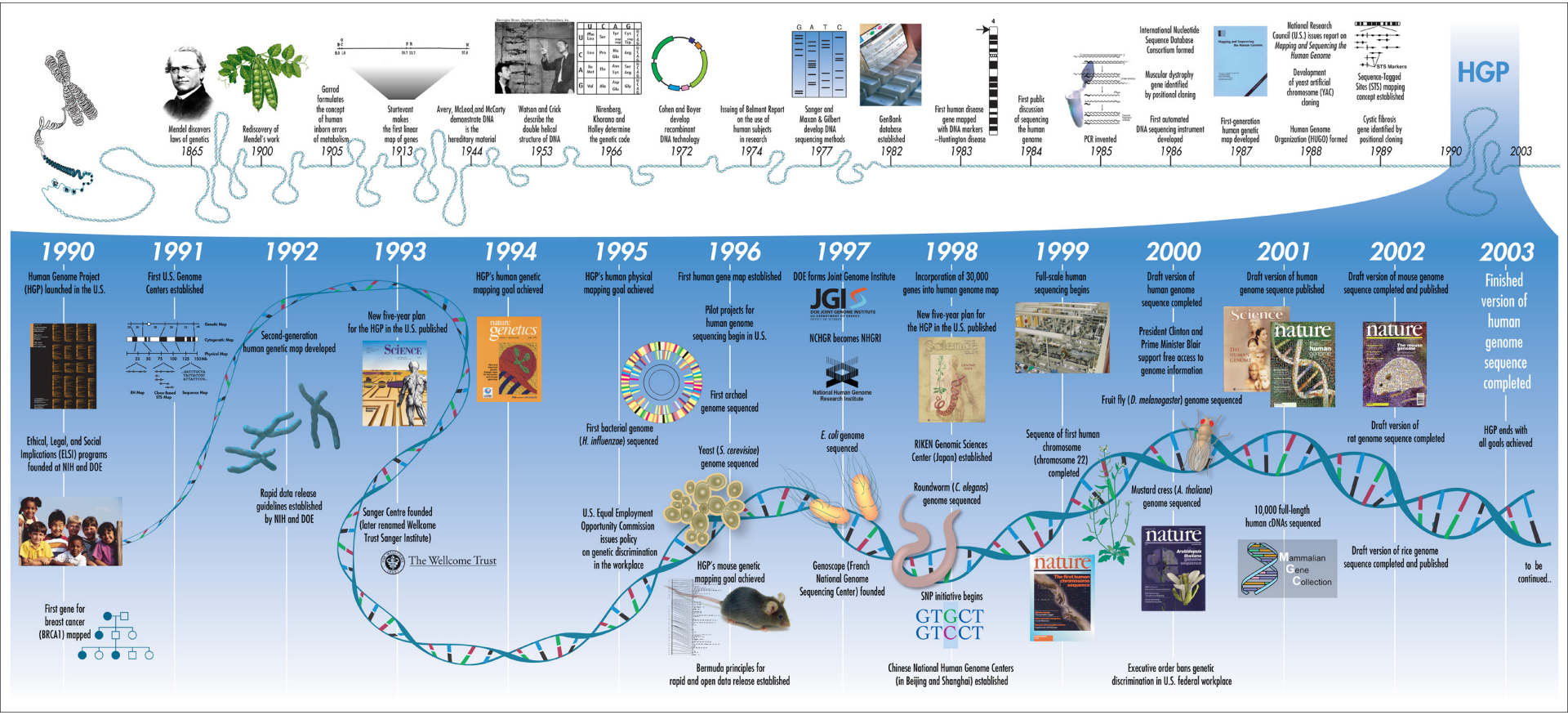
De novo Genome Assembly

Qi Zhou
Doris Bachtrog lab
CGRL seminar
zhouqi@berkeley.edu



10 years, billions of dollars, scientists from all over the world

Platform summary

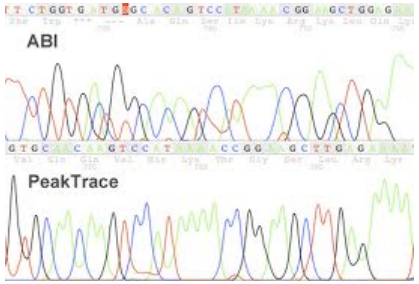
	Amplification	Sequencing	Read	Gbp/day	Cost $/Mb	Status
Sanger	cloning	synthesis	1 kb	0.006	~500	Standard
454	emPCR	synthesis (multi-nt)	450 bp	0.5	~20	Standard
Solexa	PCR on slide	synthesis (single-nt)	2 x 100 bp	25	~0.5	Standard
SOLiD	emPCR	ligation	2 x 50 bp	10	~0.5	Standard
Ion Torrent	emPCR	semiconductor	100 bp	0.1?	~5?	Market 2011
PacBio	none	synthesis (real-time)	>10 kb	>100?	NA	First results

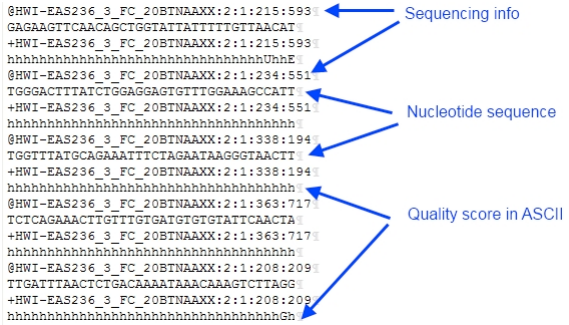
Characteristics of major NGS platforms as of February 2012

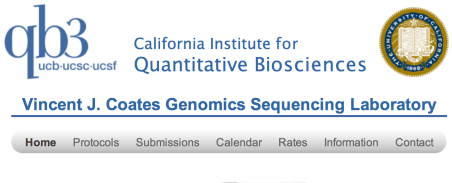
	Platform	Read length (bp)	Throughput & time per run	Technique	Dominan
	HiSeq 2000[a]	36 50 100	105–600 Gb 2–11 days	Reversible terminator	Substitutic
ystems	5500 SOLiD™ System[b]	356 075	7–9 Gb/day	Sequencing by ligation	–
enomics		35	–	Ligation based	–
ciences	HeliScope SMS[c]	25–55	21–35 Gb –	**Single molecule sequencing**	Insertion
nces	GS FLX Titanium XL+[d]	≤1000	700 Mb 23 h	Sequencing by **synthesis**	Insertion
	Ion PGM Sequencer 318[e]	>200	>1 Gb 2 h	Ion semiconductor sequencing	Insertion
iences	PacBio RS	1 k–10 k	–	**Single molecule sequencing**	Insertion

: Performance and Specifications. http://www.illumina.com/systems/hiseq_2000/performance_specifications.ilmn (11 Febru
d). [b]5500 SOLiD™ System: Specifications. http://media.invitrogen.com.edgesuite.net/solid/pdf/COI8235-5500-Series-
ruary 2012, date last accessed). [c]tSMS™ Performance. http://www.helicosbio.com/Technology/TrueSingleMolecu
erformance/tabid/151/Default.aspx (11 February 2012, date last accessed). [d]GS FLX Titanium XL+. http://my454.com/pr
x.asp (11 February 2012, date last accessed). [e]Ion Personal Genome Machine™ Sequencer: Performance. http://www.io
how-does-it-perform/ (11 February 2012, date last accessed).

From Yang et al. 2012 Briefings in Bioinformatics







Reads

Assembler

Genome

```
>gi|15611071|ref|NC_000921.1| Helicobacter pylori J99, complete genome
AGTGATTAGTGATTACAGCATCATTTTTTAAATTTAGGCATAAAACGCCCTTAAATCAAGGGTTTTTGAG
CGAGCTTTTTGCTCAAAGAATCCAAGATAGCGTTTAAAAATTTAGGGGTGTTAGGCTCAGCGTAGAGTTT
GCCAAGCTCTATGCATTCATTGATGATGATAGGGTTTTGTGTGGGCGTGAAGCCAATTTCATACGCTCCT
AAGCGTAAAATCGCCTTTTCCATGCTCCCTAATCGCTTGAAATCCCAGTCTTTTAAATGCGGTTCGATGA
GAGCGTCAATCTCATTGATTTTTTCTAACACGCCATTAAAAAGGCTTAAAGCGAAAGCGAGCTGGTTGTT
TTTAATCTTTTTTTCTTCTAACATGCTGGAAGCGATTTTTTTAATTTCTTCATTACCGCTCTCAAACGCA
TACAACAATTCAACCACAGCCCCCCTGGCTTGAGTTCGTGTCGCCATTTTAATCCTTGAGAGTTTGGCAC
AAGCTCAATAATTCAATGAGGGTGCTCATCGCTTCAAAGCCCTTATTACCGGCTTTACTGCCCGCTCTTT
CAATCGCTTGTTCAATATTGTCTGTGGTCAGCACGCCAAAGCTTACCGGCATGCTGTATTTTAGCATCGC
ACTAGCAATGCCCTTAGTCGCTTCCGCGCTCACGTAGTCAAAATGCGGAGTCCCCCCTCTAATGATCGCT
CCTAAAACGCACACGCCATCGTATTTTCCGCTCTCTAACAATTTGTCTAAAATCAAAGGCAATTCATAAG
CCCCAGGCACTAGCACGAGATCTAAAAGCTCCTCATCGCCCCCATGCCTTTTAAAGCAATCCATCGCCCC
TTCTTTTAATCTGTCTGTGATGATATGGTTGAAGCGCGATGTTAAAATAGCGATTTTTTCATTCCCTTGT
AATTGCAATTTCCCTTCTATGATTTGCATGAAATTCCTTTAAAATAAATTTTGGATTTTTAACATGTCGG
TTACTAAGTGTTCTAGCTCGTCAGGTTTTAGCATGTTTGCTCCATCGCTTAGGGCGTTTTTAGGATCAAT
ATGCGTTTCAGCGAACAGCCCATCAATCCCCACCGCCGCCGCCGCTCTGGGTAAAATAGGGGGAAAAGAG
```

Get low amount (1ug) of DNA sample of your interest

Library prep (1~2 days), Quality check

Sequencing 2~3 weeks

assembly:1 week or longer

1 months or so, get a genome

How to get a good genome in a cost-effective way?

- What do you mean by 'good genome'?

  one chromosome one sequence?

- How much shall I sequence?

  ~30 fold illumina data, well it also depends on the assembler you want to use

- Platform to use?
- Data quality
- Assembler to choose
- A reference genome available?

- What kind of data are you getting?
- Read quality and error correction
- Assembly
- How to assess the draft genome
- Mapping and chromosomal build
- SNP calling, RNA-seq analysis etc.

- 454/Sanger : longer reads, more accurate at repeat region; cost much more

Overlap-layout-consensus:

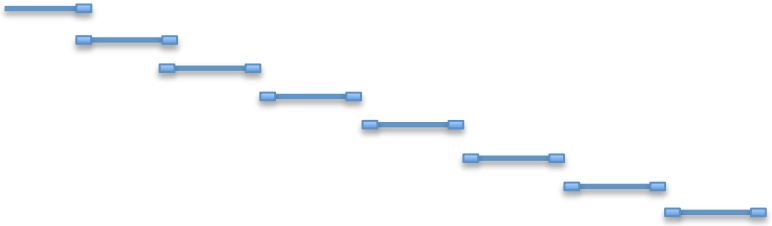
Phrap/Phred, Lasergene suite, Newbler, CABOG..

- Illumina : time/money cost-efficient

de brujin/string graph:

SOAPdenovo, allpaths-lg, SGA etc.
Seqwiki : a list of softwares

Sanger/454: overlap-layout-consensus algorism
1 run of 454 reads: 500,000 reads → $2.5*10^{11}$ pair-wised alignments



Illumina:
1 run of Hi-seq 100bp PE: 30Gb data: ~$2500: $0.08/Mb

PacBio RSII: 4.5kb long on average, ~200Mb data

Any application. Any study. Any budget.

HiSeq 2500/1500

Two run modes. High output or rapid run.

Flexibility to batch process multiple samples with high output in a single run, or get rapid results with fewer samples for time-critical studies.

	HiSeq 2500		HiSeq 1500	
Run Mode	High Output	Rapid Run*	High Output	Rapid Run*
Output (2 × 100 bp)	600 Gb	120 Gb	300 Gb	60 Gb
Run Time (2 × 100 bp)	~11 days	~27 hours	~8.5 days	~27 hours
Cluster Generation	cBot	On board	cBot	On board
Paired-end Reads	6 Billion	1.2 Billion	3 Billion	600 Million
Single Reads	3 Billion	600 Million	1.5 Billion	300 Million
Maximum Read Length**	2 × 100 bp	2 × 150 bp	2 × 100 bp	2 × 150 bp
Bases Above Q30***	> 85% (2 x 50 bp) > 80% (2 x 100 bp)			

HiSeq 2000/1000

One run mode. High output.

Generate the highest output and number of reads for batch processing multiple samples in a single run.

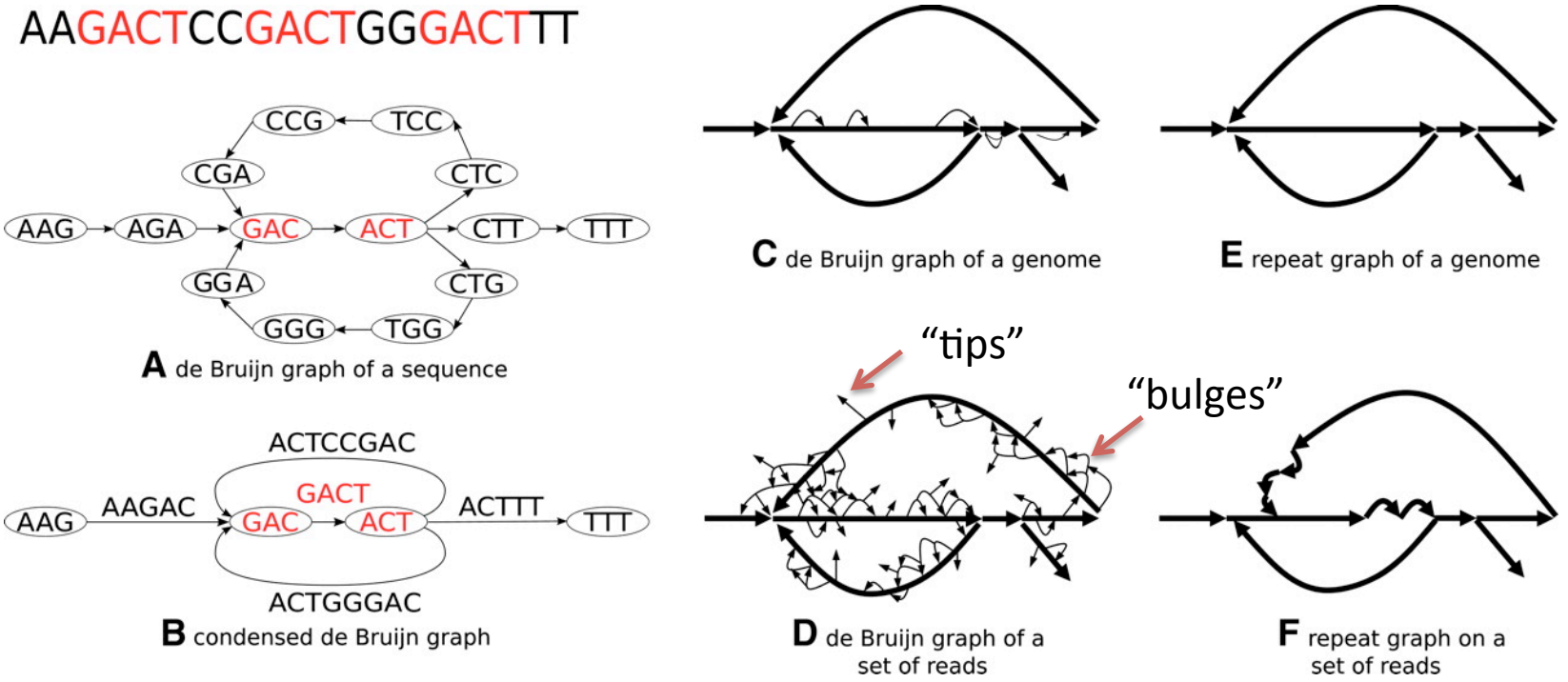
	HiSeq 2000	HiSeq 1000
Output (2 × 100 bp)	600 Gb	300 Gb
Run Time (2 × 100 bp)	~11 days	~8.5 days
Cluster Generation	cBot	cBot
Paired-end Reads	6 Billion	3 Billion
Single Reads	3 Billion	1.5 Billion
Maximum Read Length**	2 × 100 bp	2 × 100 bp
Bases Above Q30***	> 85% (2 x 50 bp) > 80% (2 x 100 bp)	

Table 1 Price comparison of benchtop instruments and sequencing runs

Platform	List price	Approximate cost per run	Minimum throughput (read length)	Run time	Cost/Mb	Mb/h
454 GS Junior	$108,000	$1,100	35 Mb (400 bases)	8 h	$31	4.4
Ion Torrent PGM						
(314 chip)	$80,490[a,b]	$225[c]	10 Mb (100 bases)	3 h	$22.5	3.3
(316 chip)		$425	100 Mb[d] (100 bases)	3 h	$4.25	33.3
(318 chip)		$625	1,000 Mb (100 bases)	3 h	$0.63	333.3
MiSeq	$125,000	$750	1,500 Mb (2 × 150 bases)	27 h	$0.5	55.5

Solution?

Break the trillions of reads into zillions of even shorter fragments (kmer)!

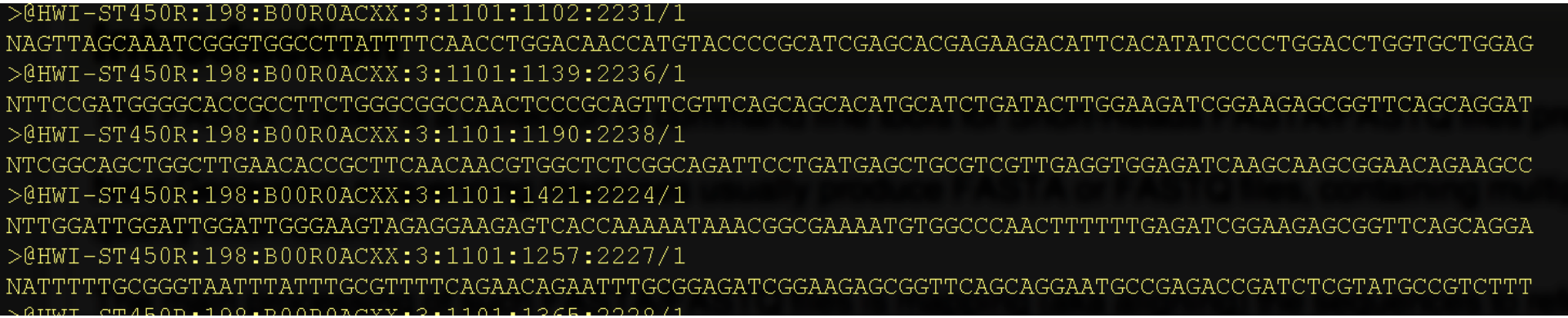


Mark J. Chaisson et al. Genome Res 2009

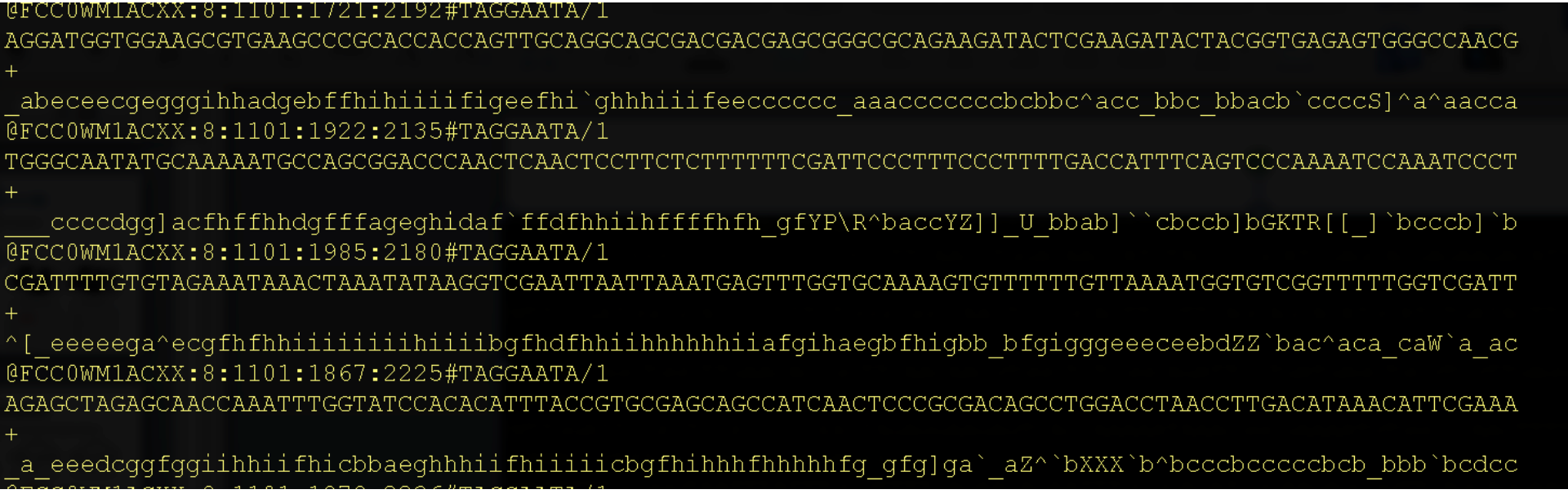
- Error correction: most of the time we're dealing with a diploid genome
- Discriminate between sequencing errors and polymorphism
- Repeat resolution: insert-size of PE library

- What kind of data are you getting?
- Read quality and error correction
- Assembly
- How to assess the draft genome
- Mapping and chromosomal build
- SNP calling, RNA-seq analysis etc.

Fasta format



Fastq format



@FCC0WM1ACXX:8:1101:1922:2135#TAGGAATA/1
TGGGCAATATGCAAAAATGCCAGCGGACCCAACTCAACTCCTTCTCTTTTTTCGATTCCCTTTCCCTTTTGACCATTTCAGTCCCAAAATCCAAATCCCT
+
___ccccdgg]acfhffhhdgfffageghidaf`ffdfhhiihfffffhfh_gfYP\R^baccYZ]]_U_bbab]``cbccb]bGKTR[[_]`bcccb]`b

- FCC0WM1ACXX: instrument name
- 8: run id
- 1101: flowcell id
- 1922:'x'-coordinate of the cluster within the tile
- 2135:'y'-coordinate of the cluster within the tile
- #TAGGAATA: index sequences
- /1: first end of the mate pair

Sequences

+

Quality vs. mapping quality→ An explanation here

$$Q = -10\log_{10}(e)$$

The Relationship Between Quality Score and Base Call Accuracy

Quality Score	Probability of Incorrect Base Call	Inferred Base Call Accuracy
10 (Q10)	1 in 10	90%
20 (Q20)	1 in 100	99%
30 (Q30)	1 in 1000	99.9%

References

1. Ewing B, Hillier L, Wendl MC, Green P. (1998): Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 8(3):175-185
2. Ewing B, Green P. (1998): Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8(3):186-194

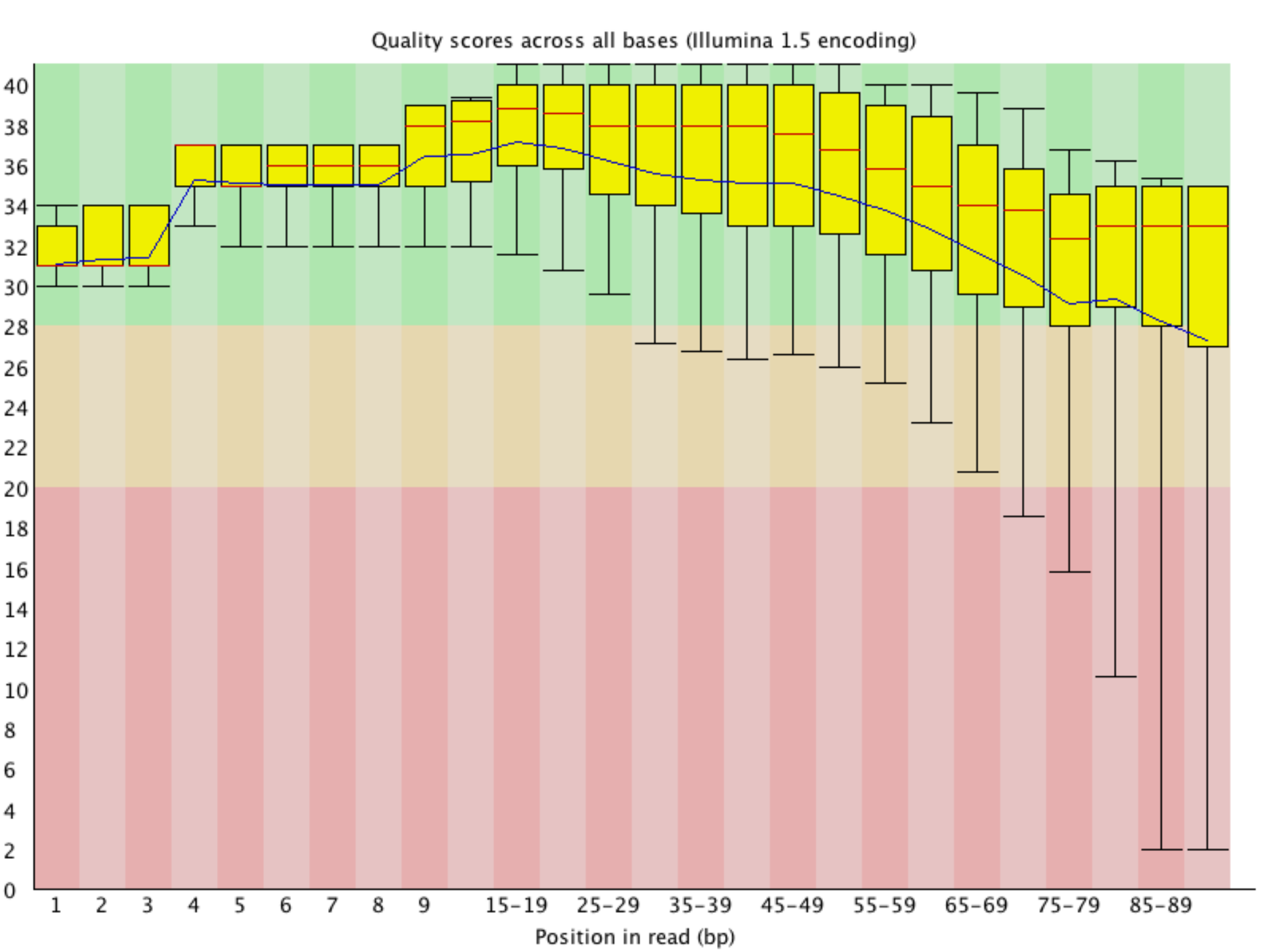
1. Each base pair has a quality score
2. mapping quality/calibrated quality is more accurate than the raw quality

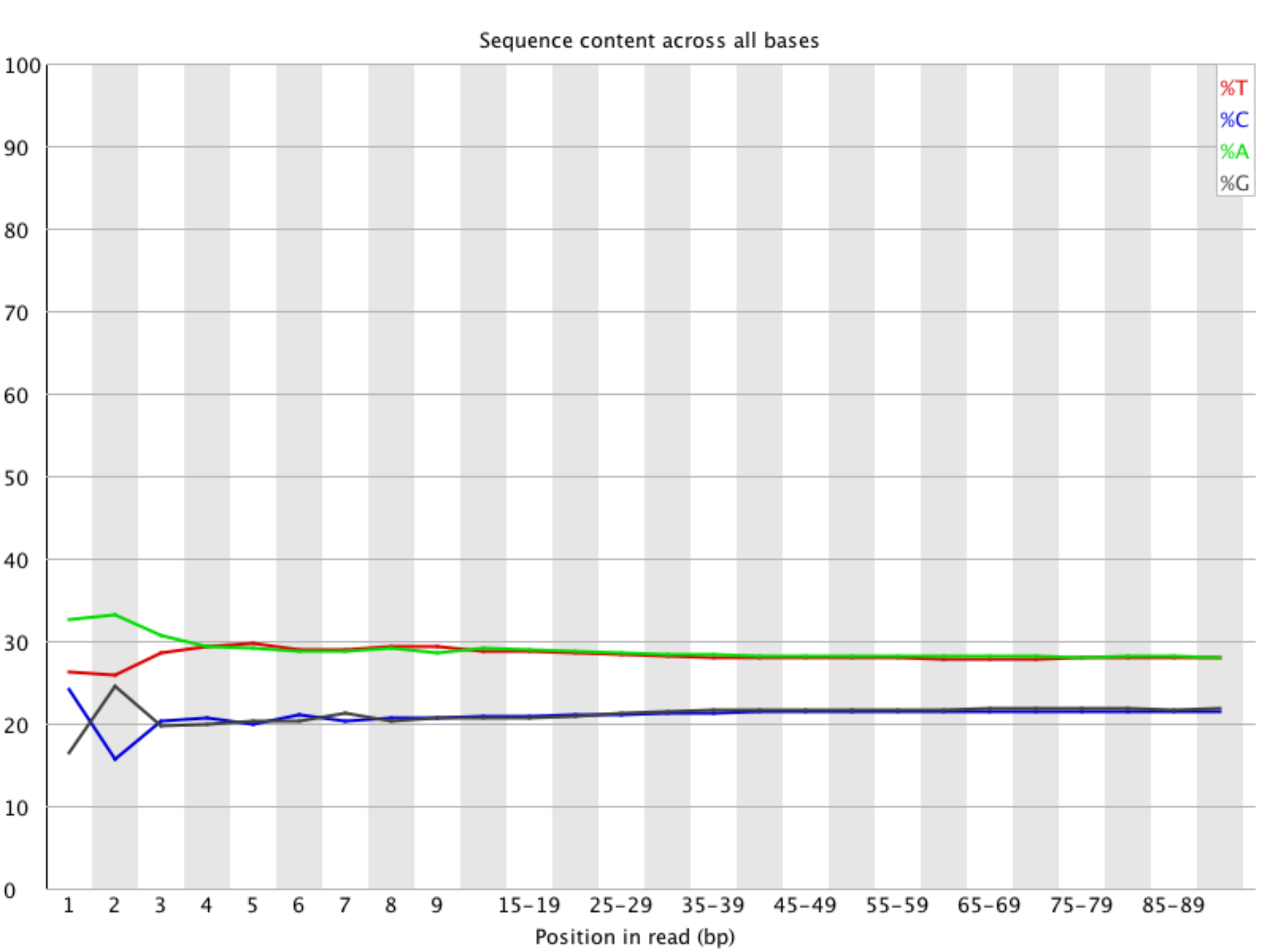
How to assess the read quality?

FastQC

Function	A quality control tool for high throughput sequence data.
Language	Java
Requirements	A suitable Java Runtime Environment The Picard BAM/SAM Libraries (included in download)
Code Maturity	Stable. Mature code, but feedback is appreciated.
Code Released	Yes, under GPL v3 or later.
Initial Contact	Simon Andrews

Download Now


Quality scores across all bases (Illumina 1.5 encoding)
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
15-19
25-29
35-39
45-49
55-59
65-69
75-79
85-89
Position in read (bp)


Sequence content across all bases
100
90
80
70
60
50
40
30
20
10
0
%T
%C
%A
%G
1
2
3
4
5
6
7
8
9
15-19
25-29
35-39
45-49
55-59
65-69
75-79
85-89
Position in read (bp)

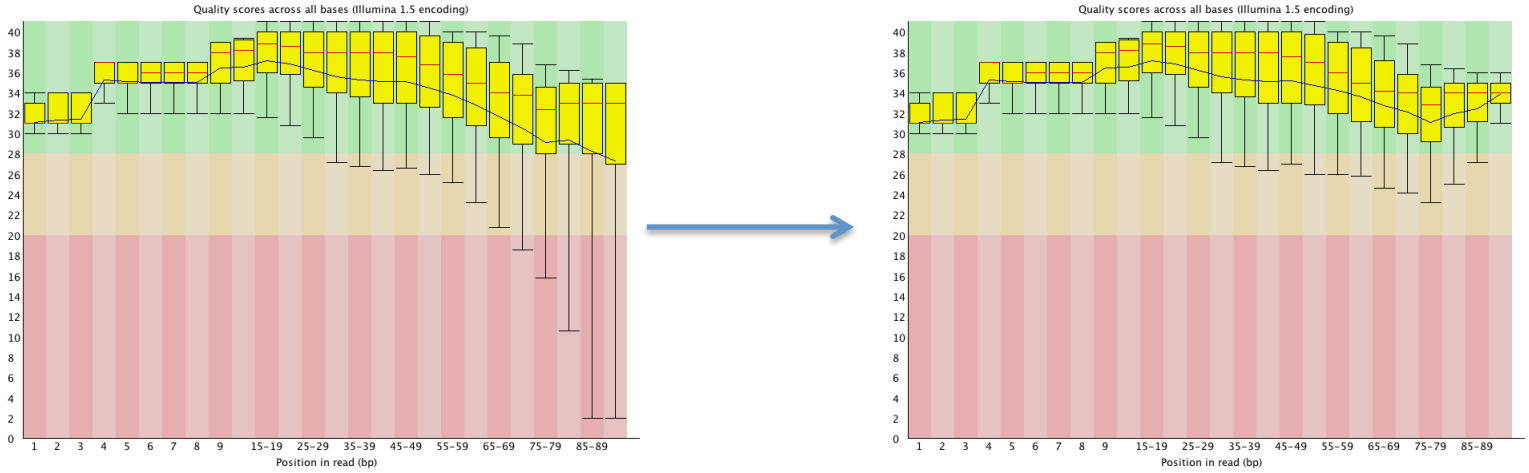
- There are a couple of reasons you may have bad quality reads:

1) Bad quality of DNA/RNA template
2) Something goes wrong with the sequencing reagent
3) Adapter contamination

- Base composition is more informative than base quality: You'd sequence adapter sequences to very high base quality…

- Chop off those base pairs with abnormal base compositions

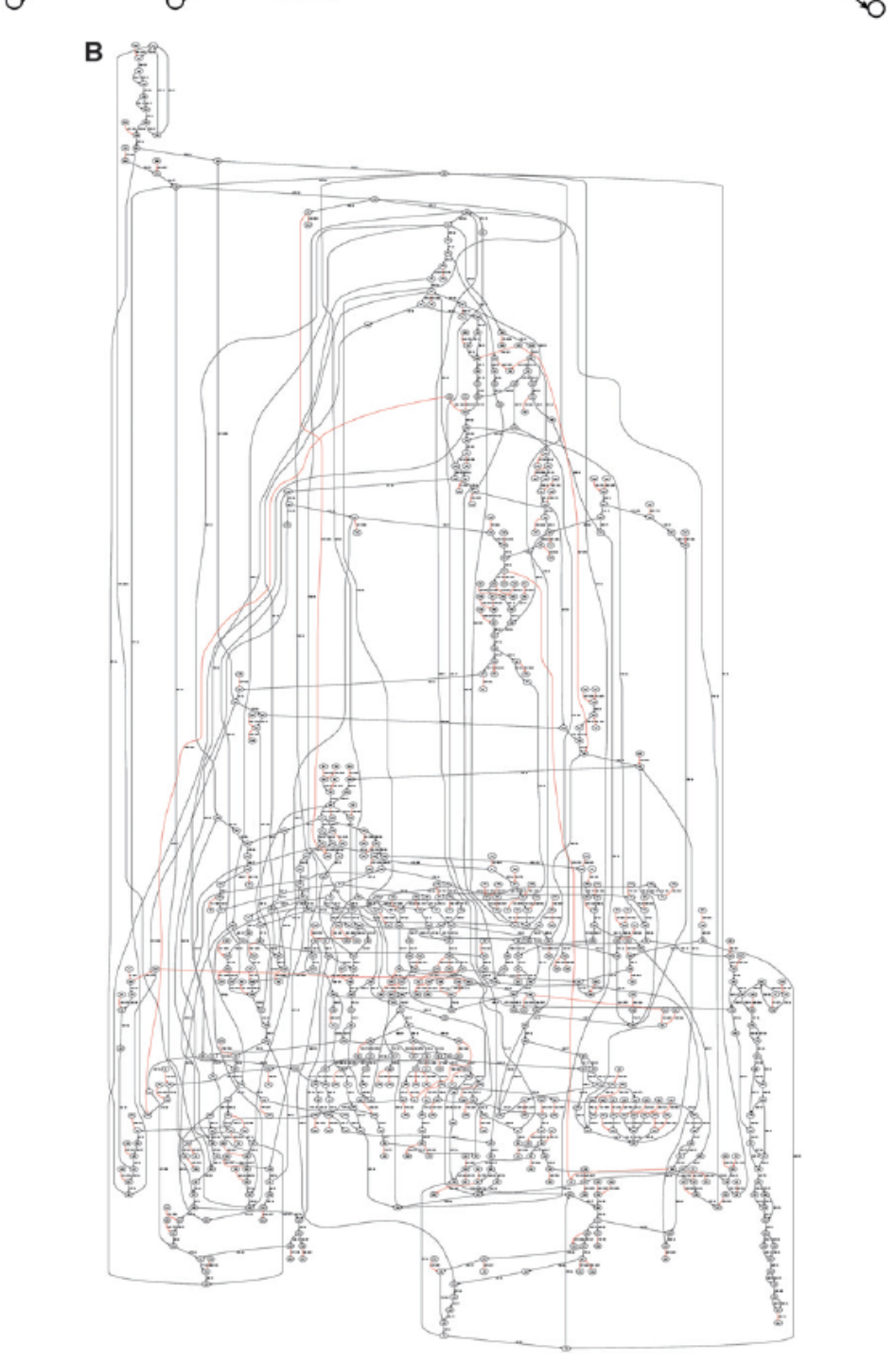
./fastq_quality_trimmer -t 30 -l 30 -i ../test_data/test.fq1 -o test.qualtrim.fq1



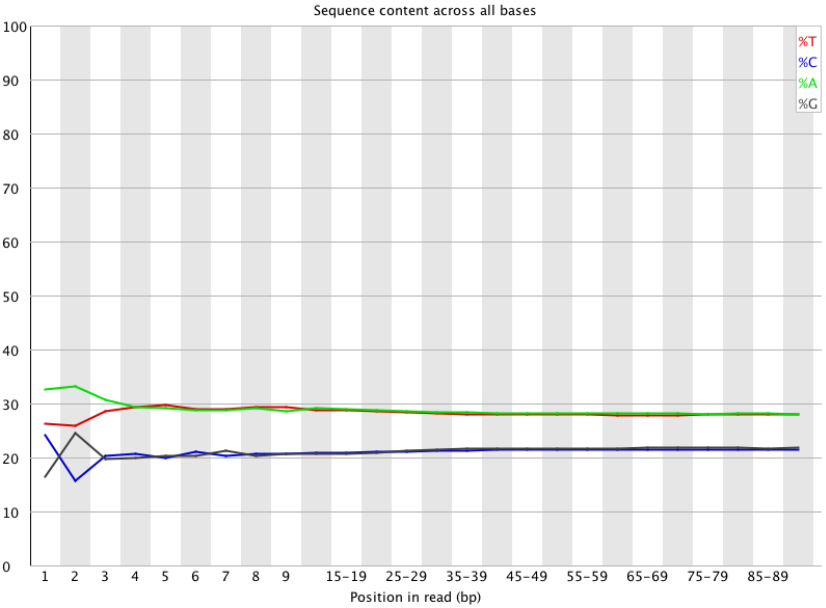
- What kind of data are you getting?
- Read quality and error correction
- Assembly
- How to assess the draft genome
- Mapping and chromosomal build
- SNP calling, RNA-seq analysis etc.

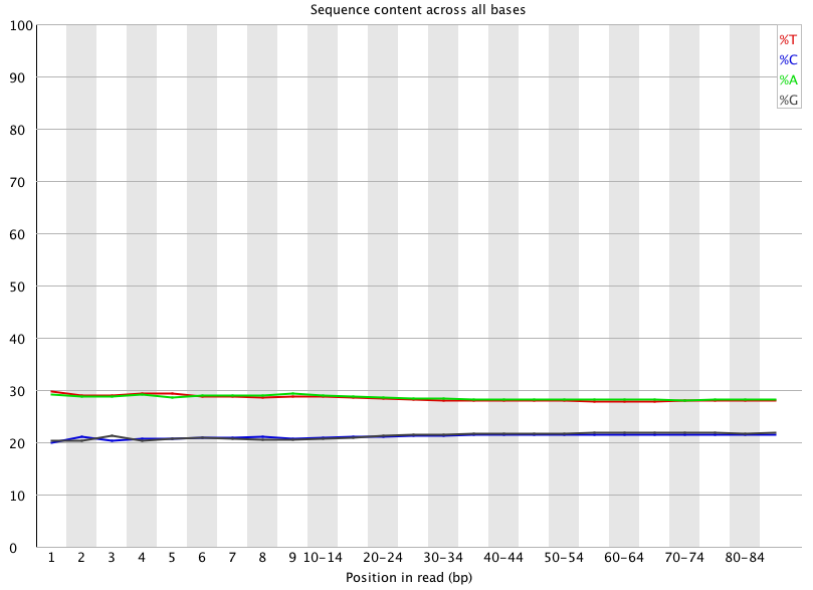
Something I'm perfectly happy there's no need to look into it by my eyes..

A genome-wide *de brujin* graph!



./fastx_trimmer -f 5 -i ../test_data/test.fq1 -o choped.first3bp.test.fq1 &





How to correct bad-quality reads?

- Lots of the 'corrector' relies on really high sequencing coverage (>50 fold) to call a non-biased distribution of *k*-mer so that they can correct reads.
- Not suitable for polymorphism/pool-sequence data

Some popular correctors

- Quake: website

Quake.py –f [fastq_file_list] –k 31 –p 4

- SOAP reads corrector: Download link

- Coral: Download link

Table 4 SOAPdenovo bee assembly

Assembly	Trimmed Only	Corrected	Removed	Contigs	N50	N90	Scaffolds	N50	N90	Reads
Uncorrected Corrected	146.0 M	-	12.9 M	312,414	2,383	198	90,201	37,138	9,960	167.3 M
SOAPdenovo Corrected	134.4 M	15.7 M	15.6 M	188,480	4,051	515	36,525	36,525	9,162	164.8 M
Quake	146.9 M	16.5 M	13.0 M	189,621	4,076	514	37,279	37,014	9,255	167.3 M

SOAPdenovo assemblies of *Megachile rotundata* 124 bp paired end reads. We trimmed the reads before correcting with SOAPdenovo, which greatly improved its performance on our experiments with simulated data. The 'Trimmed only' column includes reads trimmed before and during SOAPdenovo correction. Quake trims reads automatically during correction. Correcting the reads reduces the number of contigs and scaffolds, increases the contig sizes, and allows the assembler to include more reads. Quake corrects more reads than SOAPdenovo which results in a slightly better assembly.


Phylogeny Programs
TreeRot
FILD
genie
EDIBLE
DA
MBE
NSA
SimPlot
BAliPhy
Phylogeny
.Fr
PhyRe

Wait a minute, why there're so many assemblers?

Assembler List

Showing below up to **68** results starting with #**1**.

View (previous 250 | next 250) (20 | 50 | 100 | 250 | 500)

A
- ABBA
- ABySS
- ALLPATHS
- AMOS
- ANCHOR
- Arachne

C
- CABOG
- CLCbio Genomics Workbench
- Contrail
- Cortex
- Cufflinks
- Curtain

D
- DNA Baser

E
- EDENA
- Est2assembly
- EULER

F
- FLASH
- Forge
- Fuzzypath

G
- GeeFu
- Geneious
- Genomatix Mining Station (GMS)
- GenoMiner

G cont.
- Gk arrays

I
- ICORN
- IDBA
- IOmics
- ISSAKE

L
- Lasergene
- LOCAS

M
- Mapsembler
- Mason
- Meraculous
- MetaSim
- MIRA
- MOSAIK
- MuSICA 2

N
- Newbler
- Ngs backbone
- NovelSeq

O
- Omixon Variant Toolkit

P
- Phred Phrap Consed Cross match
- PRICE

Q
- QSRA

R cont.
- RGA

S
- SCARF
- SeqCons
- SeqMan NGen
- Sequencher
- SGA
- SHARCGS
- SHORTY
- SHRAP
- SOAPdenovo
- SOPRA
- Spiral Genetics
- SR-ASM
- SSAKE
- STADEN
- Supersplat

T
- Taipan
- TASR
- Tracembler

V
- VCAKE
- Velvet

Z
- ZORRO

Let them run!

Resource

Assemblathon 1: A competitive assessment of de novo short read assembly methods

Dent Earl,[1,2] Keith Bradnam,[3] John St. John,[1,2] Aaron Darling,[3] Dawei Lin,[3,4] Joseph Fass,[3,4] Hung On Ken Yu,[3] Vince Buffalo,[3,4] Daniel R. Zerbino,[2] Mark Diekhans,[1,2] Ngan Nguyen,[1,2] Pramila Nuwantha Ariyaratne,[5] Wing-Kin Sung,[5,6] Zemin Ning,[7] Matthias Haimel,[8] Jared T. Simpson,[7] Nuno A. Fonseca,[9] İnanç Birol,[10] T. Roderick Docking,[10] Isaac Y. Ho,[11] Daniel S. Rokhsar,[11,12] Rayan Chikhi,[13,14] Dominique Lavenier,[13,14,15] Guillaume Chapuis,[13,14] Delphine Naquin,[14,15]

Bradnam *et al. GigaScience* 2013, **2**:10
http://www.gigasciencejournal.com/content/2/1/10

(GIGA)^n SCIENCE

RESEARCH Open Access

Assemblathon 2: evaluating *de novo* methods of genome assembly in three vertebrate species

Keith R Bradnam[1*†], Joseph N Fass[1†], Anton Alexandrov[36], Paul Baranay[2], Michael Bechner[39], Inanç Birol[33], Sébastien Boisvert[10,11], Jarrod A Chapman[20], Guillaume Chapuis[7,9], Rayan Chikhi[7,9], Hamidreza Chitsaz[6], Wen-Chi Chou[14,16], Jacques Corbeil[10,13], Cristian Del Fabbro[17], T Roderick Docking[33], Richard Durbin[34], Dent Earl[40], Scott Emrich[3], Pavel Fedotov[36], Nuno A Fonseca[30,35], Ganeshkumar Ganapathy[38], Richard A Gibbs[32], Sante Gnerre[22], Élénie Godzaridis[11], Steve Goldstein[39], Matthias Haimel[30], Giles Hall[22], David Haussler[40], Joseph B Hiatt[41], Isaac Y Ho[20], Jason Howard[38], Martin Hunt[34], Shaun D Jackman[33], David B Jaffe[22], Erich D Jarvis[38], Huaiyang Jiang[32], Sergey Kazakov[36], Paul J Kersey[30], Jacob O Kitzman[41], James R Knight[37], Sergey Koren[24,25], Tak-Wah Lam[29], Dominique Lavenier[7,8,9], François Laviolette[12], Yingrui Li[28,29], Zhenyu Li[28], Binghang Liu[28], Yue Liu[32], Ruibang Luo[28,29], Iain MacCallum[22], Matthew D MacManes[5], Nicolas Maillet[8,9], Sergey Melnikov[36], Delphine Naquin[8,9], Zemin Ning[34], Thomas D Otto[34], Benedict Paten[40], Octávio S Paulo[31], Adam M Phillippy[24,25],

Resource

GAGE: A critical evaluation of genome assemblies and assembly algorithms

Steven L. Salzberg,[1,7] Adam M. Phillippy,[2] Aleksey Zimin,[3] Daniela Puiu,[1] Tanja Magoc,[1] Sergey Koren,[2,4] Todd J. Treangen,[1] Michael C. Schatz,[5] Arthur L. Delcher,[6] Michael Roberts,[3] Guillaume Marçais,[3] Mihai Pop,[4] and James A. Yorke[3]

Bioinformatics Advance Access published May 10, 2013

Original Article

GAGE-B: An Evaluation of Genome Assemblers for Bacterial Organisms

Tanja Magoc[1], Stephan Pabinger[1,2], Stefan Canzar[1], Xinyue Liu[3], Qi Su[3], Daniela Puiu[1], Luke J. Tallon[3], and Steven L. Salzberg[1,*]

[1]Center for Computational Biology, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21025, USA; [2]Division for Bioinformatics, Innsbruck Medical University, Innsbruck, Austria; [3]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD 21205, USA

Genome Assembly Gold-Standard Evaluations

Genome Assembly Gold-Standard Evaluations

Main page | Genome Assemblers | Data sets | Recipes | Results | Twitter

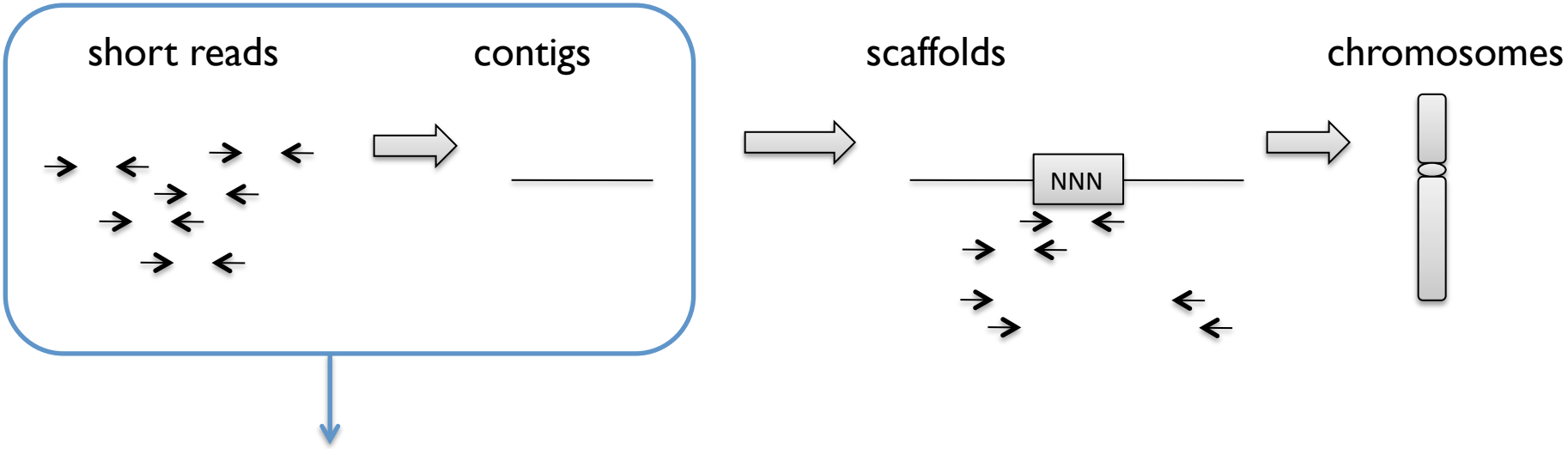
What recipes were used for each genome assembly?

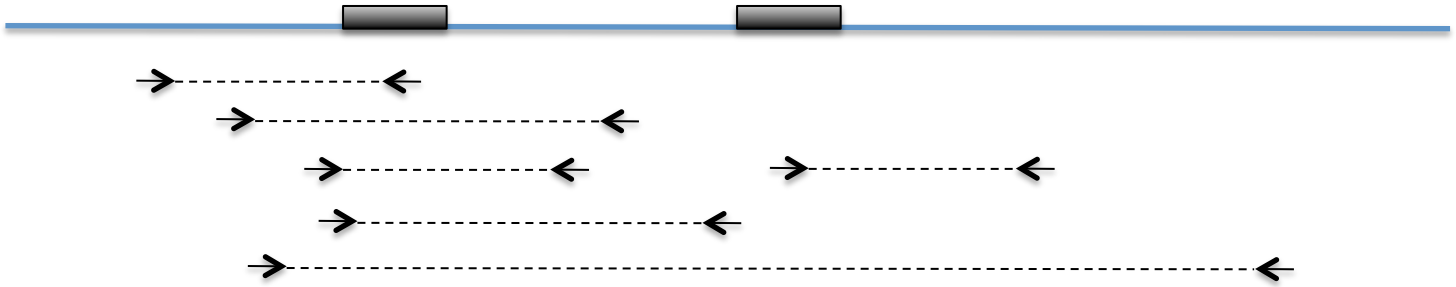
Links to recipes for each genome assembler, which include instructions and all necessary scripts to reproduce the results:

1. ABYSS recipes
2. ALLPATHS-LG recipes
3. Bambus2 recipes
4. Celera Assembler recipes
5. MSR-CA recipes
6. SGA recipes
7. SOAPdenovo recipes
8. Velvet recipes

http://gage.cbcb.umd.edu/recipes/index.html

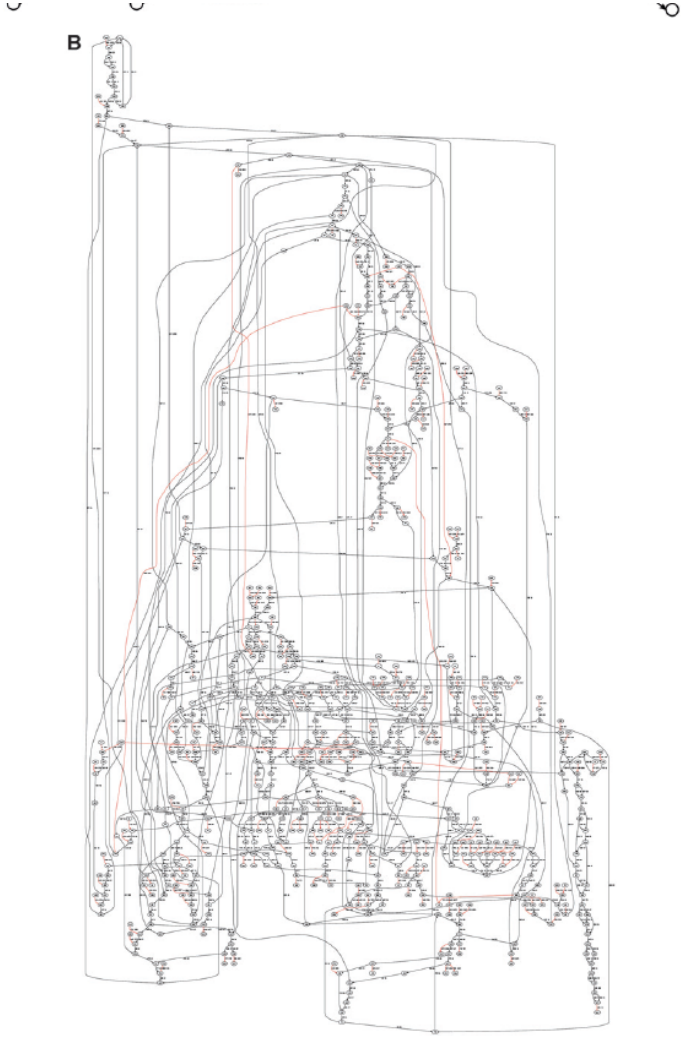
How does the assembler work?





Different insert-length would greatly help the scaffolding process

Now are you ready to do some assemblies?



- What kind of data are you getting?
- Read quality and error correction
- Assembly
- How to assess the draft genome
- Mapping and chromosomal build
- SNP calling, RNA-seq analysis etc.

- Contigs: continuous sequences without any gaps
- Scaffolds: joined by contigs through mate-pair relationship
- Superscaffolds/chromosomes: joined by scaffolds by comparing to a reference genome or using other source of linkage information (physical map, FISH..etc.)
- N50 length: how continuous is the genome
  the length N for which half of all bases in the sequences are in a sequence of length L < N.
- Gap content: integrity of the genome: gaps/kb, N%
- Accuracy: compare to a genome sequence produced by other platform or that of a related species

Table 3. Assemblies of *R. sphaeroides* (genome size 4,603,060)

	Contigs				Scaffolds			
Assembler	Num	N50 (kb)	Errors	N50 corr. (kb)	Num	N50 (kb)	Errors	N50 corr. (kb)
ABySS	1915	5.9	55	4.2	1701	9	3	5
ALLPATHS-LG	204	42.5	43	**34.4**	**34**	**3192**	**0**	**3192**
Bambus2	**177**	93.2	368	12.8	92	2439	2	2419
CABOG	322	20.2	34	17.9	130	66	5	55
MSR-CA	395	22.1	42	19.1	43	2,976	5	2966
SGA	3067	4.5	**8**	2.9	2096	51	**0**	51
SOAPdenovo	204	**131.7**	414	14.3	166	660	3	658

Table 4. Assemblies of human chromosome 14 (ungapped size 88,289,540)

	Contigs				Scaffolds			
Assembler	Num	N50 (kb)	Errors	N50 corr. (kb)	Num	N50 (kb)	Errors	N50 corr. (kb)
ABySS	51,924	2.0	**691**	2.0	51,301	2.1	**9**	2
ALLPATHS-LG	4529	36.5	2659	21.0	**225**	**81,647**	45	**4702**
Bambus2	13,592	5.9	8677	4.3	1792	324	143	161
CABOG	**3361**	**45.3**	3032	**23.7**	479	393	597	26
MSR-CA	30,103	4.9	4316	4.3	1425	893	1068	94
SGA	56,939	2.7	831	2.7	30,975	83	19	79
SOAPdenovo	22,689	14.7	5163	7.4	13,502	455	268	214
Velvet	45,564	2.3	4541	2.1	3,565	1190	9156	27

Columns are the same as in Table 2.

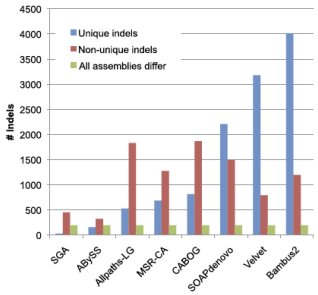


Figure 5. Comparison of insertion and deletion errors among all eight assemblers for human chromosome 14. (Blue) The indel errors >5 bp in length that are unique to each assembler. (Red bars) Indel errors made by at least one other assembler. (Green bars) Indels shared by all assemblers, which might represent true differences between the target genome and the reference.

- SOAPdenovo and ALLPATHS-lg

SOAP	ALLPATHs-lg
Quite flexible at the input data: paired-end, single-end, short insert, long insert…	Has to provide overlapping and long-insert library data
No requirement for sequencing coverage	~100 fold
Have separate modules for error correction, contiging, scaffolding, gapfilling	One button run: the program will take care of everything
At least 8CPUs, 5G memory for small genome, 150G for human genome	32Gb for small genome 512Gb for mammalian sized genome
Longer N50, but sometimes accuracy is not as good as ALLPATHs	Achieve both a good N50 and accuracy

```
ucbvpn-208-176:SOAP zhouqi$ ./SOAPdenovo31mer

Version 1.05: released on July 29th, 2010


Usage: SOAPdenovo <command> [option]
    pregraph     construction kmer-graph
    contig       eliminate errors and output contigs
    map          map reads to contigs
    scaff        scaffolding
    all          doing all the above in turn
ucbvpn-208-176:SOAP zhouqi$ ./SOAPdenovo31mer all

Version 1.05: released on July 29th, 2010


SOAPdenovo all -s configFile [-a initMemoryAssumption -K kmer -d KmerFreqCutOff -D EdgeCovCutoff -M mergeLevel -R -u -G gapLenDiff -L minContigLen -p n_cpu] -o Output
  -s ShortSeqFile: The input file name of solexa reads
  -a initMemoryAssumption: Initiate the memory assumption to avoid further reallocation
  -K kmer(default 23): k value in kmer
  -p n_cpu(default 8): number of cpu for use
  -F (optional) fill gaps in scaffold
  -M mergeLevel(default 1,min 0, max 3): the strength of merging similar sequences during contiging
  -d KmerFreqCutoff(optional): delete kmers with frequency no larger than (default 0)
  -D EdgeCovCutoff(optional): delete edges with coverage no largert than (default 1)
  -R (optional): unsolve repeats by reads (default no)
  -G gapLenDiff(default 50): allowed length difference between estimated and filled gap
  -L minLen(default K+2): shortest contig for scaffolding
  -u (optional): un-mask contigs with high coverage before scaffolding (default mask)
  -o Output: prefix of output file name
ucbvpn-208-176:SOAP zhouqi$
```

Input:

- Configuration file
- Kmer size: experimental
- Cpu number

Output:

Scaffold sequences, contig sequences

Three different version of SOAPdenovo

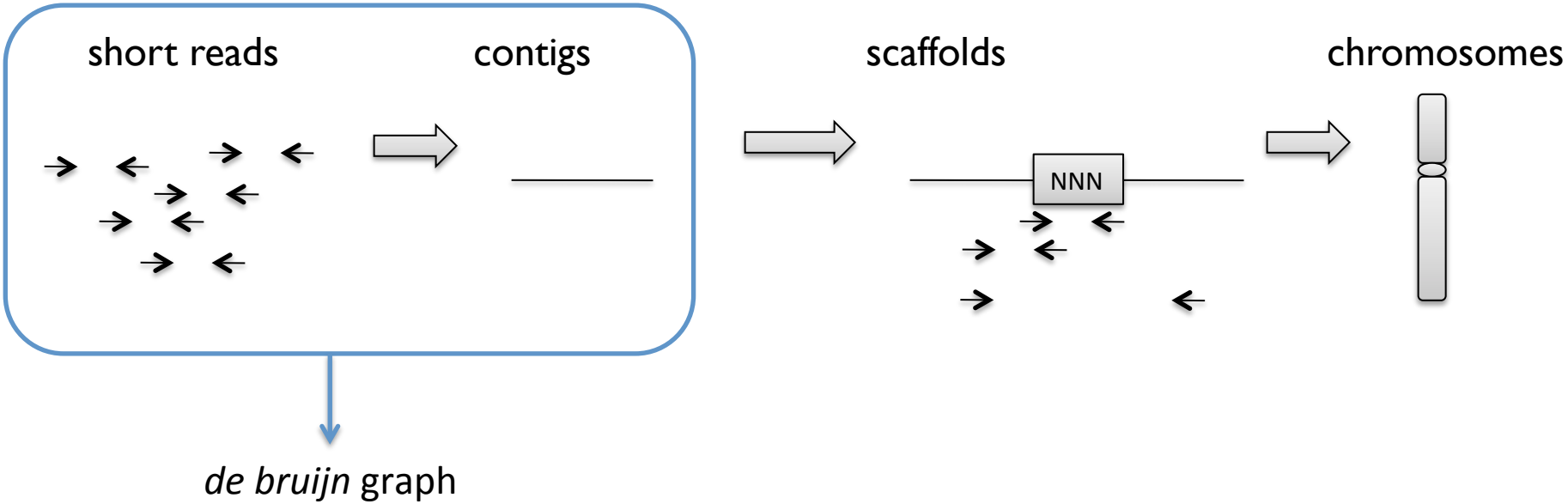
SOAPdenovo127mer, SOAPdenovo63mer, SOAPdenovo31mer

For different kmer range

An example of SOAP configuration file

```
#maximal read length
max_rd_len=100
[LIB]
#average insert size,which you'd know from the library prep
avg_ins=600
#if sequence needs to be reversed, reverse only for long-insert library
reverse_seq=0
#in which part(s) the reads are used, 0 means do nothing, 1 means do contiging, 2 means do scaffolding, 3 means do both
asm_flags=3
#in which order the reads are used while scaffolding
rank=1
#fastq file for read 1
q1=
#fastq file for read 2 always follows fastq file for read 1
q2=
#fasta file for read 1
#f1=/path/**LIBNAMEA**/fasta_read_1.fa
#fastq file for read 2 always follows fastq file for read 1
#f2=/path/**LIBNAMEA**/fasta_read_2.fa
#fastq file for single reads
#q=/path/**LIBNAMEA**/fastq_read_single.fq
#fasta file for single reads
#f=/path/**LIBNAMEA**/fasta_read_single.fa
#a single fasta file for paired reads
#p=/path/**LIBNAMEA**/pairs_in_one_file.fa
#[LIB]
#avg_ins=2000
#reverse_seq=1
#asm_flags=2
#rank=2
#q1=/path/**LIBNAMEB**/fastq_read_1.fq
#q2=/path/**LIBNAMEB**/fastq_read_2.fq
#q=/path/**LIBNAMEB**/fastq_read_single.fq
#f=/path/**LIBNAMEB**/fasta_read_single.fa
```

How does the assembler work?



Command line:
SOAPdenovo31mer all -K 31 -s test.cfg -p 32 -L 500 -o test > outlog 2> errorlog

(half an hour running time with 32 threads)

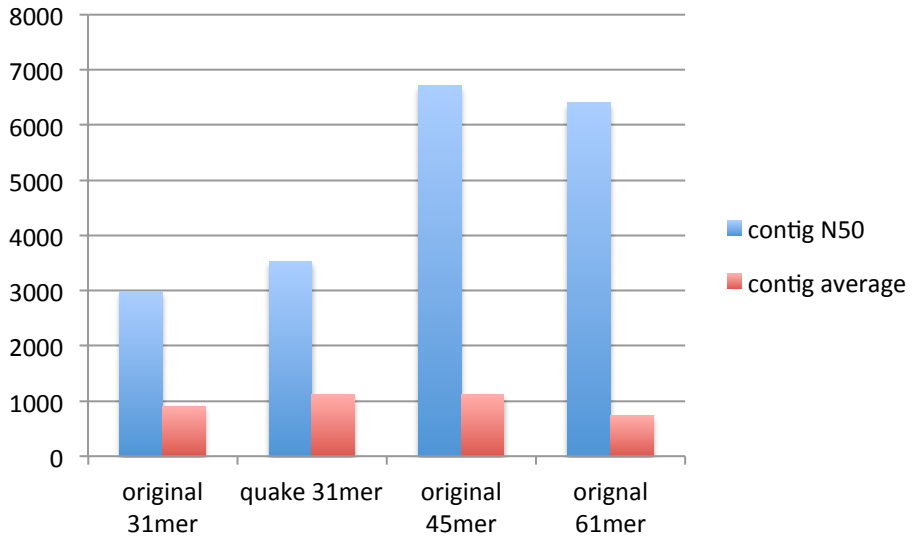
Let's see an example using 3G data of a Drosophila genome…

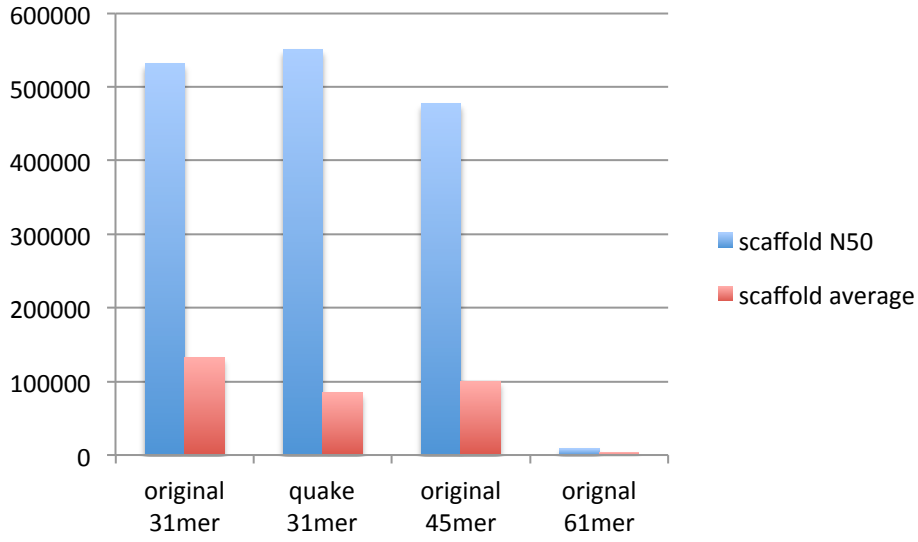
What to read from the log file

- Contig N50, average length, contig number, total size of the contigs
- Mapped reads %,
- Estimated map-pair distance: is it consistent with what you have inputed in the configuration file?
- Scaffold N50, average scaffold length, scaffold number, assembled size (genome size)

After Running SOAPdenovo..

- Adjust the *k*-mer size until getting a better N50
- Adjust the insert-length if not consistent





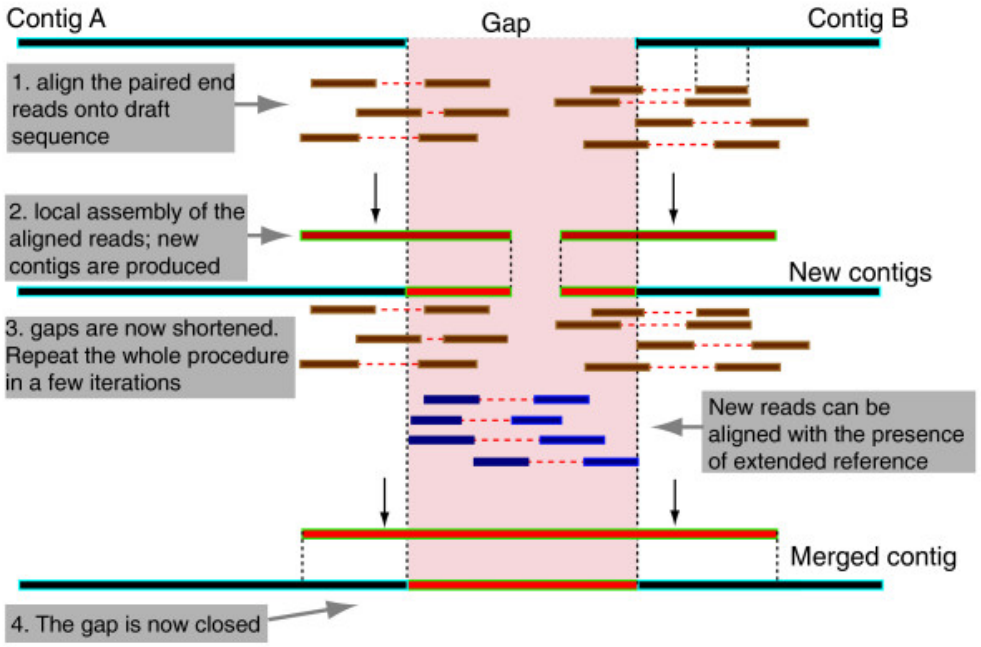
- Post-assembly gap-filling:

Popular Gap-filler: SOAP-gapcloser; IMAGE

Gapcloser –a [SOAP resulting scaffold sequences] –b [same configuration file you feed to SOAP] –o [name: Best Genome Ever] –p [kmer size] –t [thread number]

/jbods/data00/zhouqi/soft/GapCloser -a test.scafSeq -b test.cfg -o test.gapfilled -t 32 1>gapfil.out 2>gapfil.errout &

→example



Method

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Improving draft assemblies by iterative mapping and assembly of short reads to eliminate gaps

Isheng J Tsai*, **Thomas D Otto** and **Matthew Berriman**

* Corresponding author: Isheng J Tsai jit@sanger.ac.uk

Author Affiliations

Parasite Genomics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK

For all author emails, please log on.

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Software

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Toward almost closed genomes with GapFiller

Marten Boetzer and **Walter Pirovano***

* Corresponding author: Walter Pirovano walter.pirovano@baseclear.com

Author Affiliations

BaseClear BV, Einsteinweg 5, 2333 CC, Leiden, The Netherlands

For all author emails, please log on.

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Viewing options
Abstract
Full text
PDF (413KB)

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Table 1 Gap closure results obtained on the bacterial datasets

Method	Original	IMAGE	SOAPdenovo	GapFiller	GapFiller-LC
Escherichia coli					
Genome size (bp)	4,478,287	4,530,961	4,490,973	4,490,638	
Scaffolds	179	179	179	179	
Gap count	544	291	16	11	
Total gap length (bp)	12,516	2,861	16	130	
Errors (SNPs)	12	40	33	22	
Errors (indels)	4	17	25	9	
Errors (misjoins)	1	1	1	1	
N50	50,557	50,558	50,558	50,558	
Streptomyces coelicolor					
Genome size (bp)	8,558,275	8,576,331	8,557,720	8,558,333	
Scaffolds	115	115	115	115	
Gap count	158	63	60	23	
Total gap length (bp)	9,221	4,009	1,288	806	
Errors (SNPs)	299	423	406	280	
Errors (indels)	664	677	769	686	
Errors (misjoins)	12	17	18	18	
N50	173,822	173,822	173,822	173,822	
Staphylococcus aureus					
Genome size (bp)	2,880,676		2,880,926	2,881,756	2,883,448
Scaffolds	19		19	19	19
Gap count	48		27	27	22
Total gap length (bp)	9,900		1,547	5,508	1,861
Errors (SNPs)	79		260	98	173
Errors (indels)	16		53	26	37
Errors (misjoins)	4		13	7	5
N50	1,091,731		1,091,333	1,092,281	1,092,421
Rhodobacter sphaeroides					
Genome size (bp)	4,609,785		4,609,466	4,609,596	4,610,796
Scaffolds	38		38	38	38
Gap count	170		163	161	139
Total gap length (bp)	21,409		14,166	20,667	17,625
Errors (SNPs)	218		410	230	300
Errors (indels)	187		294	190	199
Errors (misjoins)	6		10	6	7
N50	3,192,334		3,192,075	3,192,215	3,192,974

Gap closure results obtained on four bacterial datasets show that the GapFiller strategy yields the most accurate finished genomes. Also, the gap count is lower compared to the other methods. The IMAGE method significantly underperforms on all quality measures and would therefore not be the preferred method to use. Differences are smaller between GapFiller and SOAPdenovo. Interestingly, whereas the gap count after closure is generally less for GapFiller, SOAPdenovo yields in three cases a shorter total gap length. This suggests the latter method is able to close larger gaps. Strikingly, however, the amount of errors is significantly higher for SOAPdenovo regardless of the source (SNPs, indels and misjoins). Even when applying less strict settings for GapFiller (GapFiller-LC: minimum coverage $o = 1$, ratio $r = 0.5$) to shorten the total gap length, our method still yields significantly less errors.

- What kind of data are you getting?
- Read quality and error correction
- Assembly
- How to assess the draft genome
- Mapping and chromosomal build
- SNP calling, RNA-seq analysis etc.

- Setup configuration file telling ALLPATHs to convert the reads format : fastq→fastb
- Runallpaths:

Log file:

Validating Fragment Libraries

Kmer Spectrum Analysis

Table 1: library names, number of pairs (N), original (L0) and new sizes (L)

Table 2: fraction of reads in each length interval

---------- AllPathsReport -> assembly_stats.report

What we get from the assembler is just a “draft genome”

- Assess the assembly: PCR or compared to reference sequences

  published EST/genome sanger sequences, compared to a sister species' genome
- Link scaffolds into chromosomal sequences
- Gene annotation with RNA-seq data/proteins of related species
- More downstream analysis: find differential expression pattern, annotate regulatory sequences, identify copy number variation, population history analysis…

There're no existent chromosomal builder yet..

- Blast/blat your draft genome against a reference genome

- Pick up a best-aligned scaffold for each site

- Link them

454/PacBio/Hybrid assembly

Illumina assembly

Gap-patch or extension by 454/ Pacbio
Tools: CABOG, PBJelly

Mix the reads together and perform hybrid assembly: CABOG, MIRA

A recipe of genome assembly

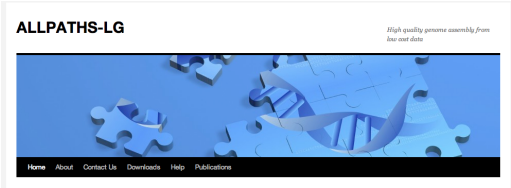
- Sequence the DNA to at least 30 fold coverage, preferably with different insert-lengths and long-insert libraries
- Assess the read-quality, trim them if necessary
- Error-correct the reads if there's sufficient coverage
- Assemble the genome with assembler program, test different parameter sets until getting a better N50, a lower gap content
- Gap-filling the draft genome
- Align the draft genome to other genomes to build chromosome
- Gene annotation, downstream analysis etc.

Resources

http://gage.cbcb.umd.edu

http://assemblathon.org

http://soap.genomics.org.cn : aligner, transcriptome-assembler



http://www.broadinstitute.org/software/allpaths-lg/blog/

http://seqanswers.com

Thank you!

Bachtrog Lab

Functional and Evolutionary Genomics



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University of California, Berkeley
Department of Integrative Biology
Center for Theoretical Evolutionary Genomics
3060 VLSB
Berkeley, CA 94720-3140
office # 4087
phone (lab): (510)-642-9271



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