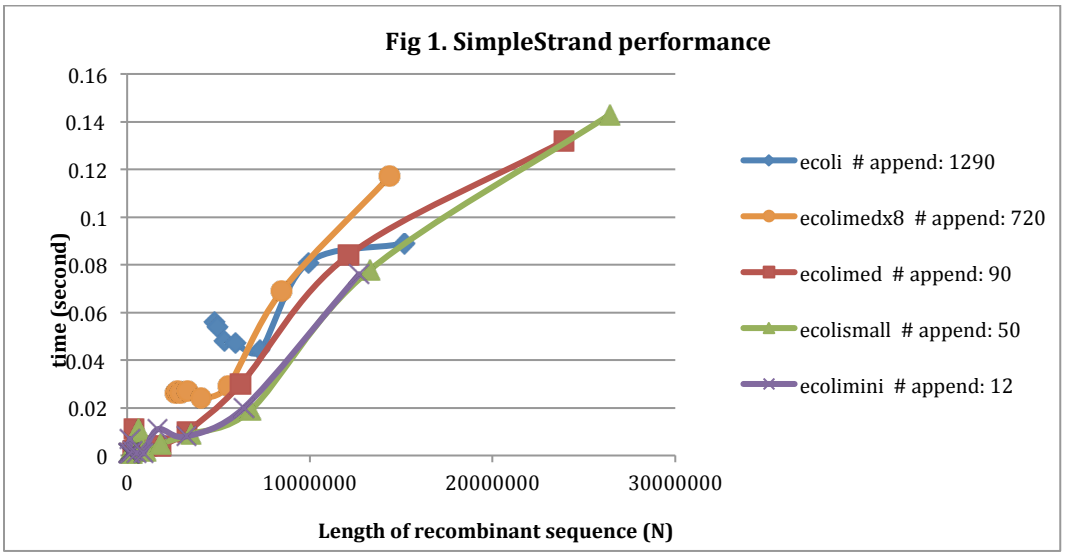
DNA Analysis

Yuanjie Jin & Junfei Liu

1) Using SimpleStrand to create the recombinant strand is an O(N) operation where N is the size of the resulting recombinant strand. As the length of splicee grows, the code takes longer to execute.

   Reasoning: In SimpleStrand, when it replaces the enzyme-recognized sequence with the splicee, the method of cutAndSplice calls append function. As in StringBuilder, such append method will copy the string to append and build on the original stored string. Therefore, the time this method takes correlates with the total length of all the strings to append, which is the length of the whole resulting recombinant strand.

   Data: Besides ecoli.dat and ecolimed.dat, we also generated new data files: ecolimedx8, which contains 8 copies of ecolimed; ecolismall, which is a small truncation of ecolimed; and ecolimini, which is an even smaller truncation of ecolimed. The specific output of running DNABenchMark on each data file is listed in Table 1 on next page. The performance of SimpleStrand is shown in **Fig 1** below.



1.1) For each data file, as the length of splicee doubles, its length of resulting recombinant strand also increases. As is shown in Fig 1, as the lengths of recombinant sequences increase, the code will take longer to execute. And the correlation between them is linear.
1.2) Comparing between different data files, although they have different append numbers, as their recombinant lengths get close, the time lengths they take to run the code are also similar.
1.3) Therefore, creating recombinant strand using SimpleStrand is an O(N) operation.

Table 1.

SimpleStrand generated recombinant of ecoli.dat			**dna length = 4,639,221**	**cutting at enzyme gaattc**
Class	splicee	recomb	time	
SimpleStrand:	256	4,800,471	0.056	# append calls = 1290
SimpleStrand:	512	4,965,591	0.054	# append calls = 1290
SimpleStrand:	1,024	5,295,831	0.048	# append calls = 1290
SimpleStrand:	2,048	5,956,311	0.047	# append calls = 1290
SimpleStrand:	4,096	7,277,271	0.044	# append calls = 1290
SimpleStrand:	8,192	9,919,191	0.081	# append calls = 1290
SimpleStrand:	16,384	15,203,031	0.089	# append calls = 1290
SimpleStrand generated recombinant of ecolimedx8.dat			**dna length = 2,561,280**	**cutting at enzyme gaattc**
Class	splicee	recomb	time	
SimpleStrand:	256	2,651,280	0.026	# append calls = 720
SimpleStrand:	512	2,743,440	0.027	# append calls = 720
SimpleStrand:	1,024	2,927,760	0.026	# append calls = 720
SimpleStrand:	2,048	3,296,400	0.027	# append calls = 720
SimpleStrand:	4,096	4,033,680	0.024	# append calls = 720
SimpleStrand:	8,192	5,508,240	0.029	# append calls = 720
SimpleStrand:	16,384	8,457,360	0.069	# append calls = 720
SimpleStrand:	32,768	14,355,600	0.117	# append calls = 720
SimpleStrand generated recombinant of ecolimed.dat			**dna length = 320,160**	**cutting at enzyme gaattc**
Class	splicee	recomb	time	
SimpleStrand:	256	331410	0.002	# append calls = 90
SimpleStrand:	512	342930	0.002	# append calls = 90
SimpleStrand:	1,024	365970	0.011	# append calls = 90
SimpleStrand:	2,048	412050	0.002	# append calls = 90
SimpleStrand:	4,096	504210	0.003	# append calls = 90
SimpleStrand:	8,192	688530	0.003	# append calls = 90
SimpleStrand:	16,384	1057170	0.004	# append calls = 90
SimpleStrand:	32,768	1794450	0.004	# append calls = 90
SimpleStrand:	65,536	3269010	0.01	# append calls = 90
SimpleStrand:	131,072	6218130	0.03	# append calls = 90
SimpleStrand:	262,144	12116370	0.084	# append calls = 90
SimpleStrand:	524,288	23912850	0.132	# append calls = 90
SimpleStrand generated recombinant of ecolismall.dat			**dna length = 200,040**	**cutting at enzyme gaattc**
Class	splicee	recomb	time	
SimpleStrand:	256	206,290	0.002	# append calls = 50
SimpleStrand:	512	212,690	0.001	# append calls = 50
SimpleStrand:	1,024	225,490	0.002	# append calls = 50
SimpleStrand:	2,048	251,090	0.001	# append calls = 50
SimpleStrand:	4,096	302,290	0.002	# append calls = 50
SimpleStrand:	8,192	404,690	0.002	# append calls = 50
SimpleStrand:	16,384	609,490	0.011	# append calls = 50
SimpleStrand:	32,768	1,019,090	0.002	# append calls = 50
SimpleStrand:	65,536	1,838,290	0.005	# append calls = 50
SimpleStrand:	131,072	3,476,690	0.009	# append calls = 50
SimpleStrand:	262,144	6,753,490	0.019	# append calls = 50
SimpleStrand:	524,288	13,307,090	0.078	# append calls = 50
SimpleStrand:	1,048,576	26,414,290	0.143	# append calls = 50
SimpleStrand generated recombinant of ecolimini.dat			**dna length = 100,020**	**cutting at enzyme gaattc**
Class	splicee	recomb	time	
SimpleStrand:	256	101,520	0.001	# append calls = 12
SimpleStrand:	512	103,056	0.001	# append calls = 12
SimpleStrand:	1,024	106,128	0.001	# append calls = 12
SimpleStrand:	2,048	112,272	0.001	# append calls = 12
SimpleStrand:	4,096	124,560	0.001	# append calls = 12
SimpleStrand:	8,192	149,136	0.007	# append calls = 12
SimpleStrand:	16,384	198,288	0.001	# append calls = 12
SimpleStrand:	32,768	296,592	0.002	# append calls = 12
SimpleStrand:	65,536	493,200	0.001	# append calls = 12
SimpleStrand:	131,072	886,416	0.001	# append calls = 12
SimpleStrand:	262,144	1,672,848	0.011	# append calls = 12
SimpleStrand:	524,288	3,245,712	0.008	# append calls = 12
SimpleStrand:	1,048,576	6,391,440	0.02	# append calls = 12
SimpleStrand:	2,097,152	12,682,896	0.076	# append calls = 12

2) Test SimpleStrand with multiple memory settings and report the power-of-two string that can be used without running out of memory with the input file ecoli.dat.

2.1) The Java runtime heap size is set using the command-line argument "-Xmx512M". As is shown in the table 2 below, the longest recombinant strand constructed using the largest possible power-of-two splicee (131,072) is 89,176,791. And this process takes around 0.464 second.

Table 2.			dna length = 4,639,221	cutting at enzyme gaattc
Class	splicee	recomb	time	
SimpleStrand:	256	4,800,471	0.056	# append calls = 1290
SimpleStrand:	512	4,965,591	0.053	# append calls = 1290
SimpleStrand:	1,024	5,295,831	0.047	# append calls = 1290
SimpleStrand:	2,048	5,956,311	0.048	# append calls = 1290
SimpleStrand:	4,096	7,277,271	0.043	# append calls = 1290
SimpleStrand:	8,192	9,919,191	0.092	# append calls = 1290
SimpleStrand:	16,384	15,203,031	0.097	# append calls = 1290
SimpleStrand:	32,768	25,770,711	0.154	# append calls = 1290
SimpleStrand:	65,536	46,906,071	0.239	# append calls = 1290
SimpleStrand:	131,072	89,176,791	0.464	# append calls = 1290

2.2) When the size of the heap available to the Java runtime is doubled (-Xmx1024M), the next power-of-two strand (262,144) is now supported. The time it takes to construct the current longest recombinant strand (173,718,231) is 0.938 second, which is roughly the doubled time length of the previous 0.464 second.

Table 3			dna length = 4,639,221	utting at enzyme gaattc
Class	splicee	recomb	time	
SimpleStrand:	256	4,800,471	0.056	# append calls = 1290
SimpleStrand:	512	4,965,591	0.055	# append calls = 1290
SimpleStrand:	1,024	5,295,831	0.051	# append calls = 1290
SimpleStrand:	2,048	5,956,311	0.044	# append calls = 1290
SimpleStrand:	4,096	7,277,271	0.044	# append calls = 1290
SimpleStrand:	8,192	9,919,191	0.051	# append calls = 1290
SimpleStrand:	16,384	15,203,031	0.101	# append calls = 1290
SimpleStrand:	32,768	25,770,711	0.113	# append calls = 1290
SimpleStrand:	65,536	46,906,071	0.236	# append calls = 1290
SimpleStrand:	131,072	89,176,791	0.37	# append calls = 1290
SimpleStrand:	262,144	173,718,231	0.938	# append calls = 1290

2.3) Further thoughts as for mathematical prove: Dna: the length of original DNA for recombination; $Splicee$: the length of the largest possible splicee given a set memory; $Append$: the number of appends; $enzyme$: the length of the enzyme site to be replaced.

Let $Dna' = Dna - enzyme * \frac{Append}{2}$

Because $$Dna' + Splicee * \frac{Append}{2} < \boldsymbol{Memory} < Dna' + (2 * Splicee) * \frac{Append}{2}$$

$$Dna' + (2 * Splicee) * \frac{Append}{2} < 2 * Dna' + 2 * Splicee * \frac{Append}{2} < 2 * \boldsymbol{Memory} < 2Dna' + 2 * 2 * Splicee * \frac{Append}{2}$$

Thus, theoretically, the doubling of memory will always be able to support the doubling of splicee length.

Also, when the original size of the DNA to be spliced is significantly smaller than Memory and could be neglected,

$$2 * \boldsymbol{Memory} < Dna' + 2 * 2 * Splicee * \frac{Append}{2}$$

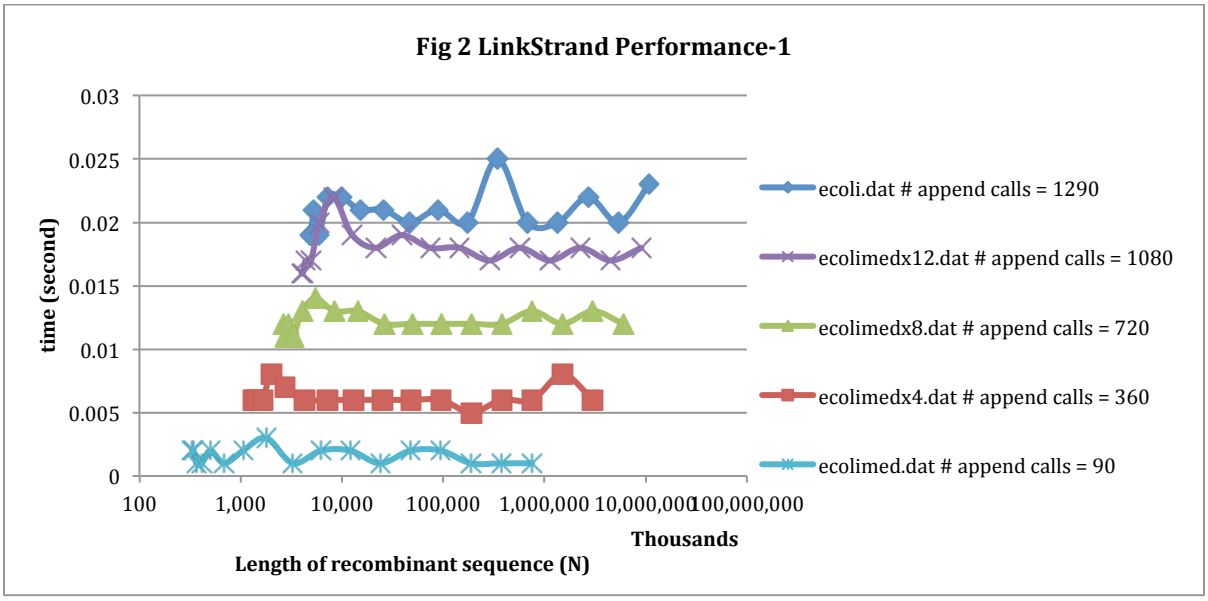
This means memory doubling will only be able to support one round of splicee doubling.

However, it also needed to be pointed out that the memory is not allocated solo for the storage of resulting recombinant strand, as other factors may affect.

3) Using LinkStrand to create the recombinant strand is an O(B) operation where B is the number of breaks/splits created by the restriction enzyme.

Reasoning: In LinkStrand, repeated nodes are created in a way that they contain pointers to the exactly same splicee string. This representation avoids recopying the splicee string over and over again. Therefore, the append method takes constant time to generate a new node regardless of the base-pair length of the splicee, i.e. it is an O(1) operation. As a result, the overall running time should be O(B), where B is the number of breaks in the original strand.

Data: Besides ecoli.dat and ecolimed.dat, we also used other data files: ecolimedx8, which contains 8 copies of ecolimed; ecolimedx4, which contains 4 copies of ecolimed; and ecolimedx12, which contains 12 copies of ecolimed. The specific output of running DNABenchMark on each data file is listed in **Table 4** on next page. The performance of LinkStrand is shown in **Fig 2** below.

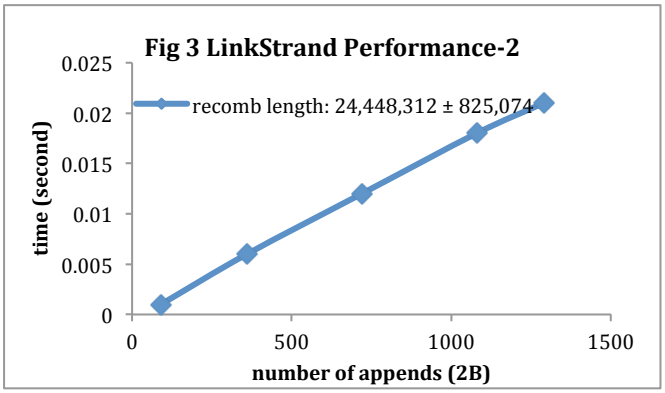


3.1) As is shown in **Fig2**, for each data file, as their resulting recombinant strands increase, their running time lengths roughly remain, with only small fluctuations.

3.2) Files with more append numbers take more time to run the code, showing correlation between time and append numbers. The append number is approximately twice the number of breaks, so here we use append number as a reference of B.

3.3) A recombinant length range from 21,533,400 to 26,152,080 is picked, and the time length vs. number of appends is plotted in **Fig 3.** The correlation is shown to be linear.

Data File	recomb	time	append
ecolimed	23,912,850	0.001	90
ecolimedx4	24,872,520	0.006	360
ecolimedx8	26,152,080	0.012	720
ecolimedx12	21,533,400	0.018	1080
ecoli	25,770,711	0.021	1290



3.4) Comparing the same data file run by SimpleStrand and LinkStrand, the latter is more efficient both in time and memory. For example, SimpleStrand generates a 15,203,031 base pair recombinant from ecoli.dat in 0.089 second, while LinkStrand can generate a 10,825,939,671 base pair recombinant from ecoli.dat in 0.023 second.

Table 4				
LinkStrand generated recombinant of ecolimed.dat dna length =320,160, cutting at enzyme gaattc				
Class	splicee	recomb	time	
LinkStrand:	256	331,410	0.002	# append calls = 90
LinkStrand:	512	342,930	0.002	# append calls = 90
LinkStrand:	1,024	365,970	0.001	# append calls = 90
LinkStrand:	2,048	412,050	0.001	# append calls = 90
LinkStrand:	4,096	504,210	0.002	# append calls = 90
LinkStrand:	8,192	688,530	0.001	# append calls = 90
LinkStrand:	16,384	1,057,170	0.002	# append calls = 90
LinkStrand:	32,768	1,794,450	0.003	# append calls = 90
LinkStrand:	65,536	3,269,010	0.001	# append calls = 90
LinkStrand:	131,072	6,218,130	0.002	# append calls = 90
LinkStrand:	262,144	12,116,370	0.002	# append calls = 90
LinkStrand:	524,288	23,912,850	0.001	# append calls = 90
LinkStrand:	1,048,576	47,505,810	0.002	# append calls = 90
LinkStrand:	2,097,152	94,691,730	0.002	# append calls = 90
LinkStrand:	4,194,304	189,063,570	0.001	# append calls = 90
LinkStrand:	8,388,608	377,807,250	0.001	# append calls = 90
LinkStrand:	16,777,216	755,294,610	0.001	# append calls = 90
LinkStrand generated recombinant of ecolimedx4.dat dna length = 1,280,640 , cutting at enzyme gaattc				
Class	splicee	recomb	time	
LinkStrand:	256	1,325,640	0.006	# append calls = 360
LinkStrand:	512	1,371,720	0.006	# append calls = 360
LinkStrand:	1,024	1,463,880	0.006	# append calls = 360
LinkStrand:	2,048	1,648,200	0.006	# append calls = 360
LinkStrand:	4,096	2,016,840	0.008	# append calls = 360
LinkStrand:	8,192	2,754,120	0.007	# append calls = 360
LinkStrand:	16,384	4,228,680	0.006	# append calls = 360
LinkStrand:	32,768	7,177,800	0.006	# append calls = 360
LinkStrand:	65,536	13,076,040	0.006	# append calls = 360
LinkStrand:	131,072	24,872,520	0.006	# append calls = 360
LinkStrand:	262,144	48,465,480	0.006	# append calls = 360
LinkStrand:	524,288	95,651,400	0.006	# append calls = 360
LinkStrand:	1,048,576	190,023,240	0.005	# append calls = 360
LinkStrand:	2,097,152	378,766,920	0.006	# append calls = 360
LinkStrand:	4,194,304	756,254,280	0.006	# append calls = 360
LinkStrand:	8,388,608	1,511,229,000	0.008	# append calls = 360
LinkStrand:	16,777,216	3,021,178,440	0.006	# append calls = 360
LinkStrand generated recombinant of ecolimedx8.dat; dna length = 2,561,280, cutting at enzyme gaattc				
Class	splicee	recomb	time	
LinkStrand:	256	2,651,280	0.012	# append calls = 720
LinkStrand:	512	2,743,440	0.011	# append calls = 720
LinkStrand:	1,024	2,927,760	0.012	# append calls = 720
LinkStrand:	2,048	3,296,400	0.011	# append calls = 720
LinkStrand:	4,096	4,033,680	0.013	# append calls = 720
LinkStrand:	8,192	5,508,240	0.014	# append calls = 720
LinkStrand:	16,384	8,457,360	0.013	# append calls = 720
LinkStrand:	32,768	14,355,600	0.013	# append calls = 720
LinkStrand:	65,536	26,152,080	0.012	# append calls = 720
LinkStrand:	131,072	49,745,040	0.012	# append calls = 720
LinkStrand:	262,144	96,930,960	0.012	# append calls = 720
LinkStrand:	524,288	191,302,800	0.012	# append calls = 720
LinkStrand:	1,048,576	380,046,480	0.012	# append calls = 720
LinkStrand:	2,097,152	757,533,840	0.013	# append calls = 720
LinkStrand:	4,194,304	1,512,508,560	0.012	# append calls = 720
LinkStrand:	8,388,608	3,022,458,000	0.013	# append calls = 720
LinkStrand:	16,777,216	6,042,356,880	0.012	# append calls = 720
LinkStrand generated recombinant of ecolimedx12.dat; dna length = 3,841,920; cutting at enzyme gaattc				
Class	splicee	recomb	time	
LinkStrand:	256	3,976,920	0.016	# append calls = 1080
LinkStrand:	512	4,115,160	0.016	# append calls = 1080
LinkStrand:	1,024	4,391,640	0.017	# append calls = 1080
LinkStrand:	2,048	4,944,600	0.017	# append calls = 1080
LinkStrand:	4,096	6,050,520	0.02	# append calls = 1080
LinkStrand:	8,192	8,262,360	0.022	# append calls = 1080
LinkStrand:	16,384	12,686,040	0.019	# append calls = 1080

LinkStrand:	32,768	21,533,400	0.018	# append calls = 1080
LinkStrand:	65,536	39,228,120	0.019	# append calls = 1080
LinkStrand:	131,072	74,617,560	0.018	# append calls = 1080
LinkStrand:	262,144	145,396,440	0.018	# append calls = 1080
LinkStrand:	524,288	286,954,200	0.017	# append calls = 1080
LinkStrand:	1,048,576	570,069,720	0.018	# append calls = 1080
LinkStrand:	2,097,152	1,136,300,760	0.017	# append calls = 1080
LinkStrand:	4,194,304	2,268,762,840	0.018	# append calls = 1080
LinkStrand:	8,388,608	4,533,687,000	0.017	# append calls = 1080
LinkStrand:	16,777,216	9,063,535,320	0.018	# append calls = 1080
LinkStrand generated recombinant of ecoli.dat; dna length = 4,639,221; cutting at enzyme gaattc				
Class	splicee	recomb	time	
LinkStrand:	256	4,800,471	0.019	# append calls = 1290
LinkStrand:	512	4,965,591	0.019	# append calls = 1290
LinkStrand:	1,024	5,295,831	0.021	# append calls = 1290
LinkStrand:	2,048	5,956,311	0.019	# append calls = 1290
LinkStrand:	4,096	7,277,271	0.022	# append calls = 1290
LinkStrand:	8,192	9,919,191	0.022	# append calls = 1290
LinkStrand:	16,384	15,203,031	0.021	# append calls = 1290
LinkStrand:	32,768	25,770,711	0.021	# append calls = 1290
LinkStrand:	65,536	46,906,071	0.02	# append calls = 1290
LinkStrand:	131,072	89,176,791	0.021	# append calls = 1290
LinkStrand:	262,144	173,718,231	0.02	# append calls = 1290
LinkStrand:	524,288	342,801,111	0.025	# append calls = 1290
LinkStrand:	1,048,576	680,966,871	0.02	# append calls = 1290
LinkStrand:	2,097,152	1,357,298,391	0.02	# append calls = 1290
LinkStrand:	4,194,304	2,709,961,431	0.022	# append calls = 1290
LinkStrand:	8,388,608	5,415,287,511	0.02	# append calls = 1290
LinkStrand:	16,777,216	10,825,939,671	0.023	# append calls = 1290