total sequencing output. |
| Replication | Replication in this study was evaluated via the inclusion of multiple samples containing the same known virus. For evaluating within-host variants (Fig. 3d), we used multiple sequencing replicates and saw high concordance. For assessing sensitivity in a dilution series, we used two replicates (Fig. 3a) and saw high concordance. |
| Randomization | No randomization was performed. Randomization was not applicable as we do not make statistical claims based on this data. |
| Blinding | Investigators were not blinded to the identities of samples containing known viruses. However, the 15 sample pools for which microbial content was uncharacterized prior to testing serve as a form of blinded evaluation since no pathogen in these samples were known to the investigator. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
| --- | --- |
| □ | Unique biological materials |
| □ | Antibodies |
| □ | Eukaryotic cell lines |
| □ | Palaeontology |
| □ | Animals and other organisms |
| ● | Human research participants |

Methods

| n/a | Involved in the study |
| --- | --- |
| □ | ChiP-seq |
| □ | Flow cytometry |
| ● | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

Unique materials are not available from commercial sources nor from the authors. All materials included in this study were collected for research purposes and included in this study with full ethical approvals. Materials are limited in volume and subject
## Human research participants

Policy information about [studies involving human research participants](#).

| Population characteristics | Population characteristics are not relevant as only viral genetic material detected within human-derived samples was analysed in this study. |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------|
| Recruitment                | Samples from human subjects included in this study were obtained from numerous studies that had been evaluated and approved by the relevant Institutional Review Boards (IRBs) or Ethics Committees. All studies recruited individuals presenting at respective recruitment locations based on study-specific inclusion and exclusion criteria. There are no relevant biases as the study focuses on viral infections present in these human-derived samples. |