Supplementary Notes

1. Supplementary Note 1: Distance metric for clustering algorithm

Assume there are 2 Poisson distributions P_1 and P_2 with density functions

$$p_1(x, \lambda_1) = \frac{e^{-\lambda_1}\lambda_1^x}{\Gamma(x+1)}$$

and

$$p_2(x, \lambda_2) = \frac{e^{-\lambda_2}\lambda_2^x}{\Gamma(x+1)}$$

The Kullback-Leibner divergence from P_2 to P_1 is defined as:

$$D_{KL}(P_1||P_2) = \int_{-\infty}^{\infty} p_1(x) \log \frac{p_1(x)}{p_2(x)} dx$$

or in other words, it is the expectation of the logarithmic difference between the probabilities P_1 and P_2, where the expectation is taken with regard to P_1.

The log ratio of the density functions is

$$\log \frac{p_1(x)}{p_2(x)} = x \log \frac{\lambda_1}{\lambda_2} + \lambda_2 - \lambda_1$$

take expectation of this expression with regard to P_1 with mean λ_1 we have

$$D_{KL}(P_1||P_2) = \lambda_1 \log \frac{\lambda_1}{\lambda_2} + \lambda_2 - \lambda_1$$

The metric we used is the distance defined as

$$D(P_1, P_2) = \frac{D_{KL}(P_1||P_2) + D_{KL}(P_2||P_1)}{2} = \frac{1}{2}(\lambda_1 - \lambda_2)(\log \lambda_1 - \log \lambda_2)$$

2. Supplementary Note 2: Coverage re-estimation

The re-estimation is basically carried out by following two steps.

1. From nodes coverage, estimate edges' value by quadratic unconstrained optimization of the least-square function:

$$\frac{1}{2}\sum_i l_i((\sum e_i^+ - c_i)^2 + (\sum e_i^- - c_i)^2$$

where l_i and c_i is the length and coverage of a node i in the graph;

$\sum e_i^+$ and $\sum e_i^-$ indicates sum of the values of incoming and outgoing edges from i respectively. The above function and be rewritten as:

$$f(x) = \frac{1}{2}x^T Q x + b^T x + r$$

and then being minimized by using gradient-based method.

2. Update nodes' coverage based on itself and its neighbor edges' measures.

The calibration is iterative until no further improvements are made or a threshold loop count is reached.

3. Supplementary Note 3: Contigs binning

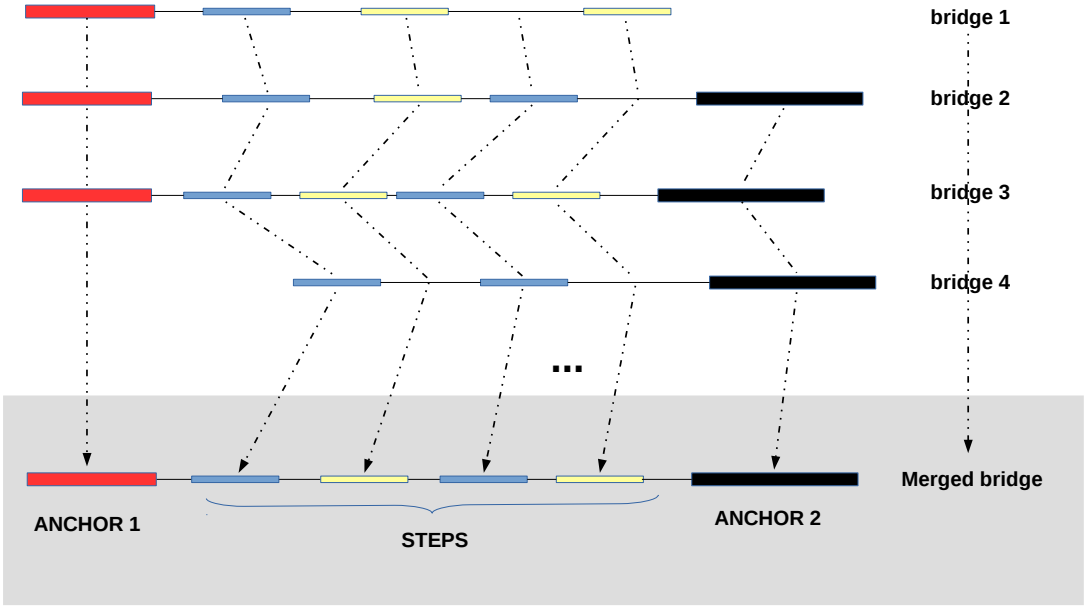
We implemented a simple binning algorithm based on the clustered contigs, the graph topology as well as statistics calculated by length and coverage of each contigs. Additional binning step is taken place in real-time using the long reads as well. In this context, the repetition or uniquess of ambigous nodes are determined with sufficient supporting evidence from the long-spanning reads. In general, the algorithm works well for isolate or simple mock metagenomics, but not robust enough for complicated community.

On the other hand, if external binning algorithm is employed, the resulting output must be converted into a text file that specifies the corresponding bin of every contigs, just like output from MetaBAT. By that, each line of the file would be: $<$ `contig_ID` $>$ $<$ `bin_ID` $>$ where `bin_ID` $= 0$ indicates unspecified binned contigs.

4. Supplementary Note 4: Constructing bridges in real-time

We implemented a data structure to represent the connection between two unique contigs in the assembly graph, namely *bridge*. A bridge has 4 levels of completion, ranging from 1 to 4 depends on how close to finish it is. When a new bridge is built with only one anchor (unique ending), it has level 1. If both anchor nodes are identified, its completion level is 2. Only then, a path finding algorithm is applied to find candidate paths of this bridge. When potential paths (more than 1) are listed successfully, the completion level is increased to 3. Finally, when the only path solution is determined out of all candidates, the bridge is assigned as complete-built with level 4.

At early stages (level 1 or 2), a bridge is constructed progressively by alignments from long reads that spanning its corresponding anchor(s). In an example from Figure 1, bridges from a certain anchor (highlighted in red) are created by extracting appropriate alignments from incoming long reads to the contigs. Each of the steps therefore is assigned a weighing score based on its alignment quality. Due to the error rate of long reads, there should be deviations in terms of steps found and spans measured between these bridges, eventhough they represent the same connection. A continuous merging phase, as shown in the figure, takes advantage of a pairwise Needleman-Wunsch dynamic programming to generate a consensus list based on weight and position of each of every stepping nodes. The spans are calibrated accordingly by averaging out the distances. On the other hand, the score of the merged steps are accumulated over time as well. Whenever a consensus bridge is anchored by 2 unique contigs at both ends and hosting a list of steps with sufficient coverage, it is ready for a path finding in the next step.



Supplementary Figure 1: Bridge merging progressively in real-time. Sequencing long reads induce alignments to the contigs where new bridges are created respectively. Bridges sharing same anchors can be merged together to form the ultimate, more comprehensive bridge (with more step nodes and better approximation of spans between them).

5. Supplementary Note 5: Path finding algorithm

However, due to false alignments from shorter contigs to the long reads, not all of the reported step nodes are neccessary to be appeared in the ultimate path resolved by the bridge. In most cases, the accumulated score of each step indicates its likelihood to be the true component of the final solution. For that reason, a strategy similar to binary searching is employed to find a path across 2 anchors of a bridge as shown in Algorithm 2.

Before that, we define Algorithm 1 to demonstrates the path finding algorithm for two nodes given their estimated distance. In which, function `shortestTree`$(\overrightarrow{vertex}, distance) : (V, Z) \rightarrow V^n$ from line 3 of the algorithm's pseudo code builds a shortest tree rooted from $\overrightarrow{v}$, following its direction until a distance of approximately d (with a tolerance regarding nanopore read error rate) is reached. This task is implemented based on Dijkstra algorithm. This tree is used on line 4 and in function $includedIn()$ on line 19 to filter out any node or edge with ending nodes that do not belong to the tree.

Basically, the algorithm keeps track of a stack that contains sets of candidate edges to discover. During the traversal, a variable d is updated as an estimation for the distance to the target. A hit is reported if the target node is reached with a reasonable distance *i.e.* close to zero, within a given tolerance (line 21). A threshold for the traversing depth is set (150) to ignore too complicated and time-consuming path searching.

Note that the $length()$ functions for node and edge are totally different. While the former returns the length of the sequence represented by the node, *i.e.* contig from short-read assembly, the latter is usually negative because an edge

Algorithm 1: Pseudo-code for finding paths connecting 2 nodes given their estimated distance.

Data: Assembly graph $G\{V, E\}$
Input: Pair of bidirected nodes $\overrightarrow{v_1}, \overrightarrow{v_2}$ and estimated distance d between them
Output: Set of candidate paths connecting $\overrightarrow{v_1}$ to $\overrightarrow{v_2}$ with reasonable distances compared to d

Function `DFS`($\overrightarrow{v_1}, \overrightarrow{v_2}$, d)**:**
  P:=new List()
  M:=`shortestTree`($\overrightarrow{v_2}, d$) `// build shortest tree from` $\overrightarrow{v_2}$ `with range` d
  if $M.contain(\overrightarrow{v_1})$ **then**
    S:=new $Stack()$ `// stack of sets of edges to traverse`
    $edgesSet$:=$getEdges(\overrightarrow{v_1})$ `// get all bidirected edges going from` $\overrightarrow{v_1}$
    $S.push(edgesSet)$
    p:=new $Path(\overrightarrow{v_1})$ `// init a path that has` $\overrightarrow{v_1}$ `as root`
    while *true* **do**
      $edgesSet$:=$S.peek()$
      if $edgesSet.isEmpty()$ **then**
        if $p.size() \leq 1$ **then**
          break `// stop the loop when there is no more edge to discover`
        $S.pop()$
        d+=$p.peekNode.length() + p.popEdge().length()$
      else
        $curEdge$:= $edgesSet.remove()$
        $\overrightarrow{v}$:=$curEdge.getOpposite(p.peekNode())$
        $S.push(getEdges(\overrightarrow{v}).includedIn(M))$
        $p.add(curEdge)$
        if *reach* $\overrightarrow{v_2}$ *with reasonable* d **then**
          $P.add(p)$
        d-=$\overrightarrow{v}.length() + curEdge.length()$
  return P

Algorithm 2: Recursive binary bridging to connect 2 anchor nodes.

Data: Assembly graph $G\{V, E\}$
Input: Brigde $B : \{\overrightarrow{v_0}, ...\overrightarrow{v_k}, ..., \overrightarrow{v_n}\}$ with $\overrightarrow{v_0}$ and $\overrightarrow{v_n}$ are two anchors, $\{\overrightarrow{v_k}\}, k = 1 \ldots (n-1)$ are steps inbetween
Output: Set of candidate paths connecting $\overrightarrow{v_0}$ to $\overrightarrow{v_2}$ that maximize the likelihood of the step list.

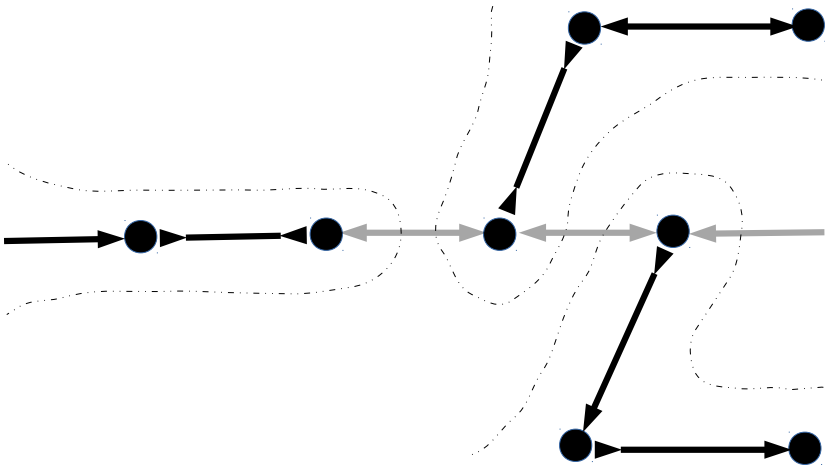
Function `BinaryBridging`(B)**:**
  `/* search for the contig with maximum score from the step list (two ends excluded) */`
  m:=$\text{argmax}_k(\overrightarrow{v_k}.score())$
  `/* if there is no step inbetween, run path finding algorithm above directly and return the result */`
  if $M.size() \equiv 2$ **then**
    return `DFS`($B.start(), B.end().B.distance()$)
  `/* divide the original bridge` B `into 2 bridges by` v_m`:` BL `and` BR `*/`
  BL:=$\{B.start(), ..., \overrightarrow{v_m}\}$
  BR:=$\{\overrightarrow{v_m}, ..., B.end()\}$
  `/* Return the join of running recursive function on two sub-bridges */`
  return `BinaryBridging`(BL) $\bowtie$ `BinaryBridging`(BR)

models a link between two nodes, which is normally an overlap (except for composite edges). For example, in a *k-mers* SPAdes assembly graph, the value of an edge is $-k+1$.

6. Supplementary Note 6: Output the results

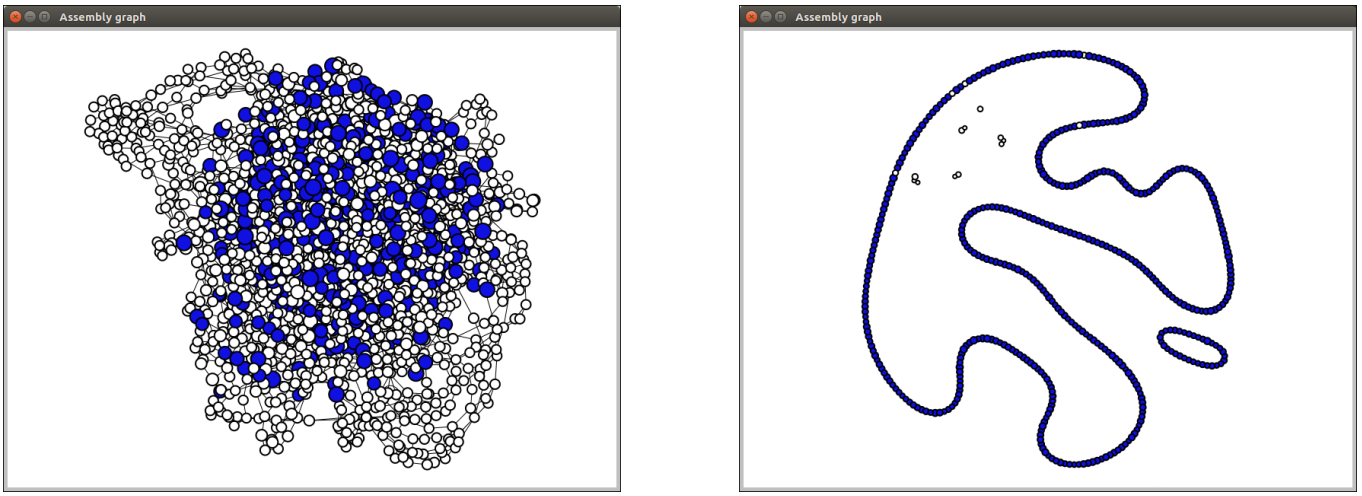
The resolved assembly graph can be reported as a whole (GFA) or sequences only (FASTA).

For the latter, longest-possible linear straight paths must be extracted from the graph for the final FASTA records. A path $p = \{v_0, e_1, v_1, \ldots, v_{k-1}, e_k, v_k\}$ of size k is considered as straight if and only if each of every edges along the path $e_i, \forall i = 1, \ldots, k$ is the only option to traverse from either v_{i-1} or v_i, giving the transition rule. To decompose the graph, the tool simply mask out all incoming/outgoing edges rooted from any node with in/out degree greater than 1 as demonstrated in Figure 2. These edges are defined as branching edges which stop straight paths from further extending.



Supplementary Figure 2: Example of graph decomposition into longest straight paths. Branching edges are masked out (shaded) leaving only straight paths (bold colored) to report. There would be 3 contigs extracted by traversing along the straight paths here.

The decomposed graph is only used to report the contigs that can be extracted from an assembly graph at certain time point. For that reason, the branching edges are only masked but not removed from the original graph as they would be used for further bridging.



(a) Initial graph

(b) Resolved graph

Supplementary Figure 3: Assembly graph of *Shigella dysenteriae* Sd197 synthetic data being resolved by `npGraph` and displayed on the GUI Graph View. The `SPAdes` assembly graph contains 2186 nodes and 3061 edges, after the assembly shows 2 circular paths representing the chromosome and one plasmid.

Supplementary Figure 3 presents an example of the results before and after graph resolving process in the GUI. The result graph, after cleaning, would only report the significant connected components that represents the final contigs. Smaller fragments, even unfinished but with high remaining coverage, are also presented as potential candidates for further downstream analysis. Further annotation utility can be implemented in the future better monitoring the features of interests as in `npScarf`.

Supplementary Figures and Tables

Supplementary Table 1: Benchmarking `npGraph` against `npScarf` versions, `hybridSPAdes` and `Unicycler` hybrid assembler with the synthetic data set.

Method	Assembly size (bp)	#Contigs	N50 (bp)	Mis-assemblies	Mismatch (per 100Kbp)	Indel (per 100Kbp)
random sequences no repeats						
npScarf	4110000	3	4000000	0	0.00	0.00
npScarf_wag	4109516	3	4000000	0	0.00	0.00
npGraph-bwa	4110000	3	4000000	0	0.00	0.00
npGraph-mm2	4110000	3	4000000	0	0.00	0.00
hybridSPAdes	4110231	3	4000077	0	0.00	0.07
Unicycler	4110000	3	4000000	0	0.00	0.00
random sequences some repeats						
npScarf	4110940	3	4002715	0	0.00	0.66
npScarf_wag	4437094	7	2795129	0	0.00	1.07
npGraph-bwa	4110000	3	4000000	0	0.00	0.00
npGraph-mm2	4110000	3	4000000	0	0.00	0.00
hybridSPAdes	4107566	3	3999364	0	0.02	0.02
Unicycler	4110000	3	4000000	0	0.00	0.00
random sequences many repeats						
npScarf	4316934	8	3963485	24	11.55	34.54
npScarf_wag	5215965	16	1515563	37	0.32	7.22
npGraph-bwa	4110000	3	4000000	0	0.32	0.15
npGraph-mm2	4110000	3	4000000	0	0.32	0.15
hybridSPAdes	4108190	3	3999621	0	0.68	0.15
Unicycler	4110000	3	4000000	0	0.32	0.15
Acinetobacter A1						
npScarf	3912299	3	3870269	4	4.74	13.02
npScarf_wag	3945166	3	3906368	1	7.00	14.02
npGraph-bwa	3918374	2	3909643	0	16.34	0.61
npGraph-mm2	3885898	2	3877167	1	18.05	1.03
hybridSPAdes	3929948	53	3353679	0	35.48	3.22
Unicycler	3917745	2	3909014	0	2.50	0.13
Acinetobacter AB30						
npScarf	4512464	7	4304628	35	57.95	72.87
npScarf_wag	5315235	13	1267710	136	73.55	8.15
npGraph-bwa	4335342	2	4148952	1	16.93	1.45
npGraph-mm2	4335790	1	4335790	0	6.97	0.25
hybridSPAdes	4337369	3	2701005	0	12.80	1.39
Unicycler	4333041	1	4333041	1	6.42	0.53
E. coli K12 MG1655						
npScarf	4649902	2	4641702	4	14.94	34.35
npScarf_wag	4687952	3	4641732	0	6.55	1.96
npGraph-bwa	4641743	1	4641743	0	4.50	0.43
npGraph-mm2	4641820	1	4641820	0	3.88	0.26
hybridSPAdes	4644555	1	4641036	0	0.62	0.09
Unicycler	4641650	1	4641650	0	3.43	0.26
E. coli O25b H4-ST131						
npScarf	5245913	3	5095571	7	7.05	18.81
npScarf_wag	5292700	3	3469617	9	9.03	1.55
npGraph-bwa	5237821	7	4049493	1	3.38	0.31
npGraph-mm2	5249799	3	5110117	0	2.40	0.15
hybridSPAdes	5252762	8	4258948	2	5.43	0.57
Unicycler	5249442	3	5109760	0	4.02	0.27

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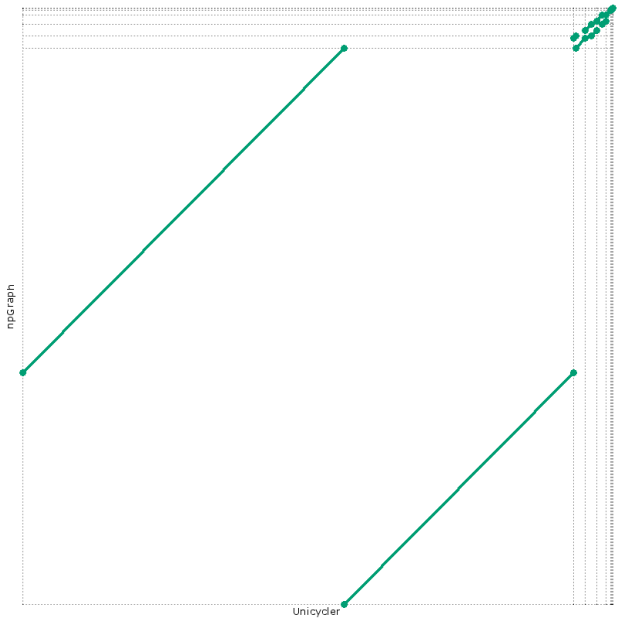
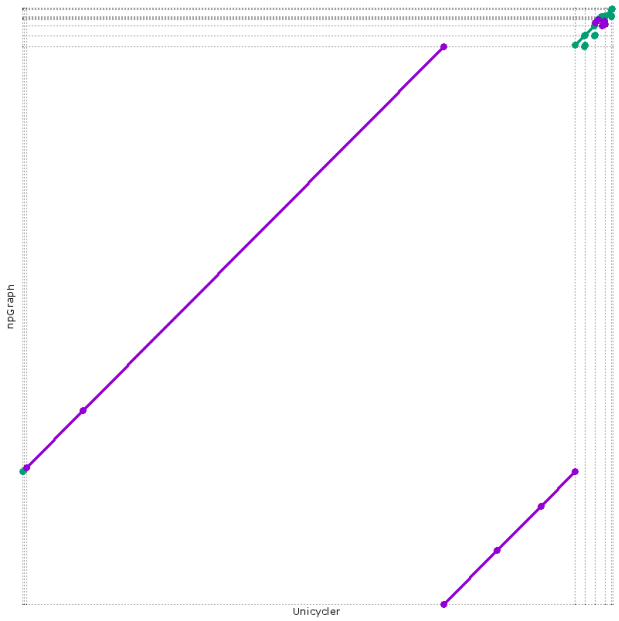
Supplementary Table 1 – *Continued from previous page*

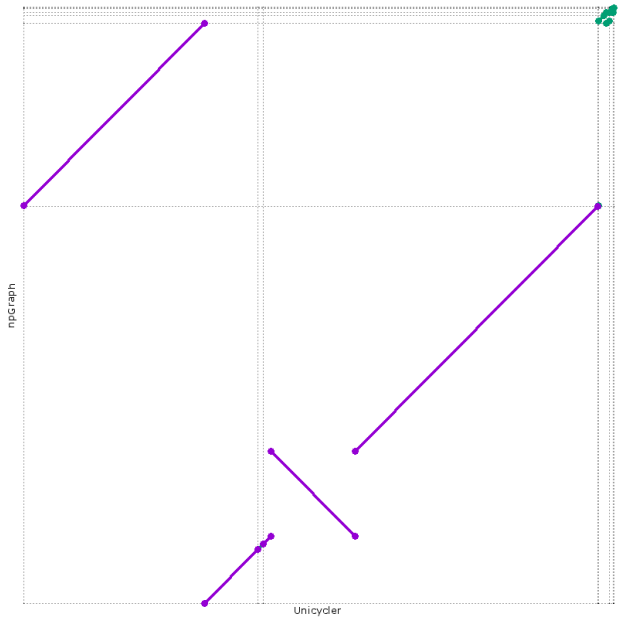
Method	Assembly size (bp)	#Contigs	N50 (bp)	Mis-assemblies	Mismatch (per 100Kbp)	Indel (per 100Kbp)
Klebsiella 30660 NJST258 1						
npScarf	5559772	7	5259053	4	17.18	13.48
npScarf_wag	5613780	7	5268535	6	1.59	1.92
npGraph-bwa	5534843	8	5263229	0	3.15	0.76
npGraph-mm2	5534878	8	5263264	0	2.75	0.74
hybridSPAdes	5545668	8	5545668	2	4.95	0.09
Unicycler	5537860	9	5263196	0	1.34	0.51
Klebsiella MGH 78578						
npScarf	5729304	5	5316429	12	14.90	20.27
npScarf_wag	5754443	5	3026286	16	8.17	2.56
npGraph-bwa	5695801	7	5311745	1	12.65	1.25
npGraph-mm2	5696302	6	5315267	0	6.06	0.44
hybridSPAdes	5706470	11	5315273	1	3.82	0.67
Unicycler	5694231	14	5315096	0	5.38	0.21
Klebsiella NTUH-K2044						
npScarf	5471696	2	5249198	6	4.82	8.55
npScarf_wag	5530559	3	5249369	2	2.25	1.35
npGraph-bwa	5472629	2	5248476	0	2.52	0.31
npGraph-mm2	5472655	2	5248503	0	1.21	0.26
hybridSPAdes	5473572	2	5248894	0	0.44	0.15
Unicycler	5472697	2	5248545	0	2.41	0.35
Mycobacterium tuberculosis H37Rv						
npScarf	4498245	4	4402238	8	5.15	2.68
npScarf_wag	4506056	4	4410942	3	6.81	2.59
npGraph-bwa	4411406	1	4411406	0	1.88	0.43
npGraph-mm2	4411532	1	4411532	0	0.68	0.00
hybridSPAdes	4413942	1	4410519	0	0.75	0.11
Unicycler	4411538	1	4411538	0	2.22	0.34
Saccharomyces cerevisiae S288c						
npScarf	11986800	24	796769	51	62.12	21.46
npScarf_wag	12003203	21	917017	21	69.14	5.47
npGraph-bwa	11921736	40	913090	3	38.04	1.94
npGraph-mm2	11920984	38	913198	2	20.66	0.95
hybridSPAdes	12027533	45	770543	5	32.58	1.94
Unicycler	11847655	72	909114	0	21.81	1.04
Shigella dysenteriae Sd197						
npScarf	4586075	173	36560	55	120.14	111.59
npScarf_wag	5462918	92	98791	105	147.48	79.28
npGraph-bwa	4564058	6	4369264	5	80.64	11.16
npGraph-mm2	4558920	6	4364264	7	75.51	10.98
hybridSPAdes	4519131	23	821249	96	9.57	1.42
Unicycler	4560901	3	4369231	0	11.88	1.05
Shigella sonnei 53G						
npScarf	6441461	20	1953896	82	164.02	219.52
npScarf_wag	-	-	-	-	-	-
npGraph-bwa	5211544	4	4988532	0	14.53	0.31
npGraph-mm2	5211527	4	4988519	0	8.56	0.17
hybridSPAdes	5223875	8	2195455	2	41.92	0.06
Unicycler	5220517	5	4988548	0	7.39	0.52
Streptococcus suis BM407						
npScarf	2183951	3	2146594	0	21.51	9.17
npScarf_wag	2289880	3	1493189	1	3.17	1.96
npGraph-bwa	2154623	6	2131479	1	5.25	0.28
npGraph-mm2	2149876	6	2146774	0	1.44	0.28
hybridSPAdes	2172703	2	2146237	0	6.82	0.09
Unicycler	2170829	2	2146250	0	2.67	0.32



(a) *Citrobacter freundii* CAV1374

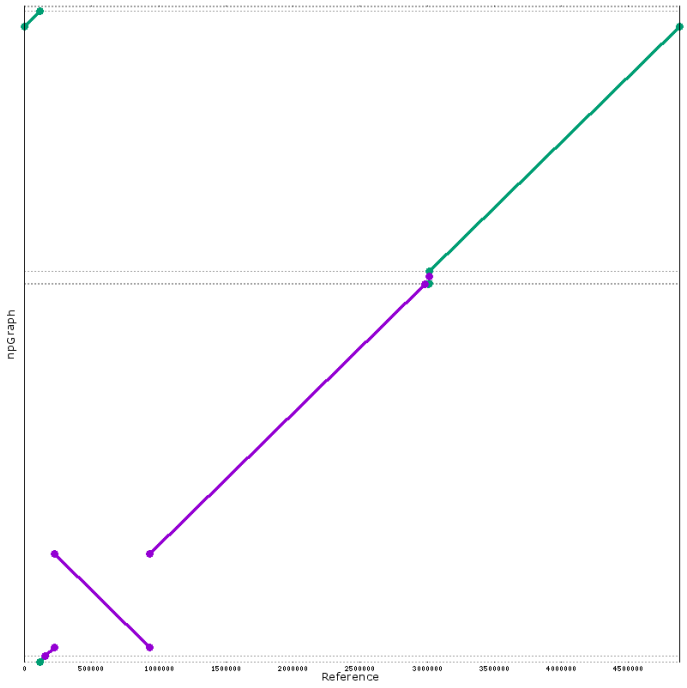


(b) *Klebsieall oxytoca* CAV1015

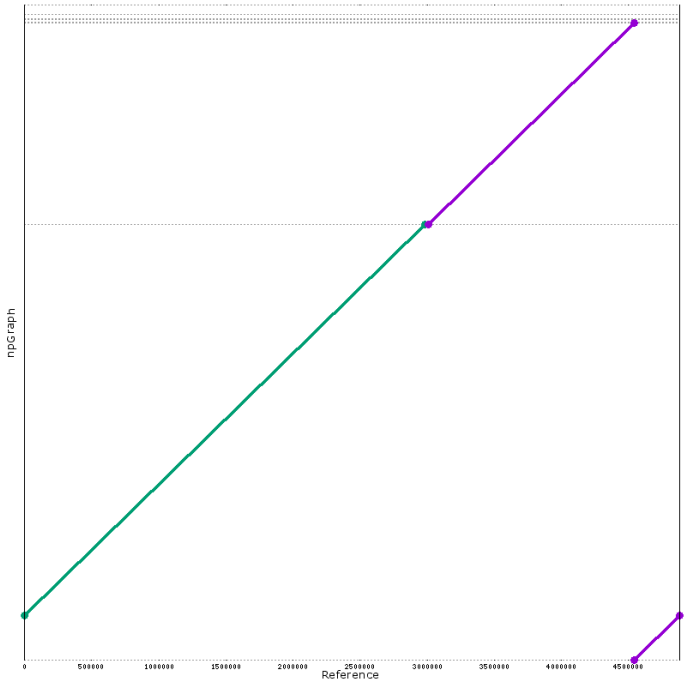


(c) *Enterobacter cloacae* CAV1411

Supplementary Figure 1: Dotplot generated by MUMmer for assembly results of `Unicycler` versus `npGraph`. Structural agreements between two methods were found in (a) *C.freundii* and (b) *K.oxytoca* assembly contigs. On the other hand, for (c) *E.cloacae* sample, there was a disagreement detected between 2 largest contigs given by two assembly algorithms. This case is investigated more thoroughly by using a reference from a same bacterial strain in Figure 2.

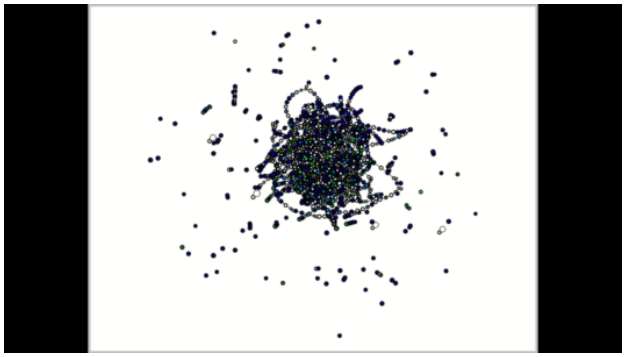


(a) *E. cloacae* `Unicycler` assembly versus reference genome

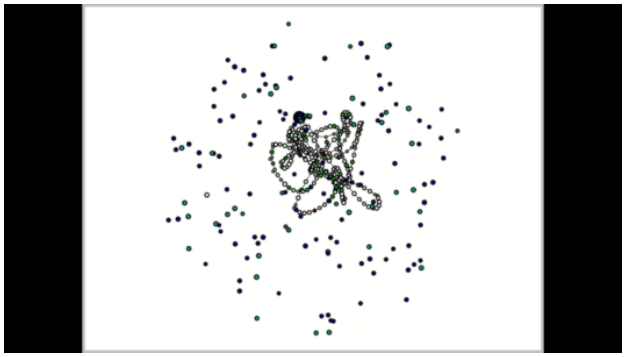


(b) *E. cloacae* `npGraph` assembly versus reference genome

Supplementary Figure 2: Alignments of an *Enterobacter cloacae* reference genome to assembly sequences generated by (a) `Unicycler` and (b) `npGraph`. While the former presents a structural variant, the latter is virtually an 1-to-1 mapping.



(a) ZymoEven assembly graph from `metaSPAdes`



(b) ZymoLog assembly graph from `metaSPAdes`

Supplementary Figure 3