Supplementary Figures

for

“Improved metagenomic analysis with Kraken 2”

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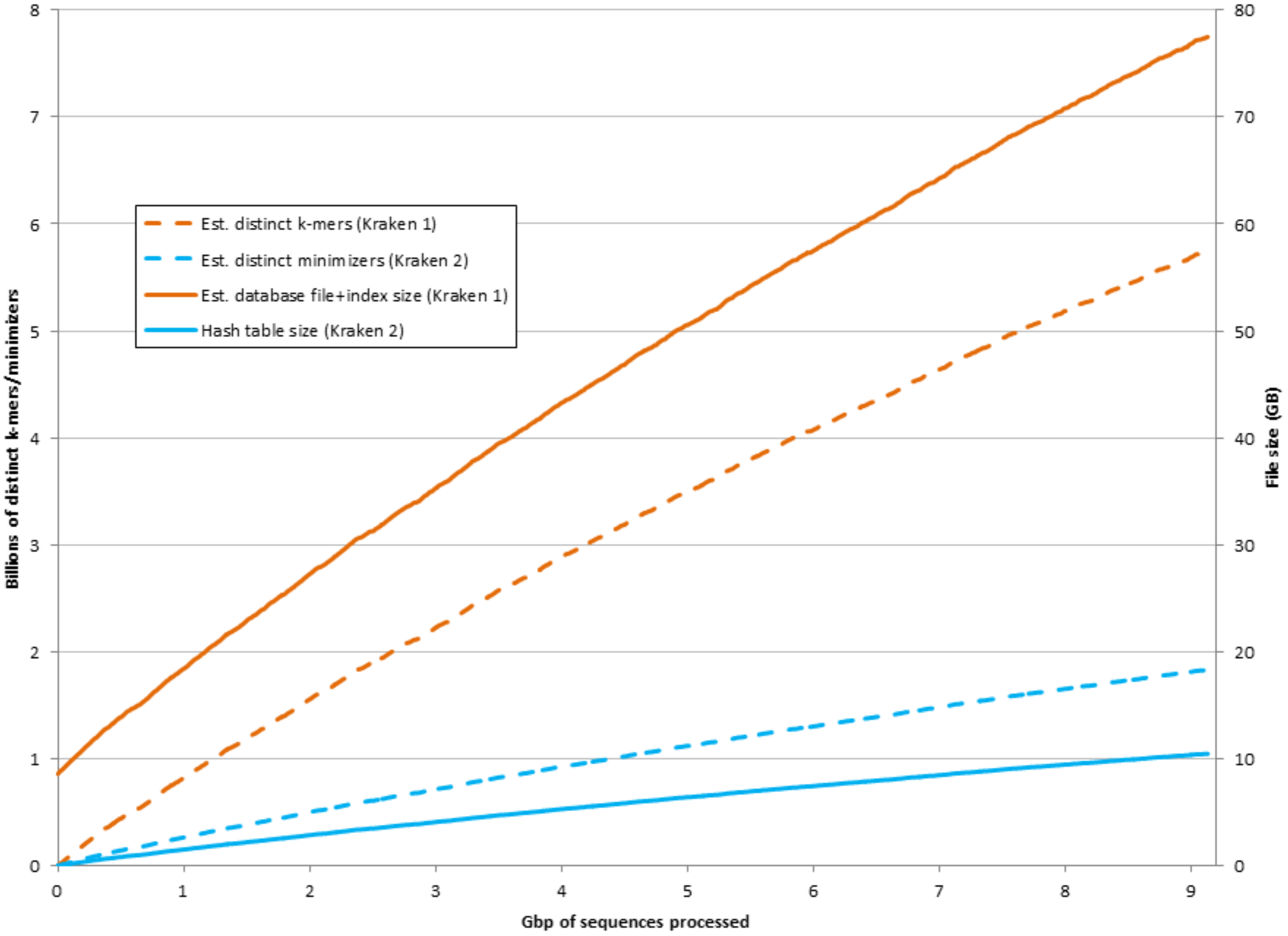
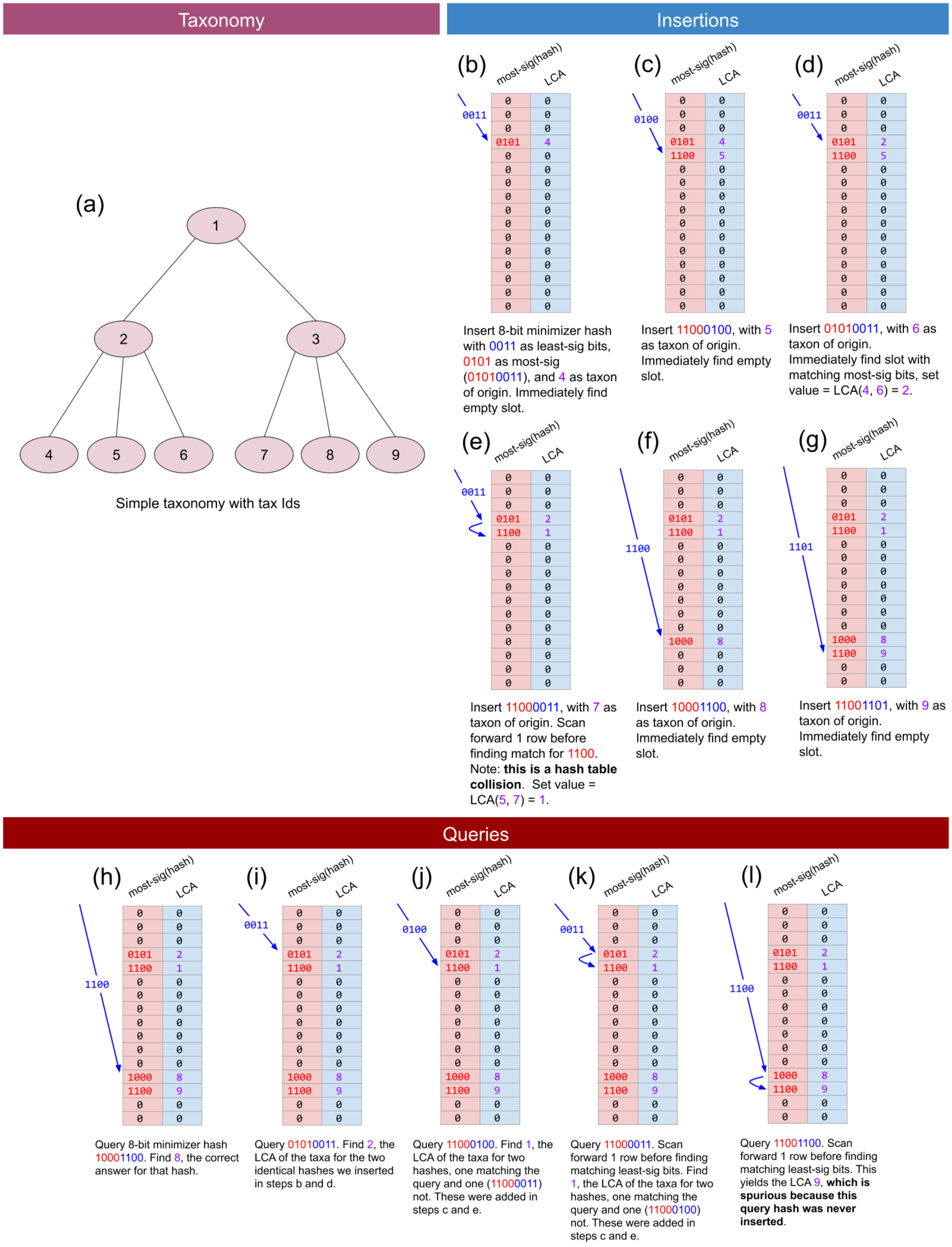


Supplementary Figure S1. Estimation of database sizes for Kraken 1 and Kraken 2 as sequences are added to the reference set. Over a shuffled set of the nucleotide sequences in our strain exclusion reference, we calculated progressively larger estimates of the number of distinct *k*-mers and minimizers. Because database sizes for Kraken 1 and Kraken 2 are functions of the numbers of distinct *k*-mers and minimizers, respectively, we also calculated the estimated database sizes for Kraken 1 and Kraken 2.



Simple taxonomy with tax Ids

(b)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	4
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0

Insert 8-bit minimizer hash with 0011 as least-sig bits, 0101 as most-sig (01010011), and 4 as taxon of origin. Immediately find empty slot.

(c)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	4
1100	5
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0

Insert 11000100, with 5 as taxon of origin. Immediately find empty slot.

(d)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	5
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0

Insert 01010011, with 6 as taxon of origin. Immediately find slot with matching most-sig bits, set value = LCA(4, 6) = 2.

(e)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0

Insert 11000011, with 7 as taxon of origin. Scan forward 1 row before finding match for 1100. Note: **this is a hash table collision**. Set value = LCA(5, 7) = 1.

(f)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1000	8
0	0
0	0
0	0

Insert 10001100, with 8 as taxon of origin. Immediately find empty slot.

(g)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1000	8
1100	9
0	0
0	0

Insert 11001101, with 9 as taxon of origin. Immediately find empty slot.

(h)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1000	8
1100	9
0	0
0	0

Query 8-bit minimizer hash 10001100. Find 8, the correct answer for that hash.

(i)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1000	8
1100	9
0	0
0	0

Query 01010011. Find 2, the LCA of the taxa for the two identical hashes we inserted in steps b and d.

(j)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1000	8
1100	9
0	0
0	0

Query 11000100. Find 1, the LCA of the taxa for two hashes, one matching the query and one (11000011) not. These were added in steps c and e.

(k)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1000	8
1100	9
0	0
0	0

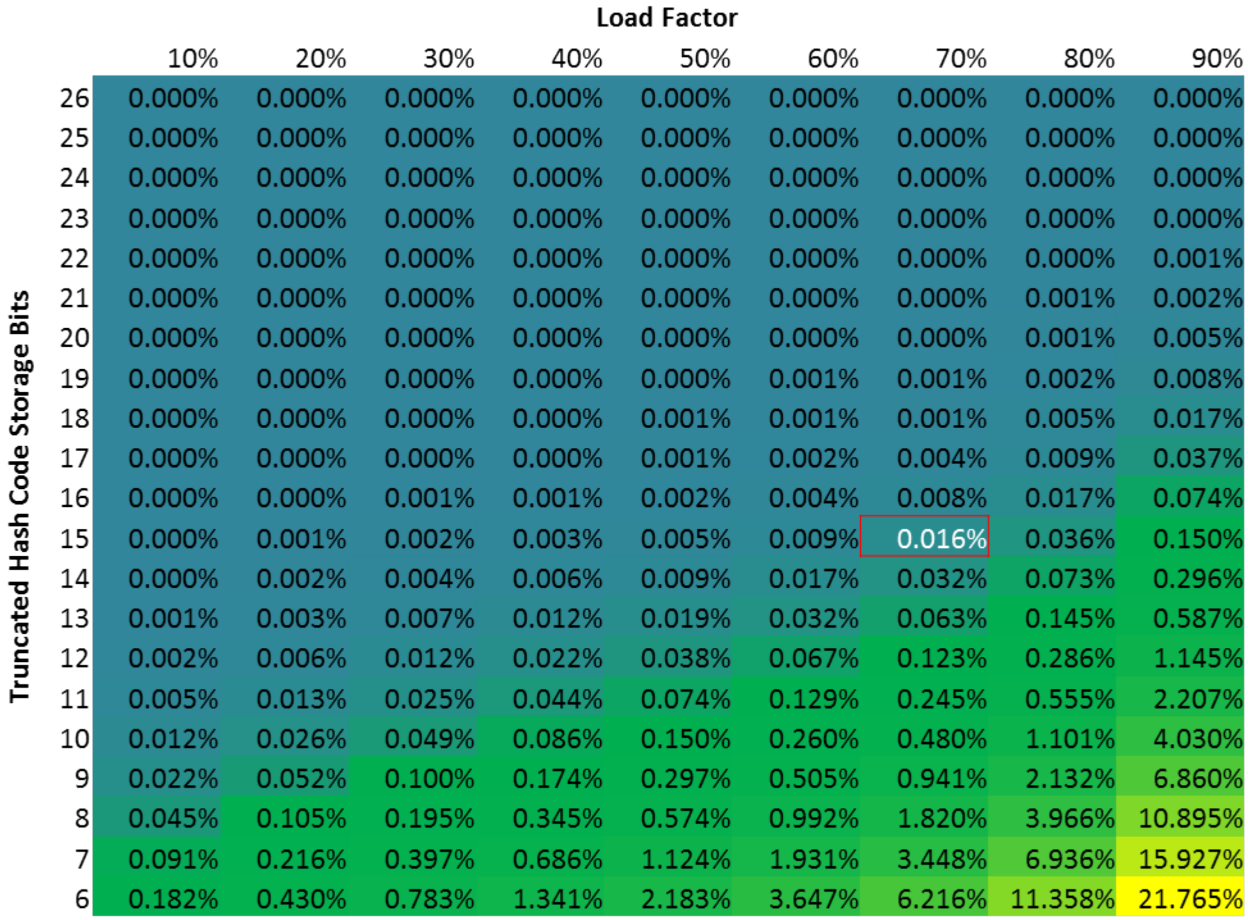
Query 11000011. Scan forward 1 row before finding matching least-sig bits. Find 1, the LCA of the taxa for two hashes, one matching the query and one (11000100) not. These were added in steps c and e.

(l)

most-sig(hash)	LCA
0	0
0	0
0	0
0101	2
1100	1
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1000	8
1100	9
0	0
0	0

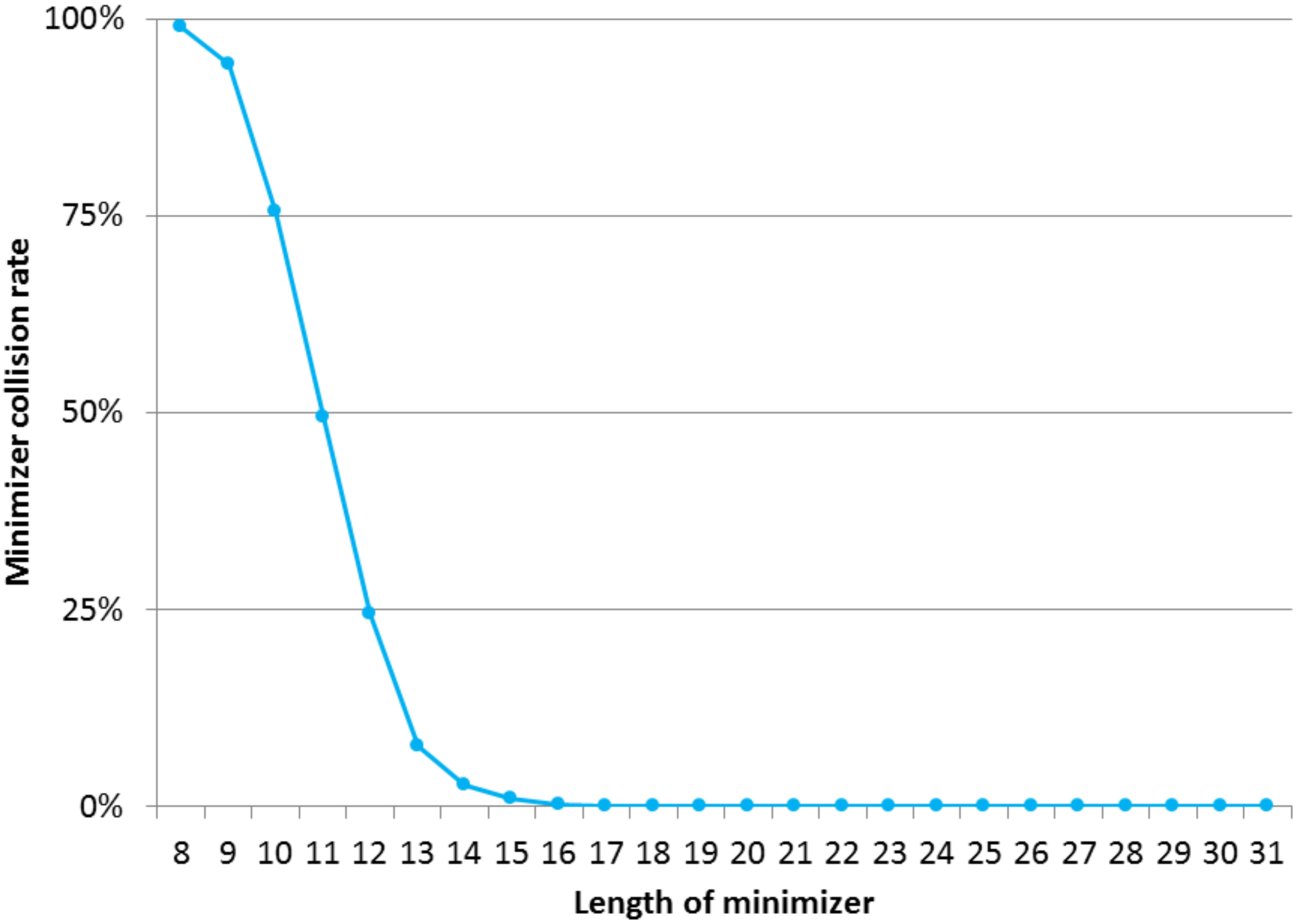
Query 11001100. Scan forward 1 row before finding matching least-sig bits. This yields the LCA 9, **which is spurious because this query hash was never inserted**.

Supplementary Figure S2. Examples of compact hash table usage with Kraken 2. (a) An example of Kraken 2's reduced internal representation of the taxonomy with sequential ID numbering via breadth-first search. **(b)-(g)** Sequential examples of Kraken 2's insertion of minimizer/LCA pairs into a compact hash table. **(h)-(l)** Sequential examples of Kraken 2's querying of a compact hash table with a minimizer. In practice, the table and the lengths of the keys are greater than shown here. Also, since the actual table does not generally have a number of rows equal to a power of 2, we use the modulo operation to obtain the search offset (blue) rather than simply taking bits from the hash value.



Truncated Hash Code Storage Bits	Load Factor								
	10%	20%	30%	40%	50%	60%	70%	80%	90%
26	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
25	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
24	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
23	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
22	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%
21	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.002%
20	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.005%
19	0.000%	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.002%	0.008%
18	0.000%	0.000%	0.000%	0.000%	0.001%	0.001%	0.001%	0.005%	0.017%
17	0.000%	0.000%	0.000%	0.000%	0.001%	0.002%	0.004%	0.009%	0.037%
16	0.000%	0.000%	0.001%	0.001%	0.002%	0.004%	0.008%	0.017%	0.074%
15	0.000%	0.001%	0.002%	0.003%	0.005%	0.009%	0.016%	0.036%	0.150%
14	0.000%	0.002%	0.004%	0.006%	0.009%	0.017%	0.032%	0.073%	0.296%
13	0.001%	0.003%	0.007%	0.012%	0.019%	0.032%	0.063%	0.145%	0.587%
12	0.002%	0.006%	0.012%	0.022%	0.038%	0.067%	0.123%	0.286%	1.145%
11	0.005%	0.013%	0.025%	0.044%	0.074%	0.129%	0.245%	0.555%	2.207%
10	0.012%	0.026%	0.049%	0.086%	0.150%	0.260%	0.480%	1.101%	4.030%
9	0.022%	0.052%	0.100%	0.174%	0.297%	0.505%	0.941%	2.132%	6.860%
8	0.045%	0.105%	0.195%	0.345%	0.574%	0.992%	1.820%	3.966%	10.895%
7	0.091%	0.216%	0.397%	0.686%	1.124%	1.931%	3.448%	6.936%	15.927%
6	0.182%	0.430%	0.783%	1.341%	2.183%	3.647%	6.216%	11.358%	21.765%

Supplementary Figure S3. Evaluation of compact hash table error rates as a function of two variables. The error rates of Kraken 2’s compact hash table are a function of the load factor and the number of bits used to store the truncated hash code. Error rates were determined by inserting the minimizers from *P. aeruginosa* UCBPP PA14 and querying with minimizers from randomly generated sequence. Kraken 2’s default database settings used 15 bits to store the truncated hash code and a load factor of 70%, which is highlighted with a red border in the figure.



Supplementary Figure S4. Evaluation of minimizer collision rates as a function of minimizer length. We examined the rate at which minimizers of 35-mers from *P. aeruginosa* UCBPP PA14 would be found as minimizers of 35-mers in randomly generated sequence. Minimizer lengths ℓ were varied from 8 to 31, with no spaced minimizers used. This demonstrates the significantly lower collision rates that occur by use of the long (ℓ=31) minimizers in Kraken 2 versus use of shorter (ℓ <16) minimizers.