Sequence assembly

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Abstract:
Despite the rapidly increasing number of sequenced and re-sequenced genomes, many issues regarding the computational assembly of large-scale sequencing data have remain unresolved. Computational assembly is crucial in large genome projects as well for the evolving high-throughput technologies and plays an important role in processing the information generated by these methods. Here, we provide a comprehensive overview of the current publicly available sequence assembly programs. We describe the basic principles of computational assembly along with the main concerns, such as repetitive sequences in genomic DNA, highly expressed genes and alternative transcripts in EST sequences. We summarize existing comparisons of different assemblers and provide a detailed descriptions and directions for download of assembly programs at: `http://genome.ku.dk/resources/assembly/methods.html`.

Keywords: Assembly methods, EST, shotgun, genomes, high-throughput sequencing.

1 Introduction

Genome sequencing is a discipline that has undergone tremendous development in the past. With the introduction of the different new massively parallel sequencing technologies the field will go through further transformations as new challenges arise. Today 567 bacterial genomes with up to 10.5 million base pairs (*Plesiocystis pacifica SIR-1*) have been sequenced and submitted to NCBI (as of October 9, 2008). In addition several eukaryote genomes with approximately three billion base pairs have been sequenced and assembled (`http://www.ensembl.org`), and many other sequencing projects are under way (`http://www.genomesonline.org`) [1].

The experimental technique used in most de novo sequencing projects of higher organisms, DNA chain termination, was developed three decades ago and remains, except for much higher levels of automation, basically the same. The introduction of new massively parallel sequencing methods, however, opens completely new fields of application. Shortly after the introduction of sequencing methods, some of the first reports of the determination and comparison of cDNA sequences were published. Late in the 1970s the bacteriophages phiX174 and Lambda [2, 3, 4] were among the first genomes to be completed together with the human mitochondrion [5, 6].

In the following decade the shotgun sequencing strategy was introduced [7, 8], and during the subsequent years it was extended by applying it to larger and larger DNA sequences cloned in plasmids (a few kilobases (kb)), cosmids (40 kb) [9], artificial chromosomes cloned in bacteria (BAC – Bacterial Artificial Chromosome) and yeast (YAC – Yeast Artificial Chromosome), with inserts of 100 to 500 kb [10]. The assembly of whole genome shotgun sequencing data was deemed to be futile until the successful WGS assembly of the 1.8Mb genome *Haemophilus influenzae* in 1994 [11]. An approximate time line of the major breakthroughs and milestones in sequencing is shown on Fig. 1.

[Figure 1]

“High throughput” sequencing (HTS) of cDNA was initiated in 1991 by Adams [12], who also introduced the term "Expressed Sequence Tag" (EST) to refer to this new type of sequence information. Collections of ESTs have given a first good approximation of the diversity of all protein coding genes in a tissue [13]. During the years ESTs have become an important tool with many applications, mostly in relation to gene analysis and gene discovery [14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38]

The amount of data generated by the different sequencing projects is overwhelming. For example, sequencing of the human genome produced 23 and 27 billion bases of raw shotgun sequences in the International Human Genome Sequencing Consortium and the Celera projects, respectively [39, 40]. However, the vast amount of fragments can not readily be concatenated to a final sequence. Only by using computers it becomes possible to carry out the assembly of the pieces, but the outcome as well as the reliability of the result for a given type of data depends on the underlying strategy implemented in the computer program. Some strategies might be more suited for one type of data than others. Also, the computational resources of some methods might not scale well with the number of sequences in the data set. Though the experimental techniques have essentially driven the computational aspect of sequence assembly, the computational aspect is still of utmost importance since any meaningful assembly needs to be computer assisted.

One of the first assemblers introduced by Staden in 1980 [41] was a computer program developed to store and manipulate DNA gel reading data obtained from the shotgun method of DNA sequencing. During the next decade several other programs were presented, among them SEQAID [42], CAP [43], PHRAP [44], and the TIGR assembler, which was used to assemble the genome of *Haemophilus influenzae* [11]. In order to assemble larger and more complex eukaryotic genomes, new assemblers have been designed and implemented. Among them the Celera Assembler (now part of AMOS) [45, 46] and GigAssembler [39], both applied to human genome data sets; the JAZZ-assembler, which was applied to both the genome of *Takifugu rubripes* (the pufferfish) [47] and *Ciona intestinalis* [48]; and the ARACHNE [49] and Phusion [50] assemblers, both applied to the mouse genome.

Several specific efforts have been undertaken in the context of EST assembly, and several tools are available. Among them are StackPack [51, 52], TIGR TGICL [53], and geneDistiller [54]. Some of the tools deal with splice variants [55] or other problems such as chimerism (and includes alternative splice variants detection) [54, 56, 57]. Approaches to incorporate rather than remove repetitive sequences are discussed in [58, 59, 60].

Along with the increasing number of completed genomes, efforts are also made in developing computational methods for comparing genomes. These include TIGRs MUMmer [61, 62], TWINSCAN, GENEWISE, GENOMESCAN [63, 64],

BLAT [65], and AVID [66, 67] used for alignment and comparison of whole genomes, and FORRepeats which is used to detect repeats on entire chromosomes and between genomes [68].

The massive effort to sequence the human genome produced a first draft version in 2001 [39], and did, as a draft sequence, contain numerous gaps. It took another 3 years of sequencing and assembly before the finished version was presented (which still contains more than 300 gaps) [69].

2 Sequencing approaches

As mentioned the choice of assembly strategy depends on the sequencing method, and the choice of sequencing method may also depend on the organism that is being sequenced. Issues that can affect the final assembly (other than the obvious quality of sequence data) are the size of the inserts, whether the sequencing was uni- or bi-directional, the library construction, the cloning vector, the selection of clones to be sequenced, and the availability of additional information (consensus genome, ESTs, known verified genes, gene maps, etc.).

Approaches for the de novo sequencing of genomes from higher organisms using Sanger sequencing [70] will be described first. In the context of genome resequencing we take a closer look on the new massively parallel sequencing technologies and their obstacles, though many of the concerns are overlapping *eg.* sequencing quality assessment.

2.1 Basic sequencing procedure

The basic procedure in sequencing has been to isolate genomic DNA or RNA (reverse transcribed into cDNA), and clone it into vectors (*eg.* plasmids, BACs) capable of stable propagation in suitable host cells such as *Escherichia coli*, see Fig. 2 for a schematic illustration of a sequencing vector. Several cloning systems with insert sizes varying from hundreds of base pairs to megabases have been developed. The ideal clone library for genomic sequencing has the following features.

1. The clones are highly redundant, covering the entire genome many times (typically 6–10).
2. The clone coverage is random and not biased towards or against specific regions of the genome.
3. The clones are stable, not subject to recombination or reorganization during the propagation process [71].

It should be noted that one of the major improvements of the new massively parallel sequencing technologies is that they do not rely on vector cloning prior to sequencing, and the concerns listed here are therefore not directly applicable to those technologies.

[Figure 2]

After propagation, the clones are selected and the sequencing is performed. An essential feature in sequencing is the attachment of quality values to the raw sequences. The quality values indicate the likelihood of each base call being correct. In the assembly stage the quality values will help to distinguish true DNA polymorphisms from sequencing errors and match end sequences of low quality [72, 73, 74, 75].

In genomic shotgun sequencing, which typically uses a single individual DNA source, sequences sharing less than 98% identity are usually assumed to come from different regions of a genome (including different repetitive elements) [76]. In contrast, EST data is usually derived from a variety of sources representing the spectrum of polymorphisms in the original samples. These will usually include a number of erroneous polymorphism which are caused by sequencing errors inherent in single pass sequencing, a relatively high rate of insertions and deletions, contamination by vector and linker sequences and the non-random distribution of sequence start sites in oligo(dT)-primed libraries. Therefore, the degree of identity in overlapping sequences from the same gene will often be lower in EST projects than in genomic sequencing projects. In addition, the patterns of overlapping sequences caused by alternative spliceforms are different from those observed in a genomic shotgun project [76].

The major tool to gather sequence information was the method introduced by Fred Sanger in the second half of the 70'ties. It uses dideoxynucleotide triphosphates (ddNTPs) as DNA chain terminators [77, 70]. The classical Sanger approach is carried out in four independent DNA polymerase reactions. Besides the DNA template and deoxynucleotides (dNTPs) a reaction mix contains either ddATP, ddCTP, ddTTP or ddGTP. Each reaction results in DNA fragments of different length terminating with the respective ddNTP. Electrophoresis of the fluorescence- or radio labeled fragments allows the recovery of the template sequence. Later, the use of dye-terminators made it possible to perform sequencing in a single reaction rather than four – the basic principle however remained the same. While the classical Sanger approach requires separate synthesis and detection steps, High Throughput Sequencing (HTS) technologies employ sequencing-by-synthesis and sequencing-by-ligation approaches, allowing for simultaneous synthesis and detection.

2.2 Shotgun sequencing

Two approaches for genome shotgun sequencing can be distinguished: whole-genome shotgun (WGS) sequencing and hierarchical shotgun sequencing.

2.2.1 Whole Genome Shotgun

Sequencing using the whole genome shotgun approach basically means that the genome is randomly broken into pieces and cloned into a sequencing vector. The inserts are subsequently processed to generate sequences of bases (referred to as reads). See illustration on Fig. 3a. During the mid 1990s several groups recognized that sequence information from both ends of relatively long inserts dramatically improves the efficiency of sequence assembly [9, 78, 79, 80, 81, 82]. In contrast to single sequence reads from one end of the shotgun clones pairs of sequence reads from both ends have known spacing and orientation. Exact knowledge of the length of the insert is not required to utilize the advantages of end sequencing in assembly [83], but good estimates of clone length will aid the assembly immensely.

[Figure 3]

2.2.2 Hierarchical shotgun sequencing

The 'Hierarchical shotgun sequencing' (also referred to as 'map-based', 'BAC-based' or 'clone-by-clone') approach involves generating and organizing a set of large insert clones (typically 100–200 kb each) covering the genome (a "minimal tiling path"), followed by separate shotgun sequencing on each clone. For illustration see Fig. 3. It is possible to establish a tiling path of overlapping BAC-clones using only BAC fingerprinting technologies [84]. However, knowledge of unique genome markers (*eg.* ESTs or sequence-tagged sites (STS)) and their location in the genome map is of great help for organizing the BAC clones in the correct order. In hierarchical shotgun sequencing the sequence information is local, therefore the risk of long-range and short-range misassembly is reduced.

2.2.3 Mixed strategy sequencing

A strategy that can be used on large complex genomes is the 'mixed strategy sequencing'. The technique utilizes both hierarchical and whole-genome shotgun. The method combines a light (x1) BAC clone coverage of the genome, with whole genome shotgun sequencing . The BAC clones act as a basic framework for WGS sequence assembly. The method was successfully applied to rat genome [85].

2.2.4 Reduced Representation Sequencing

A variant of WGS is "reduced representation sequencing" (RRS), where one selectively chooses subsets of the genome to avoid sequencing the (often much) larger regions that are not of interest. In [86], SNPs were discovered by mixing DNA from many individuals, preparing a library of appropriately sized restriction fragments, and randomly sequencing

clones. Here, the choice of the restriction fragments effectively selects only a small subset of the human genome. Several approaches to RRS have been employed for plant genomes [87, 88, 89]: Methyl-filtration (MF) sequences uses the endogenous restriction-modification system of *E. coli* to eliminate methylated DNA inserts, the RescueMu (RM) approach focuses on the gene-rich regions which are rich in mutator transposons, and High-Cot filtration avoids repetitive and low-copy sequences due to differences in the relative rates of DNA re-association. Most of the Maize and Sorghum genomes have been sequenced using MF.

Many of the applications of the new high throughput sequencing platforms are based on various RSS strategies (see 2.2.6). This includes electrophoretic size separation to enrich for small RNA molecules (*eg.* [90, 91]); reduced representation bisulphite sequencing for genome wide methylation analysis [92]; flow sorting of derivative translocation chromosomes for breakpoint mapping [93]; enrichment of DNA-fragments bound to specific proteins by chromatin immunoprecipitation of fixed, sheared DNA, for identification of transcription factor binding sites (CHiP-Seq) [94, 95, 96]; enrichment of specific parts of the genome by multiplex PCR-amplification [97] or by hybridization to custom made arrays [97, 98], *eg.* for SNP discovery [99] and in situ exon capture [100].

2.2.5 EST sequencing

Expressed Sequence Tags (ESTs) are sequences representing genes which can originate from specific tissues [12]. In EST-sequencing a single automated sequencing from one or both ends of a cDNA-inserts is performed. This single-pass approach is the major reason EST-sequencing is cost effective [101]. For additional information, see *eg.* [102] and references therein.

In most cases EST sequencing projects are aimed at establishing partial sequences of transcribed genes rather than full length cDNA sequences. However, this approach features some special challenges such as common sequence motifs, alternative transcripts and paralogous genes are challenges that potentially impact the assembly quality. These issues will be discussed further in section 4.4.3.

2.2.6 Massively parallel sequencing

Recently, a number of new sequencing technologies have emerged. The development was initiated by 454 sequencing and followed by Solexa sequencing and others [103, 104, 105, 106, 107]. The common feature of all these technologies is that they are massively parallel, *ie.* they generate a large number of different sequence reads in a single run. The generated small reads are usually aligned to a reference genome, and further analyzed, see Fig. 3d for an illustration.

The methods generally use one variant or another of fixing many sequence fragments on a substrate, cyclically adding different bases with some – technology-specific – luminal characteristics, and recording an image at each cycle. Image analysis is used to recover the all sequences at once. Sequencing of all immobilized fragments thus proceeds in parallel.

Compared to traditional sequencing a large amount of sequence data is generated at a drastically reduced cost per base. The most important disadvantage of high throughput sequencing is the significantly reduced read length, which limits their application in de novo sequencing of complex genomes (*eg.* due to repeats), at least using simple shotgun strategies. However, these new platforms have many uses in genome resequencing, especially if it is possible to align the fragments to an existing good quality reference genome.

Due to the amount of raw sequence data, high throughput sequencing is valuable in areas such as SNP finding. In EST sequencing, HTS technologies might enable a researcher to make accurate digital expression profiles, even including low abundance transcripts, and help detecting alternative splicing (depending on the platform chosen).

One of the key technologies that gave rise to the era of HTS, pyrosequencing, was introduced in 1998 [103]. This sequencing-by-synthesis method is at the very heart of GS FLX systems by 454 Life Sciences [104]. The detection is based on pyrophosphates (PPi) released during the polymerase reaction. Sulfurylase converts PPi to ATP which is subsequently consumed by luciferase to emit light in the visible spectrum. In GS FLX systems, a library of DNA templates is immobilized on DNA capture beads, amplified using emulsion PCR (emPCR) and loaded onto proprietary titer plates with several hundreds of thousands reaction wells. During a run, the four nucleotides are flowed sequentially over the

plates. The luciferase reaction triggered by nucleotides complementary to DNA templates is recorded by a CCD camera. A washing step is necessary to allow the next detection step. The GS FLX currently allows read lengths of several hundred bases. According to the manufacturer a single instrument run with two high-density plates generates information for about 20 million base pairs.

A competing technology, Solexa, now sold by Illumina (`http://www.illumina.com`), uses optically transparent surfaces to immobilize fragmented and adapter-tagged DNA. Each attached fragment is subsequently amplified $\sim$1000 fold by repeated steps of bridge amplification. The resulting clonal clusters are then sequenced using reversible terminators with removable fluorescent dyes. With approximately 30-40 bp Solexa reads are significantly shorter compared to GS FLX. However, close to 50 million clones per flow cell can be sequenced in parallel, resulting in presently >1.5 Gb of sequenced DNA in a single sequencing run. This amount can be doubled by sequencing the other end of each fragment (paired-end). Improvement in chemistry may further increase the read lengths and hence push the total amount of sequenced DNA well beyond the size of a human diploid genome.

A third synthesis-based technology, tSMS (true Single Molecule Sequencing), is currently distributed by Helicos (`http://www.helicosbio.com`). No DNA amplification is required for this approach. Instead, fragmented single stranded DNA molecules are directly immobilized on a solid surface. Similar to Solexa, tSMS works with nucleotides that carry a removable, laser light-detectable fluorescent. At the moment the system is able to sequence reads with lengths up to 55 bases at a speed of 25 to 90 million usable bases per hour.

SOLiD, a system now sold by Applied Biosystems (`http://www.appliedbiosystems.com`), is a technology that uses a sequencing-by-ligation approach. An adapter-tagged library of short DNA fragments is amplified with emPCR, immobilized on capture beads and then deposited onto high-density glass arrays. The SOLiD sequencing-by-ligation protocol uses four by four sets of 8-mer probes. In each set only two bases, fluorescently labeled, are specific. The interrogation of sequences is done in four phases. If a probe has specifically bound to the free template in the first phase, say at position 1 and 2, it is enzymatically ligated to the current $5'$ end at position 0. After the detection step 3 nucleotides of the probe along with the fluorescence label are cleaved. The next ligation step interrogates 6 and 7 and so forth. After the first phase, the ligated sequence is removed, and another set of bases are called. So in the second phase bases 2 and 3 are read, in the third 3 and 4 and so forth. The advantage of the SOLiD system is that the double base reading leads to an increased accuracy. Currently SOLiD produces read lengths of about 30-40 bp and a total of 9 Gb per single run, with read length expected to become longer in the future.

In the future, other technologies may become available, such as the use of solid state nanopores for sequencing of single DNA molecules [108]. We refer to [109] for an overview, in which several interesting ideas how this approach could be implemented in practice are presented.

3 Mapping of short high-throughput sequencer reads

Compared to de novo assembly, the mapping of resequenced reads to a template genome is a computationally easier problem. Still, efficient mapping tools are crucial (see section 4.7), and several tools for mapping of short reads are available. Most of the tools, *ie.* MAQ, SOAP, SHRiMP or Eland (proprietary), use seeding techniques that gain their speed from precomputed hash look-up tables [110, 111, 112]. Typically, seeds of fixed length allow for not more than one or two mismatches. In addition, the capability to detect insertions and deletions, as they frequently occur in 454 sequences (see section 4.4.4) is very limited,and most programs can only detect indels in subsequent alignment runs. For short sequences it would be helpful, but computationally more expensive, to incorporate indels right from the start. Current mapping tools have different additional features. The program MAQ, *eg.*, additionally supports paired-end read matching — helpful to deal with paired-end reads produced *eg.* by the GS FLX and other high throughput platforms.

4 Computational assembly

Computational assembly is the only way to efficiently assemble sequenced fragments of DNA. However, a sufficient amount of high quality sequences are required. The assembly programs should be able to handle large data sets effectively

and avoid misassemblies in the presence of large repetitive or duplicated regions and redundant sequences. To accomplish this, effective algorithms to handle large input data sets with the use of minimal computer time and memory are needed.

One of the primary difficulties in computational genome assembly is to develop an algorithmic approach capable of detecting stretches of repetitive DNA without causing misassemblies. Repetitive sequences complicate assembly as different pieces of sequence can share the same repeat sequence originating from different genomic locations. Since the pieces are put together by searching for matching overlapping nucleotides, repeats can be put together erroneously. Typically, for shotgun data, repetitive sequences are revealed by clusters containing more overlapping reads than would be expected by chance, illustrated on Fig. 4.

[Figure 4]

In EST datasets the main difficulty is to develop an algorithmic approach that, in addition to efficient assembly, can handle highly expressed genes, paralogous genes, alternative spliceforms and chimerism in the dataset.

The theoretical background for genome assembly lies in computer science, and an insight into the mathematical and theoretical background can be found in [113] and references therein.

Although pyrosequencing with a whole-genome shotgun approach has been successfully applied to bacterial genomes [104], the construction of high-quality assemblies with high-throughput sequencing data is still a non-trivial problem even for short genomes. At present, no approach has been proposed to directly assemble large animal or plant genomes directly from short sequences obtained using HTS. As described below the SHort Read Assembly Protocol (SHRAP) [114], however, comprises a protocol for high-throughput short read sequencing that differs in two respects from classical hierarchical sequencing approaches. This protocol however, expects read lengths much longer (200 nucleotides) than those produced by SOLiD or Solexa. The assembly methodology is based on the Euler engine introduced in 2004 [60]. The Euler-SR assembler, specifically designed to assemble short reads, uses an updated version of the Euler engine to reduce memory requirements. The results for real Solexa reads, however, were less convincing [115] due to the poorly understood error model and highly variable error rates across different machines and run times.

4.1 Basic principles of Assembly

For the majority of traditional assembly programs the basic scheme is the same, namely the overlap-layout-consensus approach. Essentially it consists of the following steps [44, 116]:

- Sequence and quality data are read and the reads are cleaned.
- Overlaps are detected between reads. False overlaps, duplicate reads, chimeric reads and reads with self-matches (including repetitive sequences) are also identified and left out for further treatment.
- The reads are grouped to form a contig layout of the finished sequence.
- A multiple sequence alignment of the reads is performed, and a consensus sequence is constructed for each contig layout (often along with a computed quality value for each base).
- Possible sites of misassembly are identified by combining manual inspection with quality value validation.

Prior to the assembly, the electropherogram (for Sanger sequencing, images for massively parallel sequencers) for a given sequence is interpreted as a sequence of bases (a read) with associated quality values, these values reflect the log-odds score of the bases being correct. The basecaller PHRED [117] is often used, however alternatives exist, *eg*. the CATS basecaller [118].

The reads can then be screened for any contaminant DNA such as *Escherichia coli*, cloning or sequencing vector. Low quality regions can be identified and removed [45]. Base quality values can be used in computation of significant overlaps and in construction of the multiple alignments [44, 116]. The pipeline for a typical sequence assembly is sketched on Fig. 5.

[Figure 5]

For high-throughput sequencing data, the basic proposition for SHRAP is to sample clones from the genome at high coverage, while sequencing reads from these clones at low coverage. SHRAP starts off with assembling the reads greedily to small local assemblies and subsequently to contigs on each clone. It proceeds by ordering the clones in a "clone graph", and constructing "clone contigs", which are then assembled independently. Computer simulations of the procedure show that the approach can reach a quality comparable to the current assemblies of single human chromosomes and fruit fly genomes using reads of 200nt with an error rate of not more than 1%. These are constraints that are too strict for short (Solexa or SOLiD) reads ($\approx 40bp$) and because of higher error rates challenging for real 454 reads [119]. Furthermore, for mammalian genomes the use of a hierarchical sequencing strategy might be somewhat cumbersome.

However, the use of templates might bail Solexa and SOLiD users out: In a recent study, de novo assemblies of chloroplast genomes ($\approx$ 120 kb) were improved by aligning preassembled contigs to reference genomes [120]. After de-Bruijn graph assembly of reads [121], small contigs were aligned to closely related chloroplast genomes. Between 67% and 98% of the contigs could be aligned to such templates. If alignment failed, sequences were scanned for similarity using BLASTN [122]. The authors reported that successful BLASTN matches typically contained $>$ 100 bp insertions relative to the reference genome. In the end, however, their assemblies were estimated to be 88–94% complete. Yet, the assembly of mammalian genomes or genomes without good reference sequences seems to be a considerably more difficult task. The successful de novo assembly of Chloroplasts genomes with 454 reads has been shown earlier [123].

454, SOLiD, Solexa technologies allow convenient generation of mate-pair/paired-end sequences, *ie.* the ability to sequence both ends of each DNA fragment. However, in an assembly using a hybrid dataset of real 454 reads and simulated mate-pair data, about 96% of the mate-pairs did not contribute additional information and hence did not improve the assembly [115]. Likewise, in a hybrid dataset of 454 reads and Sanger reads the vast majority of long sequences did not improve the assembly substantially, measured by N50 contig size. Hence, the authors concluded that hybrid protocols should be reviewed critically. Despite those simulation results, the latter method has already been shown to work quite well in practice [124], and one area where mate-pair/paired-end sequencing should improve the analysis dramatically is for the detection of breakpoints related to structural rearrangements, *eg.* deletions, duplications, inversions and translocations [125].

4.2 General Assembler differences

When different assemblers try to piece the DNA puzzle together they essentially work from the same input, but the assemblers differ in the way they utilize the sequence information, and in the way this is combined with additional information. In general the differences fall in the following categories.

- **Overlaps:** A lot of different methods are used to find potential overlaps between sequences. Some are based on BLAST (*eg.* geneDistiller [54, 56]), while other assemblers use various other methods to find similarities between reads.
- **Additional information:** Depending on how the sequence reads are produced some additional information might be available. This information might consist of read pair information, BAC clone information, base quality information, etc. Some assemblers use this data to impose additional structure on the assembly of the sequences (*eg.* GigAssembler [39]).
- **Short read assembly:** De novo assembly of the micro reads generated from next generation sequencing platforms is still challenging. While assemblers have been developed and applied to assemble bacterial genomes successfully [115, 126], on larger genomes the assembly is performed by mapping the micro reads to reference genomes. The major next generation sequencing platforms all have built-in software to handle this task, *eg.* GS Reference mapper, Gerald for Solexa. In SOLiD systems the mapping tool "mapreads" converts reference sequences into color space and perform the mapping in color space.

A somewhat related issue is how the sequences are cleaned of contaminant sequences (*ie.* vector sequences, repeat sequences, *etc.*). While this can essentially be considered separately and independently from the assembly itself, some assemblers incorporate cleaning in the way they process the reads (*eg.* [49]).

These basic ideas will be discussed further in the following text, and an overview on how the different assemblers applies these ideas can be found in the supplemental material (`http://genome.ku.dk/resources/assembly/methods.html`).

4.3 Overlap identification and alignment

In a whole-genome context, trillions of overlaps between reads are examined [45]. The majority of assemblers uses alignment algorithms which are general modifications of methods first introduced by Needleman and Wunsch in 1970 [127], Smith and Waterman in 1981 [128] and Gotoh in 1982 [129].

Initial overlap detection is often performed by finding exact identical subsequences (often called words, k-words or k-mers) between reads, prior to making the actual alignments. These identical subsequences are used to find pairs of potentially overlapping sequences, which can then be aligned to each other in order to check if they represent a true overlap. The size of the subsequences varies from method to method, and is dynamic in some assemblers. Furthermore, the identical subsequences are grouped and used in different ways depending on the assembler.

For almost all assemblers, a modified Smith-Waterman [128] algorithm is used to align candidate overlapping reads. The basic idea in the alignment algorithms is to use dynamic programming to construct a matrix containing scores of all subsequences, which is then analyzed to find the "optimal" alignment. Dynamic programming simply means that the alignment is calculated as extensions to already aligned subsequences. The assembly programs differ in their exact implementation of this algorithm, as (nearly) all of them use a heuristic approach to decrease the computational load, thereby increasing speed (*eg.* [116]). In the assembly of ESTs a clustering step is used to group the input sequences sharing significant regions of near identity together [130]. On Fig. 6, an assembled cluster is shown, the example is taken from the Sino-Danish pig EST sequencing project [131].

[Figure 6]

4.3.1 Multiple alignments and the consensus sequence

While the alignment of two sequences is usually straightforward, aligning more than two is not so simple. The standard Smith-Waterman algorithm can easily be extended to the task of aligning many sequences by constructing a "multi-dimensional matrix". However, the number of calculations rise exponentially with the number of sequences. This sets severe practical limits of the number of sequences that are viable to align, and therefore finding the true sequence from a number of overlapping reads becomes difficult.

Precisely how the different assemblers generate a multiple alignment and consensus sequence is only vaguely described in the literature, but a common approach is to use a heuristic greedy algorithm (see for example [132]). The greedy algorithm typically performs pairwise alignment between overlapping reads, from which a multiple alignment is build up iteratively, *ie.* adding one sequence at a time, but with this approach there is no guarantee that such a multiple alignment is correct.

After the multiple alignment has been constructed the consensus sequence is found. This would typically be the sequence generated by taking the most common base at each position in the alignment, however other methods exist. For instance geneDistiller [54], where ungapped alignments of reads is performed (thus simplifying the multiple alignment). The consensus sequence is constructed by splitting the multiple alignment in 12-mer words and analyzing the relative frequencies of these, where the presence of alternative transcripts is detected through the frequencies of the 12-mers (and displayed as stretches of 'alternate consensus').

The assumption is that the final consensus sequence correspond to the original genomic sequence where the sequenced fragment originate.

4.3.2 Eulerian Fragment Assembly

In assemblers aimed at short read assembly (*eg.* SOLiD reads) an approach based on mathematical graph theory is often used, namely the Eulerian fragment assembly method. The Eulerian fragment assembly avoids the costly computation of pairwise alignments between reads [133]. The *De Bruijn graph* of a genome has as its vertices all distinct $k-1$ tuples that occur within the sequence (where k is the word length that is used). A directed edge is inserted between s and t if there is a k tuple $\langle u_1, u_2, \ldots, u_{k-1}, u_k \rangle$ in the genome such that $s = \langle u_1, u_2, \ldots, u_{k-1} \rangle$ and $\langle t = u_2, \ldots, u_{k-1}, u_k \rangle$, *ie.*, if s and t appear shifted by single nucleotide. A sketch of a graph construction procedure is shown on Fig. 7. In practice one uses the k-tuples appearing in the collection of the sequence reads and a value of k between 6 and 9 or 10. In the error-free case, the genomic sequence can be read off directly as an Eulerian path through the De Bruijn graph (with repeats forming "tangles"). In real, error-prone data underrepresented k-tuples, *ie.* k-tuples that appear less frequently than expected from the coverage rate, indicate sequencing errors and can be omitted.

[Figure 7]

4.4 Data reliability

4.4.1 Preprocessing and cleaning

A critical aspect of any large-scale sequencing effort is the production of high quality data. To obtain this preprocessing is applied to the reads. For Sanger sequencing this includes base-calling, filtering of low quality reads, short length reads (typically less than 100 bp), identification of sequence features such as linker restriction sites, cloning vectors, polyadenylation tails, library tags, polyadenylation signals [134] and other contaminants like bacterial sequences [135].

There are different computational programs available to detect these contaminations. Most of the existing programs used for processing solely focus on a single step. While PHRED [117] deals with base-calling, cross_match [44] aims to identify and mask vector sequences in reads. Preprocessing can also be done using other programs such as LUCY [135], a sequence trimming script like SeqClean [136], or ESTprep [134].

In the Solexa system, the module for sequence alignments, Gerald, applies some filters to remove low quality base calling before the real mapping starts. As it is based on optical detection of ultra-high dense sequence clusters on surface, chastity and purity of optical signals are crucial for accessing the quality. Distance between clusters is also taken into consideration. Thresholds for these features can be customized in the program (see Illumina in-house documentation for details). Other next-generation sequencing systems employ different measures according to their methods.

4.4.2 Repeats

In mammalian genomes the repetitive content can be as high as 50%. The repeated fraction includes interspersed repeats derived from transposable elements, and long genomic regions that have been duplicated in tandem, palindromic or dispersed fashion, *eg.* ribosomal RNA genes, centromeres, heterochromatin and retrotransposons. Such features complicate the assembly into a correct finished genome sequence and have a great influence on the design of assemblers. Computationally repeats are typically handled as follows:

- **Comparing:** By comparing reads to known repeated regions in other genomes, potential repetitive sequences can be separated (and typically discarded) from the assembly.
- **Masking:** Regions which have a high depth, that is regions where many reads share the same sequence, are marked as repeats (illustrated on Fig. 4). Usually such regions are discarded by the assembler, and are not incorporated in the assembly, *eg.* by the method presented in [137].

A standard program for masking repeats is RepeatMasker [138]. It searches through curated repeat databases (*eg.* Repbase [139]) using the alignment program cross_match [44] to identify and mask repeats. The speed of cross_match can be increased by using the software wrapper MaskerAid [140].

4.4.3 Expressed Sequence Tags

Due to the way that the EST sequences are generated, there are several concerns which can severely disrupt attempts to analyze the data:

Over-clustering: This happens when ESTs from different genes are clustered together, and therefore associated with the same genetic sequence. This often arise as a result of the cloning procedure, which falsely place two originally separate sequences in the same read, *ie.* chimerism. However, paralogous genes can also be clustered together due to high sequence similarity. Using the traditional (TGICL, d2_cluster) single transitive single linkage clustering methods [141, 142], can cause all EST from both genes to be assigned to the same cluster. More stringent clustering methods such as the double linkage of geneDistiller [54] can reduce the amount of falsely clustered reads, and create more consistent assemblies and consensus sequences [56].

Highly expressed genes: In non-normalized cDNA libraries the fraction of the genes that is highly expressed, will be represented in a high number and lead to large and deep clusters, that may accidentally contain EST from more than one gene. There are several ways to handle highly expressed genes depending on the purpose of the investigation: (i) Removal of known house keeping genes: If the sequence of some house keeping genes of the organism are known, removing ESTs that originate from these genes can alleviate the problems. (ii) Adding annotated gene sequences: If a genetic sequence of an annotated gene is known, it can be used as a template for the ESTs. (iii) Seeded clustering: Known full-length transcripts can be used for 'seeded clustering', which helps to create smaller, better partitioned clusters and avoid chimeric assemblies [130].

These procedures can alleviate some of the problems, however some clusters of highly expressed genes can still contain several thousands EST sequences. Producing a consensus sequence from such a large cluster can be tricky as most assembler are not able to handle such deep clusters. Several methods have been created to deal with this problem, such as the "containment clustering" of TGICL [130], or the alignment/consensus strategy of geneDistiller [54, 56].

Other minor concerns in EST assembly are overlapping genes *eg.* they can be on opposite strand and share a UTR-tail or have common motifs. This can cause the assembly program to assign ESTs from two different genes to the same cluster [52], and will complicate analysis of the cluster.

4.4.4 Reliability of high-throughput assemblies and sequence data

Although no major comparison of assemblies generated with different HTS technologies has been published yet, preliminary analysis shows that assemblies with 454 and Solexa significantly differ from those obtained with classical sequencing reads. In a survey of assemblies for *Streptcoccus suis* from 454, Solexa and capillary data, 454 sequencing of a library with 5-fold coverage produced 5336 contigs while the Sanger method, two-fold coverage, resulted in only 1011 contigs. The length of the largest contig was 5336 for 454 and 12257 for the capillary sequencing method. Moreover, using Solexa, a ten-fold coverage was necessary to produce 8370 contigs with a maximum length of only 1687 [143]. The best results were seen for hybrid assemblies comprising data from at least two different sequencing technologies. The authors concluded that assembly methods are to be refined to address the specific shortcomings of each method [143]. As mentioned earlier, the differences are likely to be caused by very different error patterns. In the case of the Solexa technology, error rates are highly position-dependent, variable across different machines and even across different runs [115]. In a recent investigation on the quality of Solexa reads, the authors found a bias in the read coverage: significantly more reads were found in GC-rich genomic intervals. Despite the manufactures specifications for the read quality, error rates varied from 0.3% to 3.8% [144]. Compared to 454 sequences, only few insertions and deletions were found [119]. In the future a new basecalling software, *eg.* Alta-Cyclic [145], might be able to improve the quality of Solexa sequences. Additionally, it has been shown that under idealized conditions it is theoretically possible to assemble bacterial genomes (with 80x coverage of 30 nt reads) [146].

4.5 Assembly of contigs - scaffolding

While the assembly of the individual reads into contigs give some (local) information, the contigs still need to be set into the context of the whole genome. This is carried in the last phase of an assembly process: scaffolding, which is the process

where different (genomic) contigs are organized into even larger frameworks (scaffolds or super-contigs). The contigs are ordered and oriented in a consistent way, so that the scaffold build is a true representation of chromosomes, though there may still be gaps between contigs, which are dealt with in new rounds of sequencing (see finishing below).

In the scaffolding stage of an assembly, all the information usually come from other sources than the reads themselves. This information includes read-pair information, STS (Sequence Tag Sites), and other sources [147].

4.6 Finishing

When an assembly has been completed, specific parts of the assembly usually need to be reexamined, perhaps due to low quality of the data, low (or no) coverage of the sequence, sites under suspicion of misassembly, etc. The reexamination are usually dealt with in an elaborate process where manual inspection is used to analyze the ambiguous section(s) and new ways are devised to clarify the particular ambiguities.

Analysis of the assembled contigs can be performed with a number of tools. One is Consed [148], which allows navigation of the assembled contigs and reads, problematic regions can be searched for with different criteria, and regions can be tagged for further inspection. Others are Autofinish [149], BACcardi [150], and GAP4 [151], all of which has different strengths and purposes.

4.7 Genome Resequencing

Recent developments in high-throughput sequencing technologies have ignited the scientific community's imagination. Terms such as the "personal genome" or "1000$ genome" are now popular in the media [152, 153]. The growing number of publicly available reference genomes allows genome resequencing on a larger scale, as sequencing costs decrease and throughput increases [154]. However, currently even HTS only allows deep resequencing of a small number of large individual genomes [155] or of specific parts of the genome. It has been remarked that the full power of high-throughput sequencers might not be unleashed since no suitable methods to select for specific genomic subsets are available and methods for targeted amplification are more likely to be effective [97]. However, recent methods using hybrid techniques such as microarray-based genomic selection (MGS) and multiplex exon capture to narrow down the number of sequences or to focus on specific genomic locations may overcome this shortcoming [98, 97]. Thanks to the contribution of James D. Watson a first complete personal genome, sequenced using 454, was published in 2008 [156]. In this project a set of 106.5 million reads, representing 24.5 billion bases and a depth of 7.4-fold, was generated. The mapped reads in combination with 454 quality values (Q-values) were used to gather a set of 3.32 million SNPs. Several filters had to be applied to increase specificity. A read was only included if the BLAT [65] alignment (i) was spanning at least 90% of the read length, (ii) did not have alternate hits, (iii) had less than five mismatches, (iv) had less than five indels. Subsequently, the remaining 93 million reads were again realigned with PHRAP's cross_match tool. Three additional filter steps using the quality score, (see supplementary material for [156]), the ratio of the variant to total coverage (> 0.2) and the vicinity to homopolymer runs ($< 5bp$) in order to avoid false positive indels ended this complicated procedure. Finally, the authors were able to discover approximately 500 000 new putative SNPs. Additionally, approximately 2.6 million reads of novel sequence and reads with low quality alignments were assembled in 170 000 contigs spanning 48Mb. After a filter step 110000 contigs spanning 29Mb remained [69]. The authors concluded that those contigs might represent the 25Mb predicted to be absent from the current reference genome. With costs of about 1 million US$, however, the "1000$ genome" genome still seems to be a distant prospect.

Next-generation HTS has also been applied for the mapping of translocation breakpoints. HTS not only reduces the labor and time cost of traditional methods in detecting translocation breakpoints, *eg.* in situ hybridization with fluorescent dye-labeled bacterial artificial chromosome clones (BAC-FISH), but also greatly improve the resolution so that the disrupted gene can be identified by PCR cloning. Thus, mapping and sequencing breakpoints region with Solexa platform has been used to identify novel candidate genes for mental retardation [93]. Probability calculations as well as simulations suggest that current paired-end sequencing technology already provides a high probability of breakpoint detection and good resolution in localizing structural chromosomal the rearrangements [125].

5 Overview of assembly methods

Different assemblers use different information in the assembly process. Some only use sequences in fasta format and the corresponding quality values, while others can assemble without quality values. Additional information on known sequences (*eg.* genes), clones, clone sizes and the orientation of the reads (forward-reverse) might be helpful in the assembly process.

An overview of different assemblers is presented in tables 1a and 1b, which summarizes the approach each program utilizes in assembly.

[Table 1a]
[Table 1b]

5.1 Assemblers

In the following a large selection of different assemblers that have been created over time are presented. An overview with shorts presentations of the different assemblers are given on the web-page `http://genome.ku.dk/resources/assembly/methods.html`.

One of the (relatively) early assemblers is PHRAP [44], which is still in use, both in itself (for small DNA sequence sets), and as a subcomponent of WGS assemblers, *eg.* RePS [157], Phusion [50], JAZZ [158], and ATLAS [159]. Other WGS assemblers that also use some variety of the standard overlap-layout-consensus approach are, the Celera assembler [45], CAP3 [116], RAMEN [160], PCAP [161], the TIGR assembler [162], STROLL [132], and ARACHNE2 [49]. Some new approaches to assembly have been attempted, among them mira [59] and TRAP [58], which try novel ways to deal with repetitive sequences by checking the trace and quality files. An emerging approach is to use more explicit graph based programs, such as Euler [133], Partial ordered alignment (POA) [163], Velvet [121], Splicing graphs [55], ASmodeler [164], and xtract [57], where the last three are used specifically for ESTs. Other programs that analyze ESTs are TGICL [165], StackPack [13], PaCE [166], Hidden Markov Model (HMM) Sampling [167], and geneDistiller [54]. Finally, some programs are used in the scaffolding stage, where contigs are processed and put in order, *eg.* GigAssembler [168] and Bambus [147] (part of the AMOS package [46]).

5.2 Assembler Comparisons

Comparing the different assemblers is not a trivial task due to several factors. Not to mention the problems of constructing appropriate benchmark data. First the different assemblers use a variety of input data, and so comparing an assembler which uses a lot of the additional information to one which only uses a fragment of the information is inappropriate. Another aspect is evaluating the success criteria, the goal is to create a single error-free contig of each chromosome, which means that fewer gaps, longer contigs, and fewer errors are desired. However, different assemblers might do better in one area and worse in another, so weighing the performance of one assembler against another can be difficult. Still there have been a few attempts to compare assemblers.

In [132], PHRAP, TIGR Assembler, and STROLL were compared on sequence data from the bacterium *Borrelia burgdorferi*. Phusion and ARACHNE were both applied to the assembly of the Mouse genome [169, 50]. PHRAP has been compared to CAP3 in [116] (on four BAC datasets) and [76] (on EST data) where the TIGR Assembler was also included. Furthermore, a short comparison between PHRAP, Arachne, and Euler is presented in [60].

Common to these studies is that the individual performance of the assemblers depend on the data they are presented with. PHRAP is generally aggressive in joining reads and creates large contigs, though sometimes at the expense of introducing errors. This assembler would be a fairly good choice if the dataset consisted only of reads with assigned quality values. However if additional information, such as forward-reverse constraints, is available other programs (*eg.* CAP3, STROLL) would perform better. Another observation is that the performance of PHRAP degrades when it is applied to some large data sets. Additionally an updated assembler based on the Euler package [60], Euler-SR [115], is available. Euler-SR

which uses a revised version of the Euler package, is less space intensive and optimized for short Reads. Alternatives are assemblers such as Arachne or Velvet [121].

6 Applying assemblies for other analyzes

There are different possibilities for further processing of the data and thereby for finding interesting and important features for future investigation, for example searching for SNPs (Single Nucleotide Polymorphisms) and alternative splice forms, or comparing genomes with each other.

SNP detection: ESTs are the most often used data source for SNP detection, but SNPs can be found from shotgun data as well. SNPs in transcribed sequences can either be synonymous (no amino acid change), or non-synonymous (encoding a different amino acid). A variety of different computer programs are designed for SNPs analysis. Some find and predict whether a given site is polymorphic, *eg.* Polybayes [170], Polyphred [171] and novoSNP [172]. Others try to predict whether a given SNP is potentially harmful or neutral, *eg.* Polyphen [173] and SIFT [174].

Massively parallel Sequencing The new massively parallel sequencing technologies will provide a wealth of new information. As mentioned above they have already been applied for the sequencing of an individuals genome [156], and detection of genomic rearrangements [93, 125], and in the future new ways of utilizing their enormous capacity will likely appear, both with respect to the number of clones that are analyzed and the total amount of sequenced DNA.

Detection of alternative splicing: In eukaryotes, the removal of introns by splicing is a crucial step in gene expression. For some genes, splicing results in only one single type of mRNA, but studies have revealed that up to 60% of the human genes result in two or more mRNA isoforms due to alternative splicing [36, 175]. One approach to investigate alternative splicing is through assemblies of ESTs. However, assemblies of ESTs usually has multiple solutions in the presence of alternative splicing, which might end in truncated, misassembled or missing transcripts [175, 176]. Having a completed genome as a reference can help because it allows comparison of the EST to the corresponding genomic sequence. Some programs have been created which explicitly try to address the problem of assembling alternative splice variants from ESTs, among them are Splicing Graph [55] and geneDistiller [54].

Genome Comparison: Furthermore, as different sequencing project complete their respective genomes and the data become available, it becomes possible to compare differences and similarities between different species on a sequence basis. This can generate a wealth of new information, and give new insights into the evolution and biology of living organisms. Examples of how such a comparative analysis can be performed are given in [177, 67, 62].

7 Discussion

As still more genomes are studied and more sophisticated computer programs for genome assembly and analysis are developed, our knowledge of genomics will expand tremendously. Sequencing technologies have already given us a consensus sequence of *homo sapiens*, and in the future we can expect that many individual human genomes will be sequenced , which will add to the steadily growing number of genetic variations and genetic predisposition to disease that has been revealed in our specie. Furthermore, many model organisms and eventually, all species remain to be sequenced, which will give a better understanding of life and its evolution.

For mammalian genomes whole genome shotgun sequencing is likely to entail similar costs for producing a finished sequence as a hierarchical shotgun solution. The hierarchical approach has a higher initial cost than the whole-genome approach, owing to the need to create a map of clones (about 1% of the total cost of sequencing) and to identify sequence overlap between clones. On the other hand, the whole-genome approach is likely to require much greater work and expense in the final stage of the assembly, because of the challenge of resolving misassemblies.

New high-throughput sequencing technologies have rapidly emerged. However, the sequencing methods as well as the computational tools have to be further improved, to allow a complete de novo assembly for large genomes with these technologies. However, today only little data on the error models of different massively parallel sequencing technologies is available. These error models are crucial to interpret and analyze the sequence data correctly [144]. When it comes to

de novo assembly, the short read lengths of SOLiD and Solexa methodologies seem to be a momentous disadvantage and the high number of reads produced might not be able to compensate for this handicap. However, all manufacturers aim to increase the read lengths. Currently, a reasonable approach to the assembly of such short sequences could include data from low coverage Sanger sequencing. Although hybrid data set approaches are cumbersome [115], they have already been shown to produce useful assemblies [124].

The choice of sequencing strategy should also be influenced by the goal of the project. In some organisms it might be desirable to quickly generate a few contigs covering key points in the genome, while in others a broader strategy might apply. Still other projects combine whole–genome with hierarchical shotgun in a hybrid approach trying to utilize the strengths of each [159].

Other applications of sequencing and assembly are continuously being explored. For example, the growing field of environmental sequencing (or metagenomics) [178, 179, 180], will undoubtedly present new challenges to assemblers, since sequence data will no longer be known to come from a single source organism, but from several and often from a multitude of distinct organisms, with different relative abundances, different genome structures, repeat content, and so on. A somewhat related field is paleogenomics – is sequencing of fossil DNA. This field has become much more accessible with the new massively parallel sequencing methods, as the traditional Sanger sequencing is difficult and technical impractical on fossil DNA samples. The new techniques, however, have made it possible to extract genomic information from long extinct species, for example the woolly mammoth [181].

The assemblers presented in this paper show the great diversity and ingenuity that has gone into finding better ways of assembling the DNA puzzle from diverse types of data. The various strategies for overcoming the challenges revealed in assembly are also discussed. Newer assemblers (and associated programs) endeavor to surmount these challenges in novel ways, and it is likely that computational whole genome assembly will be further refined in the future. Also, it should be remembered, that a substantial fraction of the large genomes still evades sequencing/assembly with existing technology [69]. The estimated $\sim$10% of the human genome which has not been sequenced may not be without function, as exemplified by the centromeres and pericentric heterochromatic regions. Many of the tandem repeats within these regions have been sequenced at clone scale, but none have been sequenced at genome-scale, where their size exceeding many megabases preclude assembly. Why the remaining >250 smaller gaps, scattered over the euchromatic part of the human genome, with sizes ranging from 20 to 100 kb, cannot be sequenced/assembled is unknown. It is likely that this terra incognita will only be sequenced when (if) single molecule, very long read sequencing technologies have been developed.

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Figures

Figure 1 – Timeline

Figure 1: Figure showing the major breakthroughs in sequencing. The year of the different milestones is chosen to be the publication year of the first article that presented the method. Software publications are marked in cursive. On the left, the size of GenBank (in deposited basepairs) is shown, with the length and width of the bars representing the size on a logarithmic scale.

Figure 2 – Sequencing vector

Figure 2: Figure showing a schematic drawing of a sequencing vector, such as a BAC (Bacterial Artificial Chromosome). The insert can be a genomic fragment, or an cDNA (for EST sequencing). In both cases sequencing from each end will produce a read pair that can provide additional information for assemblers.

Figure 3 – Sequencing methods

Figure 3: Schematic drawing of the four different sequencing procedures. (a) Hierarchical shotgun, where a BAC clone map (tilling map) covering the genome is first created after which the BACs are sequenced. (a) Whole Genome Shotgun, where the genome is randomly split into smaller parts and sequenced. (c) EST sequencing, where mRNA is extracted from tissue and then sequenced. (d) Massively parallel sequencing where short sequence fragments are aligned to a reference genome.

Figure 4 – Repeat Contig

Figure 4: Schematic drawing of a cluster contain a likely repeat. The region on the right is covered by many more reads than would be expected by chance, and is therefore potentially a repeat region, which could be masked.

Figure 5 – Assembly pipeline

Figure 5: Figure showing the typical pipeline of a sequencing project. Sequenced reads are generated, after which they are cleaned and assembled. Following the assembly annotation and analysis can be performed.The grey line show the pipeline for massively parallel sequencing where the reads are mapped to a reference genome, while the full pipeline is for de novo sequencing ans assembly. Part of the figure is adapted from [182]

Figure 6 – Assembly example

Figure 6: Figure showing an examples of an assembled (EST) contig (cluster). The thick line at the top represents the consensus sequence produced by the applied assembler ([131]). The blowup shows a putative SNP present in the sequences. The colored stretches mark specific tri-nucleotides, 'ATG' is green and 'TAA' is red, and are drawn to show the structure of the assembly.

Figure 7 – Graph example

Figure 7: Figure showing an examples of how a graph is constructed. Two reads are mapped onto the different k-mer nodes ($k = 6$ in this example), and edges between the nodes are determined by the reads. The presence of a nucleotide difference (*eg.* sequencing error, SNP, *etc*.) between the two reads cause the graph to split up, thus causing an ambiguity in the sequence.

Fig. 1

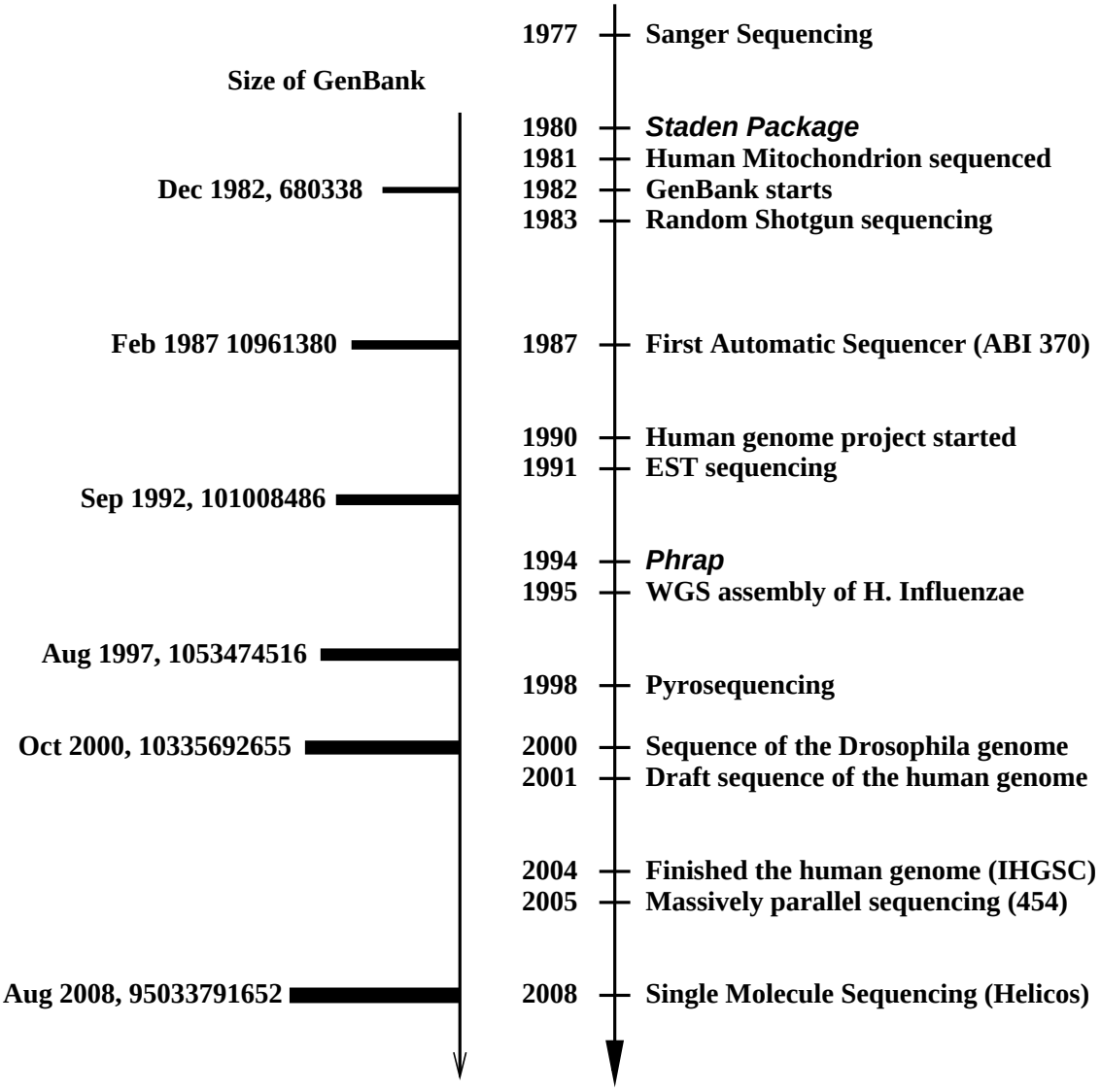
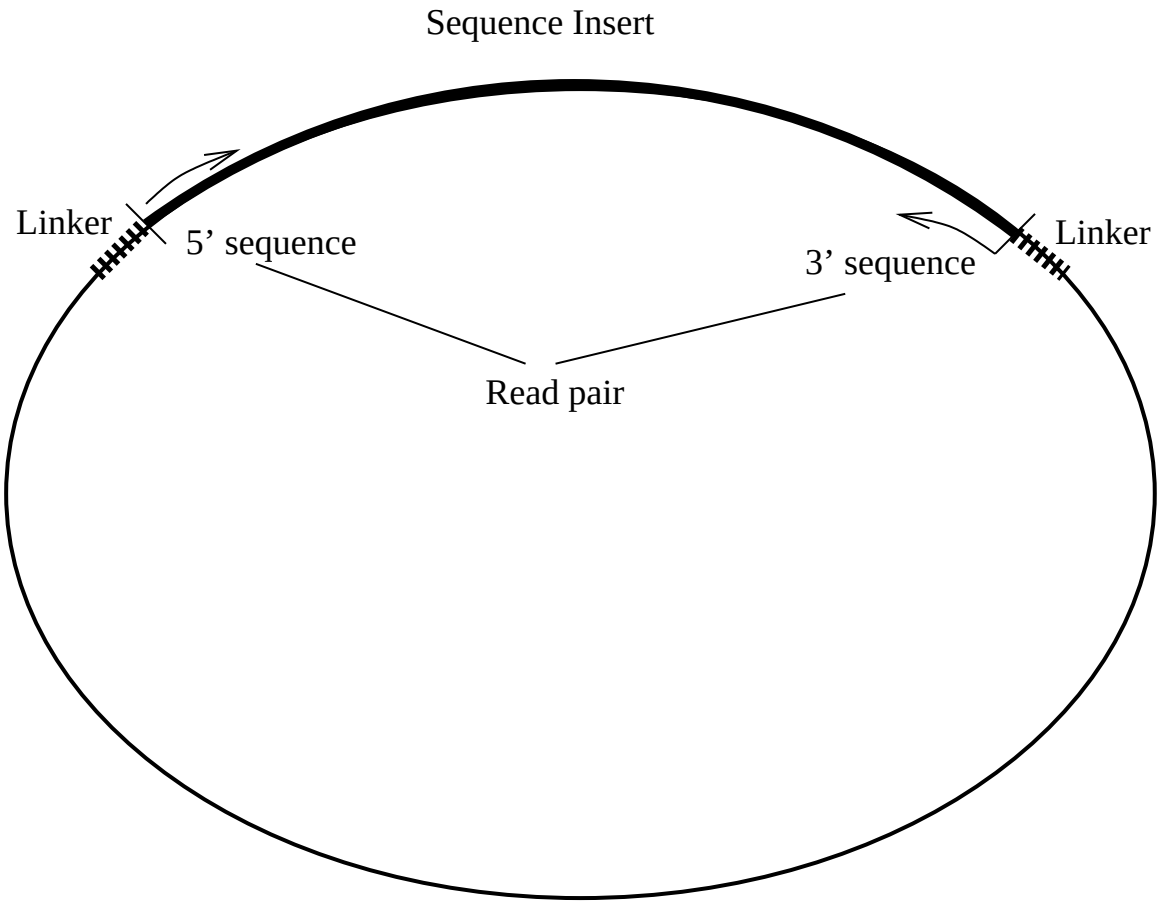


Fig. 2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Linker
5’ sequence
3’ sequence
Linker
Read pair

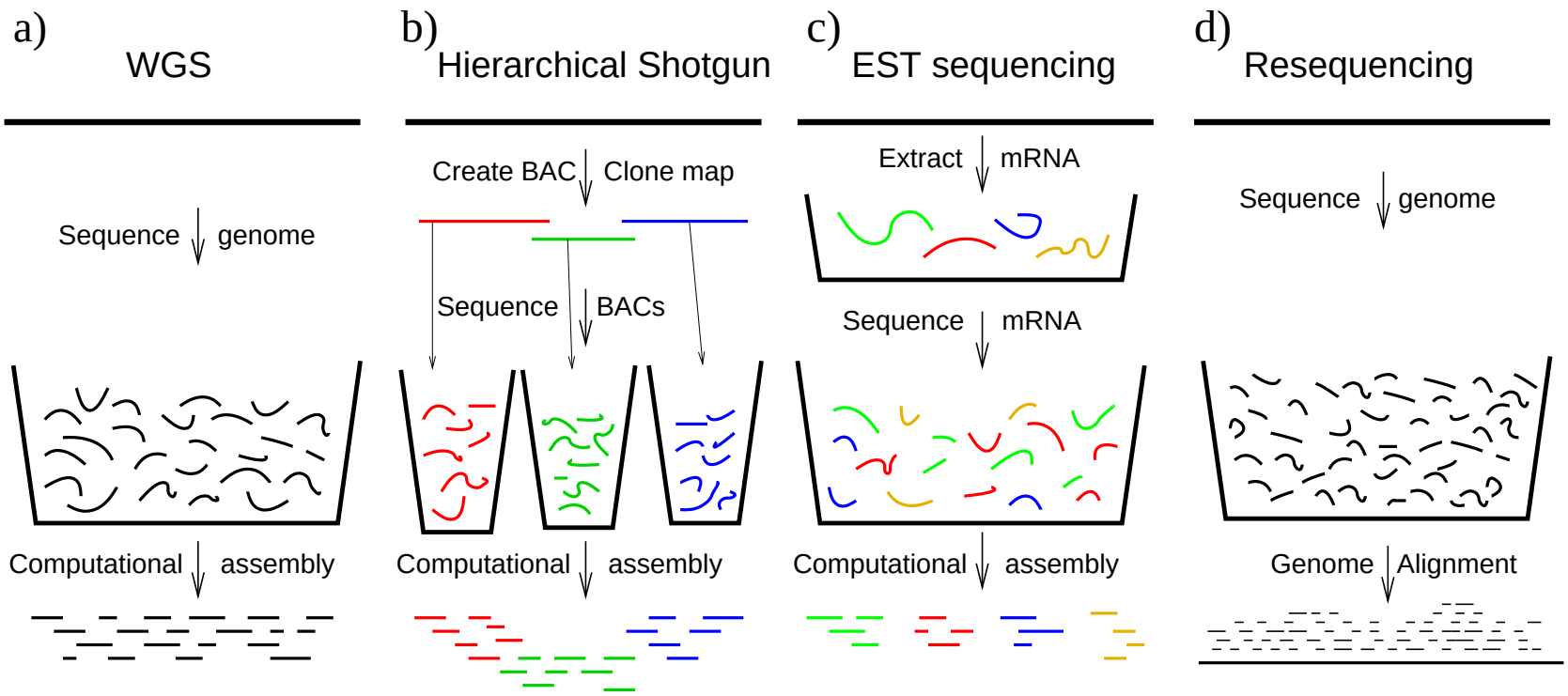

a)
WGS
Sequence
genome
Computational
assembly
b)
Hierarchical Shotgun
Create BAC
Clone map
Sequence
BACs
Computational
assembly
c)
EST sequencing
Extract
mRNA
Sequence
mRNA
Computational
assembly
d)
Resequencing
Sequence
genome
Genome
Alignment

Fig. 3

Fig. 4

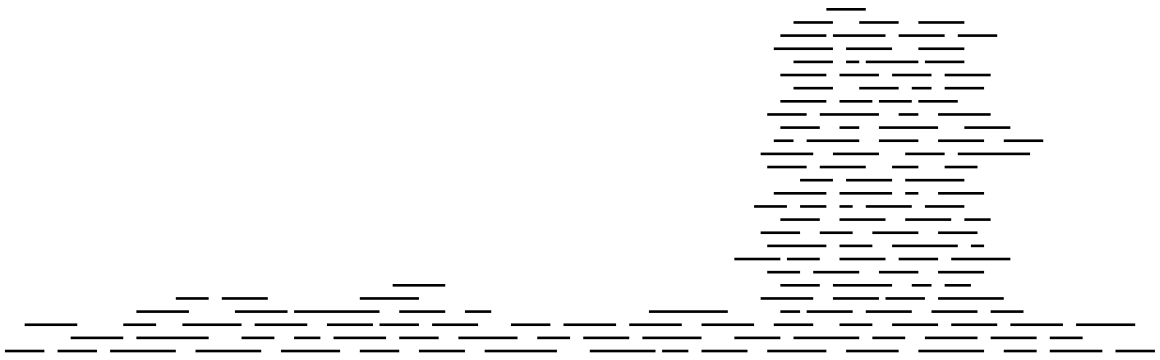
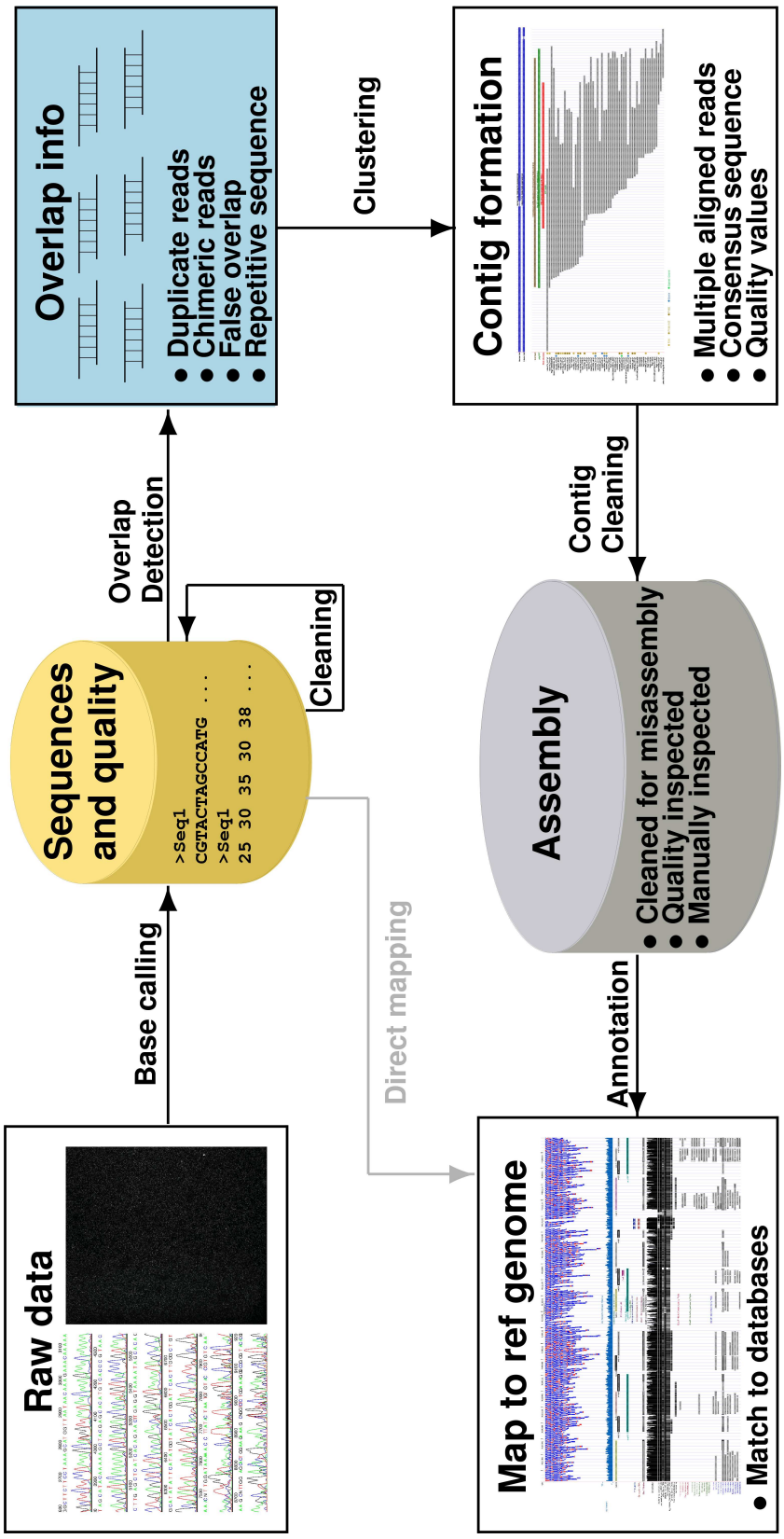


Fig. 5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Cleaning
Overlap Detection
Overlap info
• Duplicate reads
• Chimeric reads
• False overlap
• Repetitive sequence
Clustering
Contig formation
• Multiple aligned reads
• Consensus sequence
• Quality values
Contig Cleaning
Assembly
• Cleaned for misassembly
• Quality inspected
• Manually inspected
Annotation
Direct mapping
Map to ref genome
• Match to databases

Fig. 6

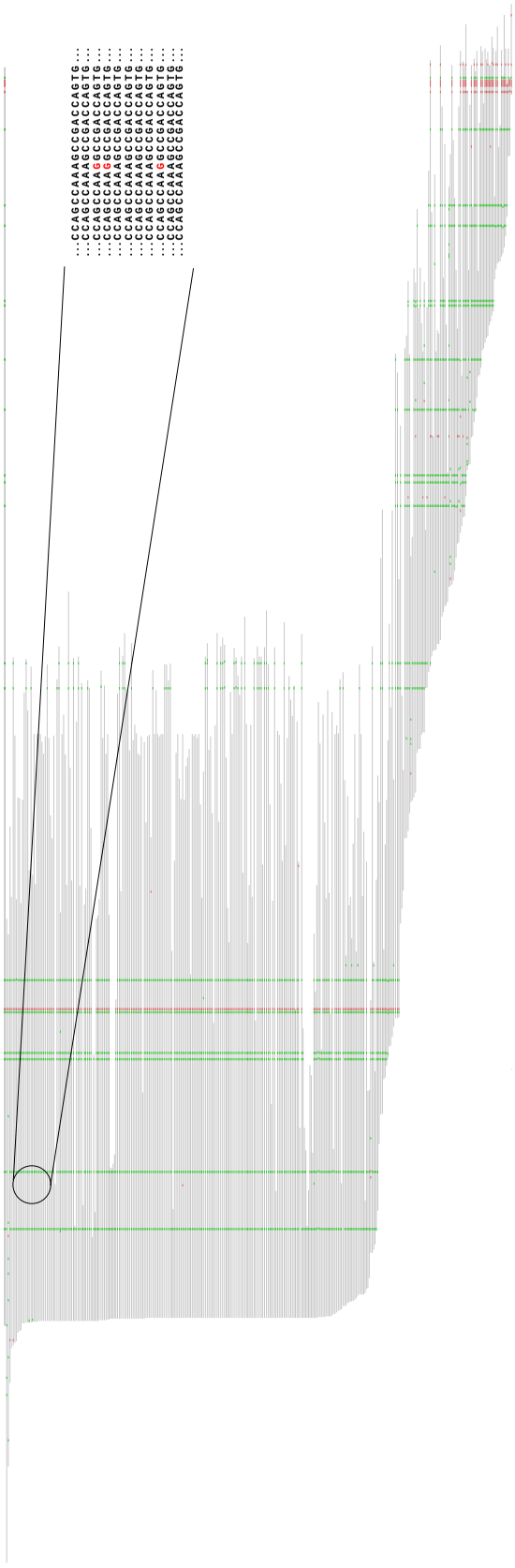
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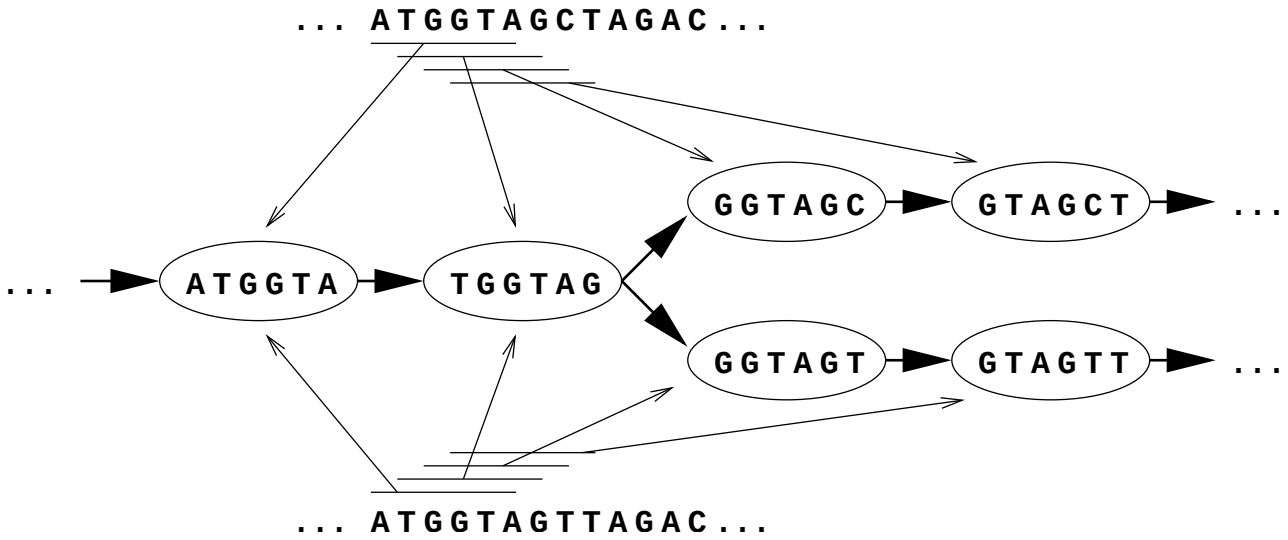
Fig. 7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Tables

Table 1a – Assemblers used primarily for shotgun data.

Assembler	Computational dependencies	Additional Information	Common Features	Reference
Phusion	RPPHRAP	PR, BAC,Q_r	P, R, K	[50]
JAZZ	banded SW, malign, PHRAP	Q_r	K	[158]
RePS	BLAST/PHRAP	PR	R, K	[157]
ARACHNE2	SW	Q_r, PR	K	[49] [183]
GigAssembler	psLayout	PR, BAC, EST, Q_r	P, R	[168]
Celera assembler	BLAST-like	PR	P	[45]
Euler	graph-based	PR	R	[133]
CAP3	banded SW	Q_r, PR	P	[116]
GAP4	CAP3, PHRAP or FAKII	Q, PR		[151]
RAMEN	banded SW	Q_r	R	[160]
ATLAS	PHRAP, banded SW	Q_r	R, K	[159]
PCAP	CAP3, banded SW		P, R	[161]
Bambus	-	contigs	P	[147]
TRAP	mod SW	Q_r	R, K	[58]
PHRAP	banded SW	Q		[44]
TIGR Assembler	mod SW	Q	R	[162]
STROLL	banded SW	Q		[132]
mira	banded SW	Q_r	R	[59]
ALLPATHS	graph-based	PR		[146]
SHARCGS	contig elongation			[184]
Velvet	graph-based	PR		[121]
SSAKE	contig elongation			[185]

Table 1a: Overview of different assembly programs (including scaffolders), some of the programs have also been used to assemble EST sequences. The additional information shows the information which a given assembler can use, besides read information. **PR**: Paired Reads information, **BAC**: Bacterial artificial Chromosome data, **Q**: quality data, **Q_r**: Quality data and trimming reads without sufficient quality. Common features are features that the assembler shares with other assemblers: **P**: Process can be run on parallel computers, **R**: Handles repeats, **K**: K-mer approach to find potential overlaps. The last four programs listed are designed primarily for short read assembly.

Table 1b – “Assemblers” designed for ESTs

Program	Computational dependencies	Additional Information	Common Features	Reference
TGICL	megablast/CAP3	known genes, Q_r	P	[141]
StackPack	PHRAP	Q_r		[13]
PaCE	Suffix tree		R	[166]
Splicing graphs	graph-based			[55]
ASmodeler	Directed acyclic graph	mRNA, EST protein sequences		[164]
HB-algorithm	HB-algorithm	EST		[186]
geneDistiller	megablast	Q_r		[54]
xtract	graph-based	Q_r		[57]

Table 1b: Overview of the programs designed for clustering, analysis and assembly of EST data. See table 1a for abbreviations.