Supplementary Materials

Parameter setting for assembly programs. *SOAPdenovo* (v1.05) is used in our experiments. For viral genome assembly, increasing the length of $k$mer used by *SOAPdenovo* will significantly increase the specificity, but its ability to capture diversity in the data will decrease. Since it is unclear which k value will yield the best performance, we tested three different values 23, 51, 99, respectively for each dataset. The results are comparable regardless of the values we are using, hence, in the comparison, we reported the result for $k = 23$.

The version 39605 of the Arachne package was used when running *AV*454. An additional component was created (available on request) to facilitate Arachne processing of paired Illumina reads with a parameter "*cov*=250", indicating a downsampling of reads such that an average of 250x genome coverage is achieved. *AV*454 module was run with the option "PIPELINE=paired" to handle Illumina paired reads, and the genome size is set as "GSIZE=10000". Other parameters were left to default settings.

The default parameter settings are used for all samples when running *VICUNA*.

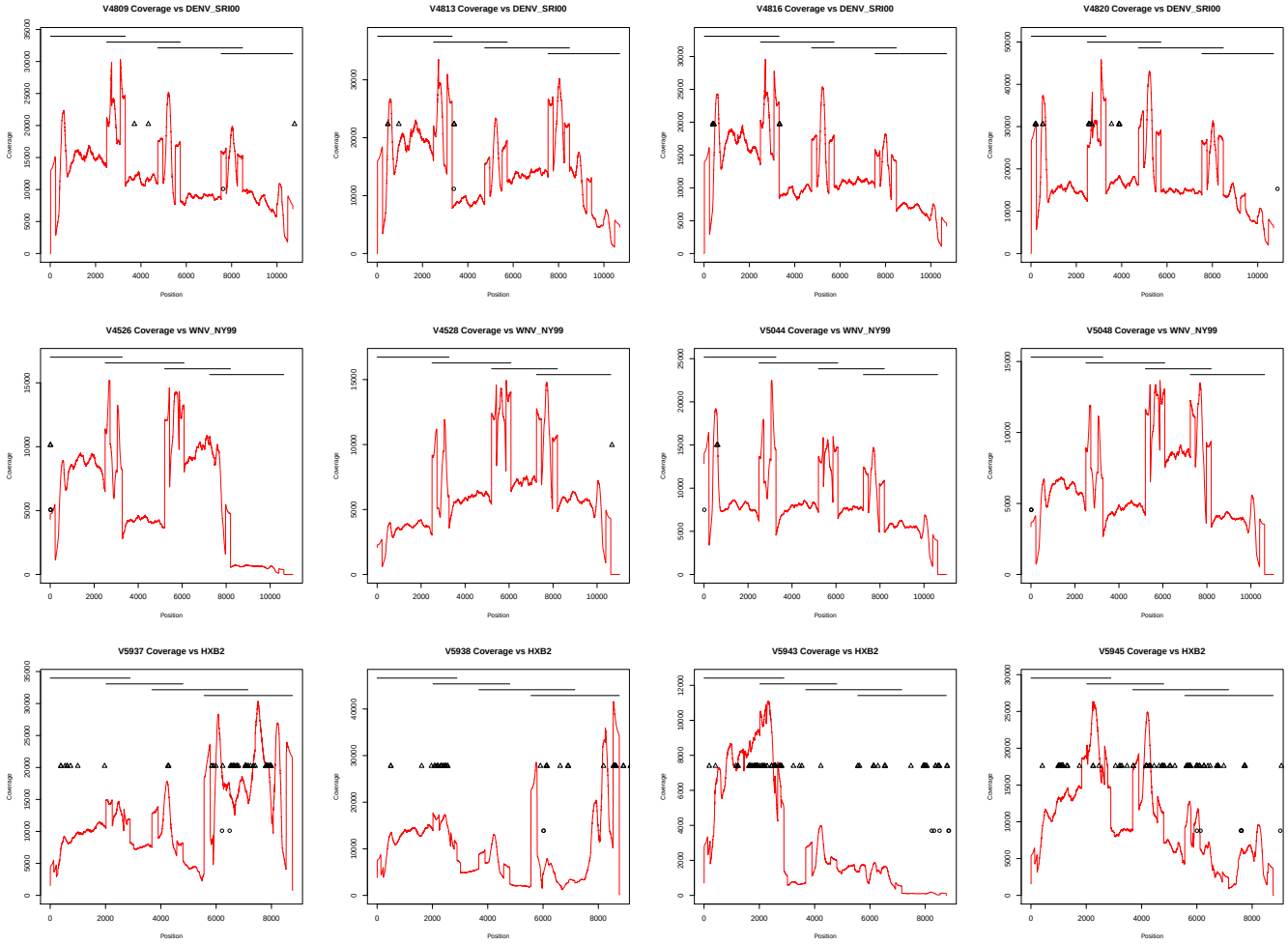
Filter creation for target-alike reads. We illustrated our method by creating a filter for HIV1B. The same method can be applied to other viral genomes. 1057 available full length HIV1B genomes were obtained from the LANL database (`http://www.hiv.lanl.gov/`), representing a wide range of genome diversity. These sequences were directly aligned using MUSCLE [1], resulting in a multiple sequence alignment (MSA) of length 21679. This is over the twice the length of the standard HIV genome size (∼10kbp). 17 sequences that create single large insertions in the alignment were removed, since they were likely misassembled. The remaining sequences were re-aligned, resulting in an alignment with a more reasonable length of 14232. We can further remove spurious genomes and re-align the remaining ones until satisfactory. The final alignment serves as the filter. Note that to create MSA for a large number of sequences is compute intensive, and is less accurate as the number of sequences increases. However, we do not require the filter to be free of mis-assembled sequences, nor do we require the filter to include all previously assembled genomes.

Profiling. The MSA filter renders each genome equivalent in length by introducing gaps in the alignment. We divide the MSA into bins, each specified by a 2-tuple $b_i = \langle s_i, e_i \rangle$, where s_i (e_i) denotes the start (end) position of the i^{th} ($0 \leq i < n_b$, n_b is a user specified parameter) bin on the MSA. To calculate b_i, first identify the longest genome G, and assign $|G|/n_b$ bases to each bin. Then $b_i = \langle |G| * i/n_b, |G| * (i+1)/n_b \rangle$ for $0 \leq i \leq n_b - 1$ if G contains no gaps. Otherwise, b_i is adjusted to include gaps. b_i determines the subsequence of each genome that belongs to the i^{th} bin, where the k-spectrum is then calculated. To account for the case that a read may overlap with two adjacent bins, we include any $k$mer that overlaps with position s_i in the k-spectrum of b_i. In addition, low frequency $k$mers are removed from consideration.

Procedure for assigning read r to bins. 1) For every $k$mer x in r^k, assign it to the i^{th} bin if its k-spectrum contains a d-neighbor of x. Note, x can be assigned to multiple bins. 2) Consider all $k$mers in r^k that were assigned to the i^{th} bin, if the total number of positions covered by these $k$mers in r divided by $|r|$ is above a given threshold t, the i^{th} bin is held as a candidate for r to be assigned. To account for the case when r spans two bins, we consider $k$mers in r^k that were assigned to adjacent bins. 3) Consider the paired reads (r, r'): assign both concurrently to bins that obey the maximum distance constraint of the paired read library size. Otherwise, we use a more stringent threshold t' ($> t$) to assign them individually.

References

1. Edgar, R. C. Muscle: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**(5), 1792–7 (2004).

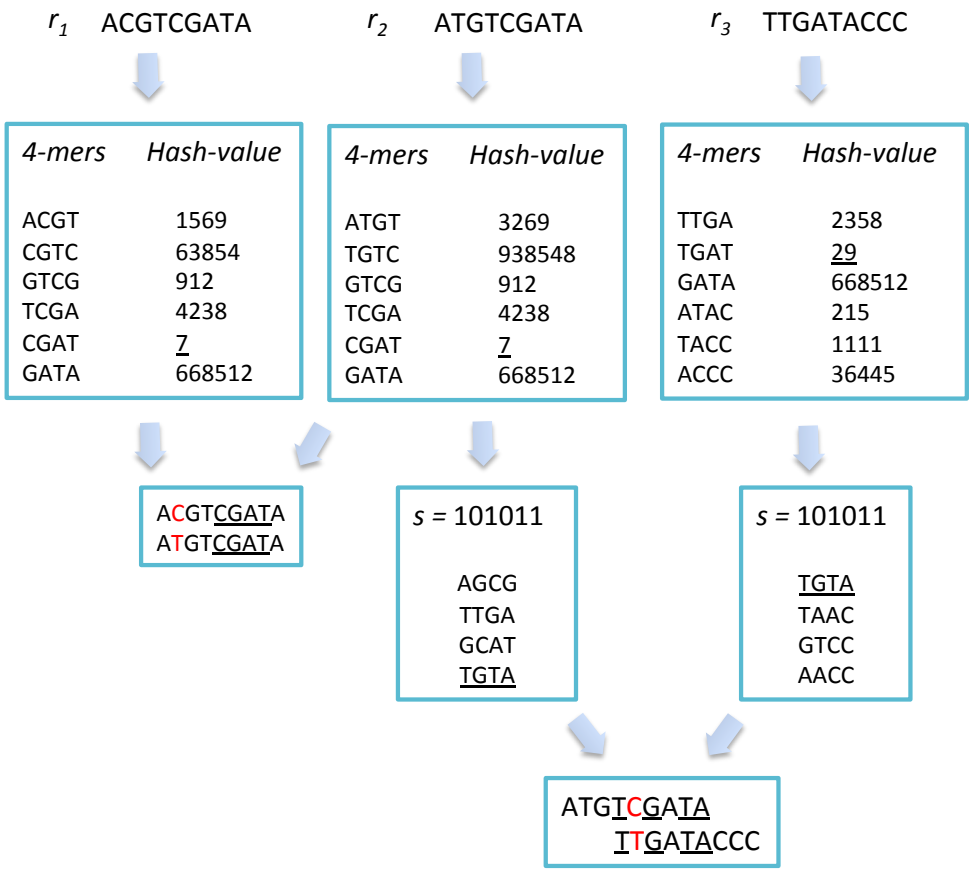


Supplementary Figure 1: Fold sequence coverage across the target region of Dengue, WNV, and HIV full length genomes. Alignments are to standard references (see Methods). Four horizontal lines on each figure represent amplicons used for generating the corresponding data set. Triangles and circles denote the non-dominant variant calls by *AV*454 and *VICUNA*, respectively, with respect to the reference genomic position.

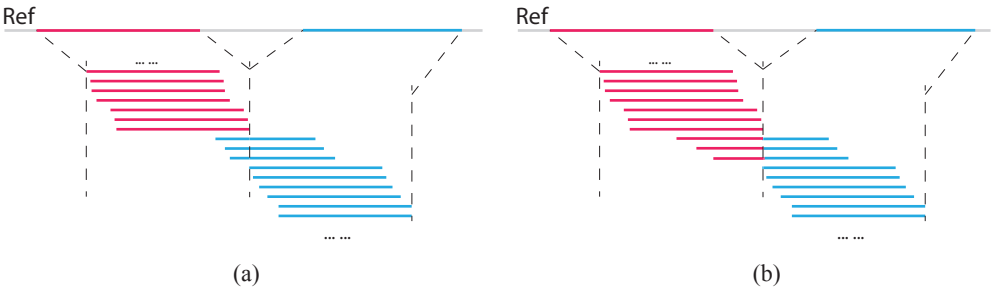
Supplementary Table 1: Datasets from Clinical WNV, DENV, and HIV Samples.

Virus	V#	NCBI SRA & *VICUNA* assembly accession	Number of reads	% Reads aligning to standard reference	% Target region covered	Average coverage	(%) Divergence between reference and sample*
WNV	V4526	XXXX	305,162	95.28	100.00	6262.0	0.214
	V4528	XXXX	322,134	95.01	100.00	6527.9	0.165
	V5044	XXXX	434,800	95.11	100.00	8744.9	0.116
	V5048	XXXX	316,880	95.04	100.00	6429.7	0.252
DENV	V4809	XXXX	645,516	93.93	100.00	12975.8	5.770
	V4813	XXXX	768,698	93.29	100.00	15456.8	5.751
	V4816	XXXX	641,024	93.77	100.00	12951.8	5.760
	V4820	XXXX	952,954	91.78	100.00	18733.4	5.829
HIV	V5937	XXXX	568,380	87.77	100.00	12451.0	6.978
	V5938	XXXX	453,648	90.52	100.00	9990.7	6.062
	V5943	XXXX	134,894	92.60	100.00	3210.9	6.410
	V5945	XXXX	440,260	89.98	100.00	10115.1	6.283

*This is calculated in the same way as non-dominant call rate.



Supplementary Figure 2: An example of contig construction. The k-spectrum ($k = 4$) of reads r_1, r_2, and r_3 are computed and hashed to an integral space. r_1 and r_2 share a common min hash value of 7, and hence can be clustered and aligned. When we further use a gapped seed, 101011, where a '0' denotes an ignored position, to generate gapped-4-mers of r_2 and r_3, a common 4-mer "TGTA" can be identified, leading to the clustering of r_2 and r_3. In both cases, the 4-mers that lead to the clustering are underlined. We can see both techniques tolerate base differences (colored red) that may due to sequencing error or true variation. Note that the illustration of min hash technique in this example is a simplified version compared to the one used in our algorithm.



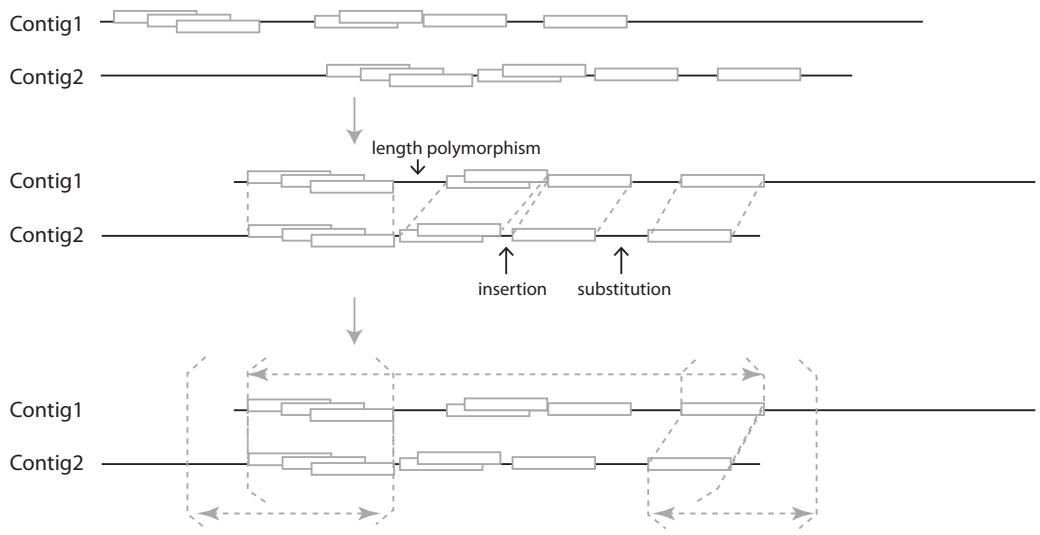
Supplementary Figure 3: Chimeric contig. Each read is represented as a short line, assigned with the same color as the fragment of the reference genome from which the read is sampled. Cause of chimeric contig due to (a) local homology among reads, or (b) chimeric reads, colored both red and blue, consisting of fragments from disjoint locations on the reference.

Supplementary Table 2: Large deletions in Clinical Samples.

V#	Virus	RefStart	RefEnd	RegionStart	RegionEnd	Observed in 454 data?
V4809	DENV	3655	5341	NS2A	NS3	no
V4820	DENV	5270	8233	NS3	NS5	no
		390	2713	Caps	NS1	no
		6236	7817	NS3	NS5	no
		651	3000	Memb	NS1	no
V5937	HIV	6085	7420	Env	Env	yes
		6309	6848	Env	Env	yes
		6302	7348	Env	Env	yes
V5938	HIV	5768	7546	Env	Env	yes
		6441	7977	Env	Env	yes
		5863	8247	Env	Env	yes
		2468	4084	Pol	Pol	no
V5943	HIV	2510	4402	Pol	Vif	yes
V5945	HIV	6060	7498	Env	Env	yes
		6362	7158	Env	Env	yes
		4496	6316	Vif	Env	yes
		2627	4546	Pol	Vif	yes

Supplementary Table 3: *VICUNA* Assembly Results for 454 Clinical Samples.

Virus	V#	# Input reads	# Output contigs (≥ 350bp)	% Target region covered	# Contigs used for reference guided merging	% Target region covered by the longest contig	% Reads aligning to consensus	Non-dominant call rate (%)
WNV	V4954	40,942	9	100	1	100	92.55	0
DENV	V4639	18,254	2	100	1	100	95.00	0
HIV	V4139	28,963	1	100	1	100	98.53	0



Supplementary Figure 4: Alignment of two contigs 1 and 2 that are represented as long lines. Common $k$mers between them are denoted as rectangles (top panel), these $k$mers are extended to form maximal common substrings (middle panel), which are forced to be aligned to each other. Inter-alignment regions may be resulted from length polymorphisms and sequencing errors (insertion, deletion and substitutions), which are further aligned using Needleman-Wunsch algorithm (bottom panel).

Supplementary Algorithm 1 Contig construction via min hash and spaced-seed.

Require: $R = \{r_1, r_2, \ldots, r_n\}$, spaced-seed s

For each read $r_i \in R$, generate two min hash values, respectively, for its forward and reverse complementary strands.
Cluster reads that share common min hash values to form initial contigs
$\mathbf{D} \leftarrow \emptyset$
for each $r_i \in R$ **do**
  $\mathcal{S}_i \leftarrow \emptyset$
  for $j = 0 \rightarrow |r_i| - |s| + 1$ **do**
    $x \leftarrow$ apply s to $r_i[j, j + |s| - 1]$
    $\mathcal{S}_i = \mathcal{S}_i \cup \{\langle x, (i, j)\rangle\}$
  end for
  if $\exists S \in \mathcal{S}_i$ such that $S.key = S'.key$, where $S' \in \mathbf{D}$ **then**
    Let r' denote the read, where $S'.key$ belongs
    Identify the two contigs C_i (may not exist) and C', where $r_i \in C_i$ and $r' \in C'$
    if neither C_i nor C' contains both r_i and r' **then**
      Add r_i to C'
    end if
  else
    $\mathbf{D} = \mathbf{D} \cup \{\mathcal{S}_i\}$
    Create a singleton contig that contains only r_i
  end if
end for

Supplementary Algorithm 2 Contig clustering via common reads.

Generate a 2-tuple $\langle id(r), id(C)\rangle$ for each read r that is contained by contig C and at least by one other contig. The results are stored in $\mathbf{M_{rc}}$
while $\mathbf{M_{rc}}$ is not empty **do**
  Sort contigs by the number of reads they contain in a decreasing order
  Flag all contigs as unprocessed
  while $\exists$ some unprocessed contig **do**
    Identify the first one in the list, let it be C
    Identify neighbors of C using $\mathbf{M_{rc}}$.
    Merge C with its neighbors and flag all contigs involved as processed
    Update $\mathbf{M_{rc}}$ by reassigning reads to new contigs when applicable
  end while
end while

Supplementary Algorithm 3 Contig validation.

Require: an input contig C, parameters max_d, max_{rt}, min_{ol}

Initialize contig list $\mathbf{C}^{\dagger}$ to be empty
repeat
  $C_{cur} \leftarrow C$
  Generate consensus for C_{cur}
  Initialize contig $C_{rem} \leftarrow \emptyset$
  repeat
    for each read r in C **do**
      Measure the distance d between r and $\mathcal{C}$
      if $d > max_d$ **then**
        $C_{cur} = C_{cur} \setminus \{r\}$ and update consensus
        $C_{rem} \leftarrow C_{rem} \cup \{r\}$
      end if
    end for
  until no change was applied to C
  Add C_{cur} to $\mathbf{C}$
  $C_{cur} \leftarrow C_{rem}$
until $C_{cur} = \emptyset$
for each contig $C \in \mathbf{C}$ **do**
  Generate the layout $(r_1, r_2, \ldots, r_{|C|})$ of C
  Calculate $\frac{n_b}{n_a}$ for each read in the layout
  Split C at r_i when either the overlap between r_i and r_{i-1} is $< min_{ol}$ or $\frac{n_b}{n_a} > max_{rt}$ for r_{i-1}
  Replace C with the resulting contigs if split occurred
end for

$^{\dagger}\mathbf{C}$ stores the resulting list of contigs.

Supplementary Algorithm 4 Contig extension.

Require: an input vector of contigs $\mathbf{C}$.

Sort $\mathbf{C}$ in an order of decreasing length
Generate a 2-tuple $\langle id(r), id(C) \rangle$ for each read r contained in contig C. The results are stored in $\mathbf{M_{rc}}$
while existing more contigs to be processed **do**
  Select target contig $C_l \leftarrow$ the first element of $\mathbf{C}$
  Get neighbors $\mathbf{N}$ of C_l via $\mathbf{M_{rc}}$
  Sort $\mathbf{N}$ in an increasing order of the number of paired-reads shared with C_l
  Compute delegates for C_l and each contig in $\mathbf{N}$
  while $\mathbf{N}$ is not empty **do**
    $C_r \leftarrow$ the last element of $\mathbf{N}$
    Compare delegate dg_l of C_l with dg_r (algorithm 5)
    if a significant prefix-suffix alignment is identified **then**
      Merge contig C_l to C_r & update dg_r
      Update $\mathbf{N}$ to include neighbors of C_r & calculate delegates for newly included contigs
      Update $\mathbf{M_{rc}}$
    end if
    Remove the last element from $\mathbf{N}$
  end while
end while

Supplementary Algorithm 5 Alignment of two sequences s_0 and s_1.

Require: parameters k, min_{ol}, min_s, max_d, max_{oh}

Identify every common $k$mer x between s_0 and s_1, and record x along with its start positions (p_s^0, p_s^1) as a 2-tuple $\langle x, (p_s^0, p_s^1)\rangle$ in array $\mathbf{A}$
Sort $\mathbf{A}$ in an increasing order with respect to p_s^0
Flag each entry of $\mathbf{A}$ as unprocessed.
for $i = 0 \rightarrow |\mathbf{A}| - 1$ **do**
  if $\mathbf{A}[i]$, the i^{th} element of $\mathbf{A}$, is flagged as unprocessed **then**
    $a \leftarrow \mathbf{A}[i]$
    Add $\langle p_s^0, p_e^0, p_s^1, p_e^1\rangle$ to $\mathbf{V}^\dagger$, where p_j^i is the start ($j = s$) or end ($j = e$) positions of $a.key$ on s_i ($i = 0, 1$)
    for $j = i \rightarrow |\mathbf{A}| - 1$ **do**
      $b \leftarrow \mathbf{A}[j]$
      if $b.key$ starts within $a.key$ **then**
        Update $\mathbf{V}$ if the two $k$mers can be joined to be a common substring of s_0 and s_1
        Flag $b.key$ as processed
      else if $b.key$ starts after $a.key$ but within max_d **then**
        Add the coordinates of $b.key$ to $\mathbf{V}$
        $a \leftarrow b$
      else
        Generate prefix-suffix alignment between s_0 and s_1 relying on $\mathbf{V}$
        If a valid alignment can be identified, accept this alignment and exit
      end if
    end for
  end if
end for

$^\dagger\mathbf{V}$, initially empty, is an array of 4-tuples: $\langle i_0, i_1, i_2, i_3\rangle$, where $s_0[i_0, i_1] = s_1[i_2, i_3]$