Genome analysis

LINKS: Scaffolding genome assemblies with kilobase-long nanopore reads

René L. Warren*, Benjamin P. Vandervalk, Steven J.M. Jones and İnanç Birol
Canada’s Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC V5Z 4S6, Canada

Received on XXXXX; revised on XXXXX; accepted on XXXXX

Associate Editor: XXXXXXX

ABSTRACT

Motivation: Owing to the complexity of the assembly problem, we do not yet have complete genome sequences. The difficulty in assembling reads into finished genomes is exacerbated by sequence repeats and the inability of short reads to capture sufficient genomic information to resolve those problematic regions. Established and emerging long read technologies show great promise in this regard, but their current associated higher error rates typically require computational base correction and/or additional bioinformatics preprocessing before they could be of value. We present LINKS, the Long Interval Nucleotide K-mer Scaffolder algorithm, a solution that makes use of the information in error-rich long reads, without the need for read alignment or base correction. We show how the contiguity of an ABySS E. coli K-12 genome assembly could be increased over five-fold by the use of beta-released Oxford Nanopore Ltd. (ONT) long reads and how LINKS leverages long-range information in S. cerevisiae W303 ONT reads to yield an assembly with less than half the errors of competing applications. Re-scaffolding the colossal white spruce assembly draft (PG29, 20 Gbp) and how LINKS scales to larger genomes is also presented. We expect LINKS to have broad utility in harnessing the potential of long reads in connecting high-quality sequences of small and large genome assembly drafts.

Availability: http://www.bcgsc.ca/bioinfo/software/links

Contact: rwarren@bcgsc.ca

1 INTRODUCTION

Long read technology has rapidly matured over the past few years, and the benefit of long reads for genome assembly is indisputable (reviewed by Koren and Phillippy, 2014). Recently, groups have shown that de novo assembly of error-rich long reads into complete bacterial genomes is possible, even without prior base correction (Berlin et al., 2014)

Portable long read sequencing technology is at our doorstep, thanks to leaps in microfluidics, electronics and nanopore technologies (Clarke et al., 2009). Expected to be a strong contender in the kilobase-long read arena, Oxford Nanopore Technologies Ltd (ONT, Oxford, UK) promises a miniature molecule “sensor” that is currently in limited early access beta-testing phase through the MiniONT™ Access Programme (MAP). At the moment, sequence reads generated by the instrument have limited utility for de novo assembly of genomes, which is mostly due to their associated high base errors and indel rates (Quick et al., 2014). Very recently, Quick et al. (2014) publicly released ONT E. coli long reads as part of the MAP. Whereas their assessment identified some of the shortcomings of the current technology, it also highlighted its great potential, including a low-cost throughput and kilobase long reads.

High quality R7 chemistry data (termed Full 2D) in the released dataset comprises reads derived from template and complementary strands. To our assessment, these reads have an average sequence identity of 77.1 +/- 10.6% (11 Mbp in 1714 reads with sequence identity of 50% or more to E. coli K-12 MG1655). Despite this low overall quality, there are still frequent continuous stretches of correct k bases in the reads when compared to the finished genome. These stretches are long enough to confer specificity, but short enough to be error free (Fig. S1, k=15). We have exploited this property of the sub-5X data (Fig. S2) to develop a genome scaffolding algorithm, LINKS, which extracts paired k-mers from the ONT reads, and uses them to link contig pairs. One advantage of the proposed implementation is its ability to iteratively refine assemblies by exploring large numbers of k-mer pair combinations for linking contigs.

2 METHODS

2.1 Sequence data, assembly, and scaffolding

E. coli K-12 substr. MG1655 Illumina MiSeq v3 TrueSeq Nano read data (paired end 301 bp, fragment length 550 bp) was downloaded from BaseSpace®, and randomly sub-sampled to ~250-fold coverage. Overlapping read pairs were merged with ABYSs-mergepairs (~q 15) and resulting ca. 550 bp pseudoreads were assembled with ABYSs v1.5.2 (Simpson et al., 2009) (k=480 t=40 s=1000) yielding 67 and 61 contigs and scaffolds ≥ 500 bp, respectively. Contigs and scaffolds (Fig. S3, Table 1A and B) were scaffolded with SSPACE v1.0 (k=15, d=4000, default parameters) using the E. coli K-12 substr. MG1655 R7 Full 2D ONT data from Quick et al. (2014; R7 chemistry ONI/ONI ERP007108), and results are shown in Table 1C, Fig. S4 and Table 1D, Fig. S5, in that order. SSPACE-LongRead v1.1 (g=200, with defaults parameters) was ran on the B assembly (Table 1E). ABYSs scaffolds were also re-scaffolded iteratively with LINKS (k=15, d=500 to 16000, 30 iterations) using the Full 2D ONT reads (Table 1F) and, in separate experiment, all available 2D reads (Table 1G, Fig. S6)

*To whom correspondence should be addressed.
and all available R.7.3 chemistry raw uncorrected reads (ERX593921/Table 1H, Fig. S7). A Baseline S. cerevisiae W303 Illumina MiSeq assembly (http://schatzlab.cshl.edu/data/nanocorr/) was rescaffolded with SSPACE (g=200), AHA (Rasko et al., 2011) and LINKS (k=15, d=2-15kbp, 27 iterations) using 262,463 raw ONT reads (Fig. S8). White spruce (Genbank:ALWZ030000000, 4.2M scaffolds) was re-scaffolded with LINKS 14 times (k=26, r=200-50 d=5-100kbp) using draft white spruce W57111 genotype assembly (Genbank: JZKD010000000, 4.1M sequences) (Fig. S9).

### 2.2 LINKS Algorithm

**Process:** ONT reads are supplied as input (–s option, fasta format) and k-mer pairs are extracted using user-defined k-mer length (–k) and distance between the 5’-end of each pairs (–d) over a sliding window (–t). Unique k-mer pairs at set distance are hashed. Fasta sequences to scaffold are supplied as input (–f), and are shredded to k-mers and paired between contigs pairing information of successful scaffolds. A log summary of k-mer pairs in the assembly is provided (–log) along with a text file describing possible issues in pairing (.pairing_issues) and pairing distribution (.pairing_distribution.csv).

### 3 RESULTS

We used a publicly available ONT data for scaffolding E. coli contigs and scaffolds derived from high-depth Illumina MiSeq 300 bp paired end reads and assembled with ABySS. These assemblies were already very contiguous (scaffold NG50=204 kbp) and of good quality, as assessed by QUAST (Gurevich et al., 2013, Table 1). First, we scaffolded ABySS contigs with only the ONT Full 2D data (k=15, d=4000), as a benchmark of the method, yielding an improved assembly that rivaled the ABySS scaffold on Illumina data (Table 1B) in assembly quality (Table 1C). The runs were fast (<1 min), and required moderate resources (~6 GB RAM). Next, we re-scaffolded E. coli ABySS scaffolds (B, using the same parameters. Running SSPACE-LongRead (E) gave similar results, but with less memory by a factor 4. Further merge opportunities are found by subsequently extracting paired k-mers at various distance intervals. Accordingly, we ran LINKS iteratively 30 times, each instance increasing the distance between k-mer pairs from 500 to 16,000 bp and gradually improving the long-range contiguity of the assembly (Table 1F and Fig. S5). Despite the relatively longer runtime (23 min.), the final assembly was in less than half the original number of sequences, and the scaffold NG50 length exceeded 600 kbp. With the same run parameters, using all available 2D ONT data, LINKS required 85 GB RAM and 2h39m to complete (Table 1G), and yielded 12 scaffolds >1 kbp that are co-linear with the E. coli genome (Fig. S6). Using raw R.7.3 ONT reads for scaffolding gave the best compromise between errors and contiguity (Table 1H). We tested LINKS on the larger S. cerevisiae ONT dataset (Goodwin et al., 2015) and obtained an assembly that compares with the Celera Assembly of Illumina-corrected ONT reads (Nanocorr) in contiguity, but with less than half the errors (Fig. S8). High RAM usage with LINKS can be mitigated by increasing the sliding window step (–t), which decreases the k-mer pair space. Doing so, re-scaffolding the colossal white spruce genome draft assembly (Birol et al., 2013) 14 times using the draft assembly of another white spruce genotype was possible with ~132 GB RAM, producing a conifer assembly whose NG50 contiguity is in excess of 110 kbp (Fig. S9).

To our knowledge, LINKS is the first publicly available scaffold designed specifically for nanopore long reads and with a general framework that could apply to scaffolding very large genomes, such as that of white spruce (20 Gbp) using another assembly draft or reference in lieu of long reads. This study highlights the present utility of ONT reads for genome scaffolding in spite of their current limitations, which are expected to diminish as the nanopore sequencing technology advances.

### ACKNOWLEDGEMENTS

This work is partly funded by Genome Canada, British Columbia Cancer Foundation, and Genome British Columbia. Research reported in this publication was also partly supported by the National Human Genome Research Institute of the National Institutes of Health under Award Number R01HG007182. The content is solely the responsibility of the authors and does not necessarily represent...
the official views of the National Institutes of Health or other funding organizations.

REFERENCES

Berlin, K. et al. (2014) Assembling Large Genomes with Single-Molecule Sequencing and Locality Sensitive Hashing. bioRxiv doi: http://dx.doi.org/10.1101/008003
Birol, I. et al. (2013) Assembling the 20 Gb white spruce (Picea glauca) genome from whole-genome shotgun sequencing data. Bioinformatics, 29(12):1492-7.
Boetzer, M. and Pirovano, W. (2014) SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information. BMC Bioinformatics. 15, 211.
Clarke, J. et al. (2009) Continuous base identification for single-molecule nanopore DNA sequencing. Nat. Nanotechnol., 4, 265-70.
Goodwin, S. et al. (2015) Oxford Nanopore Sequencing and de novo Assembly of a Eukaryotic Genome. doi: http://dx.doi.org/10.1101/013490.
Gurevich, A. et al. (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics, 29,1072-5.
Koren, S. and Phillippy, A. M. (2014) One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly. Curr.Opin.Microbiol., 23C,110-120.
Quick, J. et al. (2014) A reference bacterial genome dataset generated on the MinION™ portable single-molecule nanopore sequencer. Gigascience, 3, 22.
Rasko D.A. et al. (2011) Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. N Engl J Med., 365, 709-717.
Simpson, J.T. et al. (2009) ABySS: a parallel assembler for short read sequence data. Genome Res. 19,1117-23.
Warren, R.L. et al. (2007) Assembling millions of short DNA sequences using SSAKE. Bioinformatics, 23, 500-1.
SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Full 2D ONT long read k-mer uniqueness in the *E. coli* K-12 genome reference. *k*-mers were extracted from both the Full 2D ONT data (Quick *et al.*, 2014) and the *E. coli* K-12 substr. MG1655 (accession U00096.2) reference genome sequence. A Bloom filter (Bloom, 1970) was built from the latter and *k*-mers extracted from the former files used to query the filter for matching sequences. *k*=15 gives the best compromise of specificity, yield and uniqueness with the data set at hand.
Supplementary Figure S2. *E. coli* K-12 substr. MG1655 genome coverage analysis by Full 2D (R7 chemistry) Oxford Nanopore long reads. High-quality 2D nanopore reads (Quick et al. 2014) were aligned with blastn (Altshul et al. 1990) onto the *E. coli* K-12 substr. MG1655 reference (U00096.2), plotting only reads with sequence identity over 50% (1,713 high quality sequences out of 3,471). We identified 184 regions 1 bp and longer with no read coverage. Overall 90.3% of the 4,639,675 bp MG1655 genome was covered by at least one nanopore read.
Supplementary Figure S3. *E. coli* K-12 assembly and genome co-linearity. A baseline ABYSS assembly (Table 1B in main text) of the *E. coli* K-12 MG1655 genome yields a draft genome that despite being fragmented is co-linear with the reference. Sequence comparison was performed with MUMmer v3.23 tools, using nucmer for nucleotide sequence alignments and mummerplot for plotting (Kurtz *et al*., 2004).
Supplementary Figure S4. Full 2D ONT - LINKS scaffolds co-linearity with the MG1655 genome, single k-mer pair LINKS run. A single LINKS scaffolding round (k=15 bp, d=4000 bp) was performed on ABySS assembly sequence scaffolds (shown in Fig. S3B), bringing the number of scaffolds from 61 to 48 (Table 1D in manuscript) and harboring sequences in the correct order and orientation.
Supplementary Figure S5. Full 2D ONT-LINKS scaffolds co-linearity with the reference *E. coli* K-12 genome (multiple *k*-mer pair runs). Iterative LNKs scaffolding rounds (*k*=15, *d*=500 to 16000 bp, 30 iterations) were performed on ABysS assembly sequence scaffolds (Table 1F in manuscript), bringing the number of scaffolds further down to 27 from 61, with its underlying sequences in the exact configuration compared to the reference.
Supplementary Figure S6. LINKS scaffolds using all available R7 2D ONT reads compared to the reference *E. coli* K-12 genome (forty five k-mer pair interval runs). Iterative LINKS scaffolding rounds (*k*=15, *d*=500 to 16000 bp, 30 iterations) were performed on ABySS assembly sequence scaffolds (Table 1G in manuscript), bringing the number of scaffolds further down to 16 from 61. MUMmer co-linear analysis indicates that six large scaffolds comprise *E. coli* K-12 MG1655 re-scaffolded sequences in the correct order and orientation.
Supplementary Figure S7. LINKS scaffolds using all raw, uncorrected R7.3 ONT reads compared to the reference *E. coli* K-12 genome (forty five k-mer pair interval runs). Iterative LINKS scaffolding rounds (k=15, d=500 to 16000 bp, 30 iterations) were performed on the baseline ABySS assembly sequence scaffolds (Table 1H in manuscript), bringing the number of scaffolds down to 27 from 61. QUAST analysis reveals that re-scaffolding with the raw v7.3 ONT data produces an assembly with the best compromise, with fewer errors and highest overall contiguity.
Supplementary Figure S8. Scaffolding high-quality short read assemblies with Oxford Nanopore Technologies long reads. Publicly available ONT long reads for *E. coli* K12 MG1655 and *S. cerevisiae* W303 were recently made available (Quick et al. 2014; Goodwin et al 2015). We have used these data to re-scaffold baseline ABySS scaffold assemblies of Illumina-only data (Simpson *et al.*, 2008; Base space: Illumina MiSeq v3 TruSeq Nano read data and SRA:ERR156523) using LINKS, AHA (Rasko *et al.*, 2011) and SSPACE-LR (Boetzer and Pirovano 2014) (blue squares and red circles). Additionally, we have re-scaffolded a baseline Illumina MiSeq assembly of *S. cerevisiae* W303 (http://schatzlab.cshl.edu/data/nanocorr/) with the same tools and compared it to a Celera Assembler assembly of Illumina-corrected ONT reads (green triangles). The *E.coli* Nanocorr CA assembly was excluded from the graph for clarity; it produced a single scaffold with only 5 errors. ABYSS Illumina, Illumina-only scaffold assemblies; SSPACE-LR F2D R7, SSPACE-LR scaffolding of the base assembly using full 2D ONT reads R7 chemistry; LINKS F2D R7, LINKS 30x iterative scaffolding using full 2D ONT reads R7 chemistry; LINKS Raw R7.3, LINKS 30x iterative scaffolding using raw ONT reads.
reads R7.3 chemistry; LINKS Raw R7. LINKS iterative scaffolding using raw W303 ONT reads to rescaffold a S288c ABysS assembly (31 iterations, purple circle) or a W303 Illumina/MiSeq assembly (27 iterations, green triangle); SSPACE-LR Raw R7, SSPACE-LR scaffolding of the base assembly (S288c ABysS illumina purple circle and W303 Illumina MiSeq green triangle) using raw W303 ONT reads; CA Nanocorr, Celera Assembler assembly of Illumina-corrected W303 ONT reads; AHA, Pacific Biosciences’ A Hybrid Assembler; LINKS Nanocorr, 27x (E.coli K12), 28x (S. cerevisiae W303) or 39x (S. cerevisiae S288c) LINKS scaffolding iterations of baseline assemblies using respective Nanocorr corrected reads (http://schatzlab.cshl.edu/data/nanocorr/).
Supplementary Figure S9. LINKS re-scaffolding of the white spruce (*P. glauca*, PG29 cultivar) genome with *k*-mer pairs derived from the white spruce WS77111 genotype draft assembly. Iterative LINKS scaffolding rounds (fourteen iterations, \(k=26\), \(t=200\) to 50, \(l=5\), \(a=0.3\), \(d=5\) kbp to 100kbp, interval shown on x axis, solid black line) were performed on the PG29 V3 ABYSS assembly sequence scaffolds (Genbank: ALWZ030000000, 4.2M scaffolds \(\geq 500\)bp, dotted grey line, Birol *et al.*, 2013) using sequence data from the WS77111 V1 draft assembly (Genbank:IZKD010000000, 4.3M scaffolds \(\geq 500\)bp, dotted blue line), increasing the PG29 assembly contiguity 1.5-fold to reach an NG50 length (Earl *et al.*, 2011) of 114,888 bp (4.1M scaffolds \(\geq 500\)bp).
Supplementary References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol.*, 215, 403-10.

Bloom BH (1970) Space/Time Tradeoffs in Hash Coding With Allowable Errors, Communications of the Acm, vol. 13, pp. 422-4.

Earl D, Bradnam K, St John J, Darling A, Lin D, Fass J, Yu HO, Buffalo V, Zerbino DR, Diekhans M, Nguyen N, Ariyaratne PN, Sung WK, Ning Z, Haimel M, Simpson JT, Fonseca NA, Birol I, Docking TR, Ho Y, Rokhsar DS, Chikhi R, Lavenier D, Chapuis G, Naquin D, Mailet N, Schatz MC, Kelley DR, Phillippy AM, Koren S, Yang SP, Wu W, Chou WC, Srivastava A, Shaw TI, Ruby JG, Skewes-Cox P, Betegon M, Dimon MT, Solovyev V, Seledtsov I, Kosarev P, Vorobyev D, Ramirez-Gonzalez R, Leggett R, MacLean D, Xia F, Luo R, Li Z, Xie Y, Liu B, Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Yin S, Sharpe T, Hall G, Kersey PJ, Durbin R, Jackman SD, Chapman JA, Huang X, DeRisi JL, Caccamo M, Li Y, Jaffe DB, Green RE, Haussler D, Korf I, Paten B. (2011) Assemblathon 1: a competitive assessment of de novo short read assembly methods. *Genome Res.*, 21, 2224-41.

Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL (2004) Versatile and open software for comparing large genomes. *Genome Biology* 5, R12.

Rasko DA, Webster DR, Sahl JW, Bashir A, Boisen N, Scheutz F, Paxinos EE, Sebra R, Chin CS, Iliopoulos D, Klammer A, Peluso P, Lee L, Kislyuk AO, Bullard J, Kasarskis A, Wang S, Eid J, Rank D, Redman JC, Steyert SR, Frimodt-Møller J, Struve C, Petersen AM, Krogfelt KA, Nataro JP, Schadt EE, Waldor MK. (2011) Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med.*, 365, 709-717.