

# De novo Sequence Assembly of Second Generation Sequence Review

Sun Zhao

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## Abstract

The abstract abstract.

## 1 Before Everything

I would like to first explain the motivation of this article at the beginning. The theme of this article is about sequence assembly which is a computer aided process for constructing gene sequence. It is actually belonging to the scope of bioinformatics. As a pure computer science undergraduate student, I have started researching in this field since the summer in 2010. After reading lots of papers, discussing with biology researchers from China as well as foreign ones, trying kinds of bioinformatic tools, I approached nothing new but valuable experiences of it. In this article, I will answer the questions of “what is gene sequencing”, “what is sequence assembly and its challenge”, “how to assembly sequence”, “how to evaluate sequence assembly softwares” and my own contribution to sequence assembly in a computer science researcher’s perspective. I’m not going to tell the deep details but giving initiations about what to do and how to do about sequence assembly. Moreover, I will provide valuable references of related papers and materials to help you get a full view of sequence assembly.

## 2 What is gene sequencing

In genetics and biochemistry, sequencing means to determine the primary structure of an unbranched biopolymer. The biopolymer can be DNA, RNA, protein. In this article, I will focus on DNA and RNA sequence analysis excluding protein. DNA sequencing is the process of determining the nucleotide order of a given DNA chain. Concretely, DNA is a chain of four types nucleotide, represented by letter of ‘A’, ‘G’, ‘C’, ‘T’. DNA sequencing is trying to produce the corresponding string of ‘A’, ‘G’, ‘C’, ‘T’ for a sample DNA chain. However, the most popular biology sequencing method called shot gun, randomly cut the original DNA chain into fragments and a set of ‘A’, ‘G’, ‘C’, ‘T’ strings. Each nucleotide string which is the sequence of a fragment is called a read. To increase the read coverage and read quality, copies of DNA made by PCR amplification with a typical bacteria template are sequenced. Fig. 1 shows the shot gun process, and you may find that reads are sequenced from the two ends of DNA fragments instead of the complete one. The end sequencing phenomena is caused by biology sequencing methods, however, if particular reaction and methods are included, the distance between the two ends can be estimated. In this case, the two end reads are called pair-end reads and the distance is called insert length.

DNA molecules are double-stranded helices, consisting of two long complement strands. According to base paring principle—‘A’ complements with ‘T’ and ‘G’ complements with ‘C’, the sequence of a strand can be inferred from its opposite

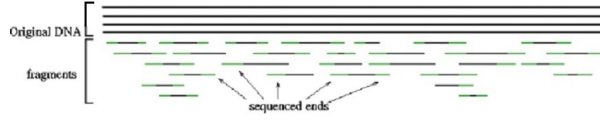


Figure 1:

one. Particular sequences denoted by 3' and 5' in the DNA strand is used to specify the orientation of the two strand, and for simplicity, if one strand is specified as 3' to 5', then the opposite one is 5' to 3'. Example 1 shows a double strain DNA fragment with pair-end reads (red string). Note that the pair-end reads are positioned on opposite strand and will be sequenced all from 3' to 5'. So the two pair-end reads string should be 'AGCTAA' and 'GCCAA'.

3'→5'  
 AGCTAATGCTATCTTGGC  
 TCGATTACGATAGAACCG  
 5'→3'

Example 1

Sequencing methods and platform is developing as time goes. The typical genome analyser of first generation is Sanger[12] producing read lengths of approximately 800bp (typically 500-600bp with non-enriched DNA). Recently, new sequencing methods have emerged [8]. Commercially available technologies include 454 Sequencing [9], Illumina genome analyser [1] and SOLiD sequencing ([www.appliedbiosystems.com](http://www.appliedbiosystems.com)). Compared to traditional Sanger methods, these technologies function with significantly lower production costs and higher throughput. However, the reads produced by these next-generation sequencing technologies are much shorter than traditional Sanger reads, currently around 400-500 base pairs (bp) for 454, 50bp for Illumina and 35bp for SOLiD. Because of their length, they must be produced in large quantities and at greater coverage depths than earlier sequencing projects.

Reads are saved as a file by sequencing chip and the most popular format of read file is FASTA and FASTQ. A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line (def-line) is distinguished from the sequence data by a greater-than (">") symbol at the beginning. It is recommended that all lines of text be shorter than 80 characters in length. An example sequence in FASTA format is shown in Fig. 2. A FASTQ file normally uses four lines per sequence. Line 1 begins with a '@' character and is followed by a sequence identifier and an optional description (like a FASTA title line). Line 2 is the raw sequence letters. Line 3 begins with a '+' character and is optionally followed by the same sequence identifier (and any description) again. Line 4 encodes the quality values for the sequence in Line 2, and must contain the same number of symbols as letters in the sequence. A minimal FASTQ file might look like this Fig. 3. For more details about the quality line, please refer introduction on [FASTQ Format](#).

The last thing to mention is RNA sequencing. As RNA is generated by transcription from DNA, the information is already present in the cell's DNA. The usual method to sequence RNA is first to reverse transcribe the sample to generate cDNA fragments. Then the cDNA are sequenced using DNA sequencing methods.

In a word, the next generation sequencing platform generates a FASTA/FASTQ format file consisting string of description, read and quality(only exists in FASTQ file) for DNA/RNA samples.

```
>AB000263 |acc=AB000263|descr=Homo sapiens mRNA for prepro cortistatin like peptide, complete
cds.len=368
ACAAGATGCCATTGCCCCGGCCTCTGCTGCTGCTCTCCGGGGCCACGGCCACCGCTGCCCTGCC
CCTGGAGGGTGGCCCCACCGGCCGAGACAGCGAGCATATGCAGGAAGCGGCAGGAATAAGGAAAAGCAGC
CTCTGACTTTCTCGCTTGGTGGTTTGAGTGACCTCCAGGCCAGTGCCGGGCCCTCATAGGAGAGG
AAGCTCGGGAGGTGGCCAGGCGGCAGGAAGGCGCACCCCCAGCAATCCGCGCGCGGGACAGAATGCC
CTGCAGGAACCTCTTCTGGAAGACCTTCTCTCTGCAAATAAACCTCACCCATGAATGCTCACGCAAG
TTTAATTACAGACCTGAA
```

Figure 2:

```
@SEQ_ID
GATTGGGGTTCAAAGCAGTATCGATCAAAATAGTAAATCCATTGTTCAACTCAGTTT
+
!' '*(((***+))%%%+)(%%%%).1***-+*'')**55CCF>>>>>CCCCCCC65
```

Figure 3:

### 3 What is sequence assembly

What the sequencing stage produces is a collection of DNA fragments's nucleotide sequence, whereas, what we need is the nucleotide sequence of the original one. Sequence assembly tries to align and merge the DNA fragments in order to reconstruct the original sequence. The intuition is to take advantage of overlap information between reads to tie them up and piece into a longer DNA sequence. Challenges listed blow makes sequence assembly a very complicated task.

- One cell contains multiple chromosomes as well as DNA chains which are sequenced together. DNA fragments from different DNA chain may overlap.
- DNA is a double-stranded structure, one strand may overlap with its opposite one.

- Sheared fragments along the genome cannot be modeled as a perfect Poisson process so that there may be some region not covered by reads.
- DNA sequence may contain repeat regions, and these regions may be incorrectly merged by overlap.
- Sequencing errors cause nucleotide deleting, replacing or inserting inside reads.
- Second generation sequencing platform produces much shorter reads so that the overlap possibility is exponentially increased.

Among those above challenges, the repeat problem is the most challenging thing for short read assembly. A simple example is shown in Fig. 4A, where the assembler incorrectly collapses the two copies of repeat A leading to the creation of two contigs instead of one Fig. 4B.

The basic strategies for assembling sequences are categorized by two groups.

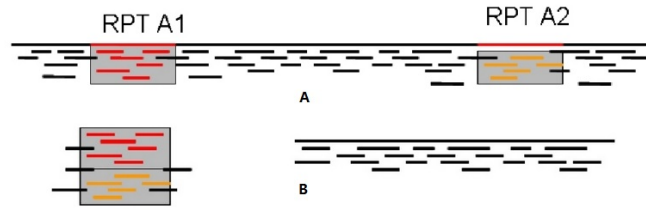


Figure 4:

One is reference based assembly, and the other one is de novo assembly. Reference based methods use a mapping tool to align reads against an existing reference sequence and build a sequence that is similar but not necessarily identical to the reference. The de novo assembly strategy does not use a reference sequence and is much useful for species whose reference sequence are unknown. This article only covers the de novo assembly and will illustrate the details in the subsequent sections.

Ideally, an assembly program should produce one contig for every chromosome of the genome being sequenced. However, because of challenge3 and challenge4, the output of the assembler is interrupted into a set of contigs. In summary, Inputting a FASTA/FASTQ read file, the assembler outputs a set of contigs.

## 4 How to assembly sequence

Overlap-consensus-layout [10] is the traditional approach for first generation sequence. It denotes each read as a separate node, where two reads presenting a clean overlap are connected by a bidirected edge. Although overlap-consensus-layout approach is both intuitive and robust, especially in the case of long reads, it is very costly for pair-wise overlap computing when assembling high quantity

short reads produced by second generation sequencing platform like SOLID and Illumina. Only one microread assembler, EDENA [5] was developed using this approach. Later, Idury and Waterman [6] introduced the use of a sequence graph to represent an assembly, and this idea has been developed into the most popular framework for short read assembly–De Bruijn graph. Sequence De Bruijn graph is constructed from a set of kmers( $k$  is a parameter) as nodes, and two kmers are connected if one kmer's last  $k-1$  nucleotides is identical to the first  $k-1$  nucleotides. kmers are produced by moving a fixed length( $k$ ) sliding window on the original read. Examples of kmers and de bruijn graph are show in Fig. 5 and Fig. 6. The sequence de bruijn graph has following properties:

- Read with length of  $n$  generates  $n-k+1$  kmers.
- Read is mapping to a unique path in the de bruijn graph.
- One node has at most 4 successors and 4 predecessors by adding 'A', 'G', 'C', 'T' at the first or last of the  $k-1$  nucleotides.

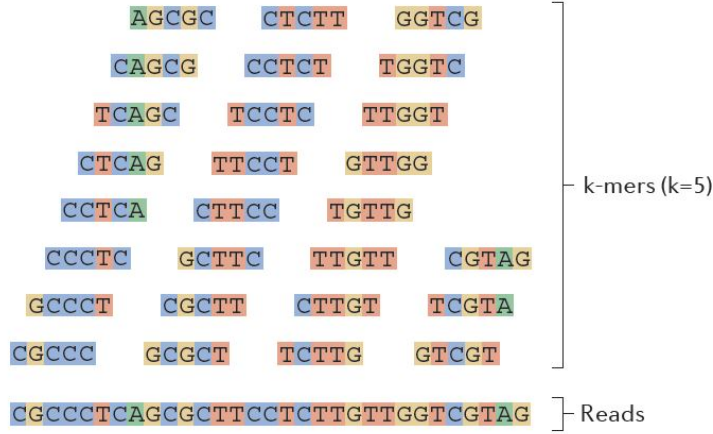
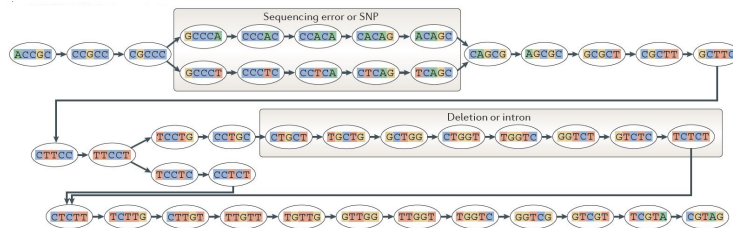


Figure 5:

According the property2 and property3, the node scale of the de bruijn graph is bounded by  $O(n-k+1)$  and the edge scale of it is bounded by  $O(8(n-k+1))$ . By the way, the length of human is about 3G base pairs whose de bruijn graph can be stored in a single server machine or cluster configured with 128GB. genome Property3 release the heavy cost of searching for all pair-wise overlap, thus making de bruijn graph approach feasible. Softwares include Velvet [15], ABYSS [14], ALLPATHS [2], SOAPDenovo [7] follows the de bruijn graph method. ABYSS and ALLPATHS claimed that they successfully assembled human's genome. A brief comparison of these softwares is show in Table 1. Software links can be found in Table 2.



I've read code and run programs of Velvet and ABYSS and provide some details about them. Velvet consists of two modules named `velveth` and `velvetg` where 'h' means hash and 'g' means graph. Velvet designed a novel graph construction algorithm and claim that it is efficient and memory saved approach on a single machine. `velveth` sequentially reads the FASTA/FASTQ read file and for each k-mer observed in the set of reads, a hash table records the ID of the first read encountered containing that k-mer and the position of its occurrence within that read. Each k-mer is recorded simultaneously to its reverse complement sequence. To ensure that each k-mer cannot be its own reverse complement, k must be odd. This first scan allows each read to be re-written as a set of new k-mers combined to overlaps with previously hashed reads. This new representation of the reads sequence is called a roadmap which is written into a file named `ROADMAP`. `velvetg` reads the `ROADMAP` file and creates a second database with the opposite information. It records, for each read, which of its original k-mers are overlapped by subsequent reads. The ordered set of original k-mers of that read is cut each time an overlap with another read begins or ends. For each uninterrupted sequence of original k-mers, a node is created. Finally, reads are traced through the graph using the roadmaps. Knowing the correspondence between original k-mers and the newly created nodes, it is possible to proceed from one node to the next, creating a new directed arc or incrementing the multiplicity of an existing one as appropriate at each step. The final output of velvet is produced by `velvetg` and saved as `config.fa`. ABYSS constructs de bruijn graph directly following the definition. The MPI version uses a hash function to determine the host machine for a node. The structure of ABYSS program is organized by a make file driver named "abyss-pe". `abyss-pe` automatically finds the dependency of abyss modules and perform a minimum number of running modules. The final output is saved as `*-contigs.fa`, `*-scanffolds.fa`, `*-unitigs.fa` and a statistic file named `*-stats`.

Table 1:

Software	Parallelism	Large Scale Assembly	Memory Cost
Velvet	OpenMP	No	High
ABYSS	OpenMP+MPI	Yes	Low
ALLPATH	?	Yes	?
SOAPDenovo	?	Yes	Low

Table 2:

Software	Software Available
Velvet	<a href="http://www.ebi.ac.uk/zerbino/velvet/">http://www.ebi.ac.uk/zerbino/velvet/</a>
ABYSS	<a href="http://www.bcgsc.ca/platform/bioinfo/software/abyss/">http://www.bcgsc.ca/platform/bioinfo/software/abyss/</a>
ALLPATH	<a href="http://www.broadinstitute.org/science/programs/genome-biology/crd">http://www.broadinstitute.org/science/programs/genome-biology/crd</a>
SOAPDenovo	<a href="http://soap.genomics.org.cn/soapdenovo.html">http://soap.genomics.org.cn/soapdenovo.html</a>

can be merged into a long node. Tips and bubbles are shown in Fig .7. Note that repeat region also creates circles in the graph, and we can not determine how many times should the repeat sequence occur. In the last step, if pair-end reads are provided, we can estimate the distance of seed nodes and find a feasible path between them.

The last thing need to mention is that RNA assembly also named transcript assembly. RNA assembly is more complex than DNA assembly, observing the reasons:

- some transcripts have low coverage, whereas others are highly expressed.
- reads with sequencing errors derived from a highly expressed transcript may be more abundant than correct reads from a transcript that is not highly expressed.
- transcripts encoded by adjacent loci can overlap and thus can be erroneously fused to form a chimeric transcript.

Directly using DNA assemblers to assemble RNA sequences only outputs rather bad results. Velvet and ABYSS have extend themselves into Velvet\_Oases [13] and Trans-ABYSS [11] separately to address the additional challenge presented by RNA assembly. Recently, Trinity [3] was specially designed for transcript assembly. Zhao et. al [16] made a early comparison of transcript assembly and give a full view of performance, result, resource usage evaluation. It shows Trinity has a relative better assembling ability than Velvet\_Oases and Trans-ABYSS but much slower and memory cost while assembling large scale sequences. A group of HPC developers [4] made efforts to accelerating trinity and reducing its memory usage which make trinity a first choice when assembling transcript sequences.



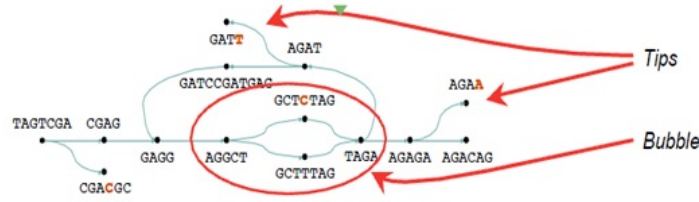


Figure 7:

## 5 How to Evaluate Assembler

To evaluate present assemblers or assemblers designed by ourselves, the first thing to do is to prepare input data. Usually, two kinds of data are used to evaluate assemblers, synthetic data and real data. `dwgsim`<sup>1</sup> is whole genome simulator based on a reference genome. Reference genome can be found on <ftp://ftp.ncbi.nlm.nih.gov/refseq/> and <ftp://ftp.ncbi.nlm.nih.gov/genomes/>. Details about refseq is explained on <http://www.ncbi.nlm.nih.gov/books/NBK50679/>. NCBI's SRA database<sup>2</sup> provides a large collection of read read sequences. Users search this database using an access code begin with SRP(studies), SRR(experiments) or SRR(runs).

## Reference

- [1] D.R. Bentley. Whole-genome re-sequencing. *Current opinion in genetics & development*, 16(6):545–552, 2006.
- [2] J. Butler, I. MacCallum, M. Kleber, I.A. Shlyakhter, M.K. Belmonte, E.S. Lander, C. Nusbaum, and D.B. Jaffe. Allpaths: De novo assembly of whole-genome shotgun microreads. *Genome research*, 18(5):810–820, 2008.
- [3] M.G. Grabherr, B.J. Haas, M. Yassour, J.Z. Levin, D.A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, et al. Full-length transcriptome assembly from rna-seq data without a reference genome. *Nature biotechnology*, 29(7):644–652, 2011.
- [4] R. Henschel, P.M. Nista, M. Lieber, B.J. Haas, L.S. Wu, and R.D. LeDuc. Trinity rna-seq assembler performance optimization. In *Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the eXtreme to the campus and beyond*, page 45. ACM, 2012.

<sup>1</sup>[http://sourceforge.net/apps/mediawiki/dnaa/index.php?title=Whole\\_Genome\\_Simulation](http://sourceforge.net/apps/mediawiki/dnaa/index.php?title=Whole_Genome_Simulation)

<sup>2</sup><http://www.ncbi.nlm.nih.gov/sra>

- [5] D. Hernandez, P. François, L. Farinelli, M. Østerås, and J. Schrenzel. De novo bacterial genome sequencing: millions of very short reads assembled on a desktop computer. *Genome research*, 18(5):802–809, 2008.
- [6] R.M. Idury and M.S. Waterman. A new algorithm for dna sequence assembly. *Journal of Computational Biology*, 2(2):291–306, 1995.
- [7] R. Li, H. Zhu, J. Ruan, W. Qian, X. Fang, Z. Shi, Y. Li, S. Li, G. Shan, K. Kristiansen, et al. De novo assembly of human genomes with massively parallel short read sequencing. *Genome research*, 20(2):265–272, 2010.
- [8] E.R. Mardis et al. The impact of next-generation sequencing technology on genetics. *Trends in genetics*, 24(3):133, 2008.
- [9] M. Margulies, M. Egholm, W.E. Altman, S. Attiya, J.S. Bader, L.A. Bemben, J. Berka, M.S. Braverman, Y.J. Chen, Z. Chen, et al. Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, 437(7057):376–380, 2005.
- [10] E.W. Myers. Toward simplifying and accurately formulating fragment assembly. *Journal of Computational Biology*, 2(2):275–290, 1995.
- [11] G. Robertson, J. Schein, R. Chiu, R. Corbett, M. Field, S.D. Jackman, K. Mungall, S. Lee, H.M. Okada, J.Q. Qian, et al. De novo assembly and analysis of rna-seq data. *Nature methods*, 7(11):909–912, 2010.
- [12] F. Sanger, GM Air, BG Barrell, NL Brown, AR Coulson, JC Fiddes, P-M Slocombe, and M. Smith. Nucleotide sequence of bacteriophage (d x174 dna. 1977.
- [13] M.H. Schulz, D.R. Zerbino, M. Vingron, and E. Birney. Oases: robust de novo rna-seq assembly across the dynamic range of expression levels. *Bioinformatics*, 28(8):1086–1092, 2012.
- [14] J.T. Simpson, K. Wong, S.D. Jackman, J.E. Schein, S.J.M. Jones, and I. Birol. Abyss: a parallel assembler for short read sequence data. *Genome research*, 19(6):1117–1123, 2009.
- [15] D.R. Zerbino and E. Birney. Velvet: algorithms for de novo short read assembly using de bruijn graphs. *Genome research*, 18(5):821–829, 2008.
- [16] Q.Y. Zhao, Y. Wang, Y.M. Kong, D. Luo, X. Li, and P. Hao. Optimizing de novo transcriptome assembly from short-read rna-seq data: a comparative study. *BMC bioinformatics*, 12(Suppl 14):S2, 2011.