

ILLUMINA

PIPELINE

by ALEX

ILLUMINA

PIPELINE

and ASSEMBLY

ILLUMINA

PIPELINE

by

ALEX

ILLUMINA

PIPELINE and

ASSEMBLY

OUTLINE:

project overview and dataflow

IGA output files

IGA quality scores

filtering for high quality reads

assembly parameters

visualization with Python Contig Viewer

Illumina GA2 high-throughput sequencing



Current performance:

10 to 20 million reads per lane

8 lanes per chip

single read - 85 to 125 nt

paired reads - 125 <-> 41+

80 to 160 million reads per slide

Conservative calculations: 100 M reads X 100 nt length = 10 Gb per run

(Illumina aiming at 90+ Gb per run - 10 Gb per lane - by the end of 2009)

Computational Facilities at the UCD Genome Center

Storage arrays - 48 Tb HD space

Computational servers with up to 128 and 512 Gb of RAM

Computational clusters with 100+ nodes

Illumina sequencing dataflow

**Illumina raw reads
on sequencing machine**

rsync/sftp
file transfer

**processed reads on
file server (storage array)**

QC and filtering for
high quality reads

**input files for downstream
assembly and analysis**

assembly using
CLC and/or Velvet

de novo assembled contigs

analysis and annotation
of assemblies

**data distribution via
sftp or web servers**



Project Goals



lettuce transcriptome sequencing

lettuce gene space sequencing

...

lettuce whole genome sequencing (~3 Gb)

Current Sequencing Scale Estimate

Cover and sequence transcriptome - 4 to 6 lanes sufficient
(1 lane by end of 2009 ?)

Gene space - 3 to 6 chips currently required
(0.5 chip - 4 lanes - by end of 2009 ?)

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Figure 6 illustrates a typical Run Folder after IPAR image analysis and Pipeline base calling and alignment, or one containing only Pipeline analysis data.

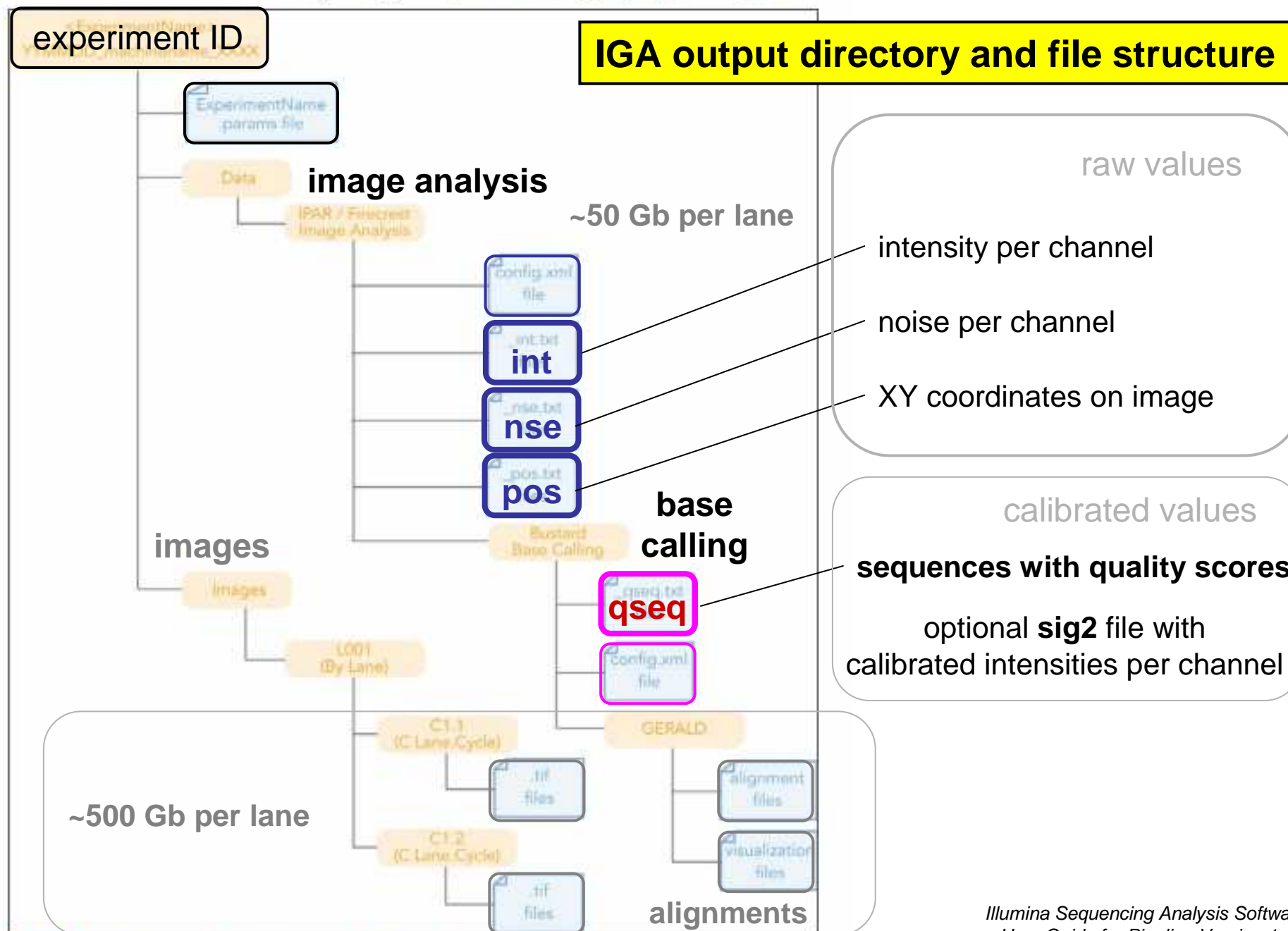


Figure 6 IPAR/Pipeline Run Folder Directory Structure

ILLUMINA Filtering Options for HQ reads

To remove the least reliable data from the analysis, the raw data can be filtered to remove any clusters that have “too much” intensity corresponding to bases other than the called base. By default, the purity of the signal from each cluster is examined over the first 25 cycles and chastity is calculated for each cycle:

$$\text{chastity} = \text{Highest_Intensity} / (\text{Highest_Intensity} + \text{Next_Highest_Intensity})$$

Chastity:

the ratio of the brightest intensity over the sum of the brightest and second brightest intensities

Filtering Options:

failed-chastity: the number of bases where the chastity is lower than the given threshold (1)

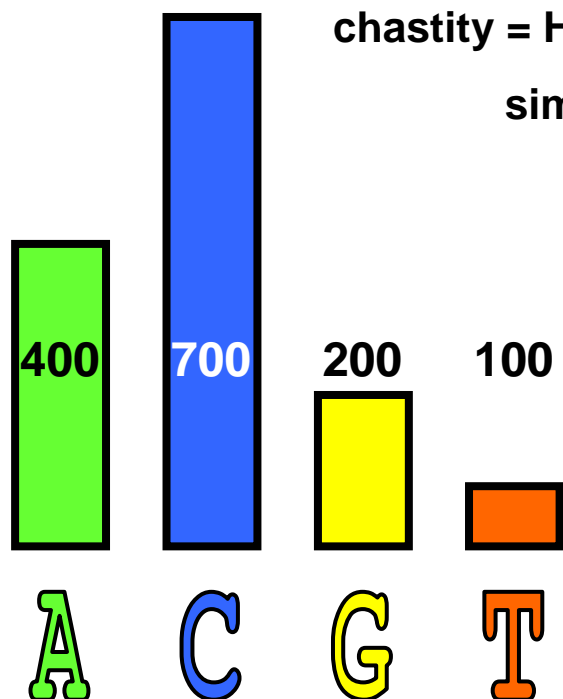
chastity: the minimum chastity (0.6)

purity: the minimum purity (25)

By default, the pipeline filters the clusters according to the relation "failed-chastity<=1", using a chastity threshold of 0.6, on the first 25 cycles. This removes all clusters with a chastity less than 0.6 on two or more bases among the first 25 bases.

```
--smt-filter failed-chastity --smt-relation le (less or equal)
--smt-threshold 1 --chastity-threshold 0.6
--pure-bases 25
```

[The new default filtering implemented in Bustard is that at most one cycle is less than the chastity threshold]



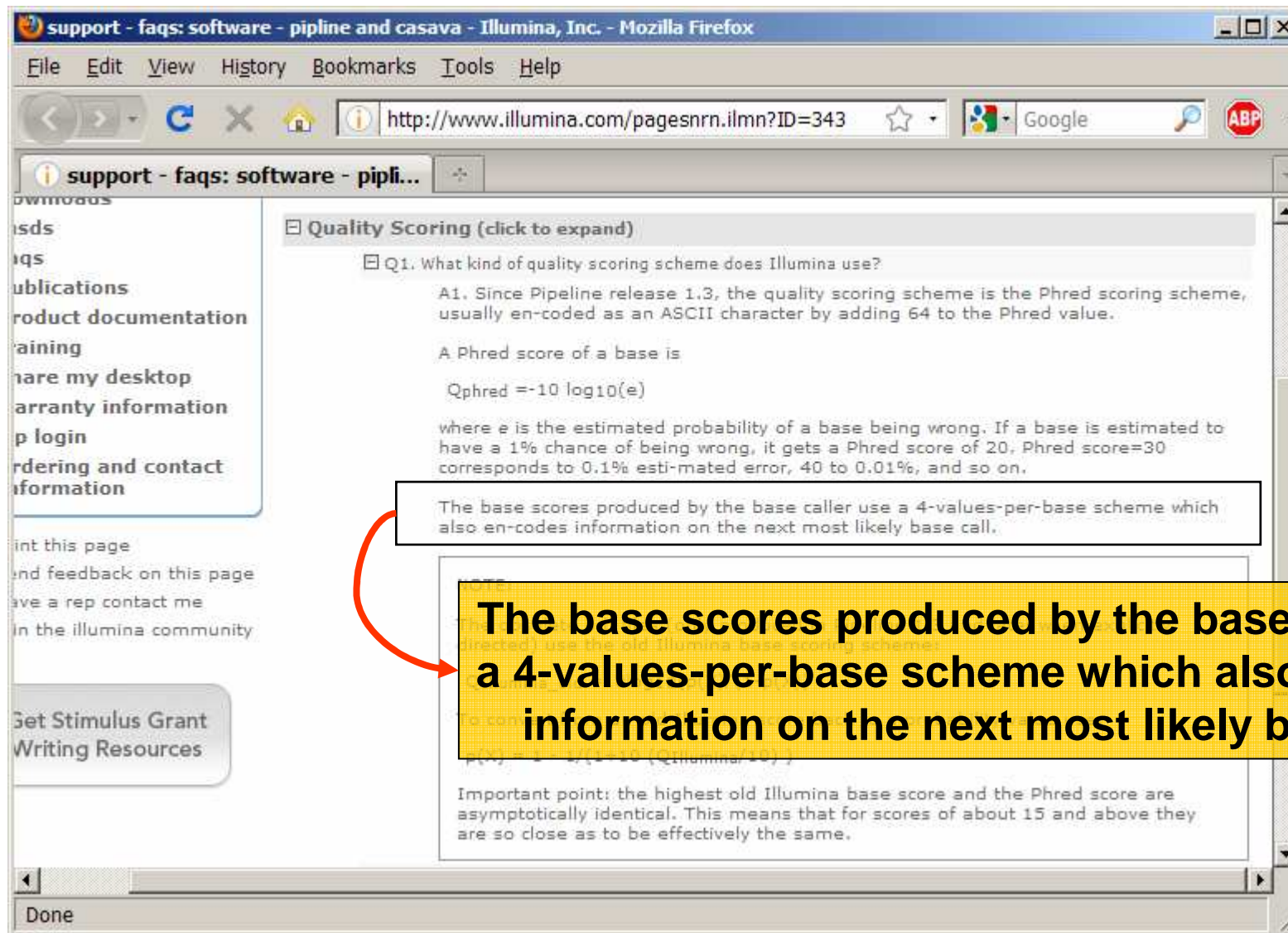
chastity = Highest_Intensity / (Highest_Intensity + Next_Highest_Intensity)

simple_ratio = Next_Highest_Intensity / Highest_Intensity

quality categories for simple ratio:

A	-	[0.0	<	Q	<=	0.2]
B	-	[0.2	<	Q	<=	0.4]
C	-	[0.4	<	Q	<=	0.6]
D	-	[0.6	<	Q	<=	0.8]
F	-	[0.8	<	Q	<=	1.0]

Intensity 1-st	1000	1000	1000	1000	1000	1000	1000
Intensity 2-nd	100	200	300	400	600	800	900
IGA chastity	0.91	0.83	0.77	0.71	0.63	0.56	0.53
(1.0 - ratio)	0.90	0.80	0.70	0.60	0.40	0.20	0.10
ratio	0.1	0.2	0.3	0.4	0.6	0.8	0.9
quality category	A (I)	A (I)	B (9)	B (9)	C (5)	D (0)	F (#)



A quality value Q is an integer mapping of p , the probability of the corresponding sequence letter being incorrect. It is calculated as follows:

$$Q_{\text{phred}} = -10 \log_{10} p$$

http://en.wikipedia.org/wiki/Phred_quality_score

Binary	Oct	Dec	Hex	Glyph
100 0010	102	66	42	B
100 0011	103	67	43	C
100 0100	104	68	44	D
100 0101	105	69	45	E
100 0110	106	70	46	F
100 0111	107	71	47	G
100 1000	110	72	48	H
100 1001	111	73	49	I
100 1010	112	74	4A	J
100 1011	113	75	4B	K
100 1100	114	76	4C	L
100 1101	115	77	4D	M
100 1110	116	78	4E	N
100 1111	117	79	4F	O
101 0000	120	80	50	P
101 0001	121	81	51	Q
101 0010	122	82	52	R
101 0011	123	83	53	S
101 0100	124	84	54	T
101 0101	125	85	55	U
101 0110	126	86	56	V
101 0111	127	87	57	W
101 1000	130	88	58	X
101 1001	131	89	59	Y
101 1010	132	90	5A	Z
101 1011	133	91	5B	[
101 1100	134	92	5C	\
101 1101	135	93	5D]
101 1110	136	94	5E	^
101 1111	137	95	5F	_
110 0000	140	96	60	`
110 0001	141	97	61	a
110 0010	142	98	62	b

http://en.wikipedia.org/wiki/FASTQ_format

A quality value Q is an integer mapping of p, the probability of the corresponding sequence letter being incorrect. It is calculated as follows:

$$Q_{\text{phred}} = -10 \log_{10} p$$

low quality

$$-10 \cdot \log_{10}(0.5) = -10 \cdot (-0.3) = 3$$

$$-10 \cdot \log_{10}(0.1) = -10 \cdot (-1) = 10$$

medium quality

$$-10 \cdot \log_{10}(0.01) = -10 \cdot (-2) = 20$$

<http://en.wikipedia.org/wiki/ASCII>

**Quality String - in symbolic ASCII format
(ASCII chracter code = quality value + 64)**

high quality

$$-10 \cdot \log_{10}(0.001) = -10 \cdot (-3) = 30$$

Illumina 1.3+ format can encode a Phred quality score from 0 to 62 using ASCII 64 to 126 (although in raw read data Phred scores from 0 to 40 only are expected).

The main output files in Bustard are the *_qseq files. They have the following format:

1. Machine name: (hopefully) unique identifier of the sequencer.
2. Run number: (hopefully) unique number to identify the run on the sequencer.
3. Lane number: positive integer (currently 1-8).
4. Tile number: positive integer.
5. X: x coordinate of the spot. Integer (can be negative).
6. Y: y coordinate of the spot. Integer (can be negative).
7. Index: positive integer. No indexing should have a value of 1.
8. Read Number: 1 for single reads; 1 or 2 for paired ends.
9. Sequence
10. Quality: the calibrated quality string.
11. Filter: Did the read pass filtering? 0 - No, 1 - Yes

```
===== qseq file =====
SOLEXA1 90707 2 1 6 88 0 1
TGTC AACAGAAAATTCAGAAATAGCTGACTTAAATCCTTTAGATTGTATTCCATTTCGCATCGGCTAATTCAGAAATTTTATCTAG
ab_bbbb]_ ^`T`aab\ a`\ba]aaaaaa`a]S]a`aaa``aXaaa]_a`aa_____ ``^W^Q][^^^[[[ ^Y^] [][[]U[[YOX 1
SOLEXA1 90707 2 1 6 333 0 1
ATTCTTGATTGCCAAACGTTGATGATGGTGCCCAATTTTAGCGGGTGGTAAAGAATTTGGGGTCGCGGGTTGGTACGGGAGCCTT
aaaabaaQ^`Y__ ^J^`_`Y`R[aG\YPIRTSGG]^`_\ZGXR`_XDTNWUBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB 0
=====
```

FASTA file:

```
>90707:SOLEXA1:2:1:6:88#0/1 891 LEN: 85
TGTC AACAGAAAATTCAGAAATAGCTGACTTAAATCCTTTAGATTGTATTCCATTTCGCATCGGCTAATTCAGAAATTTTATCTAG
```

FASTQ file:

```
@90707:SOLEXA1:2:1:6:88#0/1
TGTC AACAGAAAATTCAGAAATAGCTGACTTAAATCCTTTAGATTGTATTCCATTTCGCATCGGCTAATTCAGAAATTTTATCTAG
+90707:SOLEXA1:2:1:6:88#0/1
ab_bbbb]_ ^`T`aab\ a`\ba]aaaaaa`a]S]a`aaa``aXaaa]_a`aa_____ ``^W^Q][^^^[[[ ^Y^] [][[]U[[YOX
```

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<http://code.google.com/p/atgc-illumina/>

The screenshot shows the 'atgc-illumina' project page on Google Code, viewed in Mozilla Firefox. The browser's address bar shows the URL `http://code.google.com/p/atgc-illumina/source/browse/#svn/trunk`. The page header includes the project name 'atgc-illumina' with a logo and the tagline 'tools to process and manipulate Illumina sequences'. A search bar is present with the text 'Search projects'. Below the header, there are tabs for 'Project Home', 'Downloads', 'Wiki', 'Issues', 'Source', and 'Administer'. The 'Source' tab is active, showing a table of source files. The table has columns for 'Filename', 'Size', 'Rev', 'Date', and 'Author'. The 'trunk' directory is expanded, showing four files. Two red arrows point from the text 'illupa rectificator' to the file 'tcl_illupa_rectificator_2009_05_19_Beta.tcl'. Another red arrow points from the text 'sig2 basecalling / filtering (obsolete ?)' to the file 'tcl_solexa_sig2_base_caller_2009_05_14_Beta.tcl'. A red box highlights the last two files in the table.

Filename	Size	Rev	Date	Author
tcl_illupa_rectificator_2009_05_19_Beta.tcl	10.0 KB	r6	Jun 10, 2009	akozik
tcl_solexa_qseq_parser_2009_07_16_Beta.tcl	2.5 KB	r8	Jul 16, 2009	akozik
tcl_solexa_sig2_base_caller_2009_05_14_Beta.tcl	12.6 KB	r5	Jun 09, 2009	akozik
tcl_solexa_sig2_base_caller_2009_06_10_Beta.tcl	12.7 KB	r7	Jul 15, 2009	akozik

sig2 basecalling / filtering (obsolete ?)

qseq parser - it takes into account IGA quality scores

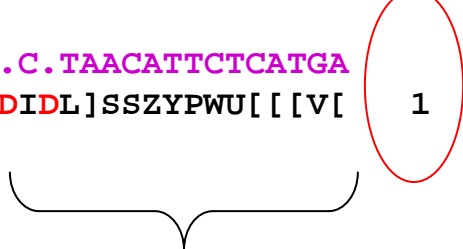
illupa rectificator - to transform raw intensities in vertical format into linear sig2 like order

qseq filtering approach summary:

qseq parser analyzes data from qseq files

filtering criteria '1'

```
SOLEXA1 90707      8      120      6      1512      0      1
AAAAACAGATGCTACTTTACTTTACTACCATATTTCCCAAACAAAGAGCTAACAGTAGAACATGACGC.C.TAACATTCTCATGA
a`aab_Z_V__aXaa\[aa`^a`[^]ab`aa^aa_a]SXZZ]`UXXS_Y`__^^S^^Y^S^GU]^]TDIDL]SSZYPWU[[[V[ 1
```



sequence after first failed call is removed

>90707:SOLEXA1:8:120:6:1512#0/1 691 LEN: 68
AAAAACAGATGCTACTTTACTTTACTACCATATTTCCCAAACAAAGAGCTAACAGTAGAACATGACGC

future plans - to analyze string of quality scores regardless of IGA default filtering criteria

qseq parser output example - *.trim.fa file

```
bash-2.03$ tclsh8.3 tcl_solexa_qseq_parser_2009_07_16_Beta.tcl
Program usage:
qseq_file_to_process, output_file, tag_length, read_length
bash-2.03$
```

Without Tag:

```
>90707:SOLEXA1:8:120:6:1333#0/1    692  LEN: 68
GAATTCCTTCGCACATCATCATAAACCTCGGTTCTCCATTTGTCAAAAAGCAAACGGGGAATTTTTG

>90707:SOLEXA1:8:120:6:1245#0/1    693  LEN: 68
GGGTGAATGTAAAGGAATATGAGGCTCTTCGACCTGACAAGCTTGCTGTGGATGGAGGCGAACAGAAT

>90707:SOLEXA1:8:120:6:236#0/1     694  LEN: 85
CGGCGATCTGATTCCGATGAGCAGTCAACCGCAAGCTCCACAGAGTAGGAGCCTGGTTATTACGGCTGGCGCAGGCGGAACCAAA

>90707:SOLEXA1:8:120:6:98#0/1     696  LEN: 85
ATCAGCTTTCCATGTAGCATAATAGTAACGCTGCCTAAGAAGTTGCAAATTCTCCCTGTCTAGAGAACTGGCTGAGAGCCTGGA
```

With Tag (bar code):

```
>PEFC42D7CAAXX:SOLEXA1:2:1:6:980#0/1  [ADPT: _GCTACAT_]    404  LEN: 54
ATAATAAAATACTTCATATCTATGTGTAAGATTAAAGGGACCATGCACTCACCT

>PEFC42D7CAAXX:SOLEXA1:2:1:6:509#0/1  [ADPT: _GCATAGT_]    405  LEN: 54
CATCATCTTGATCTTTTGCTCCACTTGGCCCTGCTCCATCATCTTGTGGATGCC

>PEFC42D7CAAXX:SOLEXA1:2:1:6:1127#0/1 [ADPT: _ACTAGCT_]    408  LEN: 54
TCCCTCCTTTAAACACCCCAAATTGCAATTTAACAATACTTGAGACTTTGGGC
```

```
#!/usr/bin/tcl
```

```
proc Process_Qseq {argv} {
```

```
    # set length_cut 60
    length_cut 40
```

```
    set f_in1  [open [lindex $argv 0] "r"]
    set f_out   [open [lindex $argv 1] "w"]
    ....
    set tag_1   [lindex $argv 2]
    set r_len   [lindex $argv 3]
```


sig2 file example

lane	tile	X coordinate	Y coordinate	calibrated intensity scores per channel											
				1-st cycle				2-nd cycle				3-d cycle			
				A	C	G	T	A	C	G	T	A	C	G	T
8	120	5	1105	-249	44	<u>523</u>	<u>315</u>	-248	<u>79</u>	25	<u>443</u>	-146	-138	<u>146</u>	<u>535</u>
8	120	5	942	-103	<u>164</u>	<u>486</u>	26	-25	<u>598</u>	-59	-34	-77	<u>564</u>	18	<u>43</u>
8	120	5	1392	-67	<u>97</u>	<u>501</u>	1	-101	<u>234</u>	-40	<u>154</u>	-128	-101	<u>306</u>	<u>238</u>
8	120	5	1114	<u>658</u>	<u>62</u>	61	52	<u>711</u>	<u>165</u>	43	-46	<u>646</u>	13	-1	<u>101</u>
8	120	5	1409	-124	<u>424</u>	<u>227</u>	-82	<u>548</u>	-92	-19	-99	<u>210</u>	25	<u>98</u>	26
8	120	5	327	-18	-69	<u>485</u>	-24	-39	<u>355</u>	-31	-72	-173	<u>450</u>	-14	-3
8	120	5	1017	-149	<u>587</u>	<u>144</u>	15	-259	<u>14</u>	-12	<u>697</u>	-67	<u>472</u>	1	<u>161</u>
8	120	5	413	<u>476</u>	-23	-44	<u>40</u>	-97	<u>88</u>	17	<u>429</u>	-95	<u>56</u>	-53	<u>493</u>
8	120	5	453	-99	<u>661</u>	-82	-37	<u>621</u>	<u>96</u>	9	-131	77	-252	<u>344</u>	<u>130</u>
					↑			↑						↑	
					C			A						G	

sig2 filtering approach summary:

sig2 basecaller analyzes calibrated peak intensities per channel

```
>SBO8_8_120_6_1512 | LINE: 692 [ FAIL: 69 ] :: LONG__Q: 24 :: CLEAN_LENGTH: 44  
[ ABCD_Q:56 F_QUAL:29 ] [ Q_FRACT:66 ]  
AAAAACAGATGCTACTTACTTTACTACCATATTTCCCAAACAANGNnCNAAcAGnANNAnAnNNCNC*N*NNnnNnnnnnNNnnN
```



low quality sequence after first failed call is removed

```
>SBO8_8_120_6_1512 | LINE: 692 [ LENGTH: 44 ] ( ____GC____: 13/44 )  
[ ABCD_Q:44 F_QUAL:0 ] [ Q_FRACT:100 ]  
AAAAACAGATGCTACTTACTTTACTACCATATTTCCCAAACAA
```

future plans - to find correlation between IGA quality scores and peak intensities

```
AAAAACAGATGCTACTTACTTTACTACCATATTTCCCAAACAANGNnCNAAcAGnANNAnAnNNCNC*N*NNnnNnnnnnNNnnN  
a`aab_Z_V__aXaa\[aa``^a`[^]ab`aa^aa_a]SXZZ]`UXXS_Y`__^^S^^Y^S^GU]^]TDIDL]SSZYPWU[[[V[
```

sig2 basecall / filtering


tcl_solexa_sig2_base_caller_2009_06_10_Beta.tcl - atgc-illumina - Project Hosting on Google Code - Mozilla Firefox

File Edit View History Bookmarks Tools Help

http://code.google.com/p/atgc-illumina/source/browse/trunk

Google

akozik@gmail.com | My favorites | Profile | Sign out

 **atgc-illumina**
tools to process and manipulate Illumina sequences

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Source path: svn/ trunk/ tcl_solexa_sig2_base_caller_2009_06_10_Beta.tcl < r6 r7

Show details

critical parameters

```
1 #!/usr/bin/tcl
2
3 proc Sig2_Table {argv} {
4
5     #### PARAMETERS ####
6     set upper_cut 180 ; # DONT CALL IF MAX VALUE BELOW THIS CUTOFF
7     set upper_dif 60 ; # ACCEPTABLE DIFF BETWEEN VALUES
8     set good_dif 100 ; # GOOD DIFF BETWEEN VALUES
9     set quality_upper_cut 1000 ; # QUALITY IN CAPITAL LETTERS ABOVE THIS VALUE
10    set quality_len_cut 20 ; # CUTOFF LENGTH OF ACCEPTABLE QUALITY SCORES
11    set good_length 24 ; # GOOD DNA LENGTH CUTOFF
12    set gc_upper 0.8 ; # UPPER GC CONTENT CUTOFF
13    set gc_lower 0.2 ; # LOWER GC CONTENT CUTOFF
14    set max_reads_debug 10000 ; # NUMBER OF SEQUENCES IN DEBUG FILE
15    set sig2_val_start 4 ; # COLUMN NUMBER WITH FIRST VALUES - QUADRO SET - COUNT FROM ZERO
16    ## END OF PARAM ##
17 }
```

Done

example of basecalling and filtering for sig2 files

	A	C	G	T		basecall			diff	ratio	qual
>SBO8_8_120_5_1105 COUNT: 636											
1:	A:-249	C:44	G:523	T:315	GOOD	BASE_CALL:->	G	FIRST_FAIL: -- MAX_DIFF: 208	RATIO: 0.60	QUAL: d	
2:	A:-248	C:79	G:25	T:443	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 364	RATIO: 0.17	QUAL: a	
3:	A:-146	C:-138	G:146	T:535	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 389	RATIO: 0.27	QUAL: b	
4:	A:-15	C:-177	G:159	T:372	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 213	RATIO: 0.42	QUAL: c	
5:	A:-241	C:-77	G:79	T:667	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 588	RATIO: 0.11	QUAL: a	
6:	A:2	C:-25	G:-10	T:558	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 556	RATIO: 0.00	QUAL: a	
7:	A:-54	C:-72	G:-1	T:561	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 562	RATIO: -0.00	QUAL: a	
8:	A:281	C:349	G:-13	T:-55	GOOD	BASE_CALL:->	c	FIRST_FAIL: -- MAX_DIFF: 68	RATIO: 0.81	QUAL: f	
9:	A:-67	C:-138	G:359	T:226	GOOD	BASE_CALL:->	G	FIRST_FAIL: -- MAX_DIFF: 133	RATIO: 0.62	QUAL: d	
10:	A:-160	C:-58	G:123	T:440	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 317	RATIO: 0.27	QUAL: b	
11:	A:-260	C:-21	G:6	T:668	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 662	RATIO: 0.00	QUAL: a	
12:	A:-17	C:-55	G:-31	T:613	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 630	RATIO: -0.02	QUAL: a	
13:	A:250	C:306	G:-35	T:-5	HOPE	BASE_CALL:->	x	FIRST_FAIL: -- MAX_DIFF: 56	RATIO: 0.81	QUAL: f	
14:	A:176	C:-13	G:-23	T:317	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 141	RATIO: 0.55	QUAL: c	
15:	A:318	C:7	G:32	T:17	GOOD	BASE_CALL:->	A	FIRST_FAIL: -- MAX_DIFF: 286	RATIO: 0.10	QUAL: a	
16:	A:194	C:303	G:69	T:-20	GOOD	BASE_CALL:->	C	FIRST_FAIL: -- MAX_DIFF: 109	RATIO: 0.64	QUAL: d	
17:	A:-86	C:13	G:-56	T:461	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 448	RATIO: 0.02	QUAL: a	
18:	A:353	C:-84	G:0	T:157	GOOD	BASE_CALL:->	A	FIRST_FAIL: -- MAX_DIFF: 196	RATIO: 0.44	QUAL: c	
19:	A:327	C:171	G:-19	T:54	GOOD	BASE_CALL:->	A	FIRST_FAIL: -- MAX_DIFF: 156	RATIO: 0.52	QUAL: c	
20:	A:149	C:-8	G:-18	T:288	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 139	RATIO: 0.51	QUAL: c	
21:	A:334	C:44	G:46	T:12	GOOD	BASE_CALL:->	A	FIRST_FAIL: -- MAX_DIFF: 288	RATIO: 0.13	QUAL: a	
22:	A:173	C:62	G:-11	T:218	HOPE	BASE_CALL:->	x	FIRST_FAIL: -- MAX_DIFF: 45	RATIO: 0.79	QUAL: d	
23:	A:130	C:-238	G:14	T:509	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 379	RATIO: 0.25	QUAL: b	
24:	A:89	C:80	G:-50	T:324	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 235	RATIO: 0.27	QUAL: b	
25:	A:-63	C:-70	G:361	T:74	GOOD	BASE_CALL:->	G	FIRST_FAIL: -- MAX_DIFF: 287	RATIO: 0.20	QUAL: b	
26:	A:41	C:-6	G:27	T:186	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 145	RATIO: 0.22	QUAL: b	
27:	A:86	C:36	G:-27	T:306	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 220	RATIO: 0.28	QUAL: b	
28:	A:-34	C:40	G:-11	T:346	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 306	RATIO: 0.11	QUAL: a	
29:	A:-147	C:80	G:-5	T:380	GOOD	BASE_CALL:->	T	FIRST_FAIL: -- MAX_DIFF: 300	RATIO: 0.21	QUAL: b	
30:	A:-101	C:-94	G:389	T:260	GOOD	BASE_CALL:->	G	FIRST_FAIL: -- MAX_DIFF: 129	RATIO: 0.66	QUAL: d	
31:	A:0	C:0	G:0	T:0	CRAP	BASE_CALL:->	*	FIRST_FAIL: 31 MAX_DIFF: 0	RATIO: X.XX	QUAL: x	
.....											
59:	A:74	C:54	G:115	T:83	NONE	BASE_CALL:->	n	FIRST_FAIL: 31 MAX_DIFF: 32	RATIO: 0.72	QUAL: x	
60:	A:173	C:-104	G:66	T:38	NONE	BASE_CALL:->	N	FIRST_FAIL: 31 MAX_DIFF: 107	RATIO: 0.38	QUAL: x	

>SBO8_8_120_5_1105 | LINE: 636 [FAIL: 31] :: SHORT_Q 22 ::
 CLEAN_LENGTH: 12 [ABCD_Q:56 F_QUAL:29] [Q_FRACT:66]
 GTTTTTTcGTTTtTACTAATAxTTGTTTTG*A*AGTGxgCCTCCCTxTGNGGATTCTTnN*GA*Gnn**t*G**nn**nNn*nnn

fasta output

sig2 basecalling and filtering - example of *.good.trim.fasta output file

```
>SB08_8_120_65_1105 | LINE: 9728 [ LENGTH: 44 ] ( ____GC____: 20/44 )
[ ABCD_Q:44 F_QUAL:0 ] [ Q_FRACT:100 ]
AACAAATGTCTCGAATCTTAACCGCACATCAAGCTGCAAGAGGG

>SB08_8_120_65_1326 | LINE: 9734 [ LENGTH: 46 ] ( ____GC____: 20/46 )
[ ABCD_Q:46 F_QUAL:0 ] [ Q_FRACT:100 ]
AGAACAAGGAGGATTATGCCAAGTTCTACGAGGCTTTCTCCAAGAA

>SB08_8_120_88_573 | LINE: 13230 [ LENGTH: 83 ] ( ____GC____: 22/83 )
[ ABCD_Q:83 F_QUAL:0 ] [ Q_FRACT:100 ]
CGAGAATATATATGAACAAGAGTTAGAACAAAAAGAAAGGATGAAAATGAAATCATACAAAATTCTGAATCATTCTCTAACT

>SB08_8_120_88_899 | LINE: 13235 [ LENGTH: 63 ] ( ____GC____: 17/63 )
[ ABCD_Q:63 F_QUAL:0 ] [ Q_FRACT:100 ]
CTCATAAGTAATTCAAAGCTTTTTCAAAC TTCATTaCAAATAATTAACGTTTCATTTC CATGG

>SB08_8_120_166_1586 | LINE: 25397 [ LENGTH: 48 ] ( LOWER_GC: 6/48 )
[ ABCD_Q:48 F_QUAL:0 ] [ Q_FRACT:100 ]
ATTCTGTTC ACTAATGGTTTATTTaTTTATTTATTTTTTTTTTTTATAA

>SB08_8_120_170_1139 | LINE: 26020 [ LENGTH: 30 ] ( UPPER_GC: 24/30 )
[ ABCD_Q:30 F_QUAL:0 ] [ Q_FRACT:100 ]
CGTCGATCCCCACCACGCCCGGCTCCGCCG
```

GC% content info



grep-able fasta header

sig2 basecalling and filtering of reads with adapter - example of *.good.trim.fastq

```
@SBN4_4_24_177_1635 _TCGTAT_ : 21
TTCAAGGATAACTTTTGCATATCGTAT
```

```
+
IIIIII99IIIIII9II599III909I9
```

```
@SBN4_4_24_178_1771 _TCGTAT_ : 19
TCCCAAATGTAGACAAAGCTCGTATGCCGTCTTCT
```

```
+
IIIIIIIIII99I5IIIIII95959II95II599II99
```

```
@SBN4_4_24_179_1454 _NOT_FOUND_ : -1
AAATGAACTCAGCCGTAACGCGATTC
```

```
+
IIII9IIIIII99I5II995I0I9II
```

I-40(73) 9-24(57) 5-20(53) 0-15(48) #-2(35)

Phred pseudo-quality scores (ASCII - 33)

```
@SBN4_4_24_181_1684 _NOT_FOUND_ : -1
AGTGGTCATGAGAAGAAAGATTGCT
```

```
+
I9II9IIII5I5II5III9II95II
```

```
@SBN4_4_24_182_1666 _TCGTAT_ : 23
GGGTGAGGGAGATAGATGGATTATCGTAT
```

```
+
999I9II59I9III9II95II99I959I9
```

```
@SBN4_4_24_183_1747 _TCGTAT_ : 24
AAGAGGGAAAAGGGCTATTAAGCTCGTAT
```

```
+
II9I9IIIIII9999IIIIII5III95II9
```

```
### TRIMMING WITH ADAPTER OPTIONS ###
set number_of_runs $cycle_last
### set trimming_with_adapter "FALSE"
set trimming_with_adapter "TRUE"
set adapter_seqs "TCGTAT"
set adapter_status "_NOT_FOUND_"
### END OF TRIMMING WITH ADAPTER ###
```

comment / uncomment options within source code

grep-able fasta header:

```
grep -A 1 " _TCGTAT_ "
```

[to extract all seqs with tag]

```
grep -A 1 " _TCGTAT_ : 21"
```

[to extract seqs with tag at pos 21]

illupa rectificator - re-format position, intensity, noise raw files into linear form

pos

-0.50	339.55
-0.47	29.30
-0.43	364.48
-0.37	653.60
-0.37	956.44
....	
1793.15	1684.18
1793.15	1756.25
1793.15	1790.75
1793.15	1850.09
1793.15	1911.18
1793.15	1955.35
1793.15	1982.12
1793.18	298.99
1793.46	1776.56
X	Y

int

-25.0	-104.9	43.2	163.0
6.2	28.5	-145.9	502.6
-12.1	-16.1	-77.0	368.6
2.9	13.9	12.9	522.6
7.8	21.2	131.9	215.9
-1.2	9.3	233.4	342.9
-2.2	-4.3	-9.6	490.6
3.6	12.4	5.1	263.3
-1.8	7.4	2.0	82.2
2.9	-23.6	4.8	451.0
6.1	19.9	-2.5	511.6
5.8	14.7	60.9	140.3
0.8	10.2	-33.7	39.9
151.3	584.0	-199.8	-74.3
188.4	623.9	0.0	-49.1
#END CYCLE 1			

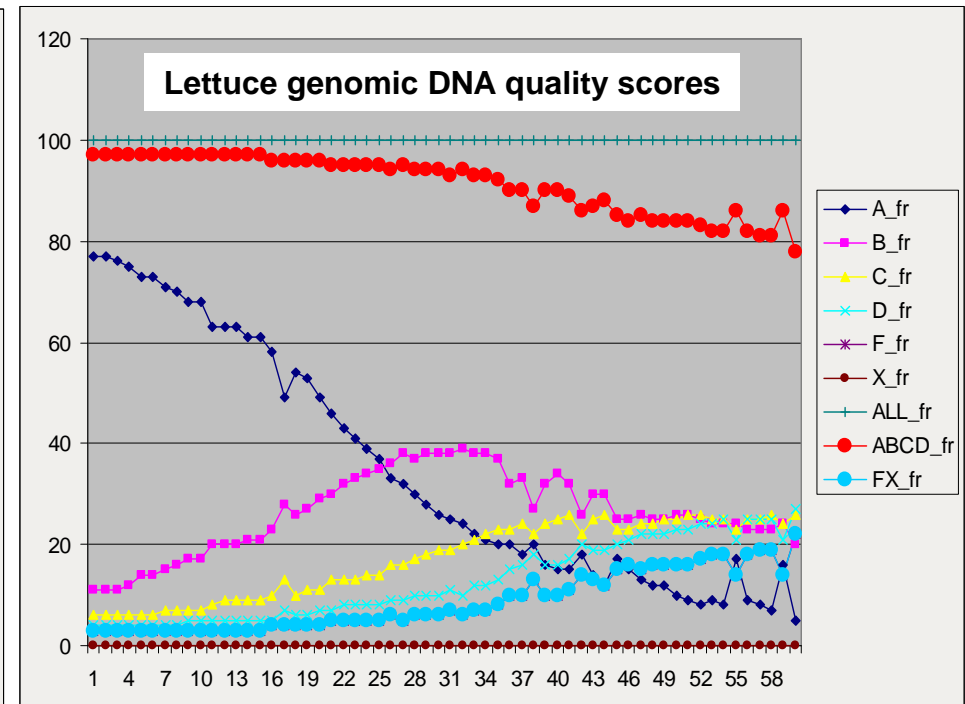
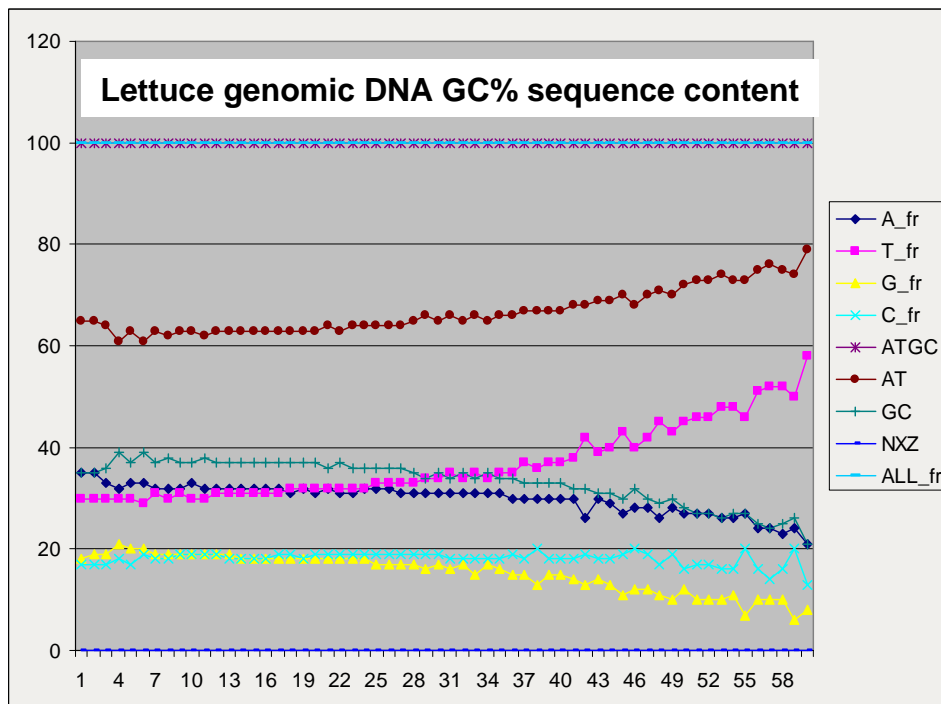
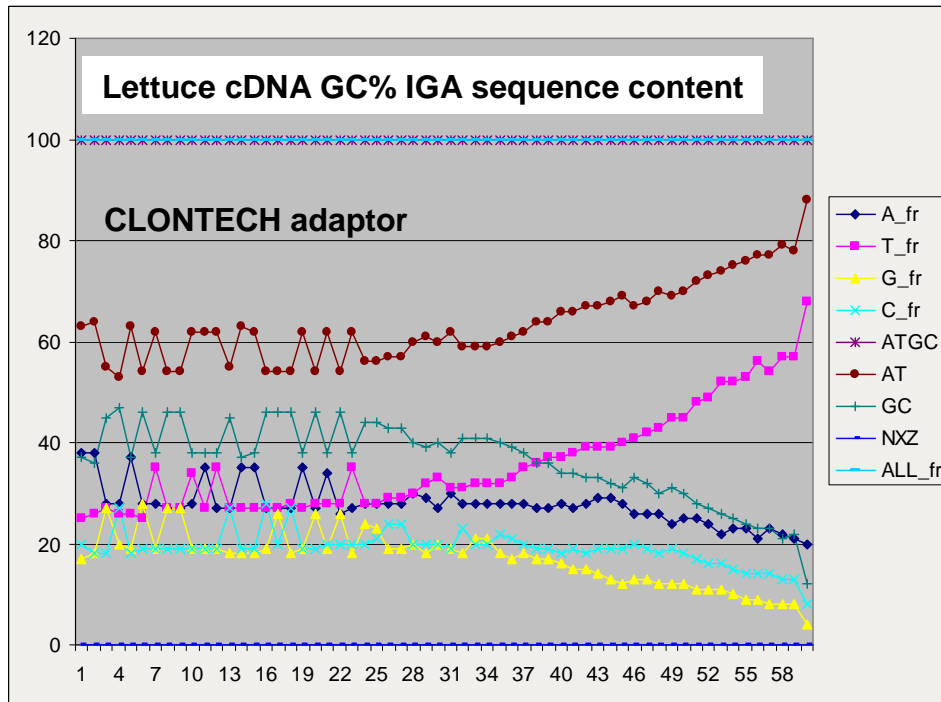
nse

3.1	5.7	2.6	3.3
3.0	5.8	2.8	5.5
3.0	5.8	2.8	5.5
3.0	5.8	2.8	5.5
3.4	6.3	3.0	3.4
3.4	6.3	3.0	3.4
2.8	5.1	2.5	5.3
2.8	5.1	2.5	5.3
2.8	5.1	2.5	5.3
3.2	5.7	2.5	4.4
3.2	5.7	2.5	4.4
2.6	5.2	2.4	3.8
2.6	5.2	2.4	3.8
3.2	6.4	2.8	4.0
2.8	5.1	0.0	5.3
#END CYCLE 1			

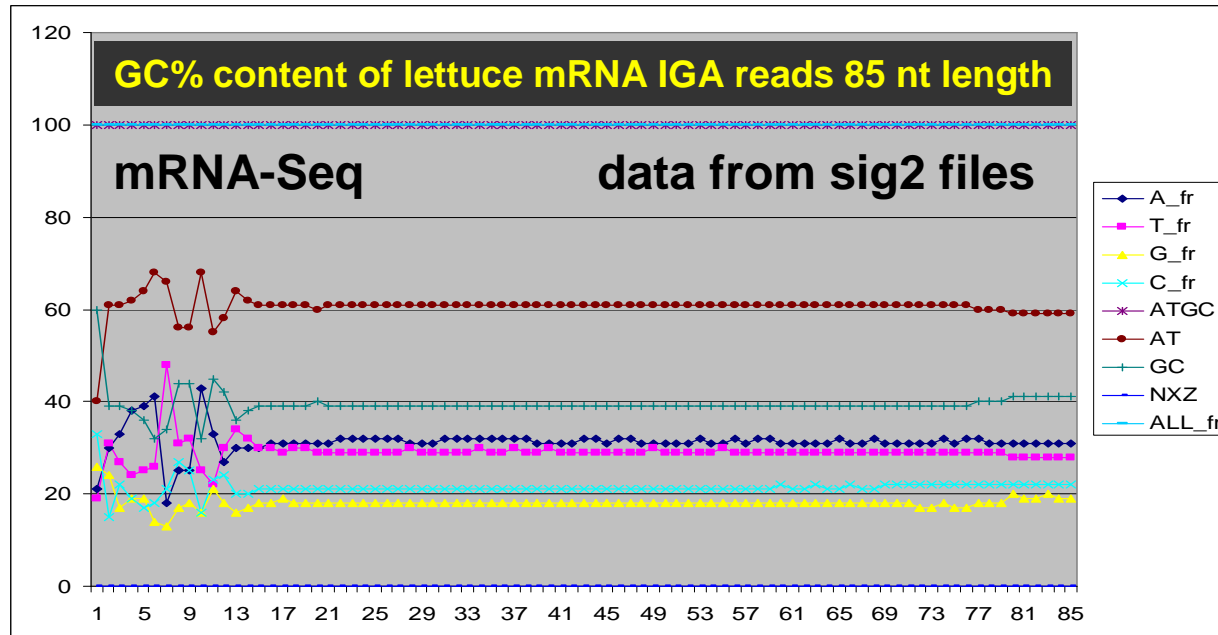
line	tile	X	Y	cycle 1				cycle 2				cycle 3				cycle 4			
				A	C	G	T	A	C	G	T	A	C	G	T	A	C	G	T
3	0012	108.61	1169.14	-10	20	22	630	9	21	894	479	13	25	756	411	182	539	2	20
3	0012	108.63	1586.81	570	685	1	9	43	37	770	545	38	45	540	394	770	529	-7	15
3	0012	108.67	1305.63	127	503	2	14	11	104	19	670	37	57	703	442	121	13	766	462
3	0012	108.72	1600.48	-98	-105	6	609	-56	528	-32	-18	676	489	-15	-32	651	407	-57	16
3	0012	108.75	1644.07	165	582	2	24	39	54	20	620	208	657	5	-13	868	574	4	-18
3	0012	108.75	1357.78	191	665	1	25	9	26	21	639	182	683	4	24	672	503	13	21
3	0012	108.76	1894.18	-26	-9	445	540	12	12	543	354	794	580	4	17	464	452	5	5
3	0012	108.78	1694.68	175	611	-7	1	-16	-29	18	569	187	633	-3	9	271	581	-16	
3	0012	108.82	1556.78	893	519	-4	18	182	545	4	3	15	17	614	343	555	374	8	12
3	0012	108.83	1365.97	148	645	2	27	908	740	3	8	773	635	6	25	694	538	2	24
3	0012	108.88	875.17	10	35	650	362	145	509	2	-43	135	453	0	21	464	364	3	14

QC for GC% content and quality scores

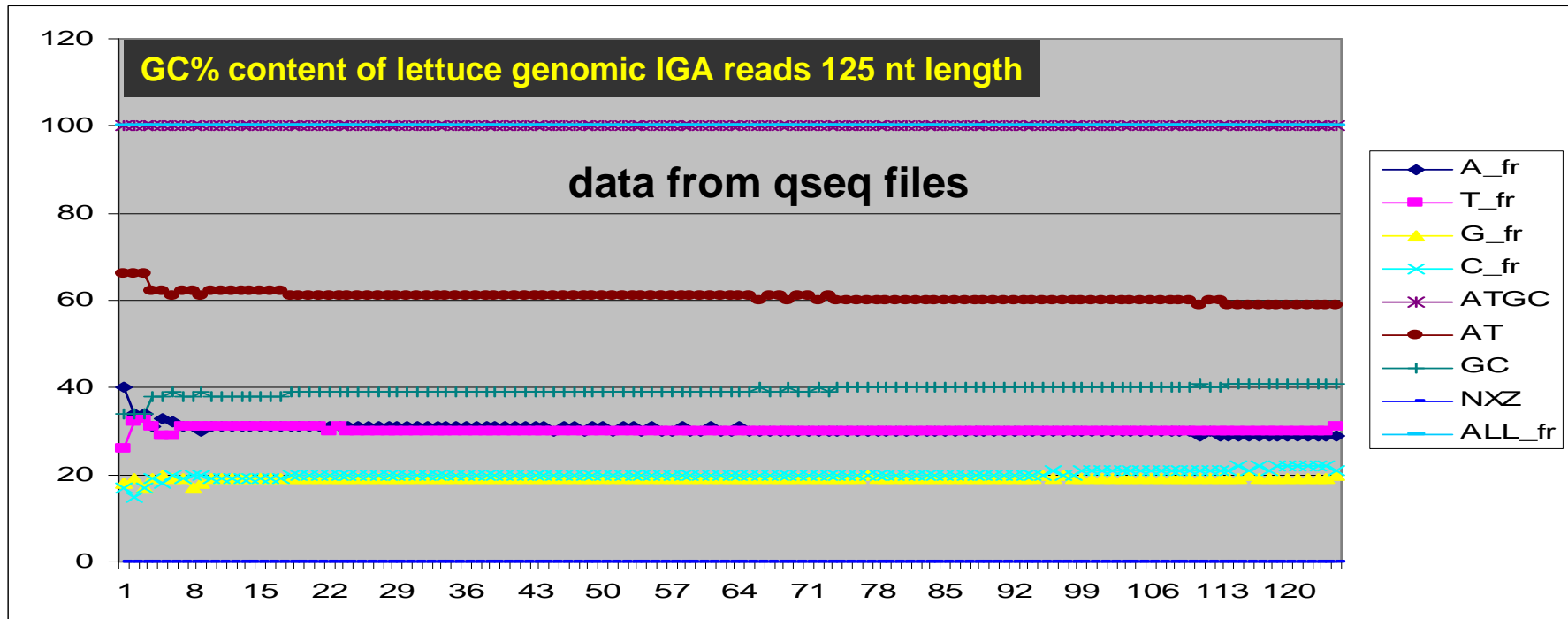
OLD CHEMISTRY

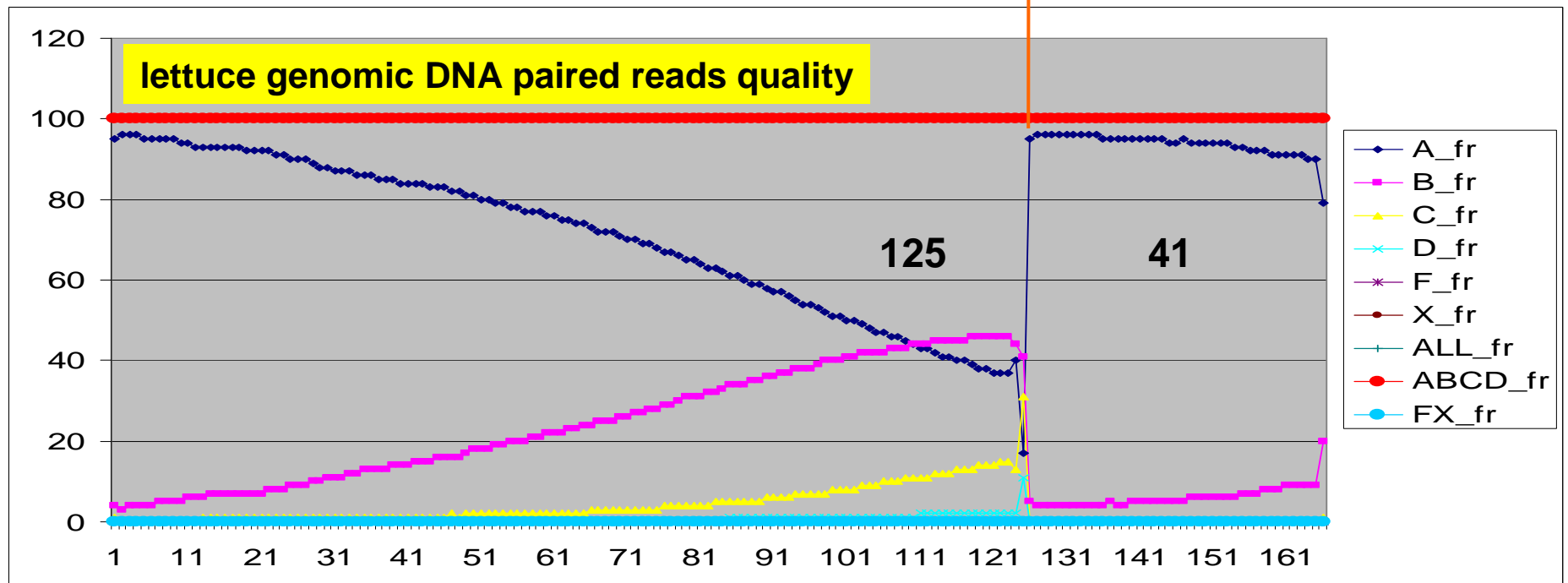
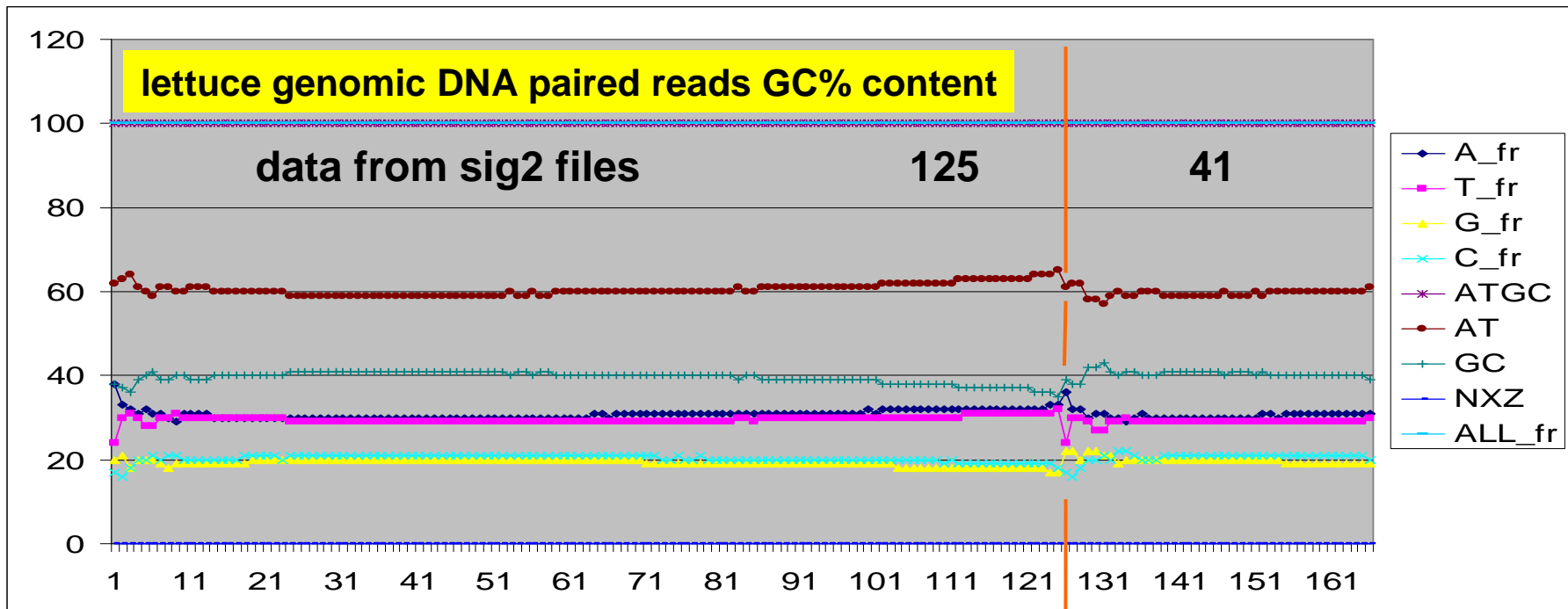


QC examples for GC% content



NEW CHEMISTRY





project overview and dataflow

IGA output files

IGA quality scores

filtering for high quality reads

assembly parameters

visualization with Python Contig Viewer

de novo assembly software -

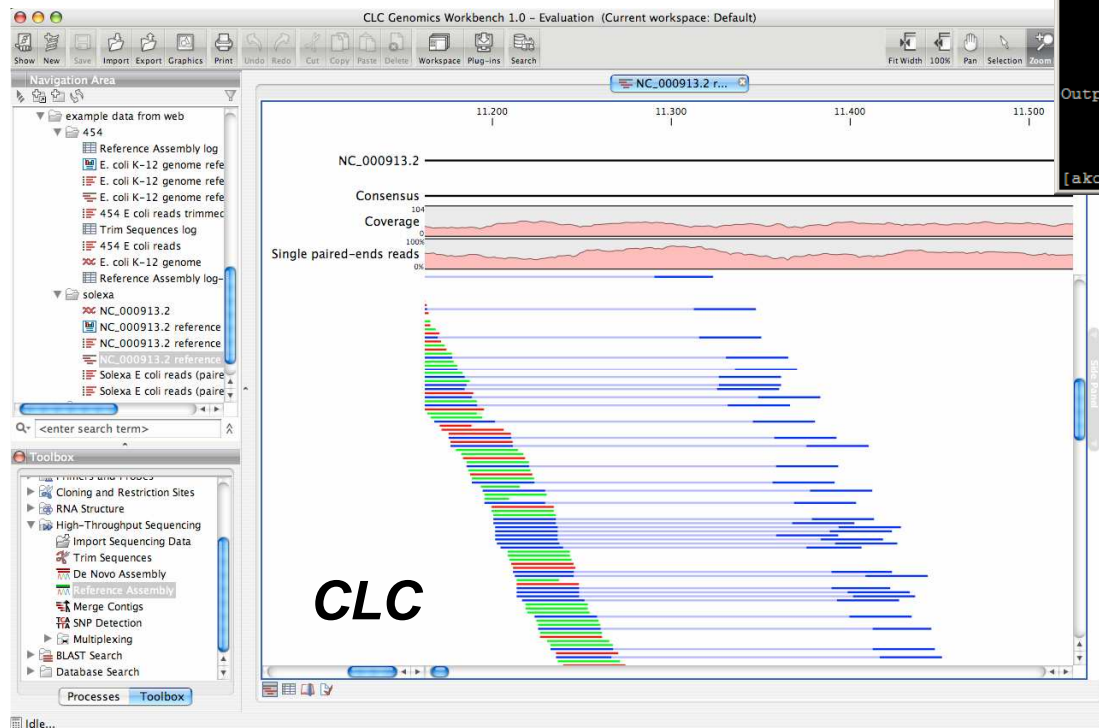
Velvet - publicly available

<http://www.ebi.ac.uk/~zerbino/velvet/>

CLC Genomics workbench - commercial

<http://www.clcbio.com/>

different algorithms - different assemblies



```
akozik@aristotle:~/_velvet_0.7.49
[akozik@aristotle _velvet_0.7.49]$ ./velveth
velveth - simple hashing program
Version 0.7.49

Copyright 2007, 2008 Daniel Zerbino (zerbino@ebi.ac.uk)
This is free software; see the source for copying conditions. There is NO
warranty; not even for MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE.

Compilation settings:
CATEGORIES = 2
MAXKMERLENGTH = 31

Usage:
./velveth directory hash_length {[-file_format][-read_type] filename}

    directory      : directory name for output files
    hash length    : odd integer (if even, it will be decremented)
    <= 31 (if above, will be reduced)
    filename       : path to sequence file or - for standard input

File format options:
-fastq
-fastq.gz
-eland
-gerald

Read type options:
-short
-shortPaired
-short2
-shortPaired2
-long
-longPaired

Output:
directory/Roadmaps
directory/Sequences
[Both files are picked up by graph, so please leave them there]
[akozik@aristotle _velvet_0.7.49]$
```

Velvet

Velvet -

- large input 120+ million reads
- low number of chimeric contigs

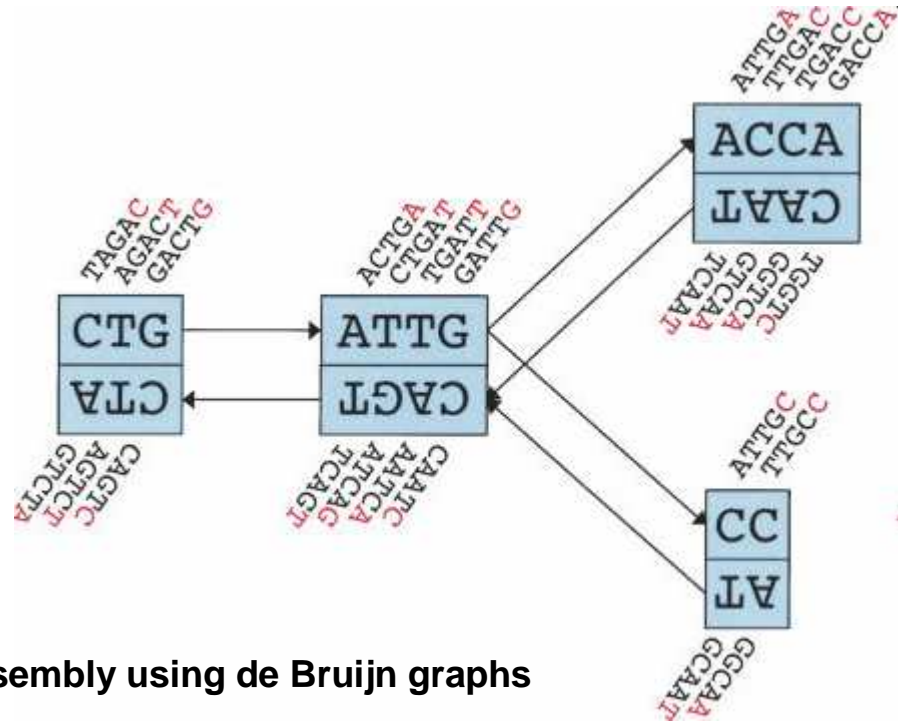
CLC -

- 20 million reads input limit
- higher number of chimeras

CLC contigs usually longer than Velvet contigs

Velvet

critical parameter K-mer value



Velvet: Algorithms for de novo short read assembly using de Bruijn graphs Daniel R. Zerbino and Ewan Birney

Figure 1.

Schematic representation of our implementation of the de Bruijn graph. Each node, represented by a single rectangle, represents a series of overlapping k-mers (in this case, $k = 5$), listed directly above or below. (Red) The last nucleotide of each k-mer. The sequence of those final nucleotides, copied in large letters in the rectangle, is the sequence of the node. The twin node, directly attached to the node, either below or above, represents the reverse series of reverse complement k-mers. Arcs are represented as arrows between nodes. The last k-mer of an arc's origin overlaps with the first of its destination. Each arc has a symmetric arc. Note that the two nodes on the left could be merged into one without loss of information, because they form a chain.

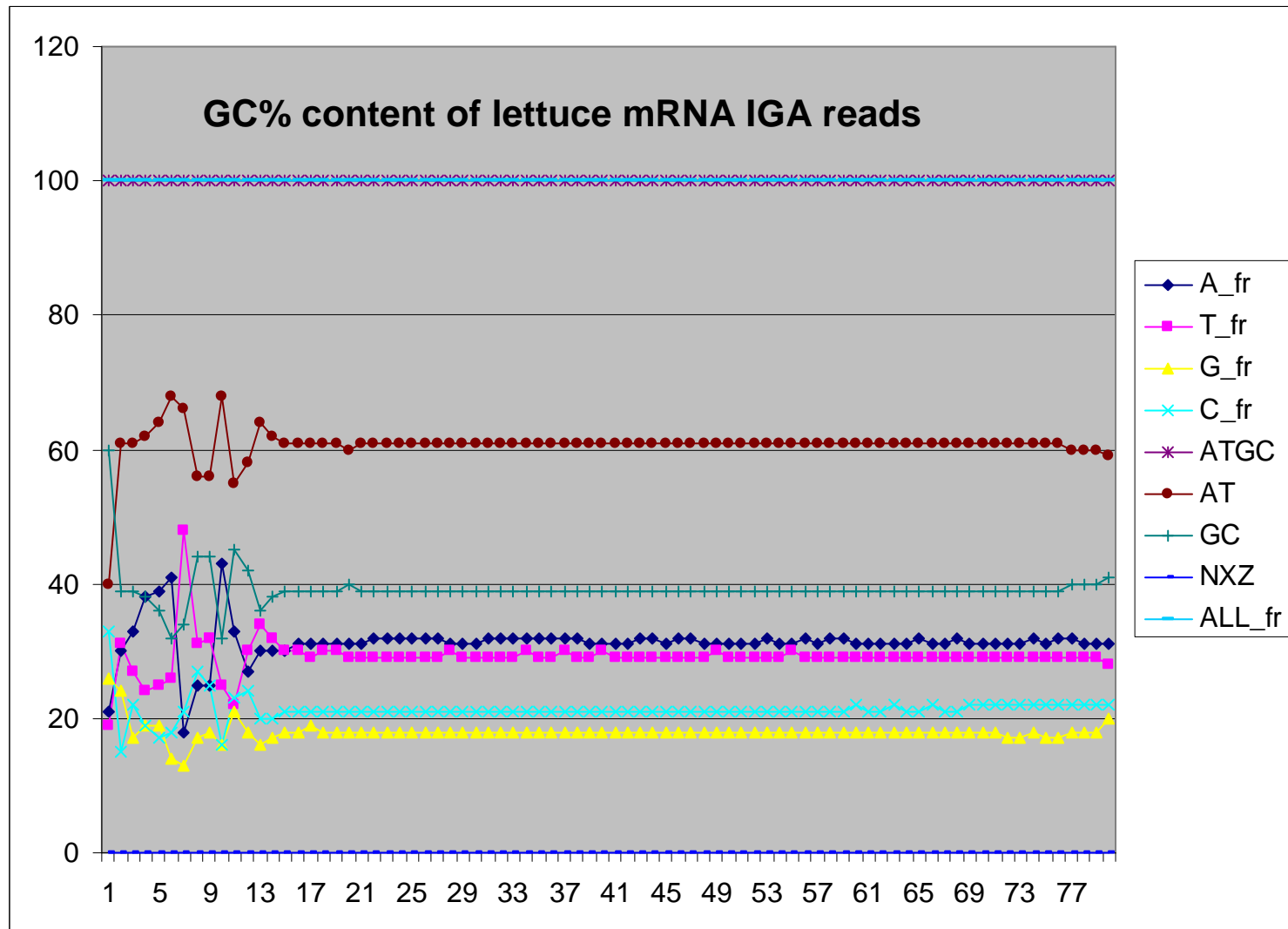
Genome Res. 2008 May; 18(5): 821–829.

doi: 10.1101/gr.074492.107.

Copyright © 2008, Cold Spring Harbor Laboratory Press

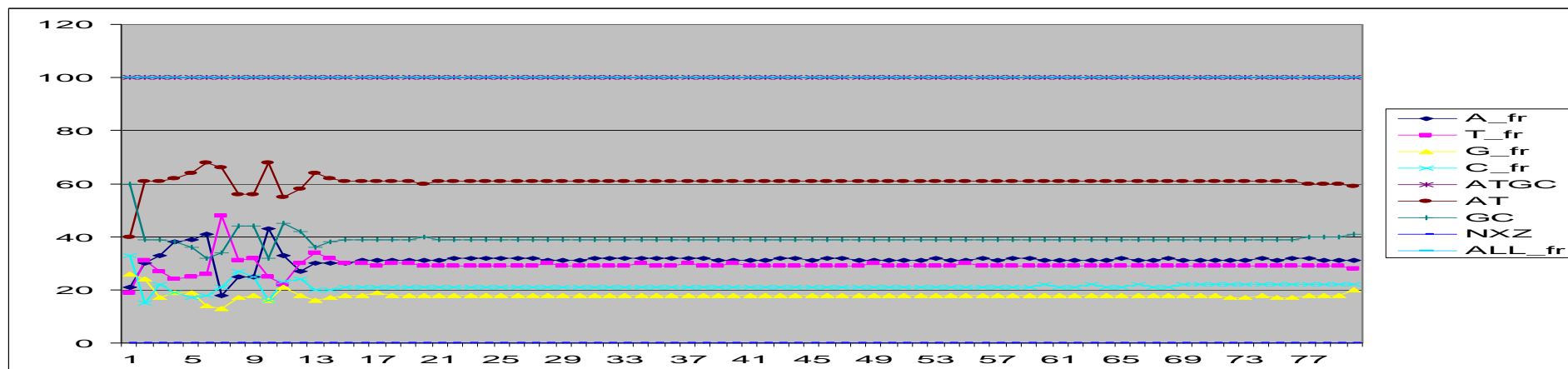
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2336801>

case study - assembly of 22.62 million mRNA reads



QC trimming and assembly with 22.62 million reads

four subsets: 80 nt, 70 nt, 65 nt and 60 nt



```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_80.fa
>SB08_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTGCTTGATGACAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_70_A.fa
>SB08_8_120_1651_1715
AACCATCTTGGACAAAACGTTTGTGCTTGATGACAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

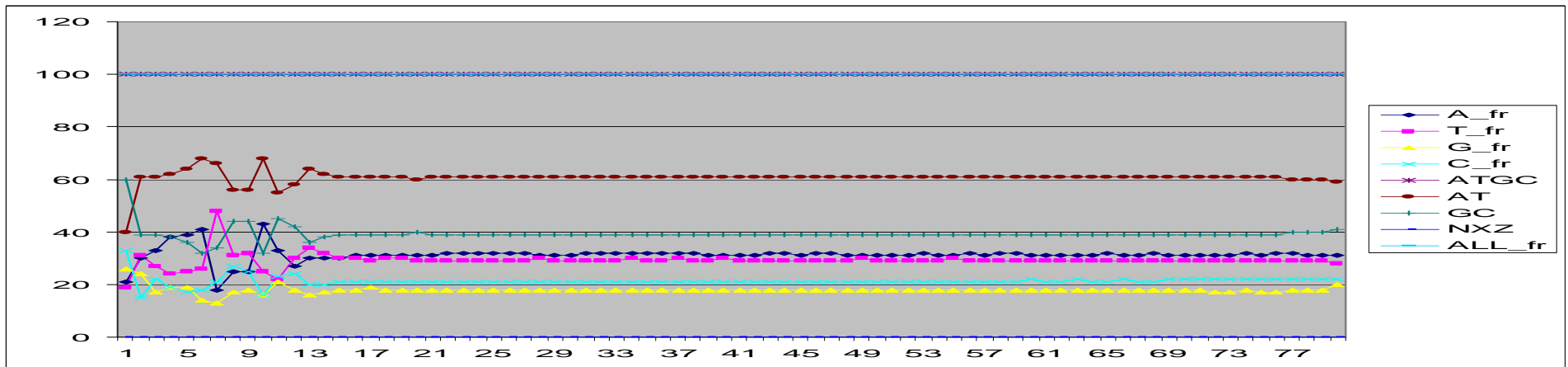
A

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_70_D.fa
>SB08_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTGCTTGATGACAACTATTATGCAAAAAGATCCGGG
```

D

set 'A' - trim left part of original sequence
set 'D' - trim right part of original sequence

10 nt trimming



```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_80.fa
>SB08_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTGCTTGATGACAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_65_A.fa
>SB08_8_120_1651_1715
TCTTGGACAAAACGTTTGTGCTTGATGACAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

A

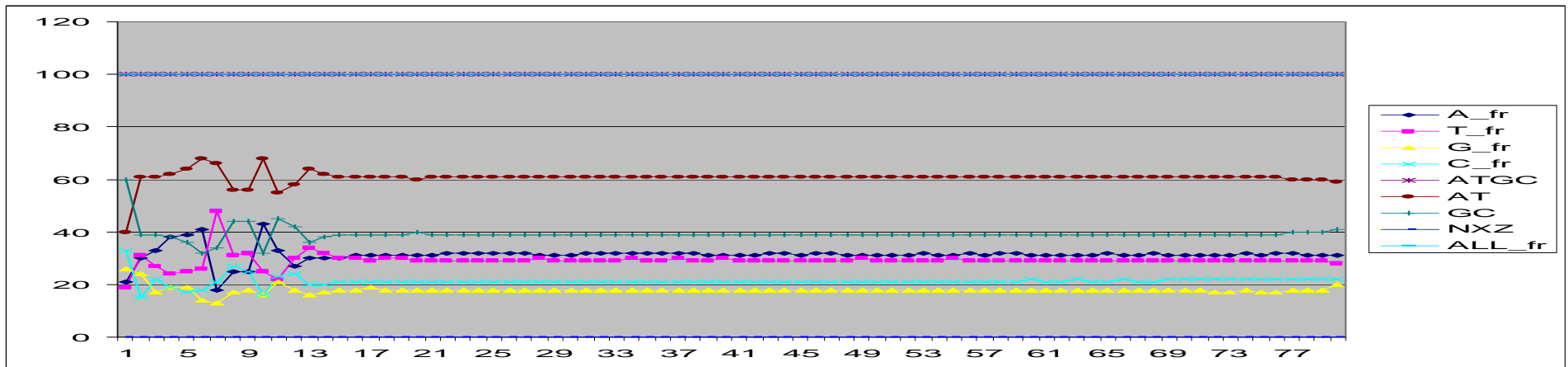
```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_65_B.fa
>SB08_8_120_1651_1715
AACCATCTTGGACAAAACGTTTGTGCTTGATGACAACTATTATGCAAAAAGATCCGGGAAGAA
```

C

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_65_D.fa
>SB08_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTGCTTGATGACAACTATTATGCAAAAAGAT
```

D

15 nt trimming



```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_80.fa
>SB08_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_A.fa
>SB08_8_120_1651_1715
GACAAAACGTTTGGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

A

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_B.fa
>SB08_8_120_1651_1715
TCTTGGACAAAACGTTTGGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAA
```

B

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_C.fa
>SB08_8_120_1651_1715
AACCATCTTGGACAAAACGTTTGGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGG
```

C

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_D.fa
>SB08_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGGTTGCTTGATGACAAACTATTATGCAAA
```

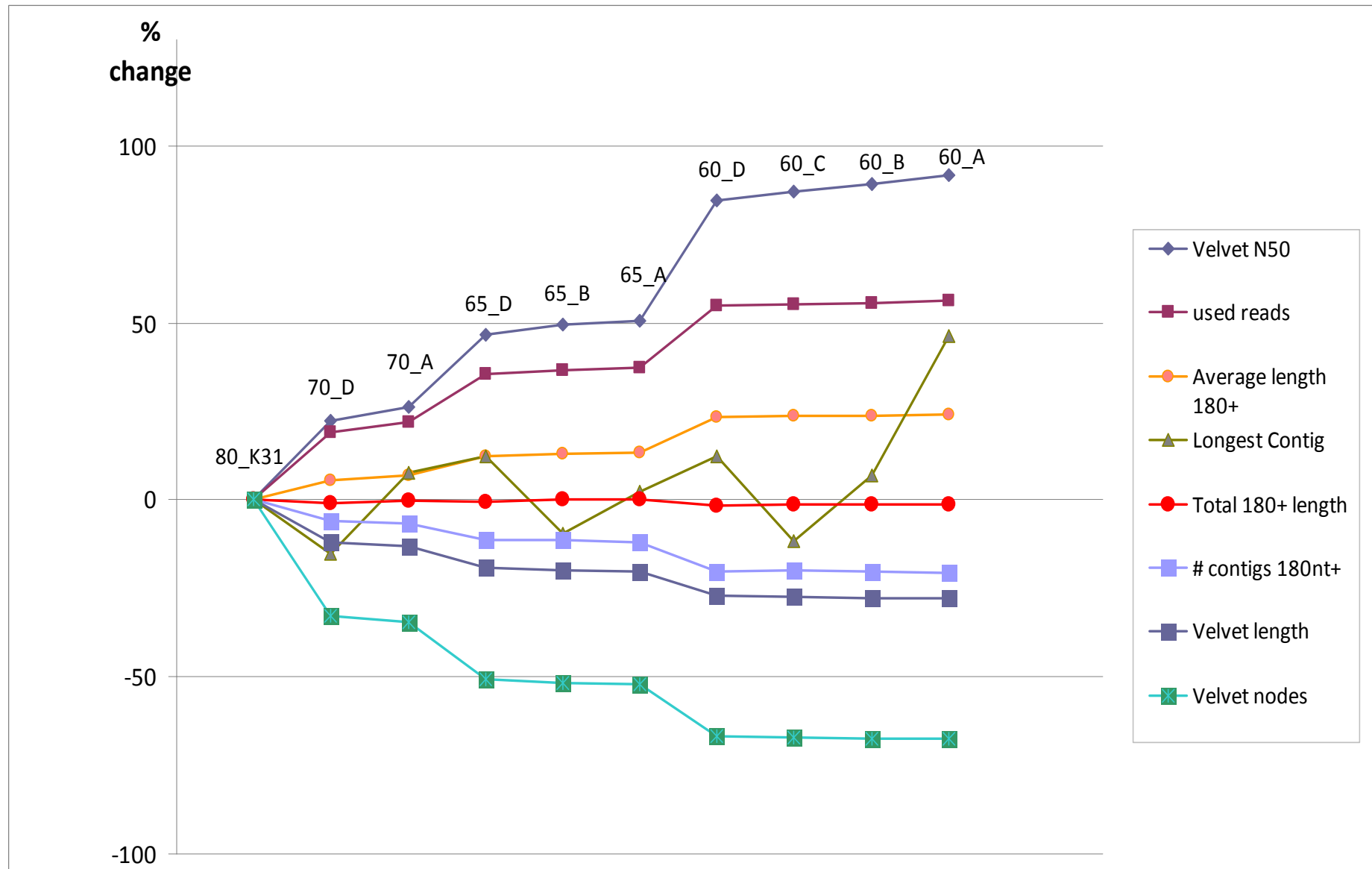
D

20 nt trimming

Trimming effect on assemblies (transcriptome)

sample	# of reads (million)	used reads (million)	velvet N50	velvet # nodes	velvet length million nt	longest	median 180 nt +	average 180 nt +	contigs 180nt +	total 180+ length million nt
80_K27	22.62	8.91	126	968,002	53.08	2428	260	312	79,889	24.94
80_K29	22.62	9.78	141	829,641	50.38	2448	262	318	82,619	26.28
80_K31	22.62	10.73	156	719,028	48.97	4417	267	326	85,123	27.76
70_D	22.62	12.78	191	483,450	43.01	3752	278	344	80,045	27.53
70_A	22.62	13.08	197	469,949	42.62	4752	279	349	79,578	27.74
65_D	22.62	14.53	229	353,683	39.51	4964	290	366	75,602	27.64
65_B	22.62	14.68	233	347,461	39.28	3995	289	368	75,387	27.76
65_A	22.62	14.75	235	343,477	39.11	4523	290	370	75,038	27.76
60_D	22.62	16.61	288	239,076	35.71	4964	307	402	67,942	27.29
60_C	22.62	16.67	292	237,207	35.57	3900	305	403	68,112	27.41
60_B	22.62	16.71	295	235,140	35.44	4720	306	404	67,836	27.41
60_A	22.62	16.76	299	233,365	35.33	6459	306	405	67,744	27.43

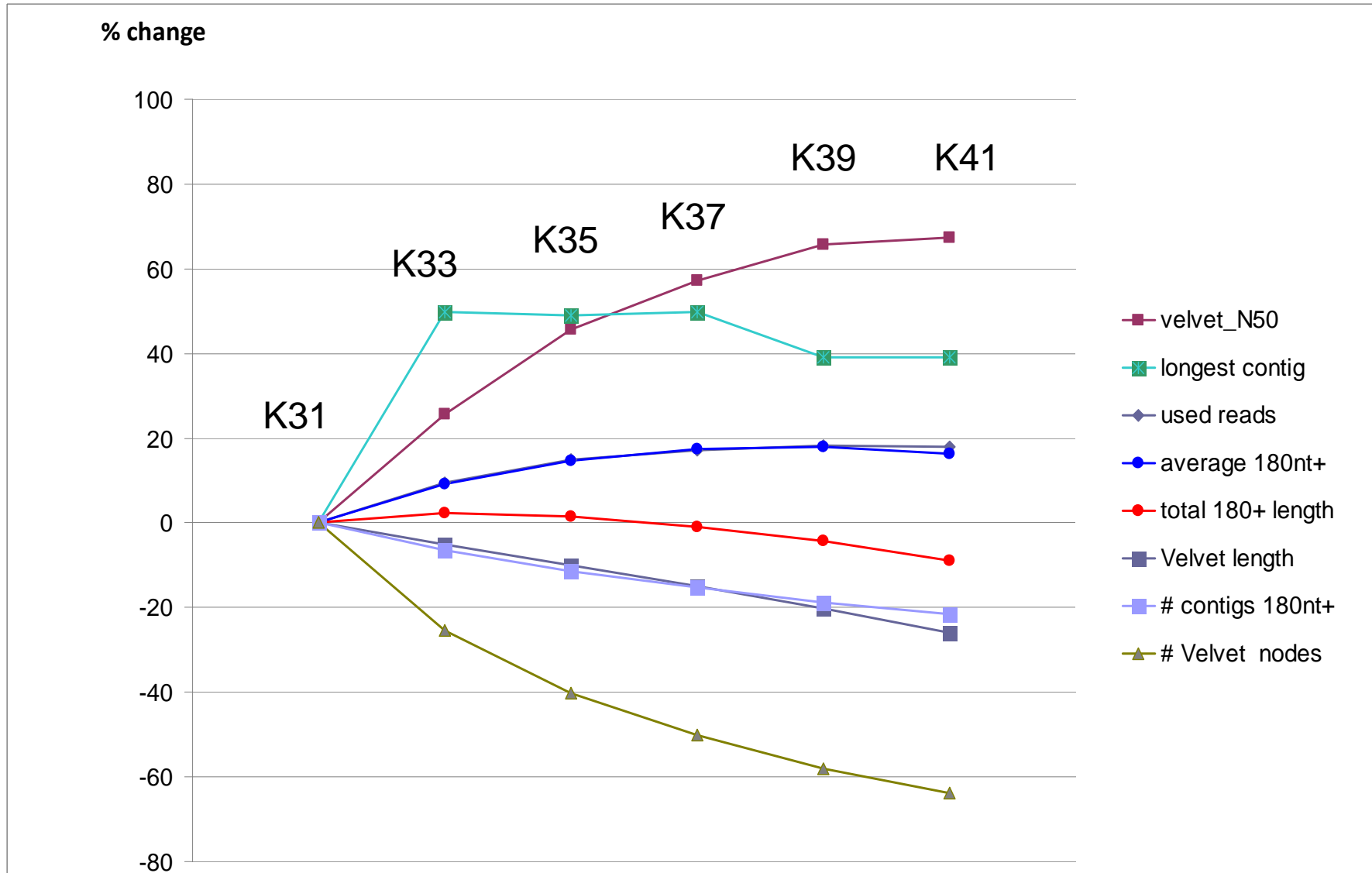
Trimming effect on assemblies (transcriptome)



Velvet K-mer effect on assemblies (transcriptome)

sample	# of reads (million)	used reads (million)	velvet N50	velvet # nodes	velvet length million nt	longest	median 180 nt +	average 180 nt +	contigs 180nt +	total 180+ length million nt
80_QC K31	47.50	17.03	126	1,160,217	60.85	4272	261	318	93,175	29.67
60_QC K31	47.50	33.44	303	325,378	42.83	5131	313	422	76,994	32.55
60_QC K33	47.50	36.64	381	242,908	40.68	7682	328	461	72,092	33.26
60_QC K35	47.50	38.39	441	194,847	38.58	7640	338	484	68,241	33.03
60_QC K37	47.50	39.17	476	162,163	36.46	7682	342	495	65,230	32.26
60_QC K39	47.50	39.58	502	136,282	34.20	7141	343	498	62,557	31.16
60_QC K41	47.50	39.47	507	117,446	31.76	7141	338	491	60,446	29.68

Velvet K-mer effect on assemblies (transcriptome):
same input 47.50 million reads of 60 nt length - different K-mer values



assembly of the assemblies

1 step

Velvet and/or CLC with different parameters and samples

2 step

CAP3 assembly with Velvet and/or CLC contigs

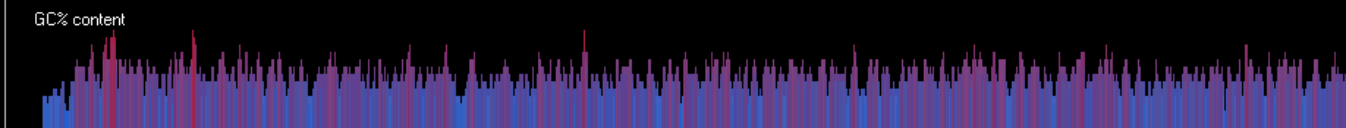
3 step

Visualization and validation

Transcriptome

CAP3 assembly of different Velvet assemblies

Assembly Identifier	Transcript Count
1	1454
182	3063
281	686
281	686
281	1787
281	686
297	1787
319	686
319	686
504	686
502	686
722	1787
722	1815
1425	1818
1425	1818
1425	1974
1425	1974
1604	1787
1604	1789
1760	1974
1760	1976
1760	2296
1760	2486
1760	2491
1760	1978
1805	3753
2006	2296
2006	3205
2006	3205
2006	2296
2006	4570
2006	6363
2006	2185
2542	3177
2542	4563
2542	6958
2544	4909
2856	3938
2941	3154
3129	4907
3177	5805
3177	5809
4549	5380
4559	4763
4549	6262
4549	6817
4564	4758
4564	5814
4927	6958
4929	6958
5366	5912
5793	6042
5798	6352
5798	6358
5798	6042
5947	6171
6016	6252
6239	6812
6346	6535
6346	6688
6346	6683
6341	6540
6351	6578
6346	6958
6394	6620
6661	6975
6666	6958
6729	6958
6724	6975
6837	7396
7396	7396



Query= SBOX_V07_399 L:7396
(7396 letters)

Database: PlantRefSeq_GB_2009_07_07.fasta
246,809 sequences; 93,580,655 total letters

>Arab_thal.NP_190607 (NP_190607.1 GI:15229738) { unknown protein } [Arabidopsis
thaliana]
Length = 3071

Score = 1771 bits (4588), Expect = 0.0
Identities = 1050/2534 (41%), Positives = 1510/2534 (59%), Gaps = 121/2534 (4%)
Frame = -2

Query: 7395 KCCLAMGQLDFFLGYSSALSLTLLLRQIQNAFSLEKAQTSTPTYDTPLVRVWDCDSSIAE 7216
KC + +G+LD YSS SL LL+ QI+ A L + + + LV D +A
Sbjct: 652 KCSMVLGKLDIVFEYSSLFSLALLIWQIEWAQKLLVDDYTGEVHSSSLV-TGGVDPEMAS 710

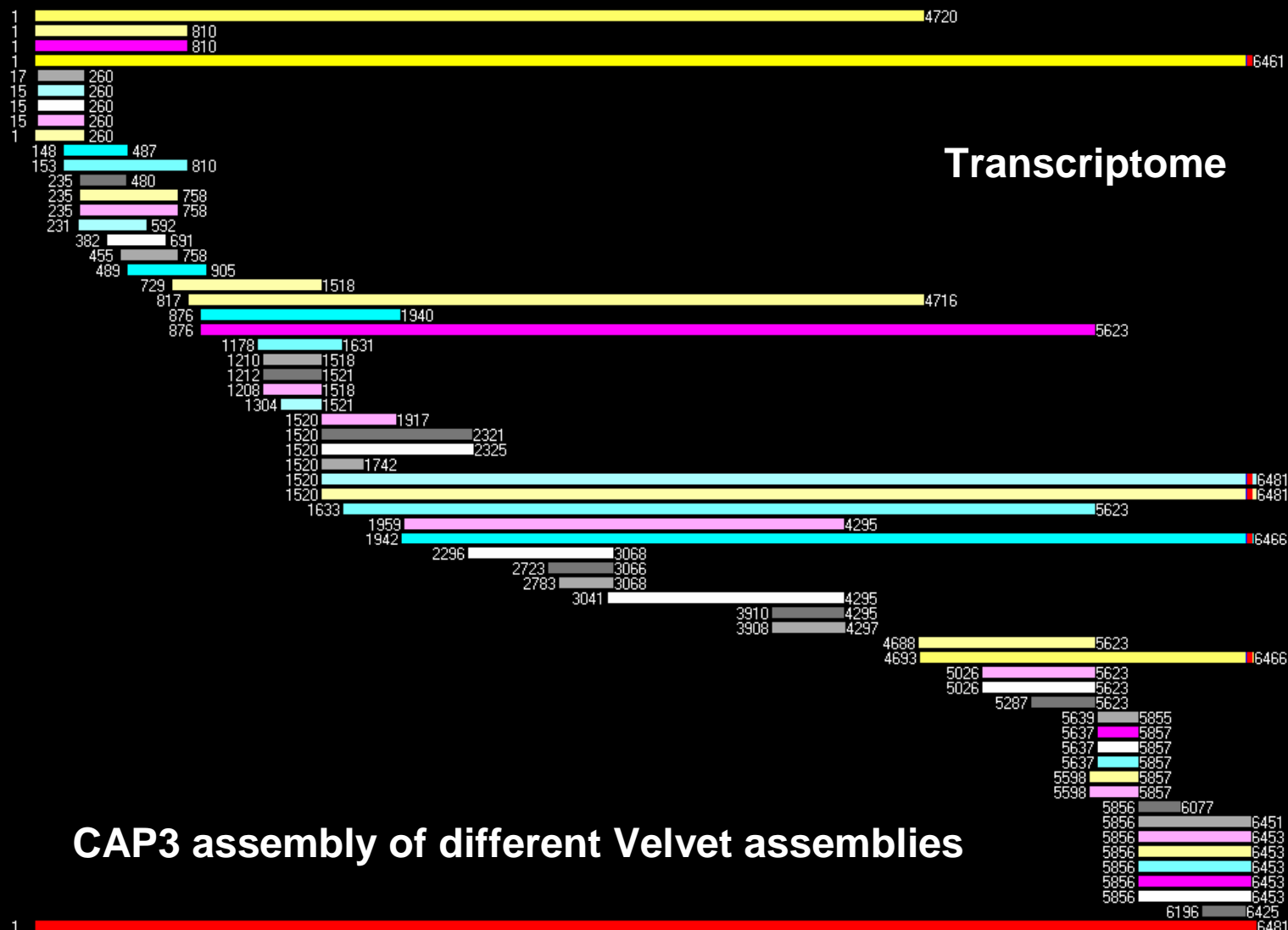
Query: 7215 -----MEKELHKVIEPEKLIIEVGVYVVGPRIRVSLRK-DSLHKAVD--DIHLSFDC 7075
+E LH+V PE+ I+VG+ + GP+I++ + K + ++ + DI L FD
Sbjct: 711 YDEYGIYRRSIELSLHRVHPERQIQVGILLGGPQIKLLVEKAAEEVNTLIGKKDI-LLFDF 769

.....

Query: 528 GDSSFHSHPLHHLRGQNEWIRIGPTILTCEHLFVNFAIGWLREQAGDL-----TAKI 376
GD S S P L G EW+IGPT+LTLCEHLFV+FAI L++ A A+
Sbjct: 2938 GDPSSSRPRRRLHGDKEWKIGPTVLTCEHLFVSFAIRILKQHATKAITSRLPKKEEAEA 2997

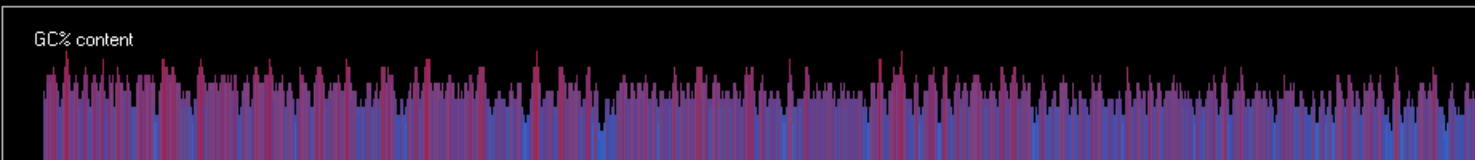
Query: 375 NWGDRFKGDPPKEIVKEEESKMSVL-KWGVGRFVVFAGMVAYIDGRLCRCIPNPVARRIV 199
D +V + + KM + K G+G FV +G+VAYIDGRLCR IPNP+ARRIV
Sbjct: 2998 ETSDSGSNTAMVPVSDNKKKKMKFMWKAGIGNFVASGIVAYIDGRLCRQIPNPIARRIV 3057

Query: 198 SGFVLSFLDKTRDK 157
SGF+LSFLDK+ ++
Sbjct: 3058 SGFLLSFLDKSSEQ 3071



SBOX_060_25290_4690_+
 SBOX_060_25419_780_2+
 SBOX_070_276999_780_+
 SBOX_060_7897_6429_1-
 SBOX0K29_469027_216_+
 SBOX_065_218711_216_+
 SBOX0K31_413415_216_+
 SBOX_070_285555_216_+
 SBOX_060_149245_230_+
 SBOX_065_101366_310_+
 SBOX_065_29339_628_2+
 SBOX0K27_226562_220_+
 SBOX_060_25455_498_2+
 SBOX_070_34736_498_3+
 SBOX_065_103099_332_+
 SBOX0K31_48180_280_3+
 SBOX0K29_478568_276_+
 SBOX_065_29091_387_2+
 SBOX_060_41787_763_1+
 SBOX_060_31902_3870_+
 SBOX_065_107198_1035-
 SBOX_070_69275_4722_+
 SBOX_065_50685_424_2+
 SBOX0K29_95719_281_4+
 SBOX0K27_172257_284_+
 SBOX_070_98422_281_3-
 SBOX_065_80054_188_2-
 SBOX_070_54762_368_3-
 SBOX0K27_444429_780_+
 SBOX0K31_160404_780_+
 SBOX0K29_290661_195_+
 SBOX_065_62868_4934_+
 SBOX_060_7863_4934_1-
 SBOX_065_37597_3965_+
 SBOX_070_98033_2311_+
 SBOX_065_57742_4493_+
 SBOX0K31_348853_745_+
 SBOX0K27_421181_318_+
 SBOX0K29_110583_258_+
 SBOX0K31_328363_1229-
 SBOX0K27_562493_360_+
 SBOX0K29_371060_362_+
 SBOX_060_34025_910_1+
 SBOX_060_7894_1742_1-
 SBOX_070_76197_572_1+
 SBOX0K31_106568_572_+
 SBOX0K27_333743_311_+
 SBOX0K29_443902_189_+
 SBOX_070_149416_191_+
 SBOX0K31_391750_191_+
 SBOX_065_119779_191_+
 SBOX_060_89982_230_2-
 SBOX_070_245901_230_+
 SBOX0K27_603341_196_+
 SBOX0K29_534598_570_+
 SBOX_070_140659_572_+
 SBOX_060_7958_572_15-
 SBOX_065_110543_572_+
 SBOX_070_319527_572_+
 SBOX0K31_470038_572_+
 SBOX0K27_337924_204_+
 SBOX_V07_1

CAP3 assembly of different Velvet assemblies



Query= SBOX_V07_1 L:6481
(6481 letters)

Database: PlantRefSeq_GB_2009_07_07.fasta
246,809 sequences; 93,580,655 total letters

>Viti_vini.XP_002267871 (XP_002267871.1 GI:225430631) { PREDICTED: hypothetical protein } [
Vitis vinifera]
Length = 2543

Score = 3073 bits (7968), Expect = 0.0
Identities = 1577/2092 (75%), Positives = 1801/2092 (86%), Gaps = 2/2092 (0%)
Frame = -3

Query: 6272 EPWVSLASSIPTSSSTKKRIRIFRNEIPSILLNSEMSDSTSQVLVDLIFTTLYIYDDRGSR 6093
E ++A S+ T STKKR+RIFR+EIP IL NSEMS++ S LVD+IF TLYIYDD GSR
Sbjct: 6 ESLAAIAGSVSTVSTKKRVRIFRDEIPPILTNSEMSAELASLLVDIIFNTLYIYDDHGSR 65

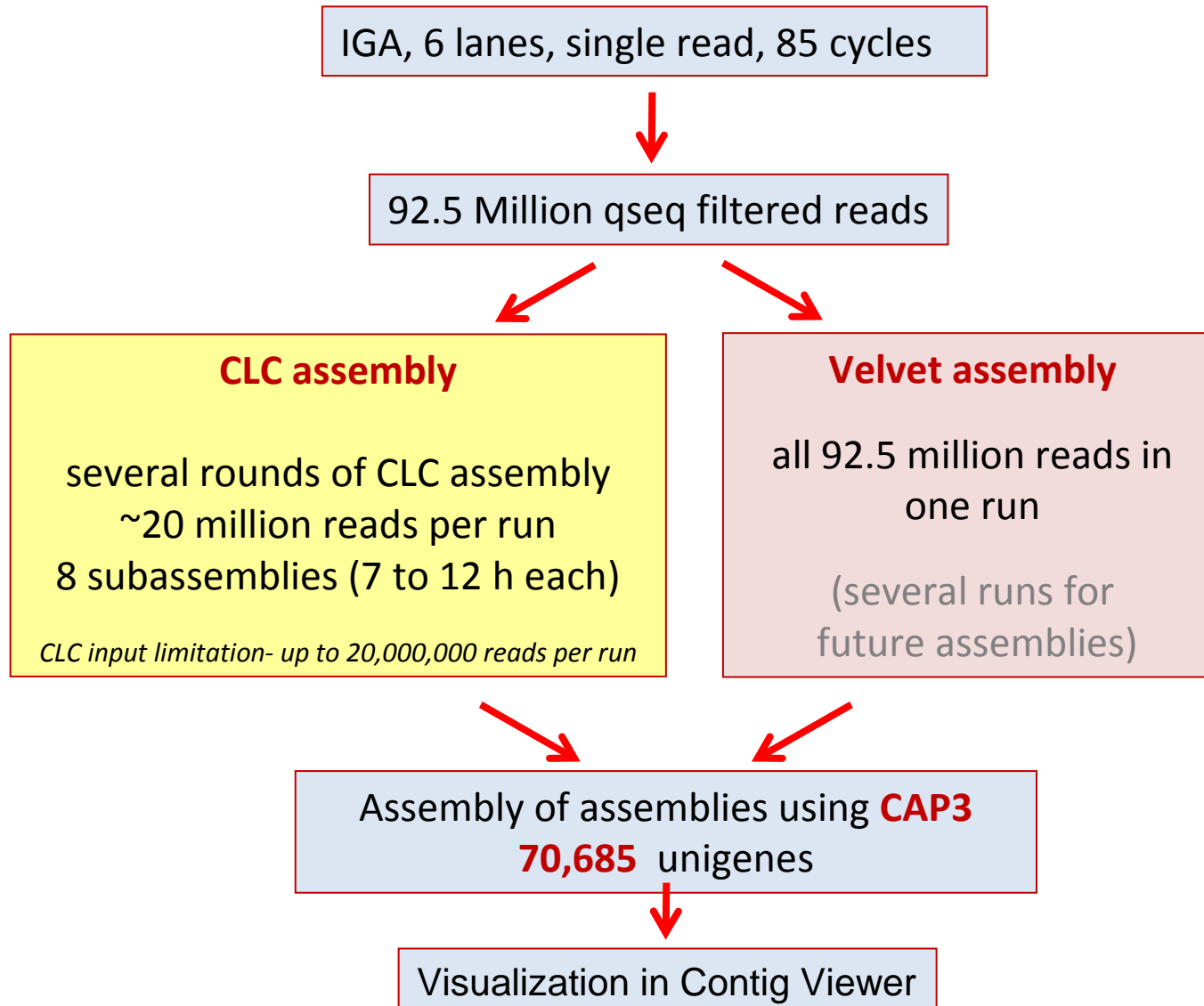
Query: 6092 KAVDDLIIKSLSEVVFMTFAAALVQVMDKQLKVQSHVGCSRLMSWSCILLCKTQFISAS 5913
KAVDD+I K+L EV+FMK+FAA LVQ M+KQ K QS++GC RL+ WSC+LL K++F S S
Sbjct: 66 KAVDDVISKALGEVIFMKSFAATLVQFMEKQSKFQSNIGCYRLKWSCLLLSKSRFASVS 125

.....

Query: 338 LAFSTLYKSAGMQAIDEIVPTLLRALEDDDMADTALDGLKQILSVRTAAVLPHILPKLVH 159
LAFSTLYKSAGMQAIDEIVPTLL +LEDD +DTALDGLKQILSVRT AVLPHILPKLVH
Sbjct: 1901 LAFSTLYKSAGMQAIDEIVPTLLHSLEDDQTSDTALDGLKQILSVRTTAVLPHILPKLVH 1960

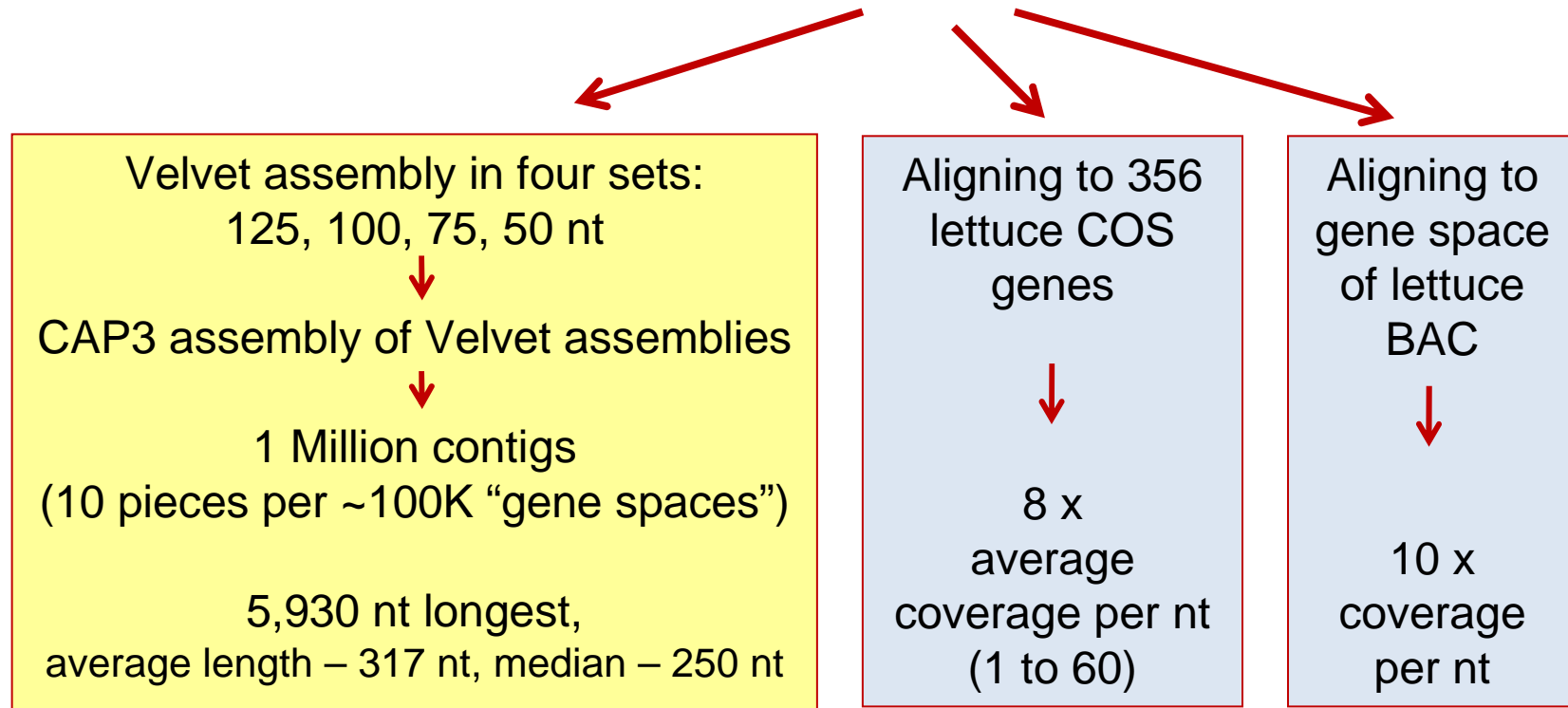
Query: 158 LPLSAFNAHAIGAVAEVAGAGLVHLSTVLPALLSAMGDDDHAEVQNLA KKA 3
LPL+AFNAHA+GA+AEVAG GLN HL VLPALLSAM DDD +VQ LAKKA
Sbjct: 1961 LPLTAFNAHALGALAEVAGPGLNFHLGIVLPALLSAMSDDD-TDVQKLAKKA 2011

Lettuce Transcriptome Sequencing and Assembly Pipeline



Lettuce Gene Space Sequencing

55 million, 125 nt single reads
6 GB (= 2x coverage w/o repeat reduction)

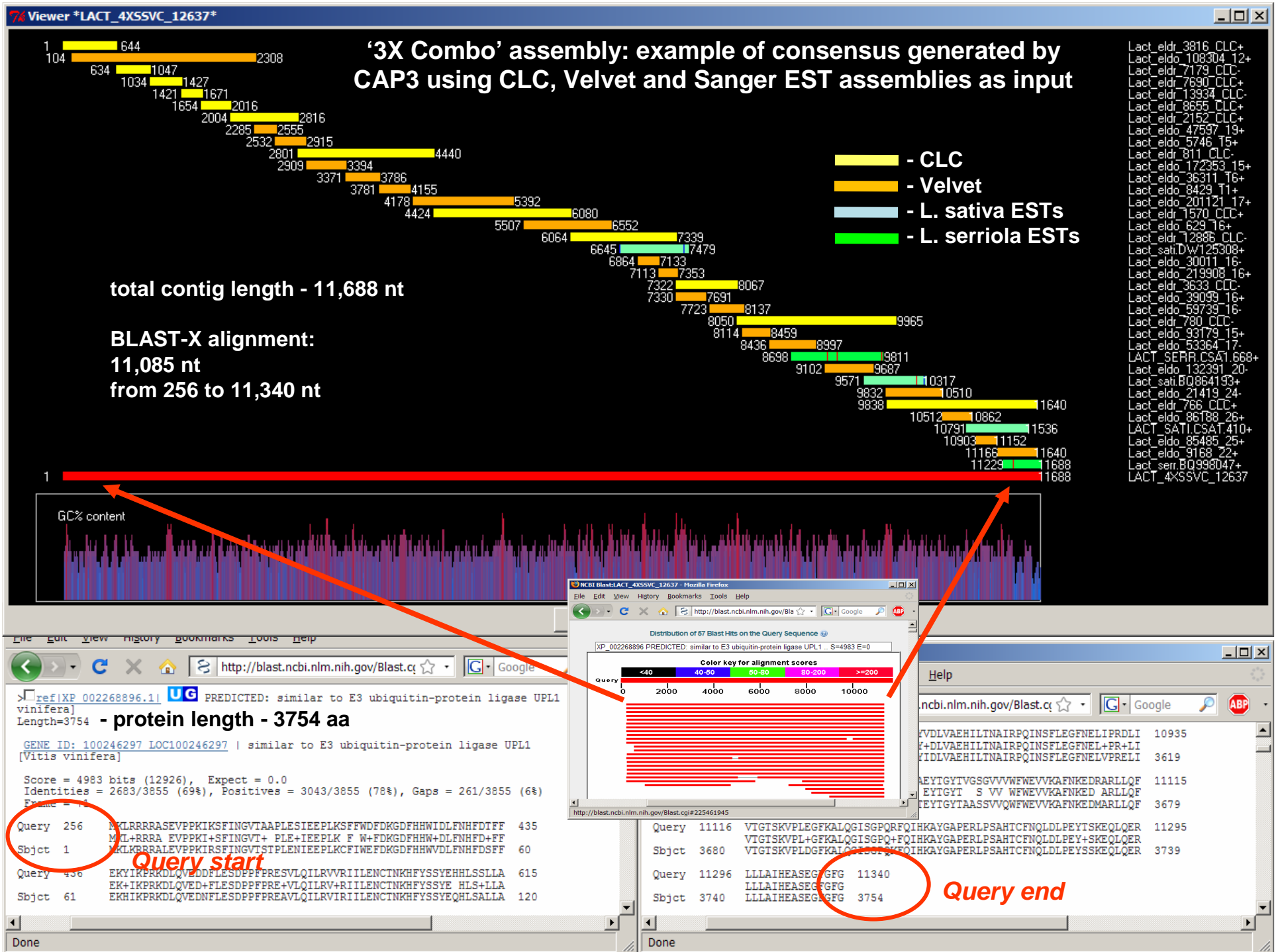


QC Analysis of Transcriptome Assembly

BLASTX versus Plant RefSeq Database: out of 8,300 longest contigs only 83 (1%) have no hits (similarity) to known protein sequences (expectation cutoff 1e-10)

QUERY	SUBJECT	HIT	Query_length_nt	Hit_Alignment_Length_aa	
LSat_DN_X01_992	Arab_thal.NP_186875	(NP_186875.2 GI:30678519) { BIG (BIG); binding / ubiquitin-protein ligase/ zinc ion binding } [Arabidopsis thaliana]	15382	5096	
LSat_DN_X01_25623	Arab_thal.NP_001154245	(NP_001154245.1 GI:238480786) { unknown protein } [Arabidopsis thaliana]	13037	4214	
LSat_DN_X01_20163	Viti_vini.XP_002274761	(XP_002274761.1 GI:225437162) { PREDICTED: hypothetical protein } [Vitis vinifera]	12279	3562	
LSat_DN_X01_15972	Popu_tric.XP_002327756	(XP_002327756.1 GI:224134102) { predicted protein } [Populus trichocarpa]	12155	3881	
LSat_DN_X01_9498	Viti_vini.XP_002267079	(XP_002267079.1 GI:225425575) { PREDICTED: hypothetical protein } [Vitis vinifera]	12112	508	chimeric?
LSat_DN_X01_1	Viti_vini.XP_002268896	(XP_002268896.1 GI:225461945) { PREDICTED: similar to E3 ubiquitin-protein ligase UPL1 } [Vitis vinifera]	11800	3754	
LSat_DN_X01_2	Viti_vini.XP_002283711	(XP_002283711.1 GI:225459044) { PREDICTED: hypothetical protein } [Vitis vinifera]	11743	1948	
LSat_DN_X01_6	Viti_vini.XP_002274451	(XP_002274451.1 GI:225432116) { PREDICTED: similar to vacuolar protein sorting 13C protein-like } [Vitis vinifera]	11434	3718	
LSat_DN_X01_11175	Popu_tric.XP_002327931	(XP_002327931.1 GI:224132994) { predicted protein } [Populus trichocarpa]	11311	3060	
LSat_DN_X01_35703	Viti_vini.XP_002283711	(XP_002283711.1 GI:225459044) { PREDICTED: hypothetical protein } [Vitis vinifera]	10588	3651	
LSat_DN_X01_37	Viti_vini.XP_002283711	(XP_002283711.1 GI:225459044) { PREDICTED: hypothetical protein } [Vitis vinifera]	10568	1948	

Majority (over 80 - 90%) of longest contigs produce uninterrupted ORFs of expected size

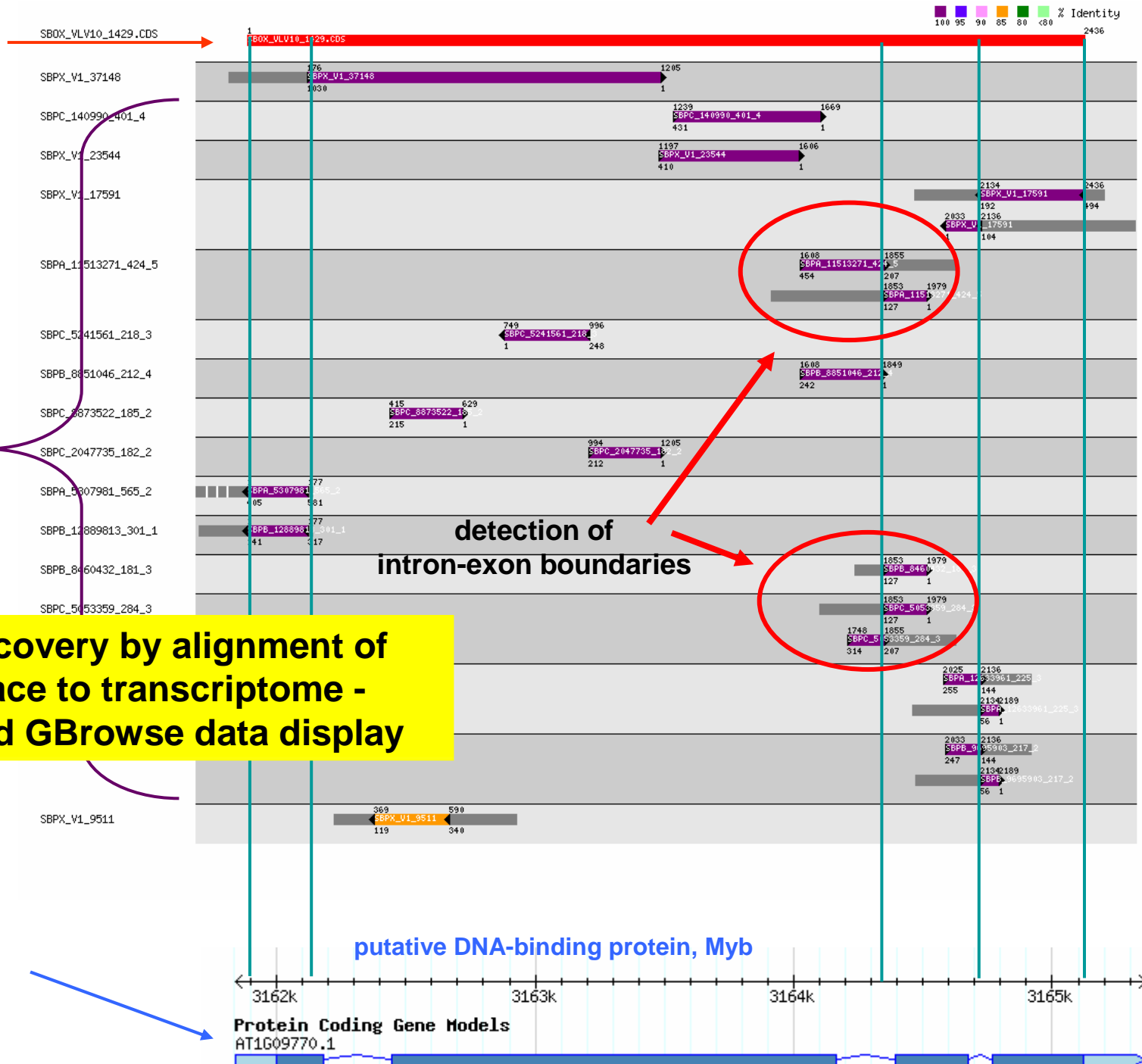


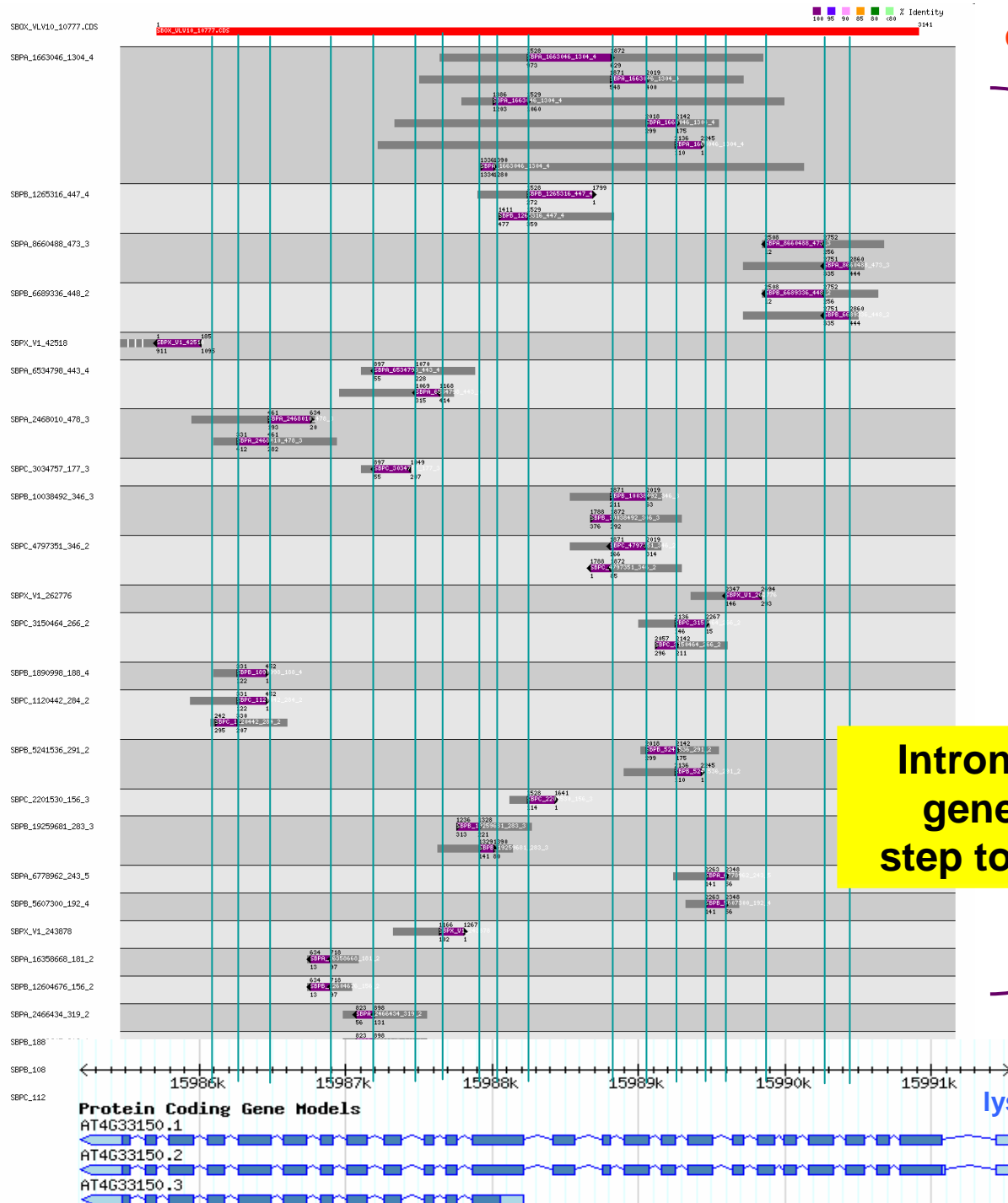
de novo
transcriptome
assembly

de novo
gene space
assemblies

Intron discovery by alignment of
gene space to transcriptome -
step toward GBrowse data display

Arabidopsis
gene model





de novo transcriptome assembly

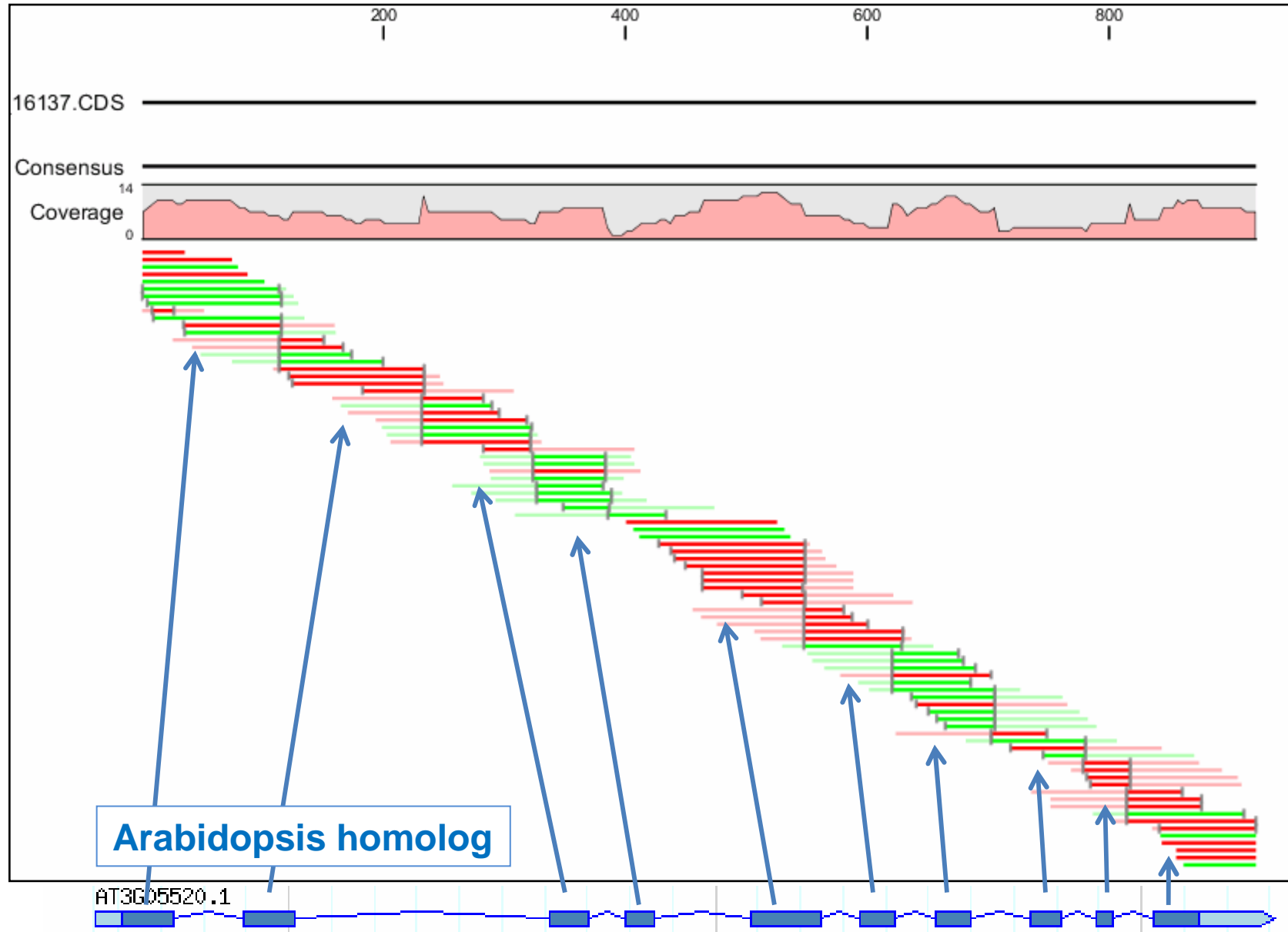
de novo gene
space assemblies

Intron discovery by alignment of
gene space to transcriptome -
step toward GBrowse data display

lysine-ketoglutarate reductase/saccharopine
dehydrogenase (LKR/SDH)
Arabidopsis gene model

How Low Can We Go to Figure the Exon-intron Structure?

E.g. gene covered by only 6.6 genomic reads per nt of coding sequence



project overview and dataflow

IGA output files

IGA quality scores

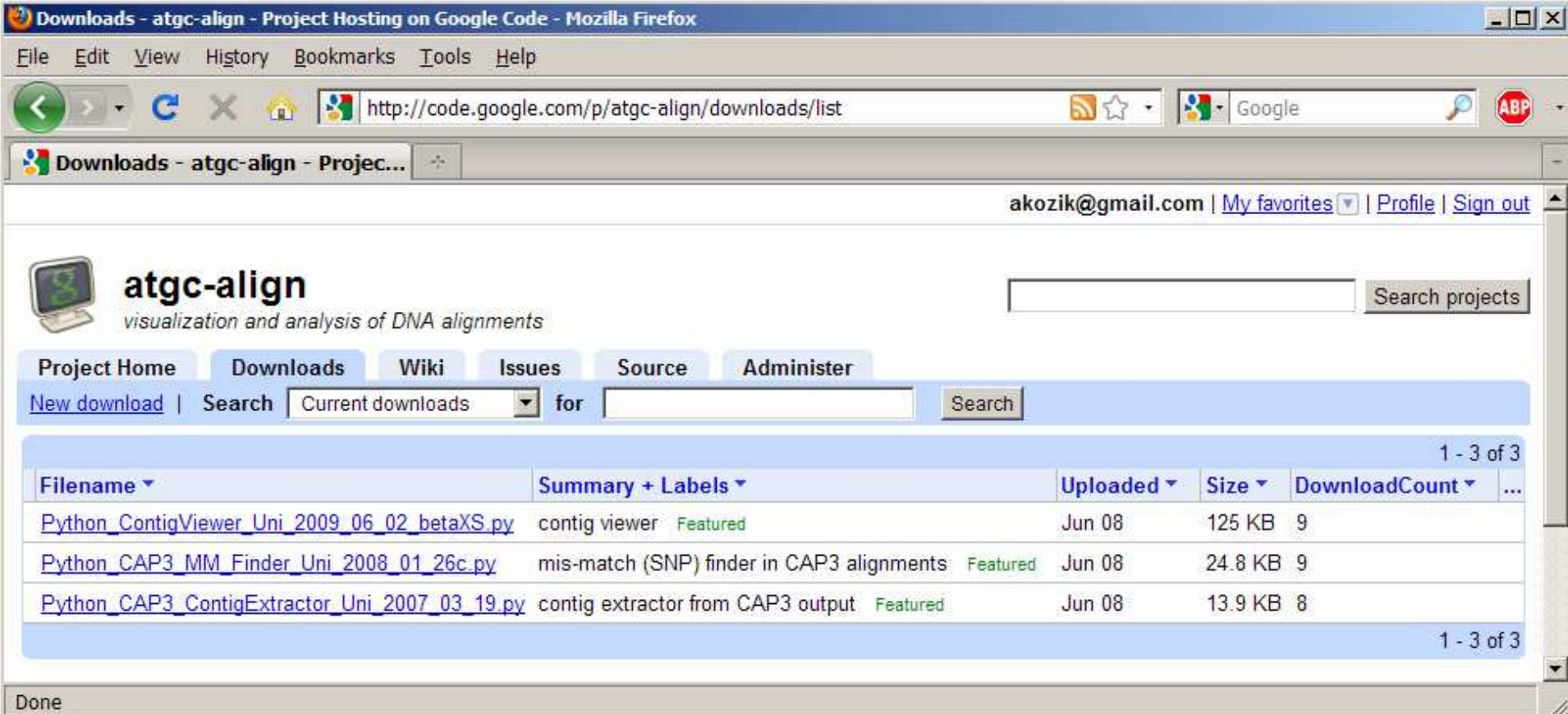
filtering for high quality reads

assembly parameters

visualization with Python Contig Viewer

<http://code.google.com/p/atgc-align/>

post-processing of CAP3 output and assembly visualization with Python Contig Viewer



The screenshot shows the 'Downloads' page of the 'atgc-align' project on Google Code. The browser window is titled 'Downloads - atgc-align - Project Hosting on Google Code - Mozilla Firefox'. The address bar shows the URL 'http://code.google.com/p/atgc-align/downloads/list'. The page header includes the user 'akozik@gmail.com' and links for 'My favorites', 'Profile', and 'Sign out'. The project name 'atgc-align' is displayed with the tagline 'visualization and analysis of DNA alignments'. Below the navigation tabs (Project Home, Downloads, Wiki, Issues, Source, Administer), there is a search bar and a dropdown menu set to 'Current downloads'. A table lists three files, each marked as 'Featured'.

Filename	Summary + Labels	Uploaded	Size	DownloadCount
Python_ContigViewer_Uni_2009_06_02_betaXS.py	contig viewer Featured	Jun 08	125 KB	9
Python_CAP3_MM_Finder_Uni_2008_01_26c.py	mis-match (SNP) finder in CAP3 alignments Featured	Jun 08	24.8 KB	9
Python_CAP3_ContigExtractor_Uni_2007_03_19.py	contig extractor from CAP3 output Featured	Jun 08	13.9 KB	8

```
LSat_DN_X01_2012.aln - WordPad
File Edit View Insert Format Help

LVK31B_73738_10+
SLNA_CLC2_9733-
SLNB_CLC2_17+
SLN2_CLC1_15388+
SLNA_CLC2_7411+
SLN6_CLC1_12360-
SLNA_CLC2_6050-
SLN2_CLC1_13630-
SLN7_CLC1_14614-
LVK31B_315329_13-
SLNA_CLC2_5251-
LVK31B_136615_14-
SLNA_CLC2_1312+
SLN2_CLC1_21081+
SLNB_CLC2_8044+
SLNA_CLC2_2307+
SLN7_CLC1_21128-
SLNA_CLC2_4570-
SLN6_CLC1_560+
SLN8_CLC1_10755+
SLN34_CLC1_7899+
SLN2_CLC1_2363+
SLN7_CLC1_12930+
SLNB_CLC2_1589-
LVK31B_3747_21+
SLNA_CLC2_6098-
SLN2_CLC1_1363+
SLN8_CLC1_4683+
SLN7_CLC1_3141+
SLN6_CLC1_2802+
SLNC_CLC3_1350+

-----
GTGAATTTCTGATCTCATCTTTAAACACTCATTATTGGGCATTGAAAGTGTTCATAATGTACTCAGCTCTTCCCATTCATGCTCAATTTTACCTAATACGTTTACTCAAAATCTGGTCAGGATACACCGTTTGATGTTCTTAGTTCAGACCACT
GTTTCATAATGTACTCAGCTCTTCCCATTCATGCTCAATTTTACCTAATACGTTTACTCAAAATCTGGTCAGGATACACCGTTTGATGTTCTTAGTTCAGACCACT
CTCAATTTTACCTAATACGTTTACTCAAAATCTGGTCAGGATACACCGTTTGATGTTCTTAGTTCAGACCACT

LSat_DN_X01_2012
GTGAATTTCTGATCTCATCTTT
For Help, press F1
```

CAP3 Usage: cap3 File_of_reads [options]

cap3 my_fasta_file.fa -o 80 -p 90 > my_fasta_file.cap3.out &

processing of CAP3 output with
Python_CAP3_ContigExtractor_Uni_2007_03_19.py:

1. - Info table
2. - Alignment files

```
ATGATGTCATCGATCTTTCTTGTCGGTTTGATTTCTGGATCAAAATGATGCTCTCATCGAGGCCAGTATTTTCAGCACTTATTAAGCTTGGAAAGACCAAGCTTTAAGAAAGTTAGATATGTTAAACTCCGGGGTCATTGATAATTGCTTCTTACTCCCAACTGCCTCAAGTGGTTT

ATGATGTCATCGATCTTTCTTGTCGGTTTGATTTCTGGATCAAAATGATGCTCTCATCG

ATGATGTCATCGATCTTTCTTGTCGGTTTGATTTCTGGATCAAAATGATGCTCTCATCGAGGCCAGTATTTTCAGCACTTATTAAGCTTGGAAAGACCAAGCTTTAAGAAAGTTAGATATGTTAAACTCCGGGGTCATTGATAATTGCTTCTTACTCCCAACTGCCTCAAGTGGTTT
ATGATGTCATCGATCTTTCTTGTCGGTTTGATTTCTGGATCAAAATGATGCTCTCATCGAGGCCAGTATTTTCAGCACTTATTAAGCTTGGAAAGACCAAGCTTTAAGAAAGTTAGATATGTTAAACTCCGGGGTCATTGATAATTGCTTCTTACTCCCAACTGCCTCAAGTGGTTT
TATTAAGCTTGGAAAGACCAAGCTTTAAGAAAGTTAGATATGTTAAACTCCGGGGTCATTGATAATTGCTTCTTACTCCCAACTGCCTCAAGTGGTTT

ATGATGTCATCGATCTTTCTTGTCGGTTTGATTTCTGGATCAAAATGATGCTCTCATCGAGGCCAGTATTTTCAGCACTTATTAAGCTTGGAAAGACCAAGCTTTAAGAAAGTTAGATATGTTAAACTCCGGGGTCATTGATAATTGCTTCTTACTCCCAACTGCCTCAAGTGGTTT
For Help, press F1
```

Info table - Contig Complexity information

SBOX_V07_10677	99	SBOD_Q65_46506_491_1-(1,521) SBOA_Q70_63067_184_1+(11,224) ...
SBOX_V07_15959	99	SBOD_Q60_38311_405_1+(1,435) SBOB_Q65_71217_151_6+(11,191) ...
SBOX_V07_33750	98	SBO80K31_405877_272_+(1,302) SBO80K29_460109_274_+(1,302) ...
SBOX_V07_2653	96	SBOC_Q60_29343_843_2-(1,873) SBOA_Q60_29209_863_2-(1,893) ...
SBOX_V07_27418	96	SBOB_Q60_23920_307_1-(1,337) SBOC_Q60_24036_283_2-(22,332) ...
...		
SBOX_V07_9935	18	SBO80K29_44764_509_4+(1,537) SBO80K27_49894_511_4+(1,537) ...
SBOX_V07_9961	18	SBOD_Q65_112060_507_+(1,537) SBOD_Q60_84313_508_8+(1,537) ...
SBOX_V07_10000	17	SBOC_Q60_49726_506_8+(1,536) SBOA_Q60_49428_287_1+(11,327) ...
SBOX_V07_10018	17	SBOD_Q70_259498_506_+(1,536) SBOB_Q65_196026_486_+(16,523) ...
...		

contig ID

sequence position and orientation in contig

number of sequences in contig

Python_CAP3_ContigExtractor_Uni_2007_03_19.py usage:

```
bash-2.03$
```

```
bash-2.03$ python Python_CAP3_ContigExtractor_Uni_2007_03_19.py
```

```
What type of output do you want? (1/2): 1
```

```
Enter the SOURCE file name: my_fasta_file.cap3.out
```

← option '1' to extract Info table

```
Enter the DESTINATION file name: my_fasta_file.cap3.out.Info
```

```
Default contig file name prefix is Contig
```

```
Enter the contig file name prefix: ASSY_V1_
```

```
read data from the input file my_fasta_file.cap3.out ...
```

```
bash-2.03$
```

```
bash-2.03$ python Python_CAP3_ContigExtractor_Uni_2007_03_19.py
```

```
What type of output do you want? (1/2): 2
```

```
Enter the SOURCE file name: my_fasta_file.cap3.out
```

← option '2' to extract alignments

```
Enter the DESTINATION directory with alignments: my_fasta_file.cap3.out.Align
```

```
Default contig file extension is aln
```

```
Enter the contig file extension :
```

```
Default contig file name prefix is Contig
```

```
Enter the contig file name prefix: ASSY_V1_
```

```
read data from the input file my_fasta_file.cap3.out ...
```

Color selection in Python Contig Viewer

ignore

Basic functions of Contig Viewer:

Path http://

Prefix_Type_1 (nine_letter_code_1)
Prefix_Type_2 (nine_letter_code_2)
Prefix_Type_3 (nine_letter_code_3)
Prefix_Type_4 (nine_letter_code_4)

Contig ID: CICH_2CDS.CSA1.1112

File Extension: .aln
.align
.alignment
.4aln.out.fa.x.aln

Get Data Remotely

Choose file from Hard Disk

prefix type

```
if prefix_type == 4:
```

prefix size

```
seq_type = (seq.getSeqName()) [0:4]
```

color defined by prefix of sequence ID

```
barcolor = '#FFFF00'
```

```
barcolor = '#AAFFFF'
```

corresponding color

```
barcolor = '#FFFF00'
```

```
barcolor = '#FF00FF'
```

```
barcolor = '#FFFFFF'
```

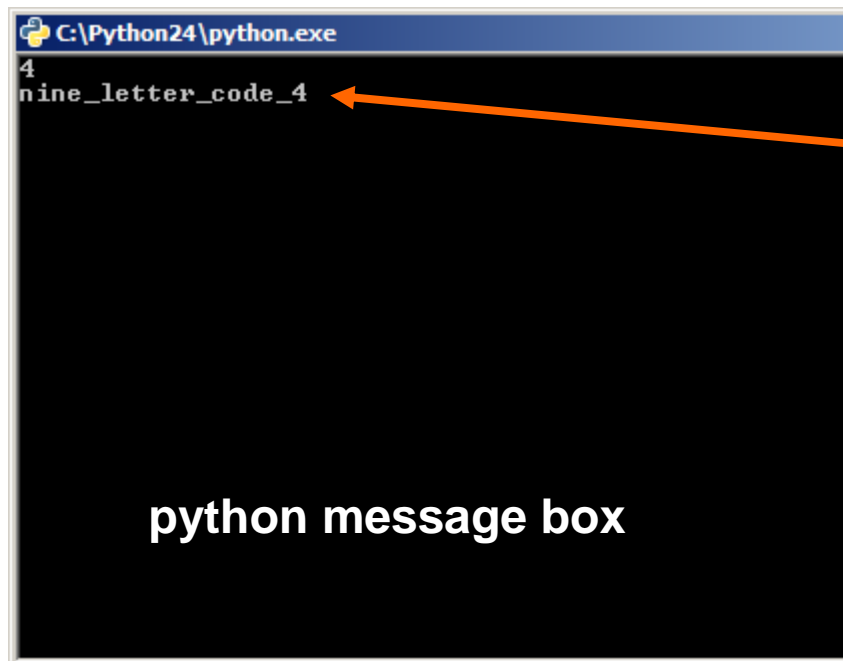
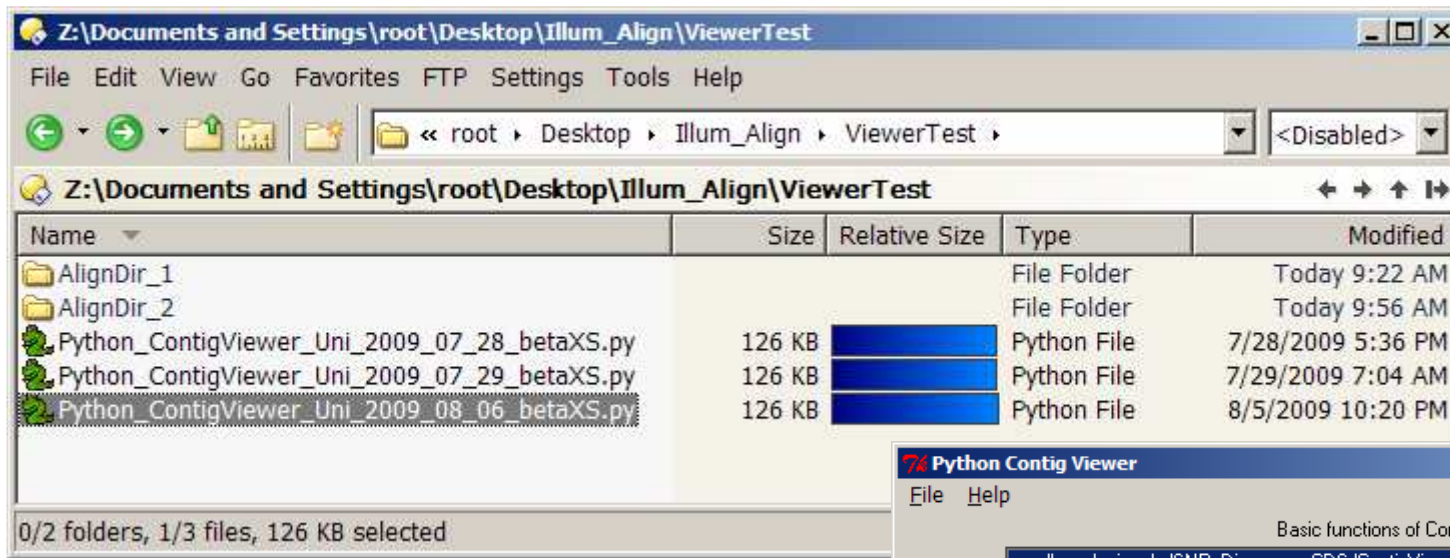
“#FFFF00”

red
green
blue
yellow

RGB - yellow

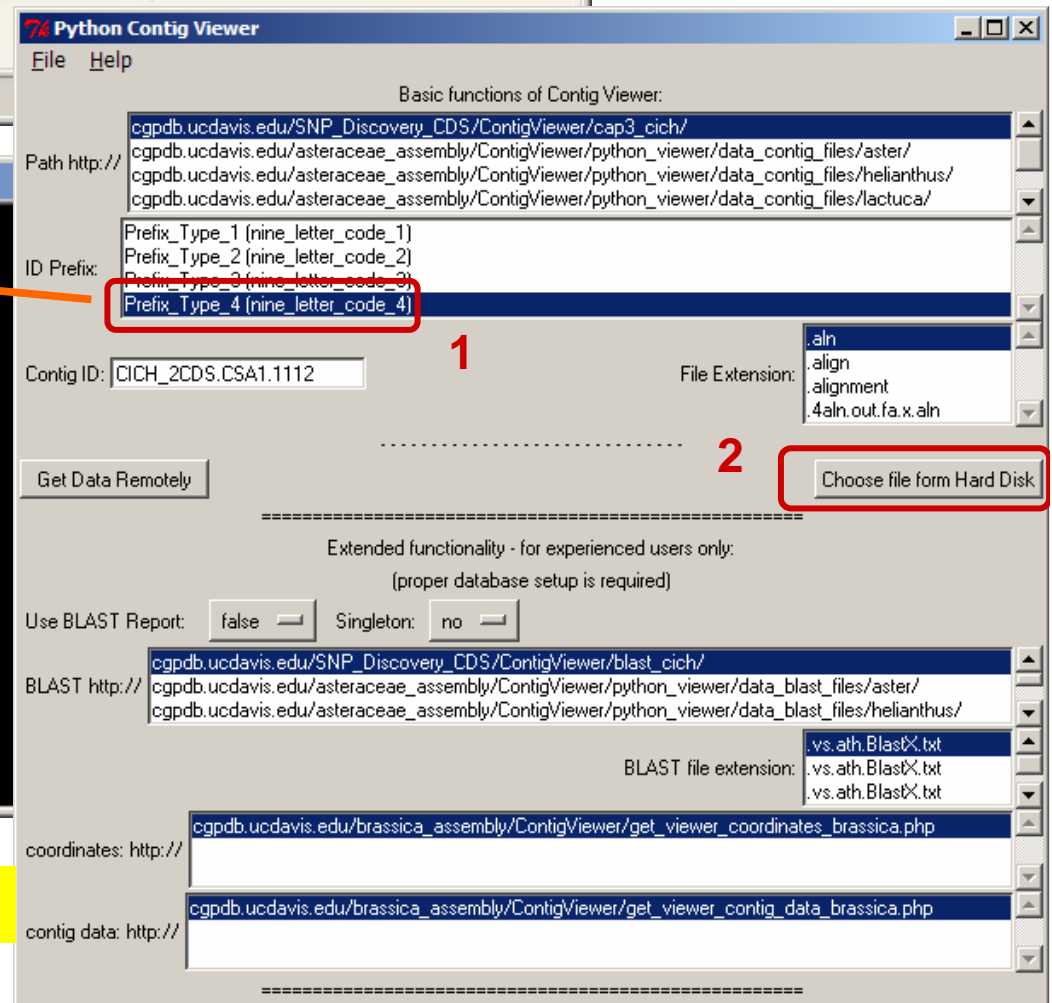
RGB - purple

cross-platform
application



python message box

Python Contig Viewer input selection

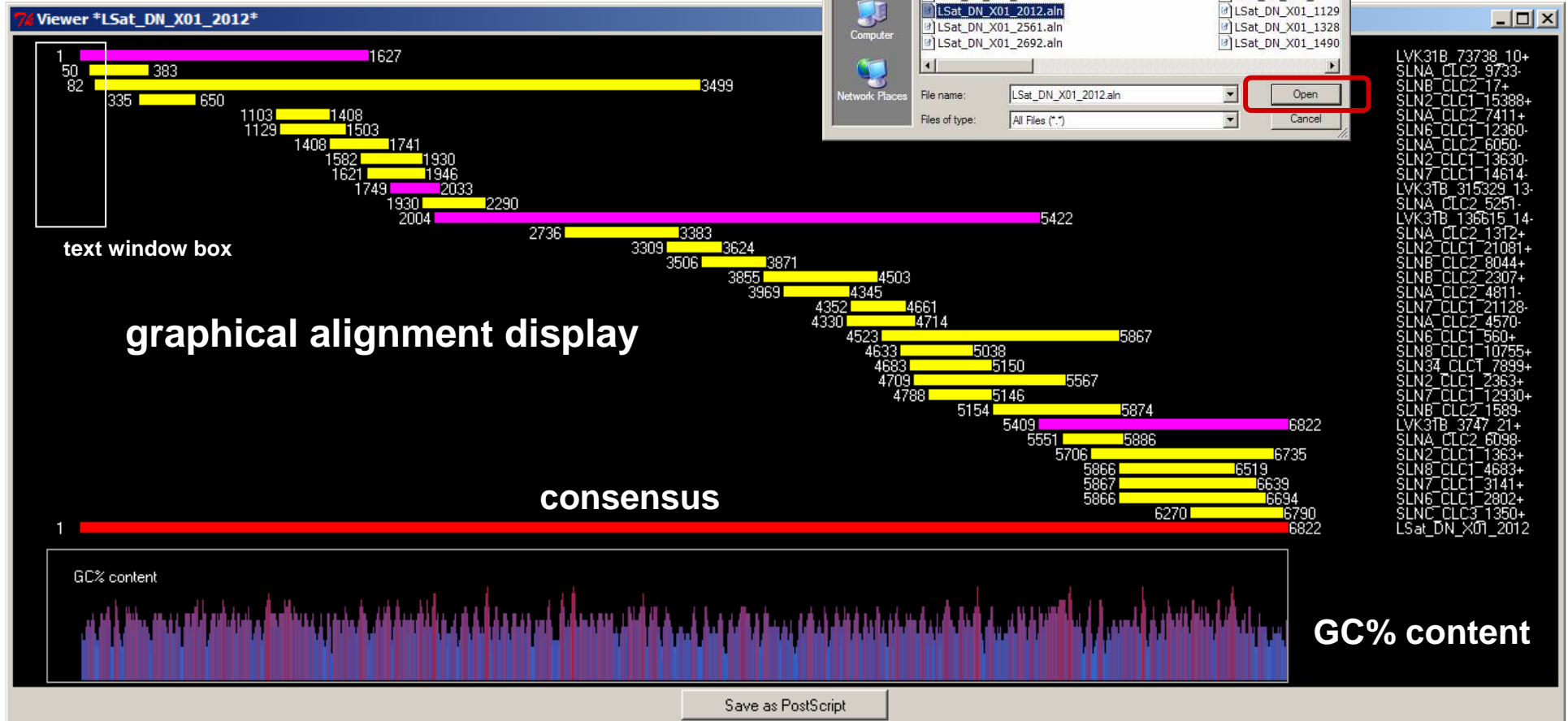
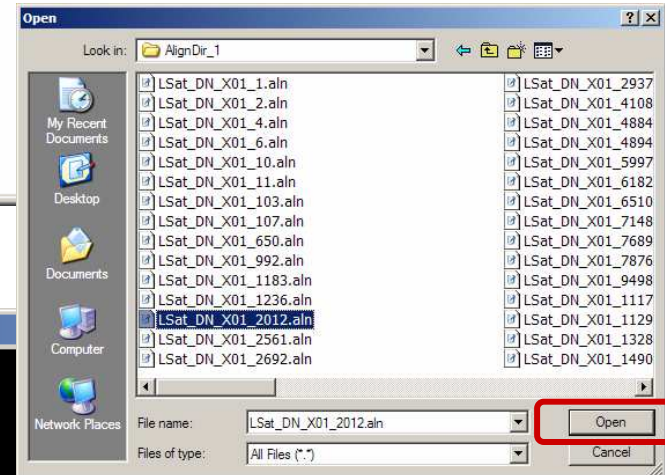


7% Sequence Text *LSat_DN_X01_2012*

```
LVK31B_73738_10+      GTGAATTTCTGATCTCATCTTTAAACACTCATTATTGGGCATTGAAGTCGTTTCATAATGTACTCAGCTCTTCCCATTCATGCTCAATTTTACCTAAT.  
SLNA_CLC2_9733-      GTTCATAATGTACTCAGCTCTTCCCATTCATGCTCAATTTTACCTAAT.  
SLNB_CLC2_17+      CTCAATTTTACCTAAT.  
SLN2_CLC1_15388+  
SLNA_CLC2_7411+  
SLN6_CLC1_12360-  
SLNA_CLC2_6050-  
SLN2_CLC1_13630-  
SLN7_CLC1_14614-  
LVK31B_315329_13-  
SLNA_CLC2_5251-  
LVK31B_136615_14-
```

prefix defines the color

text box



last remarks

UNIX tricks

fasta header modifications:

*regular expressions and
perl /find/replace/ function*

>NODE_24_length_783_cov_47.872288

perl -p -i -e 's/NODE_/SBPD_/' my_fasta_file.txt

perl -p -i -e 's/_length_/_/' my_fasta_file.txt

perl -p -i -e 's/_cov_/_/' my_fasta_file.txt

perl -p -i -e 's/\\...*//' my_fasta_file.txt

>SBPD_24_783_47

trimming:

>SB08_8_120_1640_1715

CCAAGAACGACATATAAACATTTTACATAATCTGGGGAACACTTGAAGAACATCAAAAGCTGGAAAAATGGATGAAAAACAGCTC

perl -p -i -e 's/\\>SB0/XXXXXXXXXXXXXXXX\\>SB0/' my_fasta_file.orig.txt

XXXXXXXXXXXXXXXXXX>SB08_8_120_1640_1715

CCAAGAACGACATATAAACATTTTACATAATCTGGGGAACACTTGAAGAACATCAAAAGCTGGAAAAATGGATGAAAAACAGCTC

cut -c16-75 my_fasta_file.orig.txt > my_fasta_file.trim.txt

>SB08_8_120_1640_1715

AAACATTTTACATAATCTGGGGAACACTTGAAGAACATCAAAAGCTGGAAAAATGGATGA

Unix 'cut' command

Troubleshooting

```
pgfmars.ucdavis.edu - PuTTY

Lact_sati.DY960922+ TTCCCAGG
Lact_eldr_2423_CLC- TTCCCAGG
Lact_eldr_5493_CLC- TTCCCAGG

consensus TTCCCAGG

Lact_sati.DY960922+ CTCCTGTT
Lact_eldr_2423_CLC- CTCCTGTT
Lact_eldr_2434_CLC- CTCCTGTT

consensus CTCCTGTT

Lact_sati.DY960922+ CATTCAGC
Lact_eldr_2423_CLC- CATTCAGC
Lact_eldr_5493_CLC- CATTCAGC

consensus CATTCAGC

Lact_sati.DY960922+ ACAAACAC
Lact_eldr_2423_CLC- ACAAACAC
Lact_eldr_5493_CLC- ACAAACAC

consensus ACAAACAC

Lact_sati.DY960922+ TTTCCGATAACAAACAAACCGA
Lact_eldr_2423_CLC- TTTCCGATAACAAACAAACCGA
Lact_eldr_5493_CLC- TTTCCGATAACAAACAAACCGA

consensus TTTCCGATAACAAACAAACCGA
```

```
pgfmars.ucdavis.edu - PuTTY

Lact_eldr_5531_CLC- GTGAGGTTTATCGTTCA^MGAATGGAATGGAAGTGAAGGTTTATGAAC
Lact_eldr_24344_CLC- AAGTTTATGAAC

consensus GTGAGGTTTATCGTTCA^MGAATGGAATGGAAGTGAAGGTTTATGAAC

Lact_eldr_5531_CLC- CAGGATATATC-AGGCGAT^MGCACATAACTCAGTTTAAAAGCGAAGTTGAGATCATGTTGA
Lact_eldr_24344_CLC- CAGGATATATC^MAGGCGAT-GCACATAACTCAGTTTAAAAGCGAAGTTGAGATCATGTTGA

consensus CAGGATATATC^MAGGCGATNGCACATAACTCAGTTTAAAAGCGAAGTTGAGATCATGTTGA

Lact_eldr_5531_CLC- GGTGAGACATCC-TAATATT^MGTTCCTTTTCATGGGAGCAGTTACTCGTCCGCCAAATCT
Lact_eldr_24344_CLC- GGTGAGACATCC^MTAATATT-GTTCCTTTTCATGGGAGCAGTTACTCGTCCGCCAAATCT

consensus GGTGAGACATCCNTAATATTNGTTCCTTTTCATGGGAGCAGTTACTCGTCCGCCAAATCT

Lact_eldr_5531_CLC- A^MTATTCTACCAAGGGGTAGTTTATTTAAATTATTGCATCGTTTCA
Lact_eldr_24344_CLC- A^MTATTCTACCAAGGGGTAGTTTATTTAAATTATTGCATCGTTTCA

consensus TTCCATACTTACAGANATTTCTACCAAGGGGTAGTTTATTTAAATTATTGCATCGTTTCA

Lact_eldr_24344_CLC- GTATCCAAGTTGATGA^MAAAGAGACGAATGCGTATGGCTCTTGATGTGGCCAAGGGGATG

consensus GTATCCAAGTTGATGANAAAGAGACGAATGCGTATGGCTCTTGATGTGGCCAAGGGGATG

Lact_eldr_24344_CLC- AACTATTTGCACACTAG

consensus AACTATTTGCACACTAG

Lact_eldr_24344_CLC- TAGTTTCAATTCCGTTGCG-TCTTCCGCTTCCATTCA
Lact_eldr_24344_CLC- TAGTTTCAATTCCGTTGCG-TCTTCCGCTTCCATTCA

consensus TTTCCGATAACAAACAAACCGANTAGTTTCAATTCCGTTGCGTCTTCCGCTTCCATTCA
```

corrupted output because of 'new line' problem
<http://en.wikipedia.org/wiki/Newline>

visual inspection of output

record and keep all Log files, run parameters

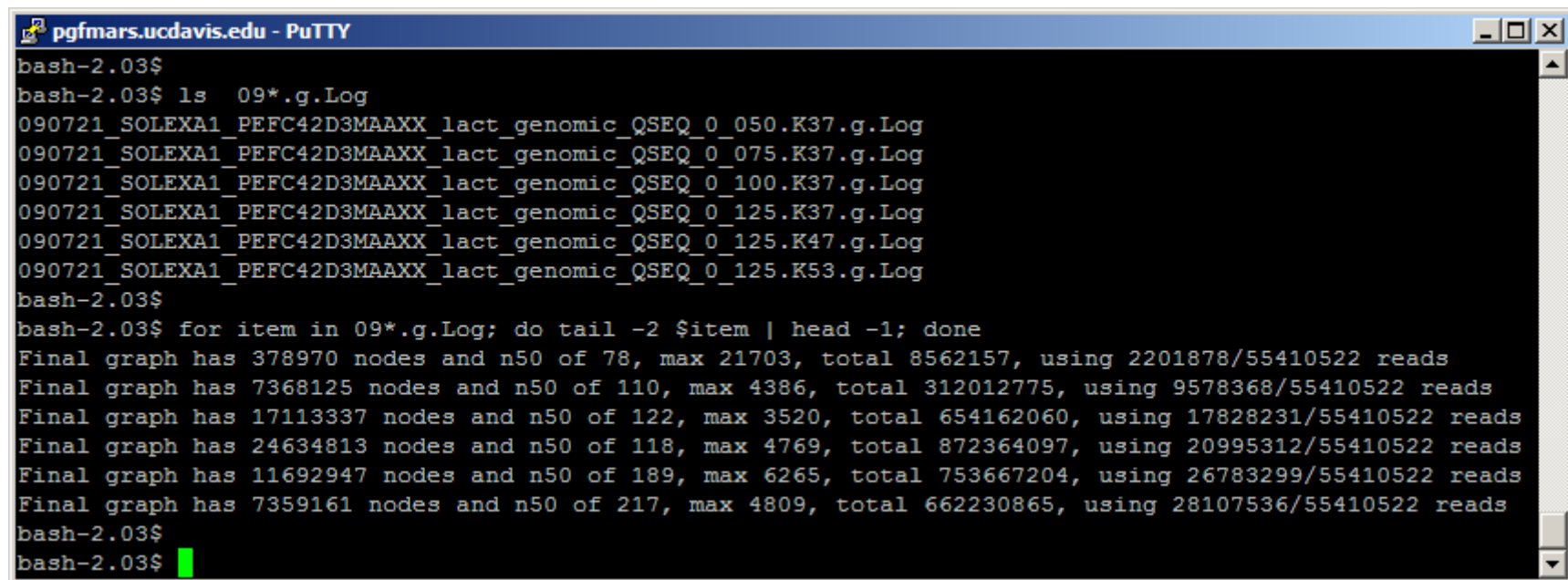
```
bash-2.03$ less 090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_125.K53/Log
```

```
Thu Sep  3 03:07:09 2009
```

```
velvetg.7.49.K53.exec 090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_125.K53 53 -fasta -short  
090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_125.K37/Sequences
```

```
Thu Sep  3 09:02:46 2009
```

```
velvetg.7.49.K53.exec 090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_125.K53 -min_contig_lgth 180  
-unused_reads yes -amos_file yes -read_trkg yes
```



The screenshot shows a PuTTY terminal window titled "pgfmars.ucdavis.edu - PuTTY". The terminal displays the following commands and output:

```
bash-2.03$  
bash-2.03$ ls 09*.g.Log  
090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_050.K37.g.Log  
090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_075.K37.g.Log  
090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_100.K37.g.Log  
090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_125.K37.g.Log  
090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_125.K47.g.Log  
090721_SOLEXA1_PEF42D3MAAXX_lact_genomic_QSEQ_0_125.K53.g.Log  
bash-2.03$  
bash-2.03$ for item in 09*.g.Log; do tail -2 $item | head -1; done  
Final graph has 378970 nodes and n50 of 78, max 21703, total 8562157, using 2201878/55410522 reads  
Final graph has 7368125 nodes and n50 of 110, max 4386, total 312012775, using 9578368/55410522 reads  
Final graph has 17113337 nodes and n50 of 122, max 3520, total 654162060, using 17828231/55410522 reads  
Final graph has 24634813 nodes and n50 of 118, max 4769, total 872364097, using 20995312/55410522 reads  
Final graph has 11692947 nodes and n50 of 189, max 6265, total 753667204, using 26783299/55410522 reads  
Final graph has 7359161 nodes and n50 of 217, max 4809, total 662230865, using 28107536/55410522 reads  
bash-2.03$  
bash-2.03$
```

```
bash-2.03$ for item in 09*.g.Log; do tail -2 $item | head -1; done  
Final graph has 378970 nodes and n50 of 78, max 21703, total 8562157, using 2201878/55410522 reads  
Final graph has 7368125 nodes and n50 of 110, max 4386, total 312012775, using 9578368/55410522 reads  
Final graph has 17113337 nodes and n50 of 122, max 3520, total 654162060, using 17828231/55410522 reads  
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Final graph has 11692947 nodes and n50 of 189, max 6265, total 753667204, using 26783299/55410522 reads  
Final graph has 7359161 nodes and n50 of 217, max 4809, total 662230865, using 28107536/55410522 reads  
bash-2.03$
```

SUMMARY

ILLUMINA output data format

criteria for filtering

trimming and sampling effect on assemblies

assembly parameters

**assembly validation and
exon-intron prediction by alignment**

visualization with Python Contig Viewer

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