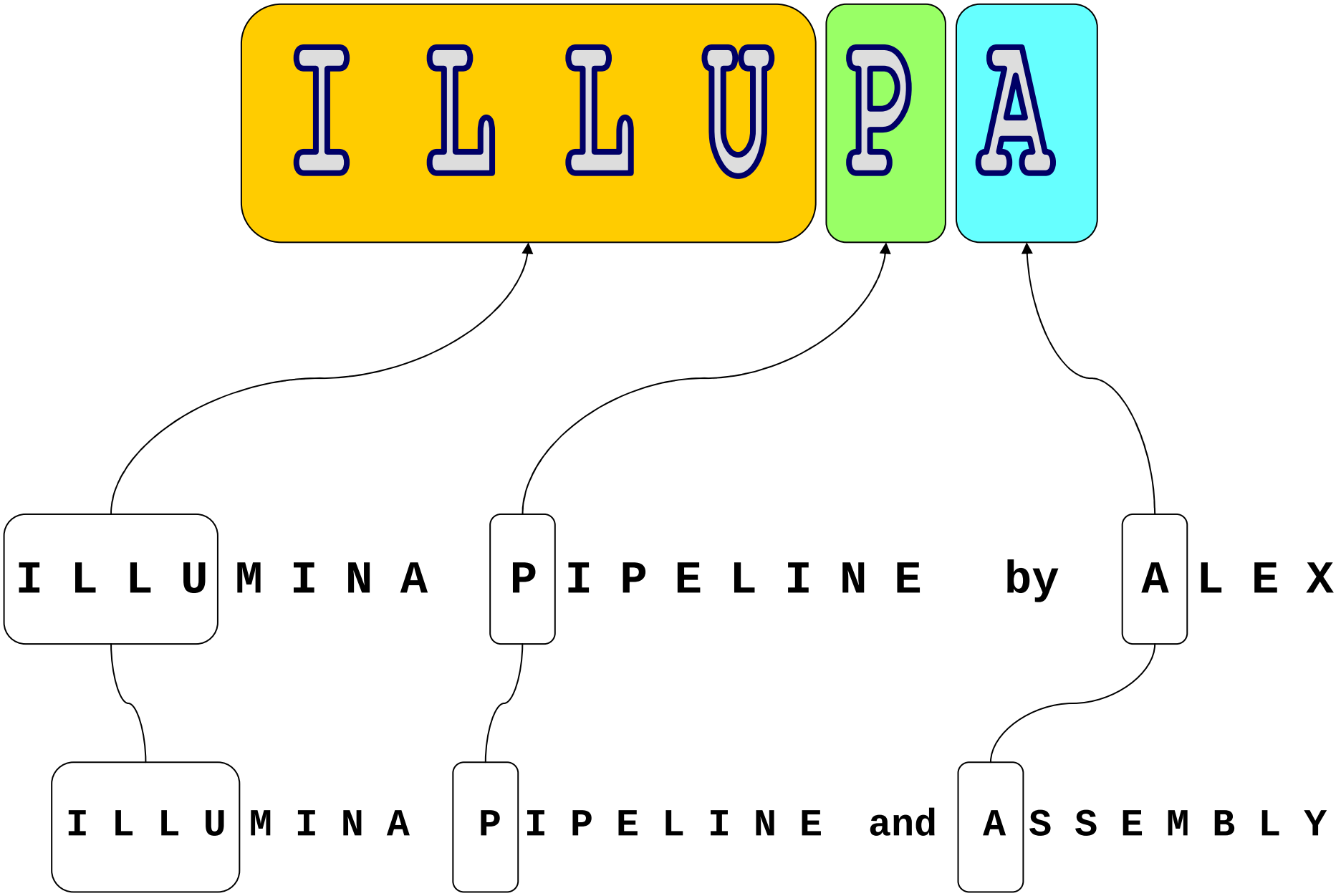
ILLUPA
ILLUMINA PIPELINE by ALEX
ILLUMINA PIPELINE and ASSEMBLY

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 Mb)

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.

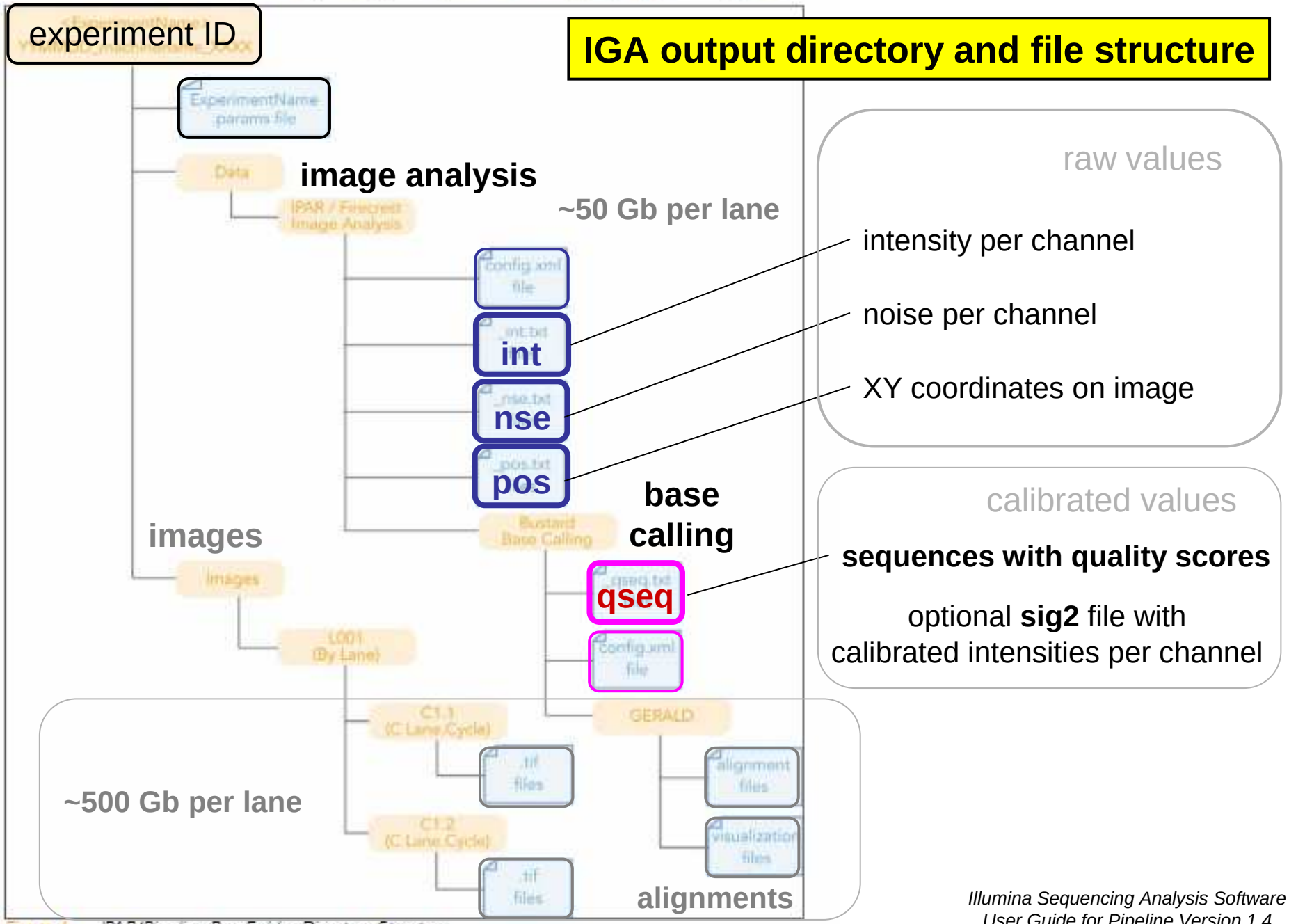


Figure 6 IPAR/Pipeline Run Folder Directory Structure

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:

chastity = Highest_Intensity / (Highest_Intensity + 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)

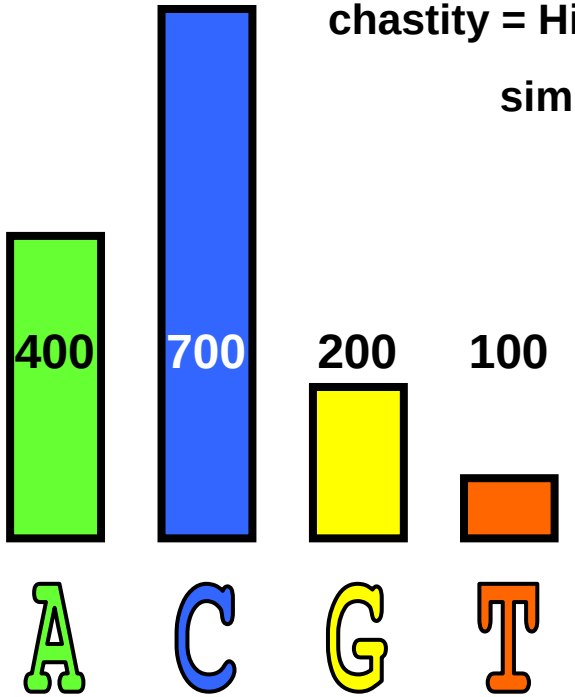
chastity: the minimum chastity (0.6)

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

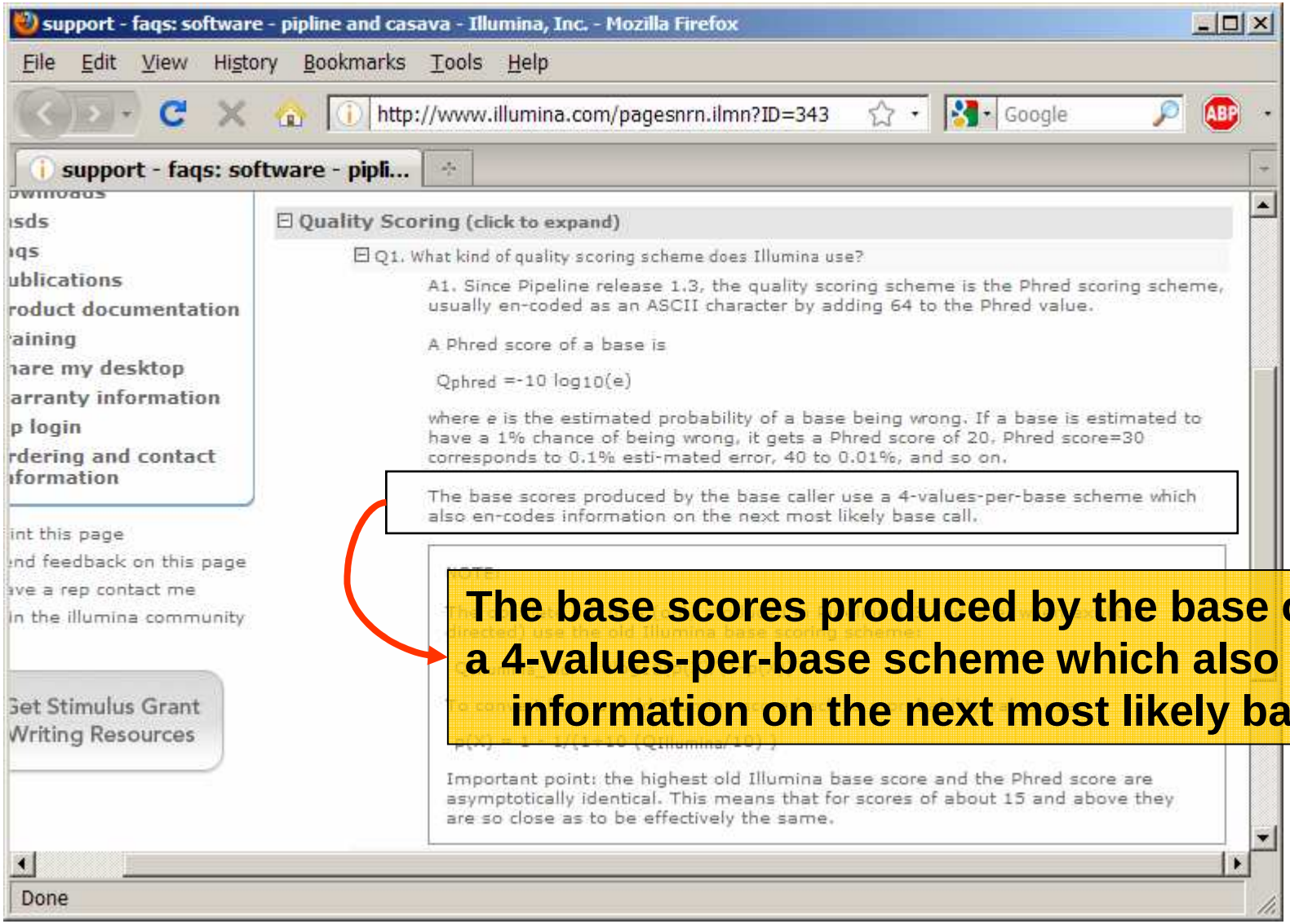
quality categories for simple ratio:

```
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** (#)

ILLUMINA Quality scores

http://www.illumina.com/pagesnrn.ilmn?ID=343



The base scores produced by the base caller use a 4-values-per-base scheme which also en-codes information on the next most likely base call.

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

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

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

Binary	Oct	Dec	Hex	Glyph	
100 0010	102	66	42	B	2
100 0011	103	67	43	C	3
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	10
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	20
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	^	30
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

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

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

medium quality

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

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

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

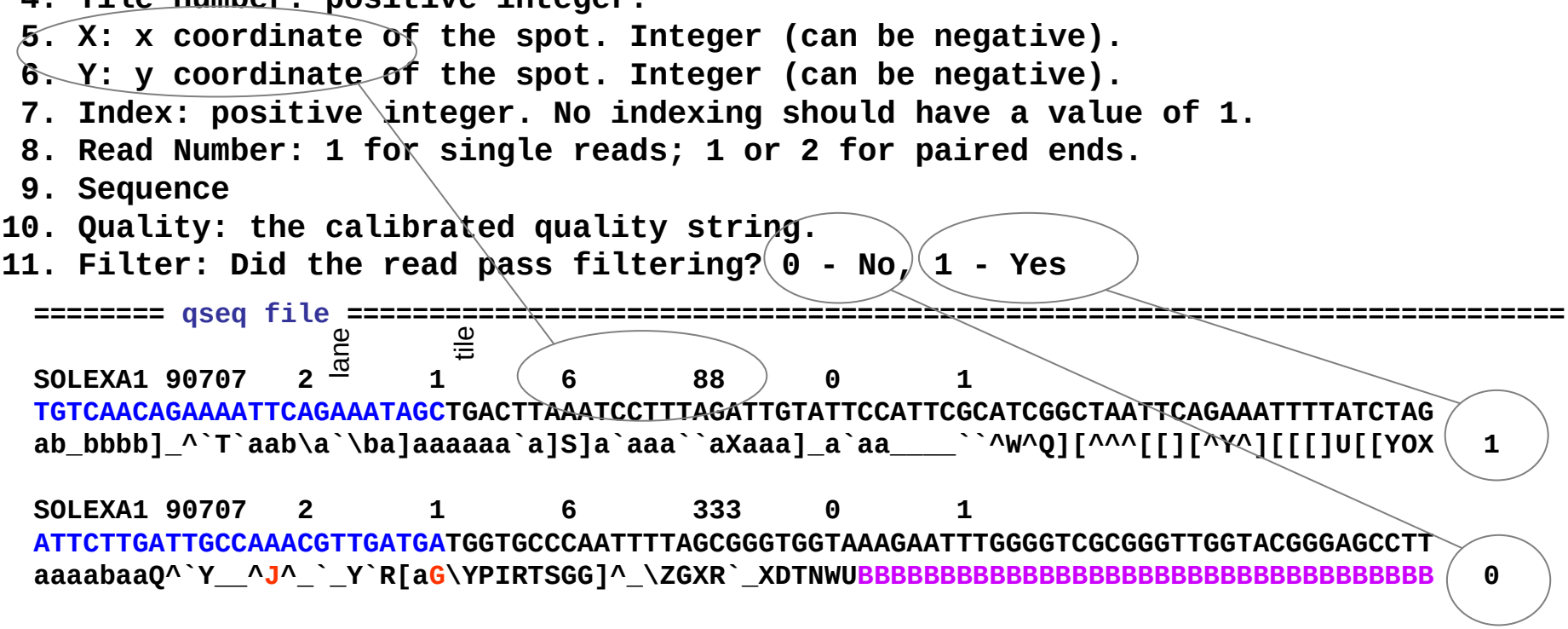
high quality

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

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
TGTCAACAGAAAATTCAGAAATAGCTGACTTAAATCCTTTAGATTGTATTCCATTCGCATCGGCTAATTCAGAAATTTTATCTAG
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`_XDTNWUBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB   0

================================================================================

FASTA file:
>90707:SOLEXA1:2:1:6:88#0/1   891  LEN: 85
TGTCAACAGAAAATTCAGAAATAGCTGACTTAAATCCTTTAGATTGTATTCCATTCGCATCGGCTAATTCAGAAATTTTATCTAG

FASTQ file:
@90707:SOLEXA1:2:1:6:88#0/1
TGTCAACAGAAAATTCAGAAATAGCTGACTTAAATCCTTTAGATTGTATTCCATTCGCATCGGCTAATTCAGAAATTTTATCTAG
+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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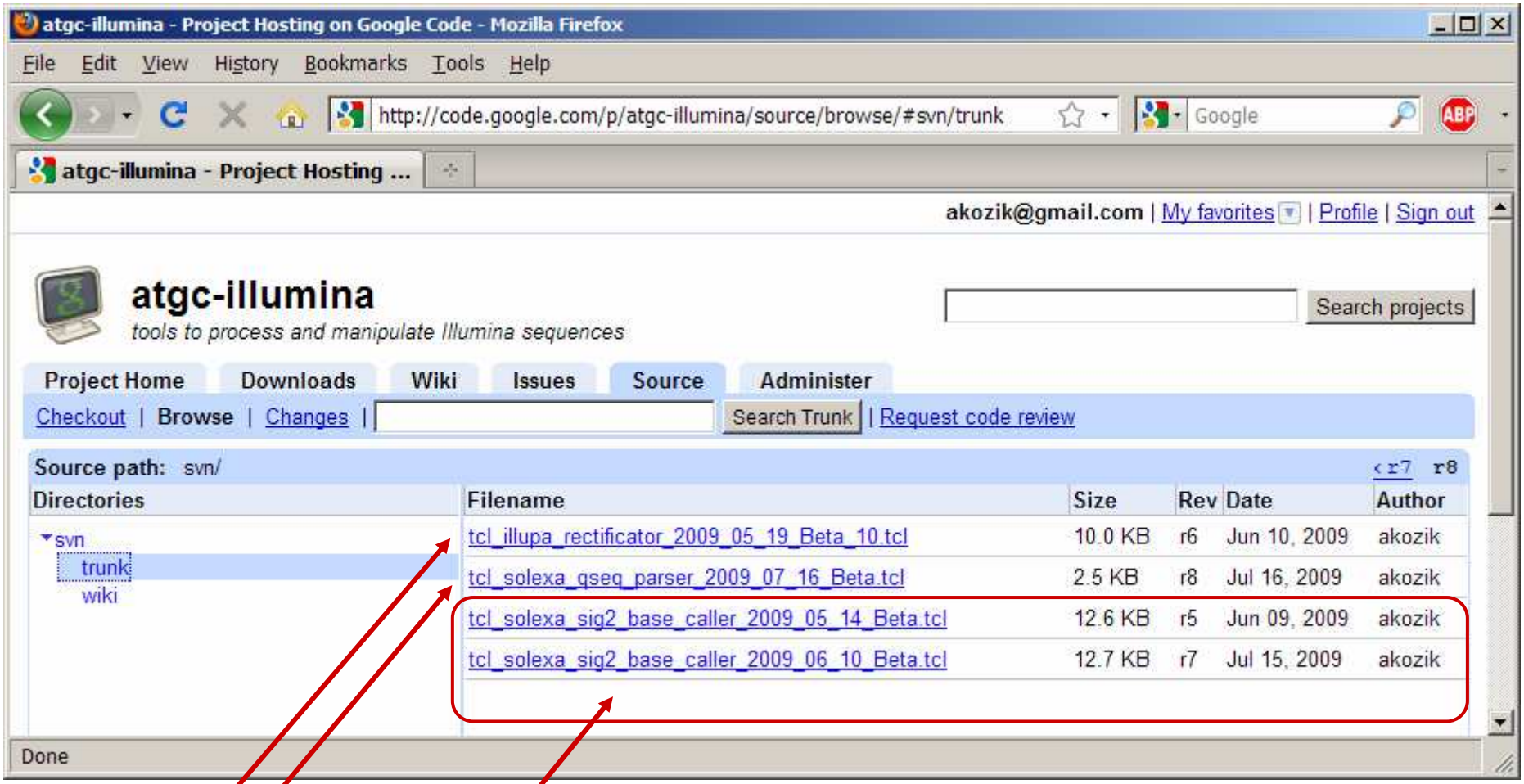
http://code.google.com/p/atgc-illumina/



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 parser output example - *.trim.fa file

```
#!/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_l  [lindex $argv 2]
    set r_len  [lindex $argv 3]
```

```
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
GAATTCCTTCGCACATCATCATAAACCTCGGTTCCTCCATTTGTCAAAAAGCAAACGGGGAATTTTTG

>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
ATCAGCTTTCCATGTAGCATAATAGTAACGCTGCCTAAGAAGTTGCAAATTCTCCCTGTCTAGAGAAACTGGCTGAGAGCCTGGA
```

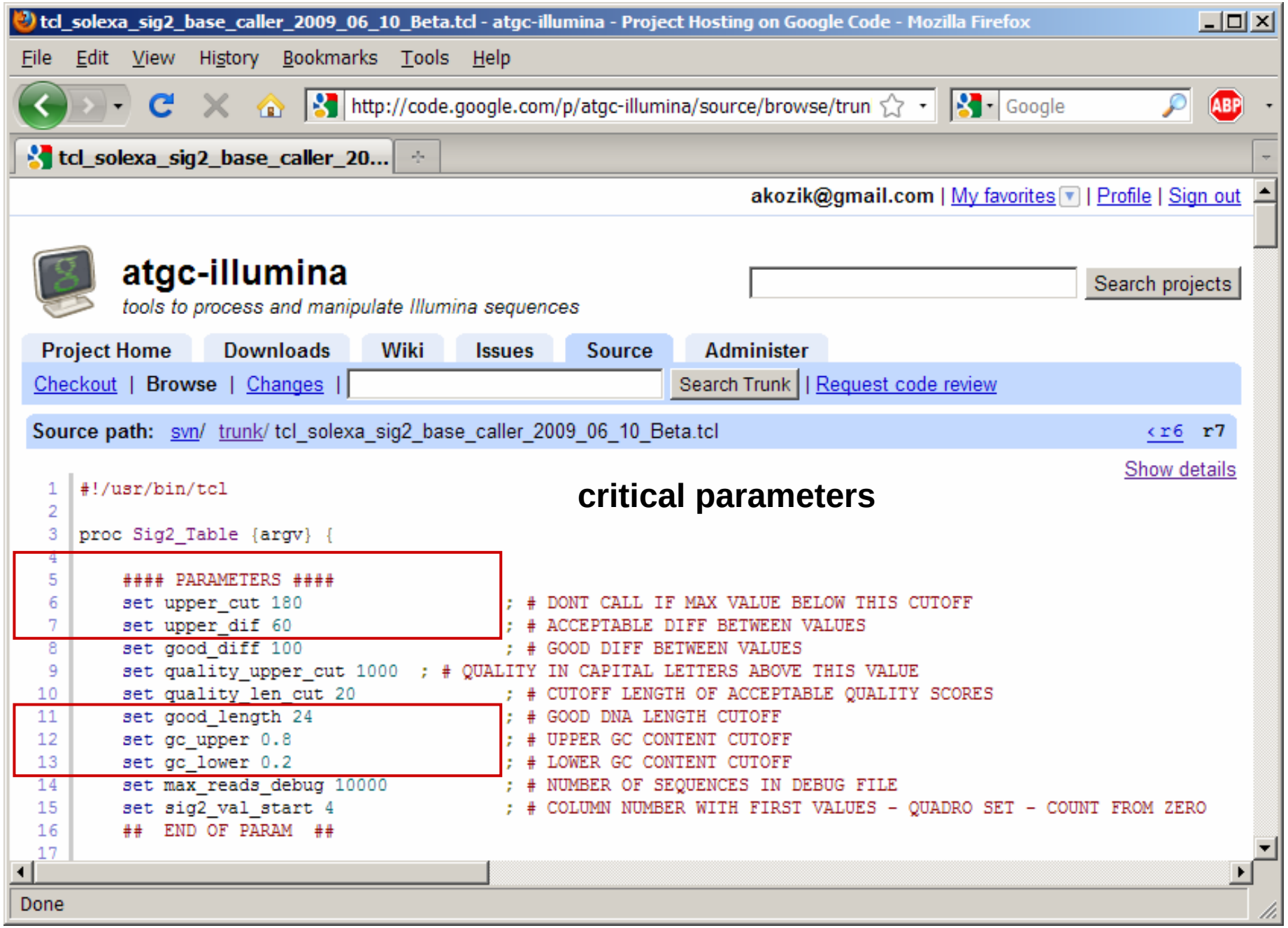
With Tag (bar code):

```
>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
TCCCTCCTTTAAAACACCCCAAATTGCAATTTAACAATACTTGAGACTTTGGGC
```

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

sig2 basecall / filtering



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]
GTTTTTTcGTTTxTACTAATAxTTGTTTTG*A*AGTGxgCCTCCCTxTGNGGATTCTTnN*GA*Gnn**t*G**nn**nNn*nnn

fasta output

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

```
>SBO8_8_120_65_1105  |  LINE: 9728  [ LENGTH: 44 ]  ( ___GC___: 20/44 )
 [ ABCD_Q:44  F_QUAL:0 ]  [ Q_FRACT:100 ]
AACAAATGTCTCGAATCTTAACCGCACATCAAGCTGCAAGAGGG

>SBO8_8_120_65_1326  |  LINE: 9734  [ LENGTH: 46 ]  ( ___GC___: 20/46 )
 [ ABCD_Q:46  F_QUAL:0 ]  [ Q_FRACT:100 ]
AGAACAAGGAGGATTATGCCAAGTTCTACGAGGCTTTCTCCAAGAA

>SBO8_8_120_88_573  |  LINE: 13230  [ LENGTH: 83 ]  ( ___GC___: 22/83 )
 [ ABCD_Q:83  F_QUAL:0 ]  [ Q_FRACT:100 ]
CGAGAATATATATGAACAAGAGTTAGAACAAAAAGAAAGGATGAAAATGAAATCATACAAAATTCTGAATCATTTCTCTAACT

>SBO8_8_120_88_899  |  LINE: 13235  [ LENGTH: 63 ]  ( ___GC___: 17/63 )
 [ ABCD_Q:63  F_QUAL:0 ]  [ Q_FRACT:100 ]
CTCATAAGTAATTCAAAGCTTTTTCAAACTTCATTaCAAATAATTAACGTTTCATTTCCATGG

>SBO8_8_120_166_1586  |  LINE: 25397  [ LENGTH: 48 ]  ( LOWER_GC: 6/48 )
 [ ABCD_Q:48  F_QUAL:0 ]  [ Q_FRACT:100 ]
ATTCTGTTCACTAATGGTTTATTTaTTTATTTATTTTTTTTTTTATAA

>SBO8_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
+
IIIII99IIIIII9II599III909I9

@SBN4_4_24_178_1771  _TCGTAT_ : 19
TCCCAAATGTAGACAAAGCTCGTATGCCGTCTTCT
+
IIIIIIII99I5IIIII95959II95II599II99

@SBN4_4_24_179_1454  _NOT_FOUND_ : -1
AAATGAACTCAGCCGTAACGCGATTC
+
IIII9IIIIII99I5II995I0I9II

@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
AAGAGGGAAAAGGGCTATTAAGCTTCGTAT
+
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

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

Phred pseudo-quality scores (ASCII - 33)

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

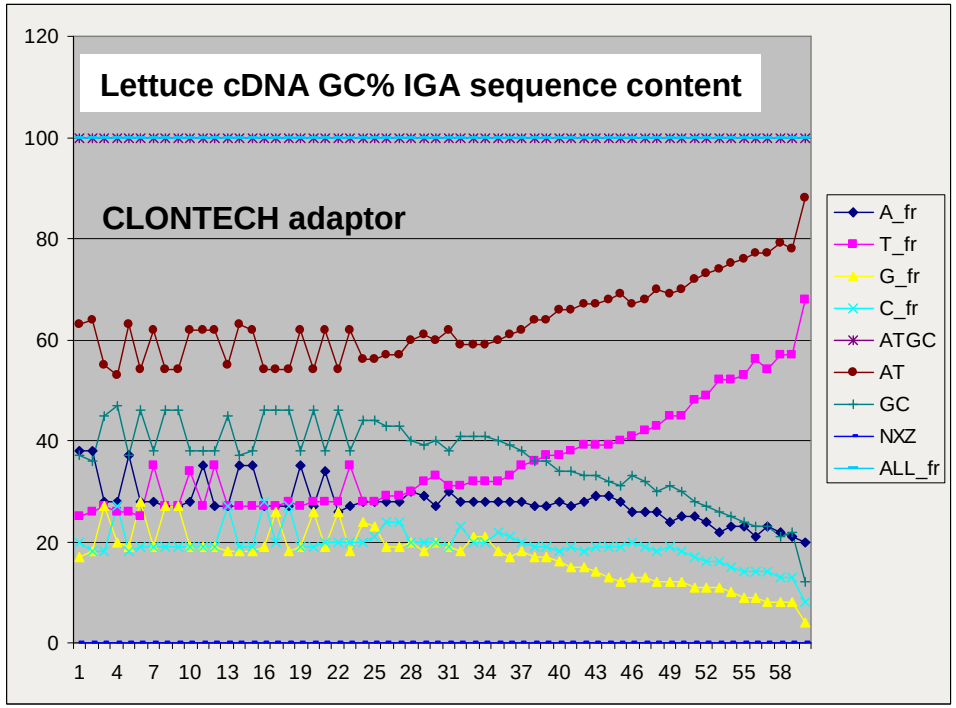
```
 -25.0 -104.9   43.2  163.0
   6.2   28.5 -145.9  502.6
 -12.1  -16.1  -77.0  368.6
   2.9   13.9   12.9  522.6
   7.8   21.2  131.9  215.9
  -1.2    9.3  233.4  342.9
  -2.2   -4.3   -9.6  490.6
   3.6   12.4    5.1  263.3
  -1.8    7.4    2.0   82.2
   2.9  -23.6    4.8  451.0
   6.1   19.9   -2.5  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

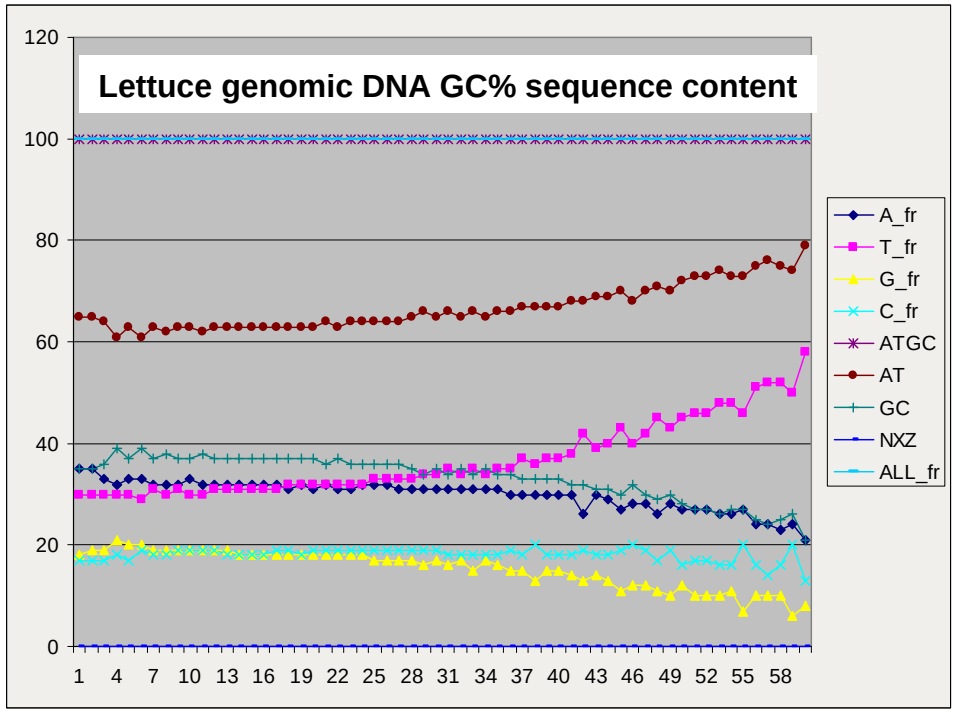
```
   3.1    5.7    2.6    3.3
   3.0    5.8    2.8    5.5
   3.0    5.8    2.8    5.5
   3.0    5.8    2.8    5.5
   3.4    6.3    3.0    3.4
   3.4    6.3    3.0    3.4
   2.8    5.1    2.5    5.3
   2.8    5.1    2.5    5.3
   2.8    5.1    2.5    5.3
   3.2    5.7    2.5    4.4
   3.2    5.7    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
```

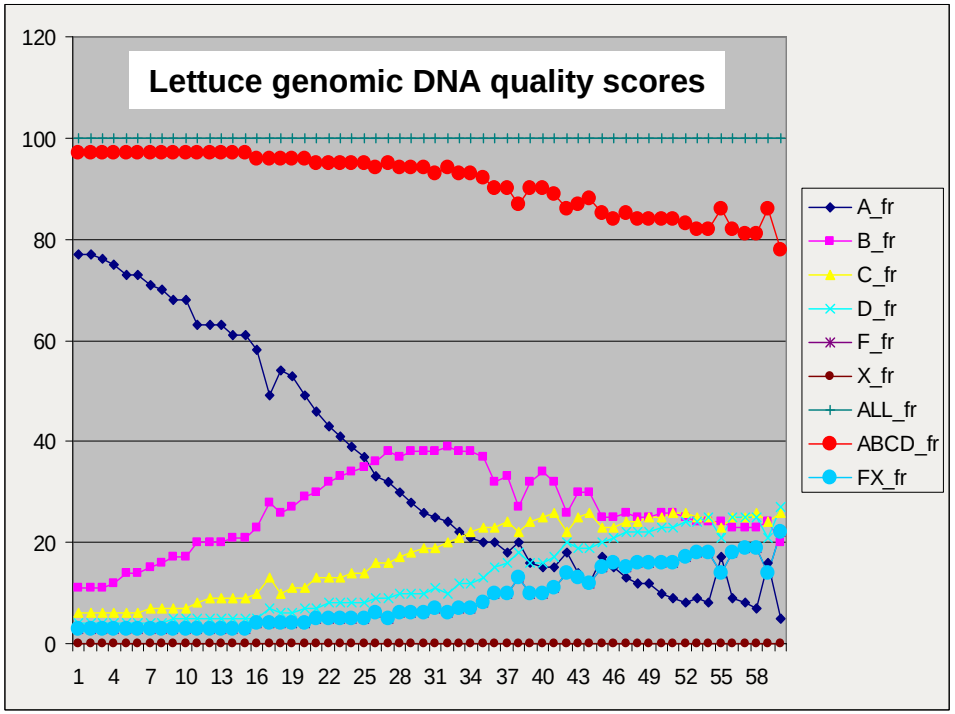
line	tile	X	Y	cycle 1 A C G T	cycle 2 A C G T	cycle 3 A C G T	cycle 4 A C G T
3	0012	108.61	1169.14	-10 20 22 630	9 21 894 479	13 25 756 411	182 539 2 20
3	0012	108.63	1586.81	570 685 1 9	43 37 770 545	38 45 540 394	770 529 -7 15
3	0012	108.67	1305.63	127 503 2 14	11 104 19 670	37 57 703 442	121 13 766 462
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

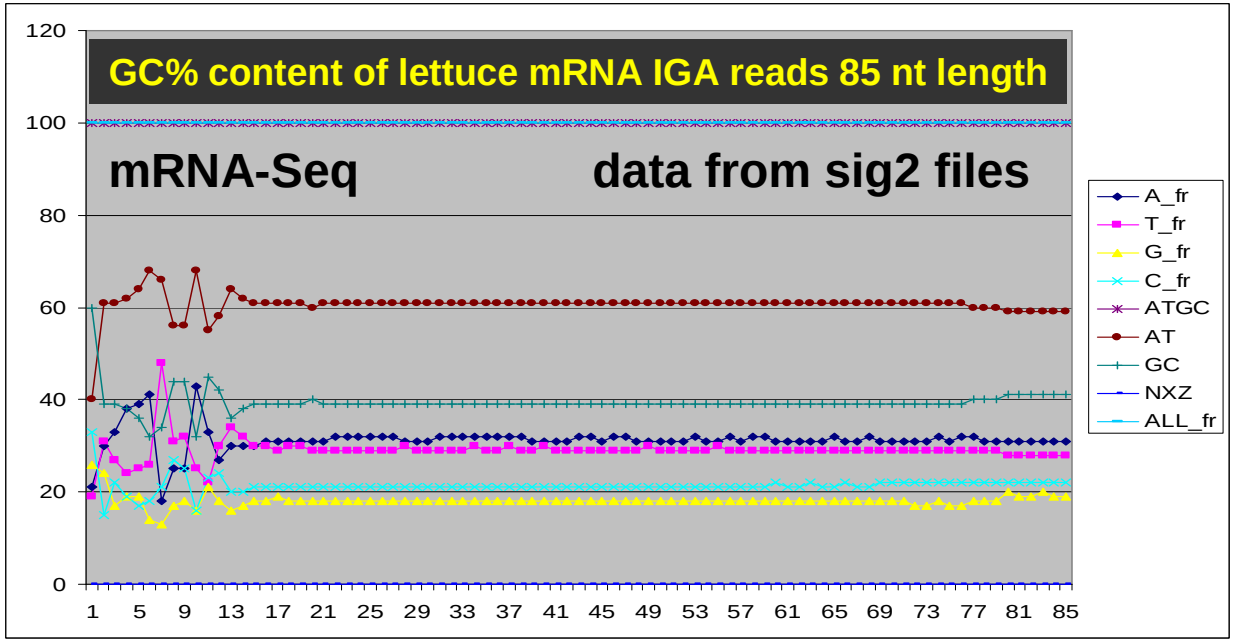


OLD CHEMISTRY

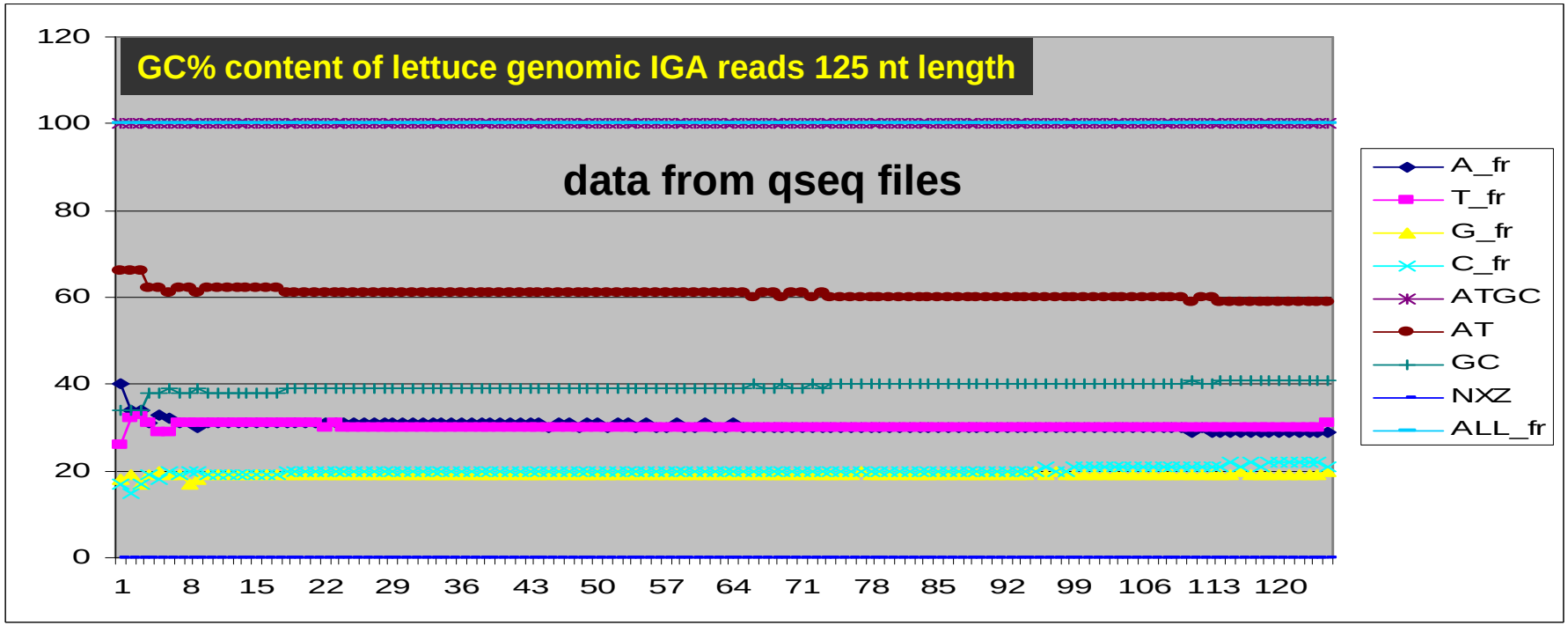


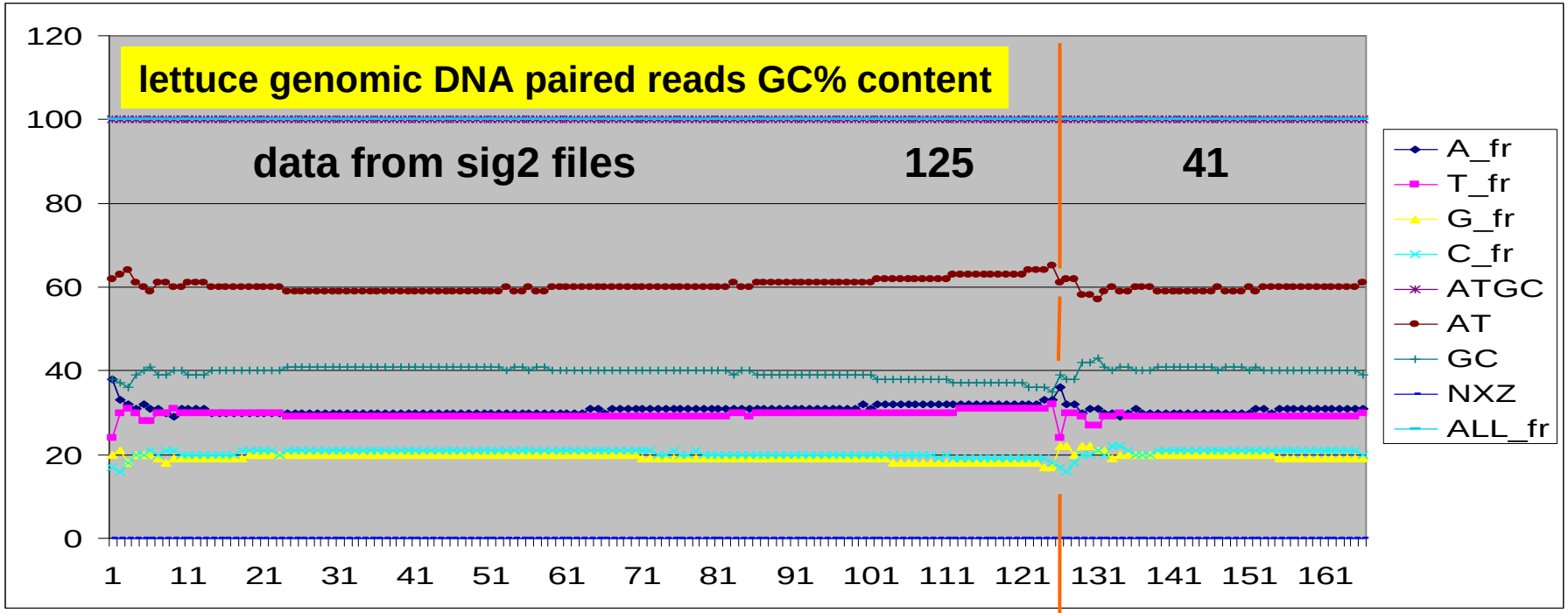


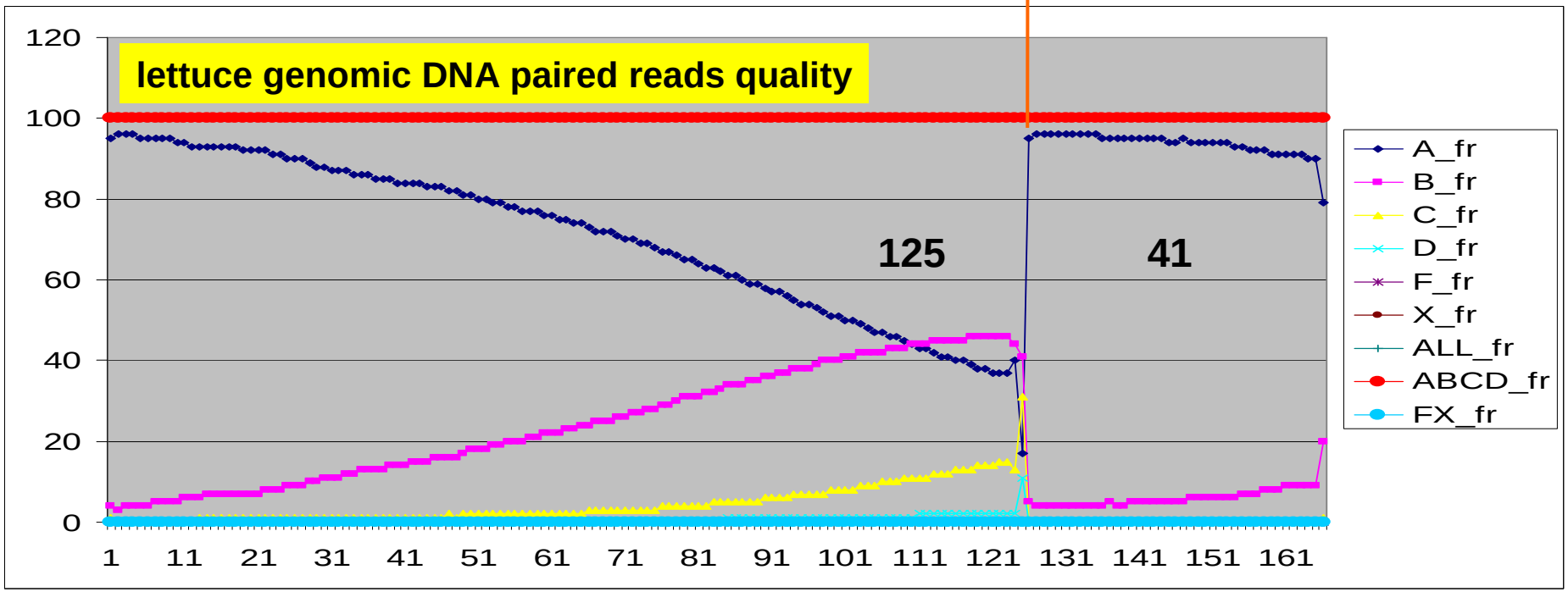
QC examples for GC% content



NEW CHEMISTRY




lettuce genomic DNA paired reads GC% content
data from sig2 files
125
41
120
100
80
60
40
20
0
1
11
21
31
41
51
61
71
81
91
101
111
121
131
141
151
161
A_fr
T_fr
G_fr
C_fr
ATGC
AT
GC
NXZ
ALL_fr


lettuce genomic DNA paired reads quality
125
41
120
100
80
60
40
20
0
1
11
21
31
41
51
61
71
81
91
101
111
121
131
141
151
161
A_fr
B_fr
C_fr
D_fr
F_fr
X_fr
ALL_fr
ABCD_fr
FX_fr

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 -

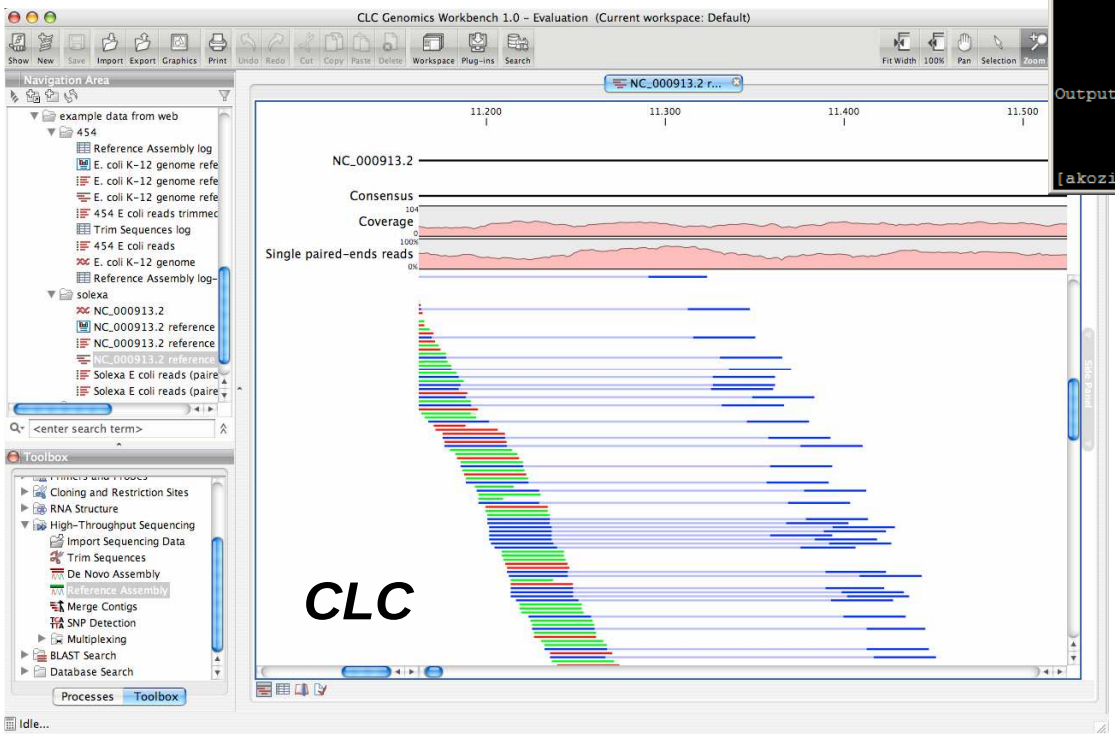
Velvet - publicly available
http://www.ebi.ac.uk/~zerbino/velvet/

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:
       -fasta
       -fastq
       -fasta.gz
       -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



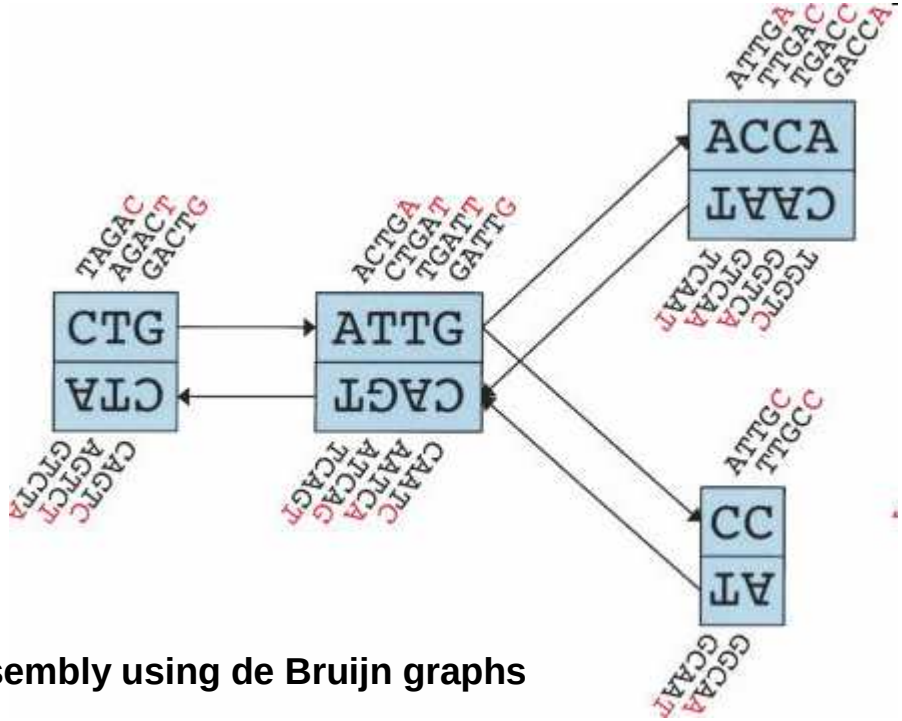
Velvet -
- large input 120+ million reads
- low number of chimeric contigs

CLC -
- 20 million reads input limit
- higher number of chimeras

CLC contigs usually longer than Velvet contigs

Velvet

critical parameter K-mer value



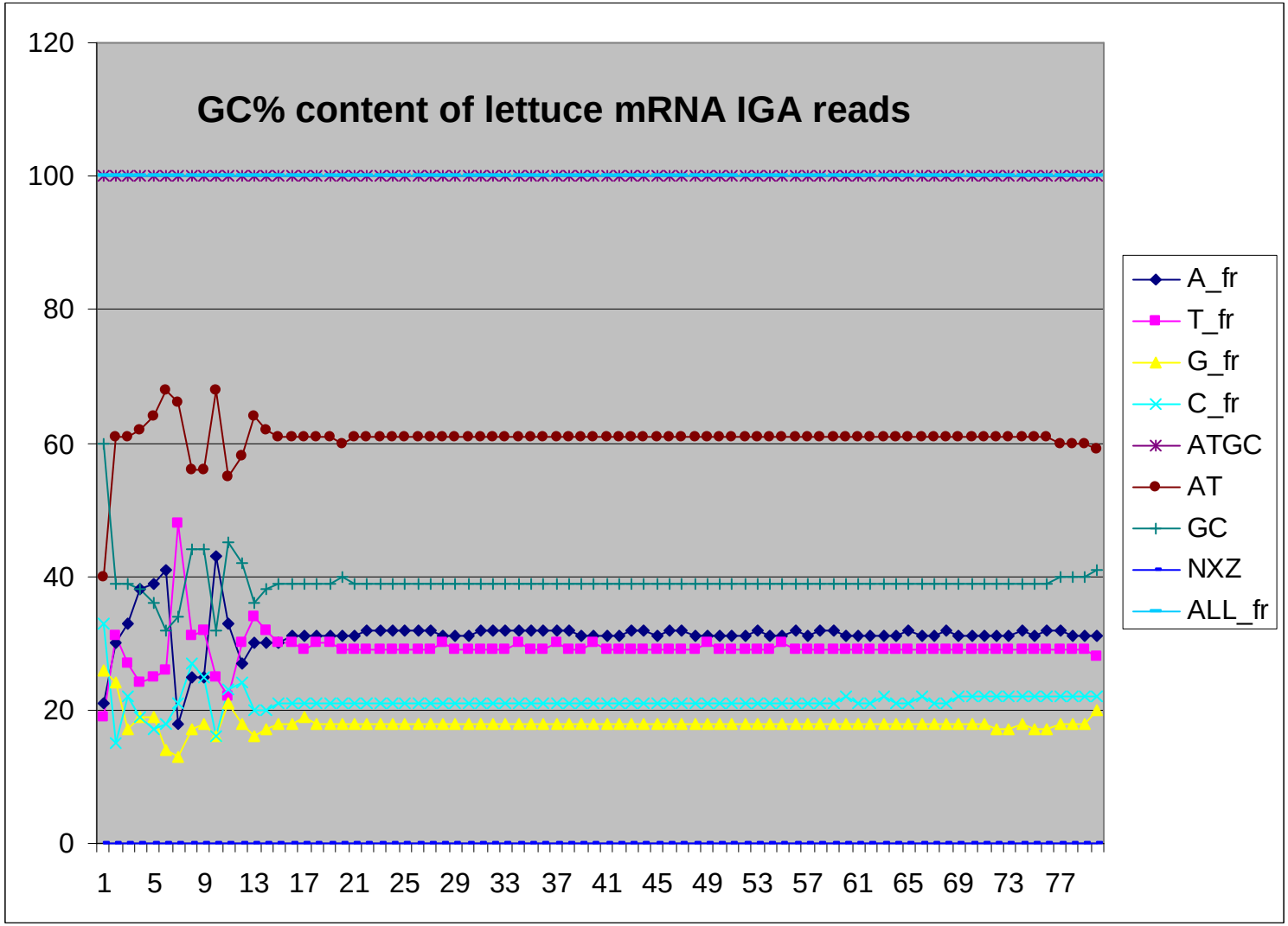
Velvet: Algorithms for de novo short read assembly using de Bruijn graphs
Daniel R. Zerbino and Ewan Birney

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.

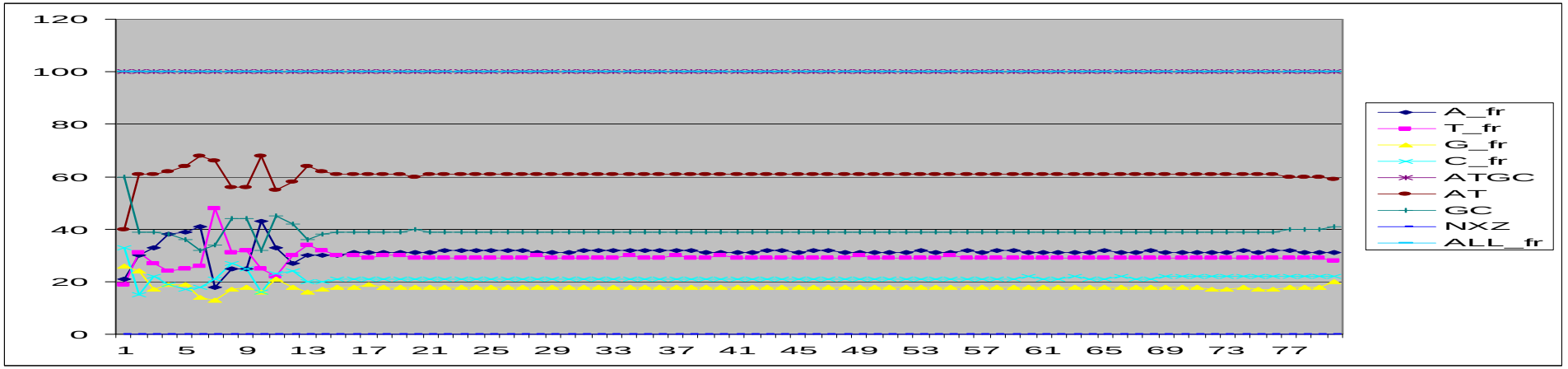
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2336801

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
>SBO8_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_70_A.fa
>SBO8_8_120_1651_1715
AACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

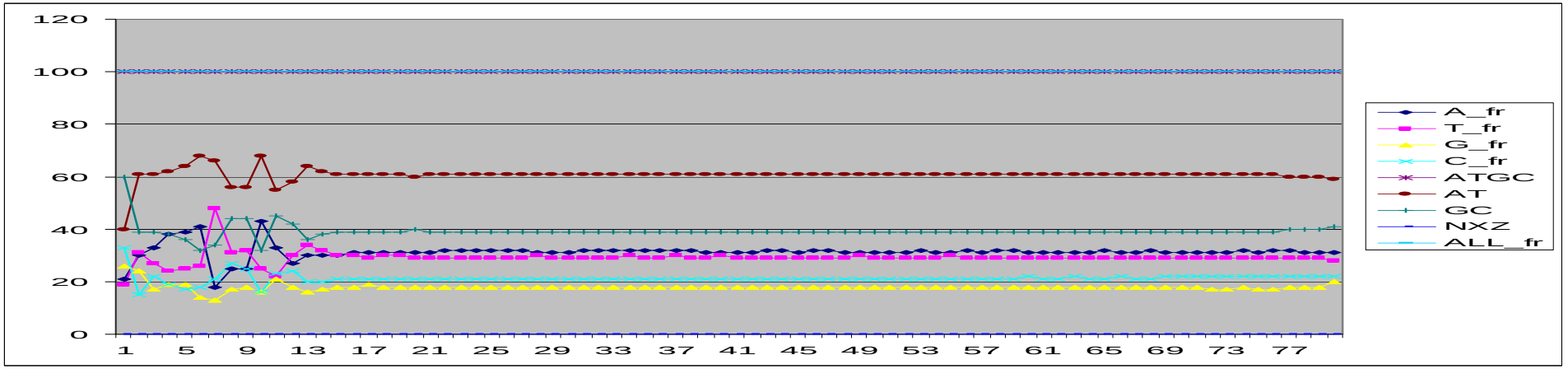
A

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_70_D.fa
>SBO8_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGG
```

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
>SBO8_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_65_A.fa
>SBO8_8_120_1651_1715
TCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

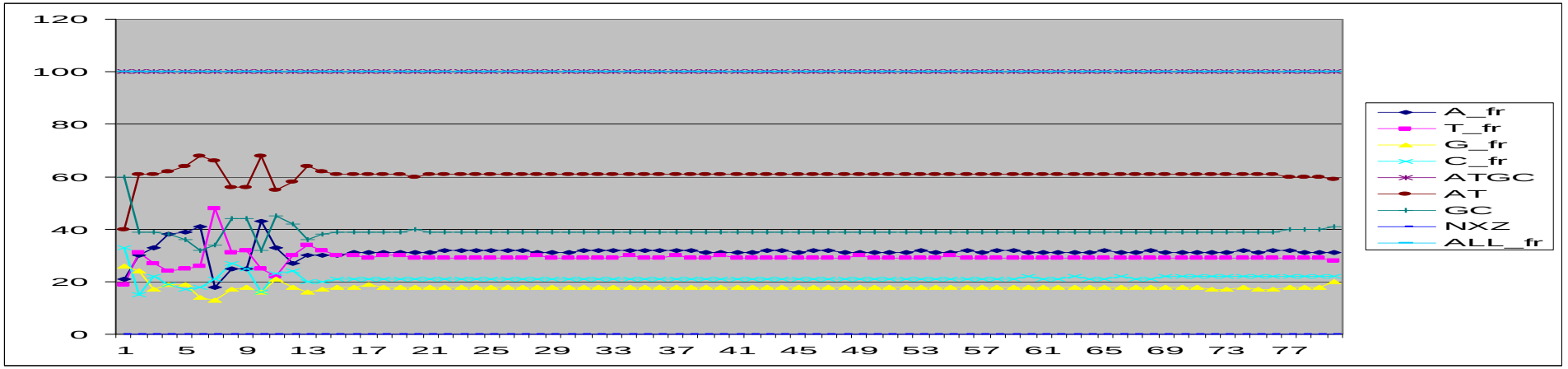
A

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_65_B.fa
>SBO8_8_120_1651_1715
AACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAA
```

C

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_65_D.fa
>SBO8_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGAT
```

D **15 nt trimming**




```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_80.fa
>SBO8_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_A.fa
>SBO8_8_120_1651_1715
GACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAAGCAGA
```

A

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_B.fa
>SBO8_8_120_1651_1715
TCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGGAAGAA
```

B

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_C.fa
>SBO8_8_120_1651_1715
AACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAAAAGATCCGGG
```

C

```
bash-2.03$ tail -2 090707_SOLEXA1_FC42EK3AAXX_lettuce_cDNA.Clean.GC.Q_60_D.fa
>SBO8_8_120_1651_1715
CAACAAGGCAAACCATCTTGGACAAAACGTTTGTTGCTTGATGACAAACTATTATGCAAA
```

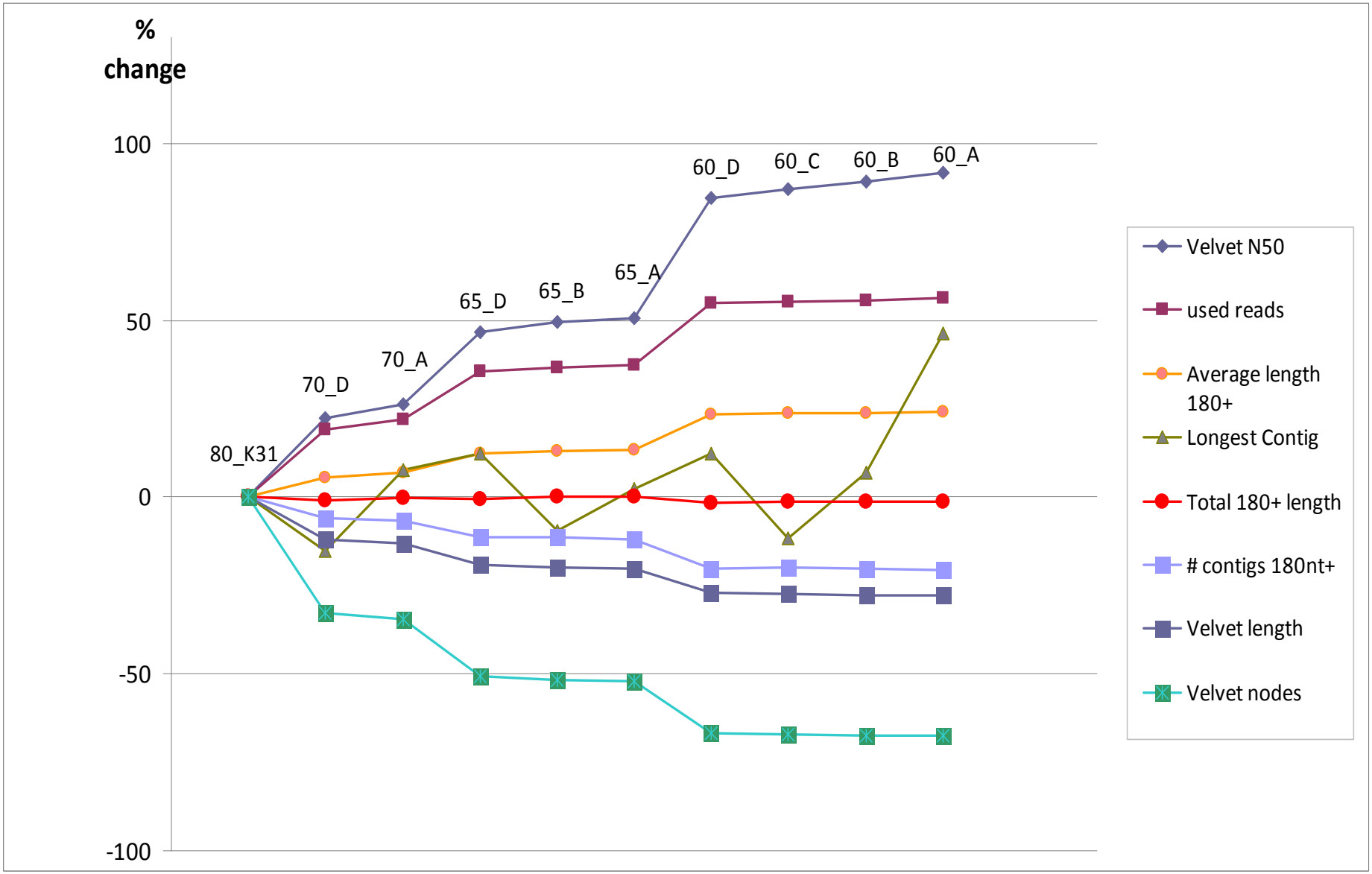
D

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**

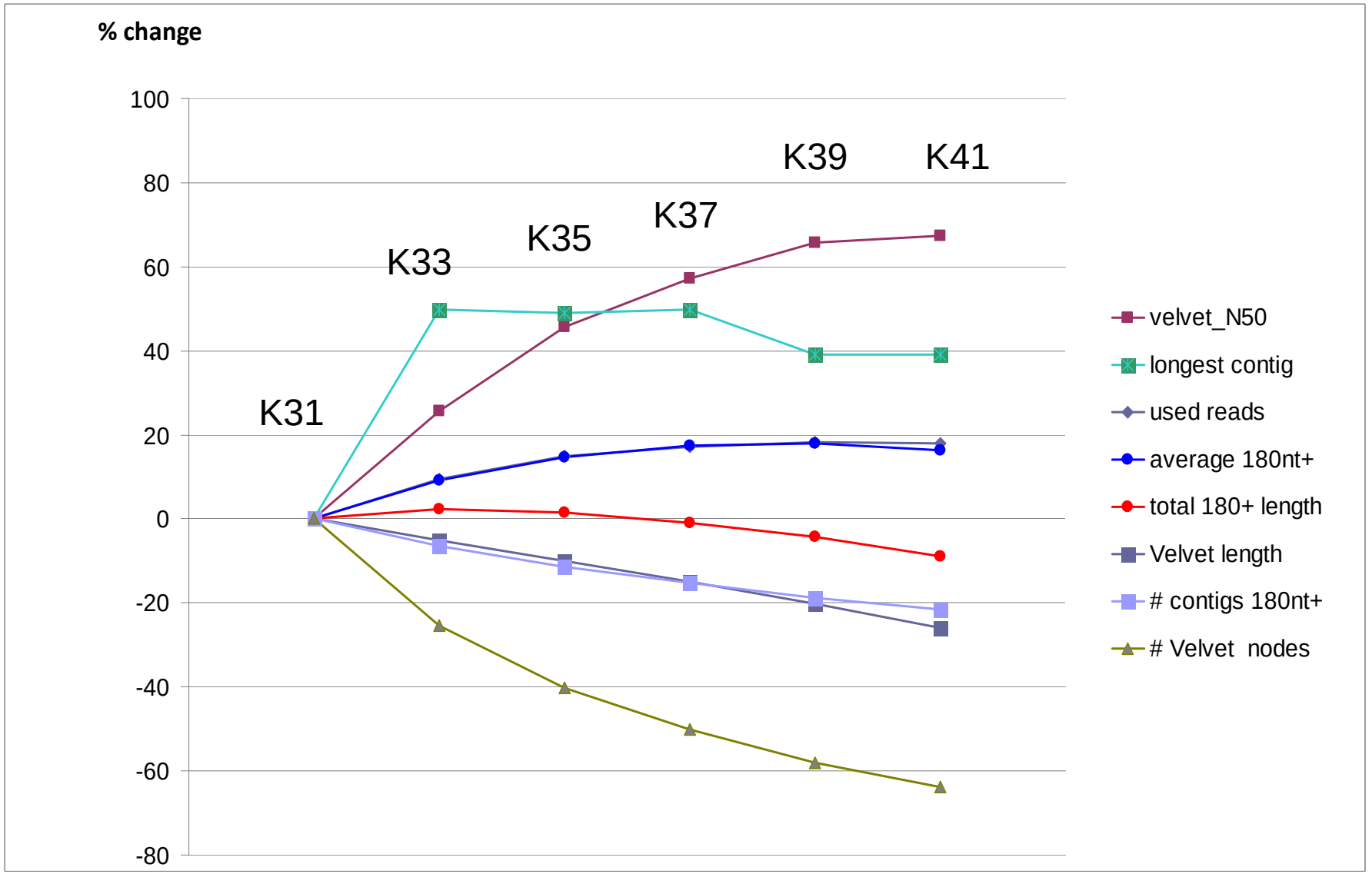
Trimming effect on assemblies (transcriptome)



Velvet K-mer effect on assemblies (transcriptome)

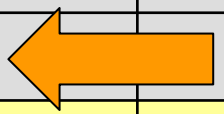
sample	# of reads (million)	used reads (million)	velvet N50	velvet # nodes	velvet length million nt	longest	median 180 nt +	average 180 nt +	contigs 180nt +	total 180+ length million nt
80_QC K31	47.50	17.03	126	1,160,217	60.85	4272	261	318	93,175	29.67
60_QC K31	47.50	33.44	303	325,378	42.83	5131	313	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

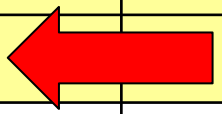
Velvet K-mer effect on assemblies (transcriptome): same input 47.50 million reads of 60 nt length - different K-mer values

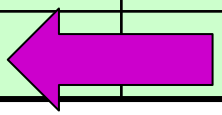


Velvet K-mer effect on assemblies (gene space)

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
050_K31	55.41	NA	71	2,412,943	52.68	5610				
075_K31	55.41	NA	86	15,931,390	433.43	3680				
100_K31	55.41	NA	83	28,268,865	723.08	3906				
125_K31	**55.41**	**NA**	**80**	**37,395,673**	**890.06**	**4247**				
050_K37	55.41	2.20	78	378,970	8.56	21703				
075_K37	55.41	9.58	110	7,368,125	312.01	4386				
100_K37	55.41	17.83	122	17,113,337	654.16	3520				
125_K37	**55.41**	**21.00**	**118**	**24,634,813**	**872.36**	**4769**				
125_K47	55.41	26.78	189	11,692,947	753.67	6265				
125_K53	**55.41**	**28.11**	**217**	**7,359,161**	**662.23**	**4809**				







assembly of the assemblies

1 step

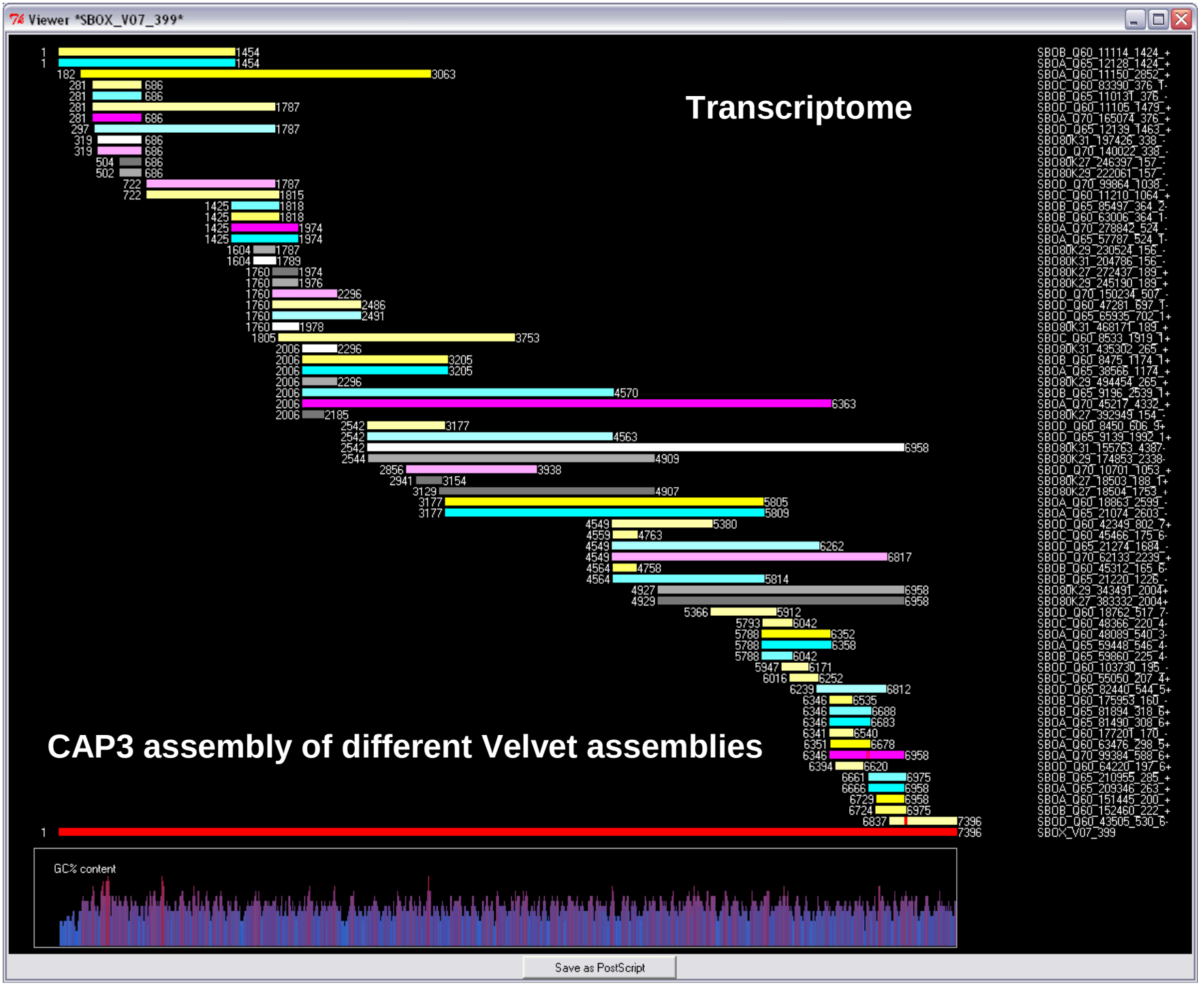
Velvet and/or CLC with different parameters and samples

2 step

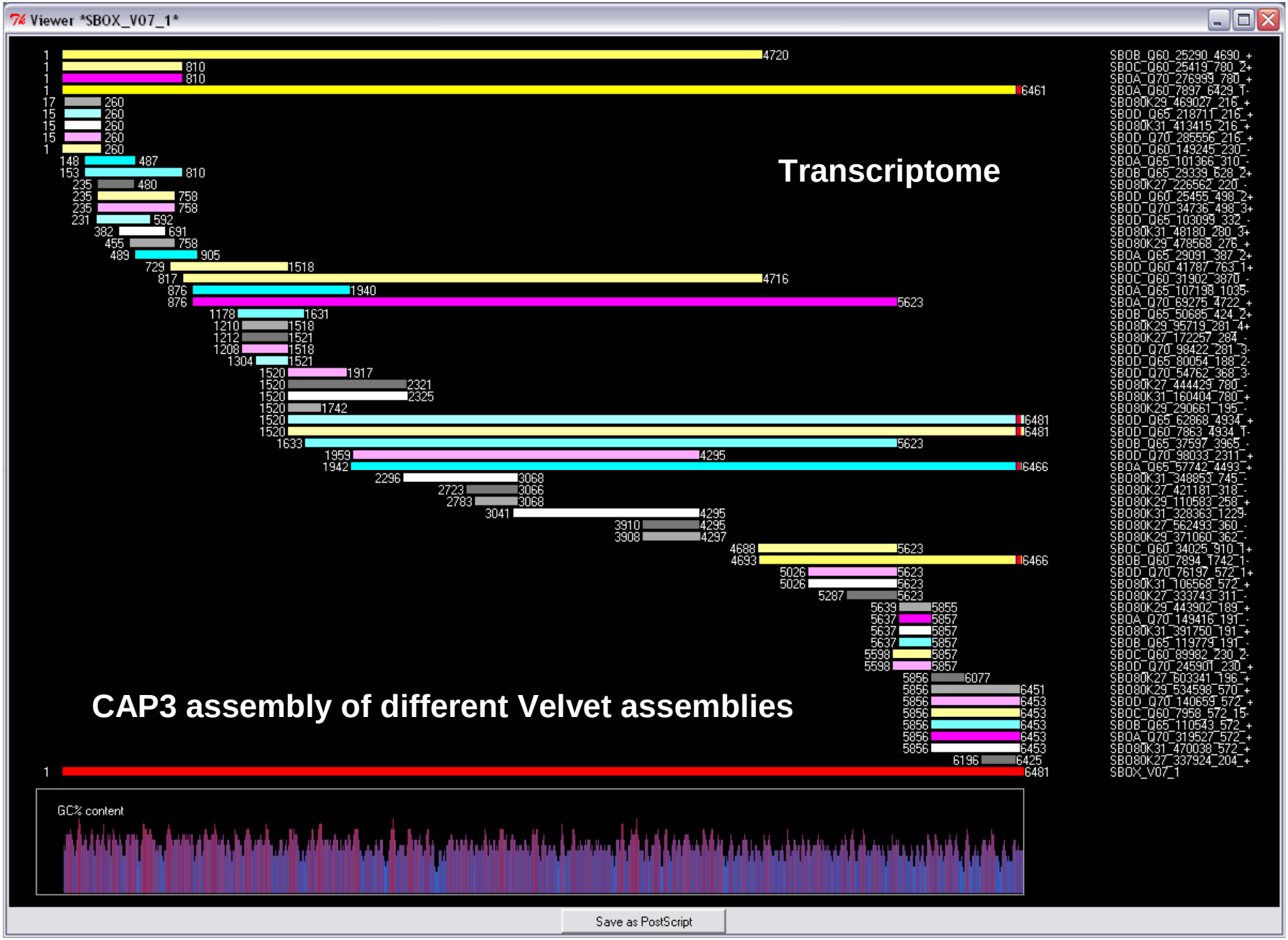
CAP3 assembly with Velvet and/or CLC contigs

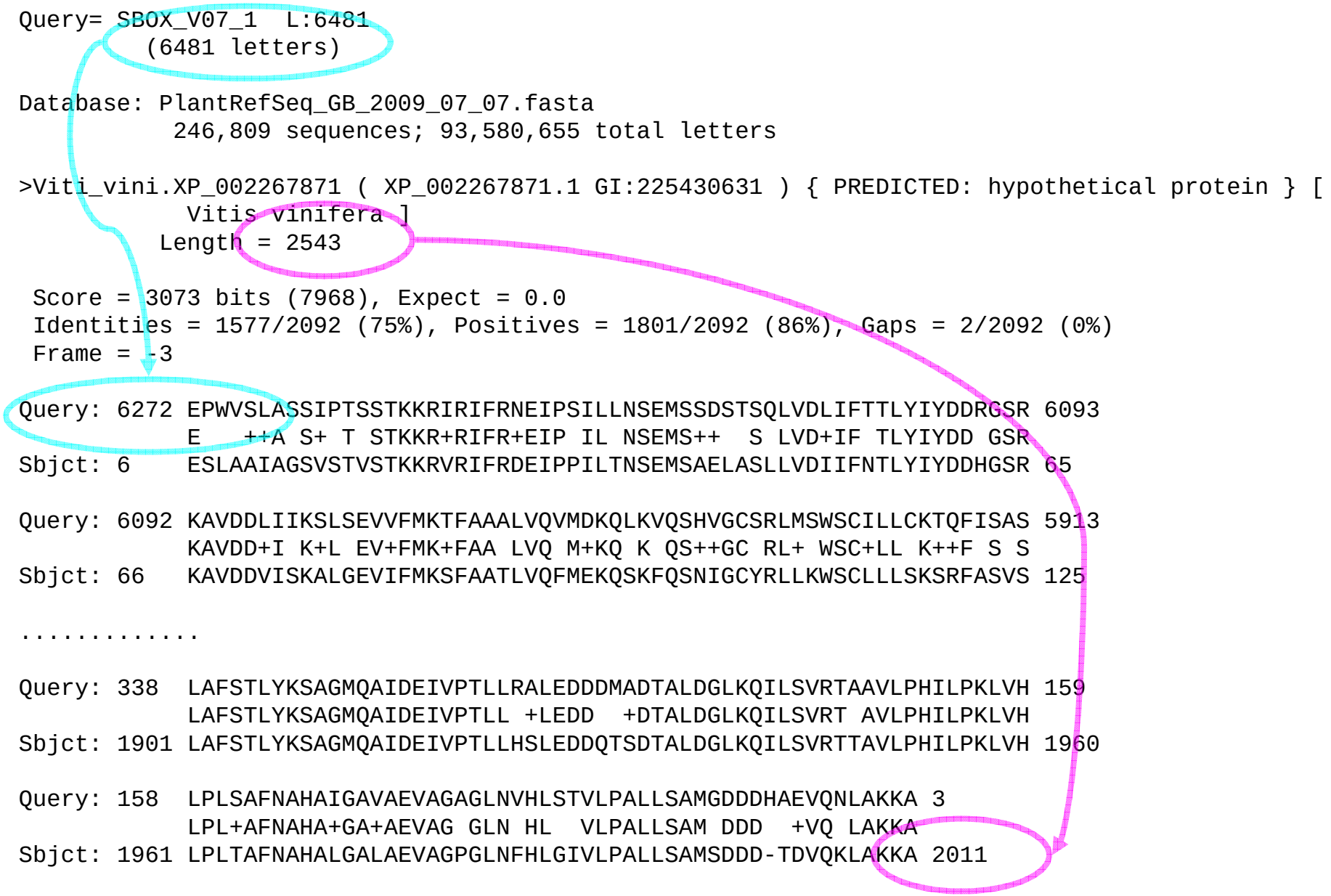
3 step

Visualization and validation

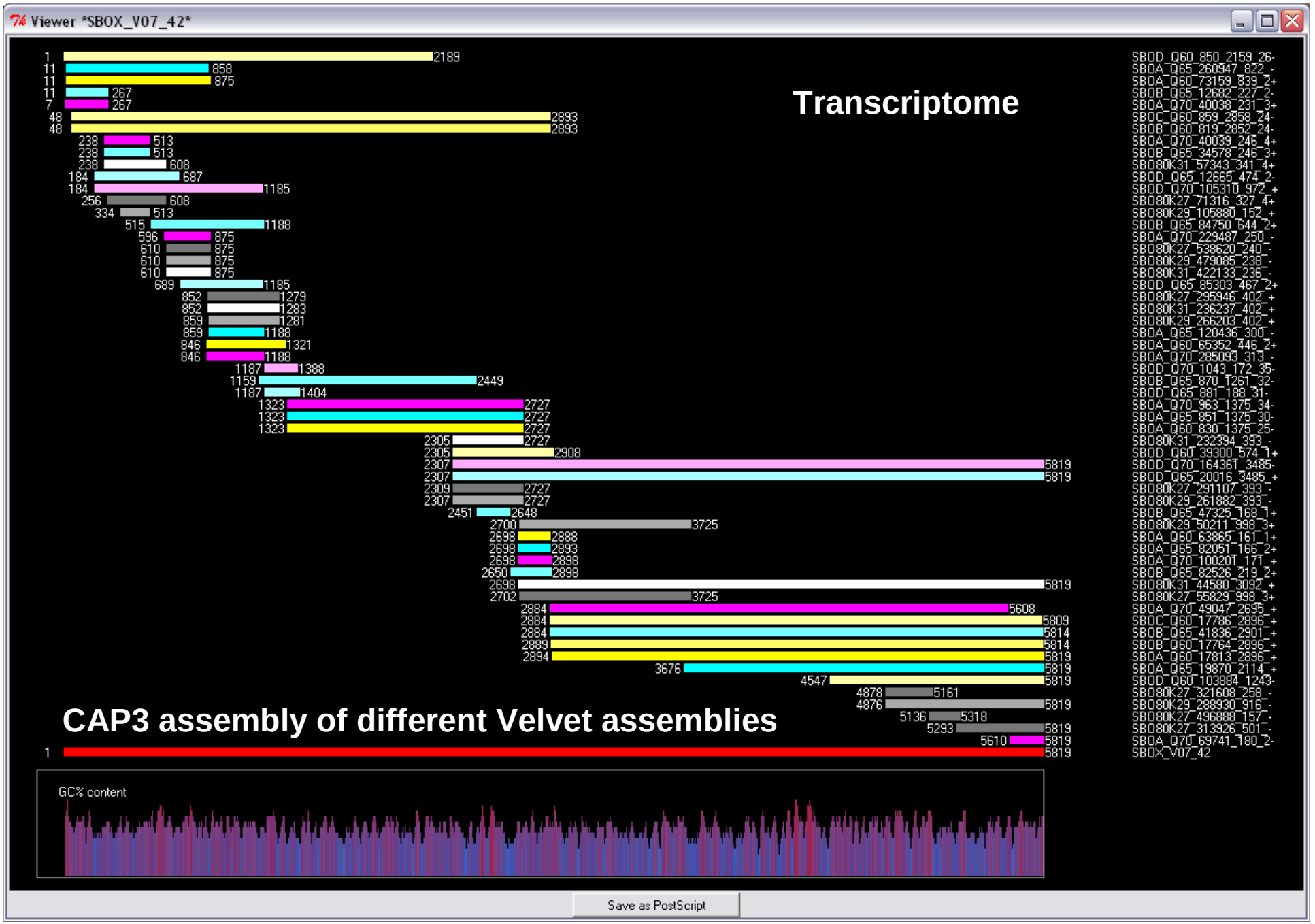

Viewer *SBOX_V07_399*
Transcriptome
CAP3 assembly of different Velvet assemblies
GC% content
Save as PostScript

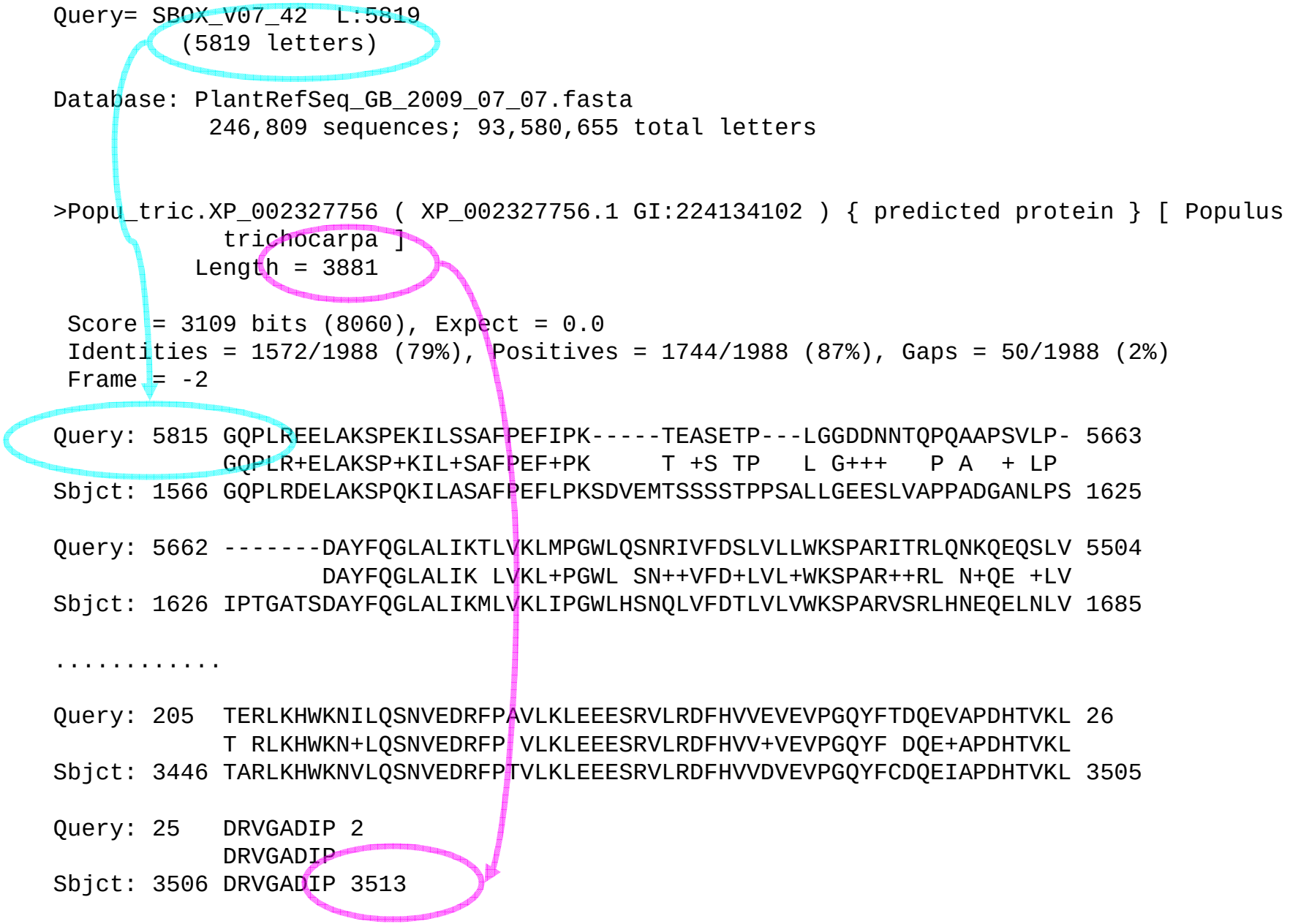
```
Query= SBOX_V07_399  L:7396
       (7396 letters)

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 ----------MEKELHKVIPEKLIEVGVYVVGPRIRVSLRK-DSLHKAVD--DIHLSFDC 7075
                     +E  LH+V PE+ I+VG+ + GP+I++ + K + ++  +   DI L FD
Sbjct: 711  YDEYGIYRRSIELSLHRVHPERQIQVGILLGGPQIKLLVEKAEEVNTLIGKKDI-LLFDF 769

..................

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

Query: 375  NWGDRFKGDPPKEIVKEEESSKMSVL-KWGVGRFVFAGMVAYIDGRLCRCIPNPVARRIV 199
               D         +V + +  KM  + K G+G FV +G+VAYIDGRLCR IPNP+ARRIV
Sbjct: 2998 ETSDSGSNTAMVPVVSDNKKKKMKFMWKAGIGNFVASGIVAYIDGRLCRQIPNPIARRIV 3057

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


Viewer *SBOX_V07_1*
Transcriptome
CAP3 assembly of different Velvet assemblies
GC% content
Save as PostScript



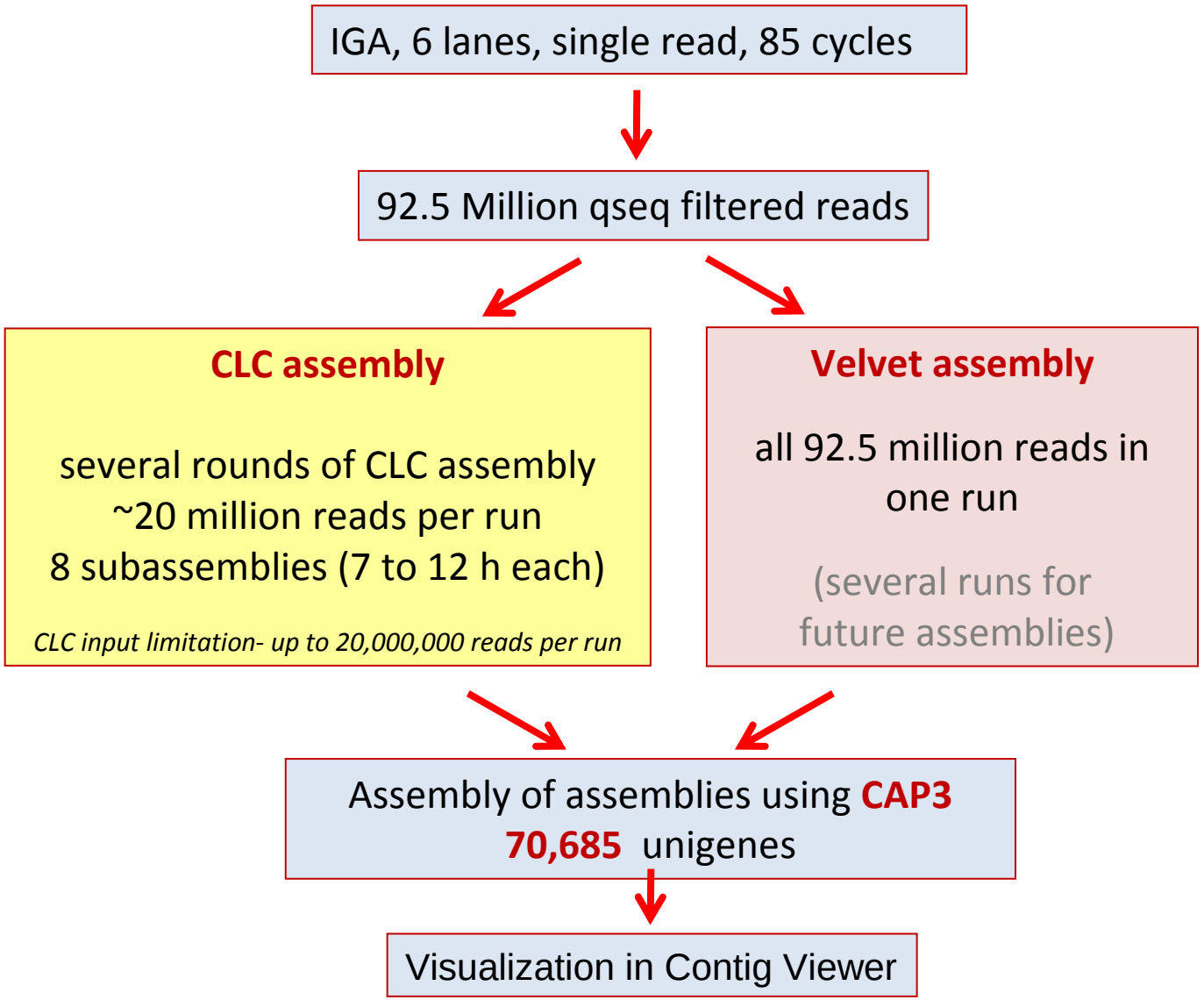
```
Query= SBOX_V07_1  L:6481
       (6481 letters)

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

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

.............

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

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


Viewer *SBOX_V07_42*
Transcriptome
CAP3 assembly of different Velvet assemblies
GC% content
Save as PostScript



```
Query= SBOX_V07_42  L:5819
       (5819 letters)

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


>Popu_tric.XP_002327756 ( XP_002327756.1 GI:224134102 ) { predicted protein } [ Populus
            trichocarpa ]
          Length = 3881

 Score = 3109 bits (8060), Expect = 0.0
 Identities = 1572/1988 (79%), Positives = 1744/1988 (87%), Gaps = 50/1988 (2%)
 Frame = -2

Query: 5815 GQPLREELAKSPEKILSSAFPEFIPK-----TEASETP---LGGDDNNTQPQAAPSVLP- 5663
          GQPLR+ELAKSP+KIL+SAFPEF+PK     T +S TP   L G+++   P A  + LP
Sbjct: 1566 GQPLRDELAKSPQKILASAFPEFLPKSDVEMTSSSSTPPSALLGEESLVAPPADGANLPS 1625

Query: 5662 -------DAYFQGLALIKTLVKLMPGWLQSNRIVFDSLVLLWKSPARITRLQNKQEQSLV 5504
                   DAYFQGLALIK LVKL+PGWL SN++VFD+LVL+WKSPAR++RL N+QE +LV
Sbjct: 1626 IPTGATSDAYFQGLALIKMLVKLIPGWLHSNQLVFDTLVLVWKSPARVSRLHNEQELNLV 1685

.............

Query: 205  TERLKHWKNILQSNVEDRFPAVLKLEEESRVLRDFHVVEVEVPGQYFTDQEVAPDHTVKL 26
            T RLKHWKN+LQSNVEDRFP VLKLEEESRVLRDFHVV+VEVPGQYF DQE+APDHTVKL
Sbjct: 3446 TARLKHWKNVLQSNVEDRFPTVLKLEEESRVLRDFHVVDVEVPGQYFCDQEIAPDHTVKL 3505

Query: 25   DRVGADIP 2
            DRVGADIP
Sbjct: 3506 DRVGADIP 3513
```

Lettuce Transcriptome Sequencing and Assembly Pipeline



Lettuce Gene Space Sequencing

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

Velvet assembly in four sets:
125, 100, 75, 50 nt

↓

CAP3 assembly of Velvet assemblies

↓

1 Million contigs
(10 pieces per ~100K “gene spaces”)

5,930 nt longest,
average length – 317 nt, median – 250 nt

Aligning to 356 lettuce COS genes

↓

8 x average coverage per nt (1 to 60)

Aligning to gene space of lettuce BAC

↓

10 x coverage per nt

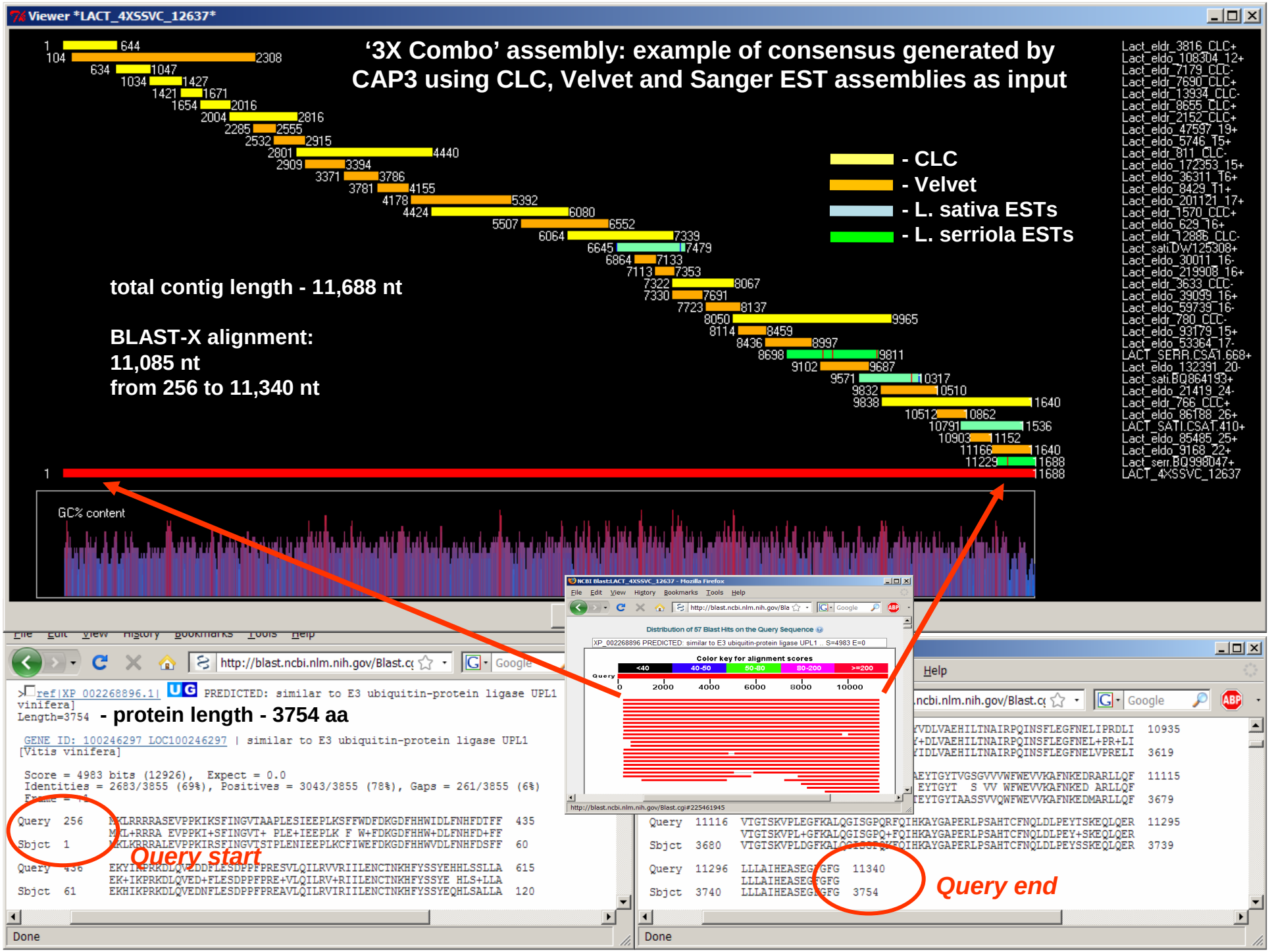
QC Analysis of Transcriptome Assembly

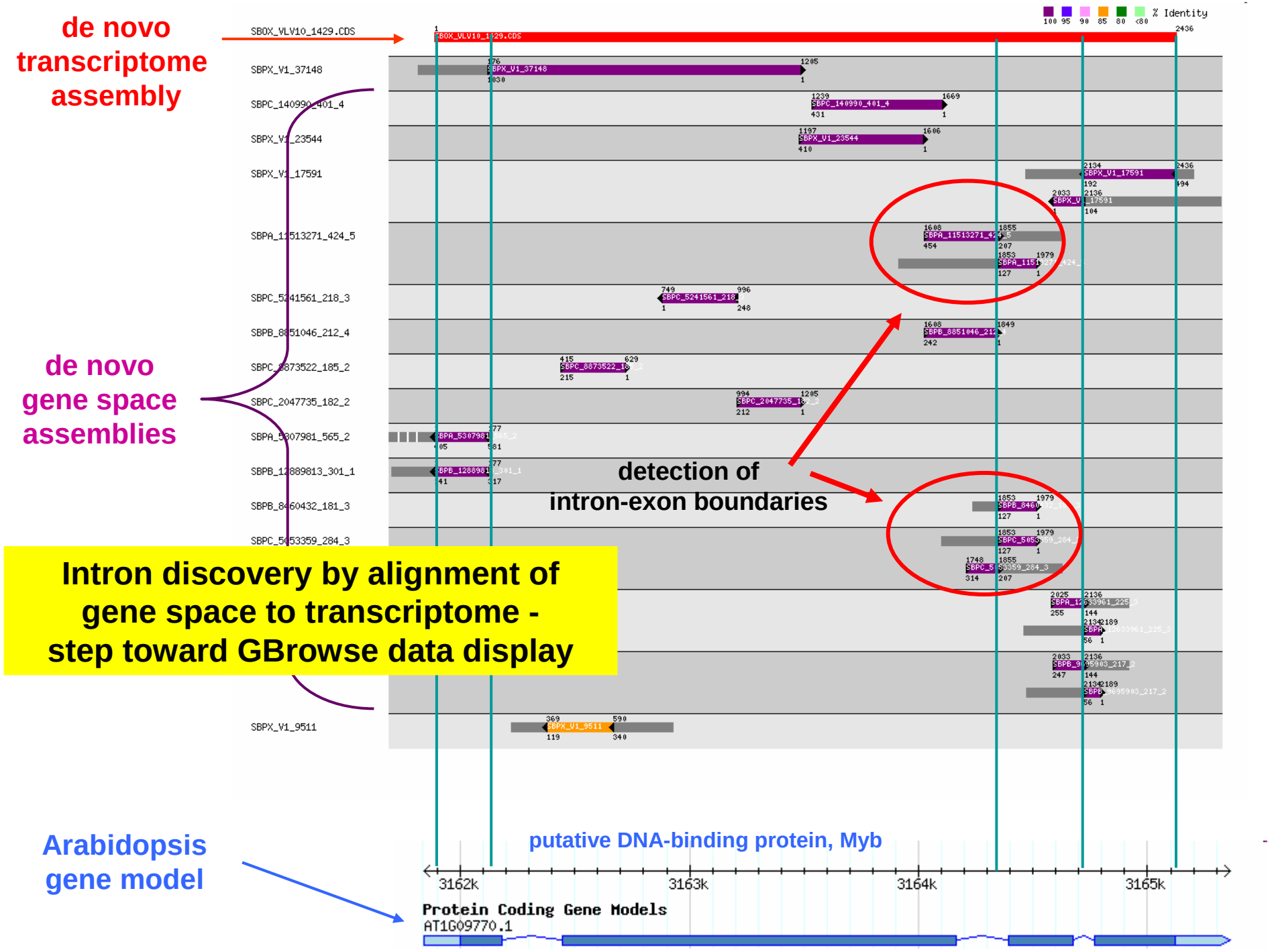
BLASTX versus Plant RefSeq Database: out of 8,300 longest contigs only 83 (1%) have no hits (similarity) to known protein sequences (expectation cutoff 1e-10)

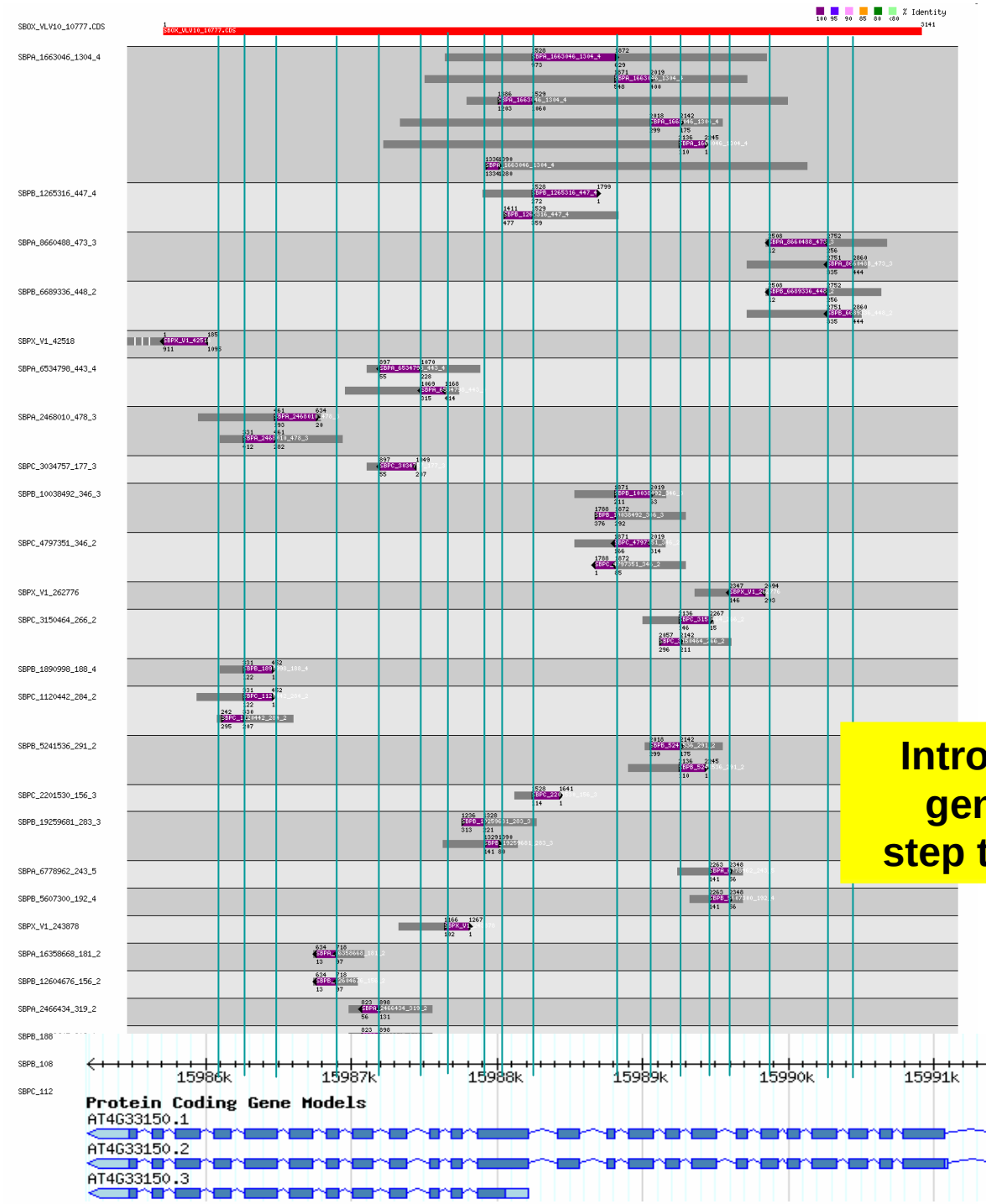
QUERY	SUBJECT	HIT	Query_length_nt	Hit_Alignment_Length_aa
LSat_DN_X01_992	Arab_thal.NP_186875	(NP_186875.2 GI:30678519) { BIG (BIG); binding / ubiquitin-protein ligase/ zinc ion binding } [Arabidopsis thaliana]	15382	5096
LSat_DN_X01_25623	Arab_thal.NP_001154245	(NP_001154245.1 GI:238480786) { unknown 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
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

chimeric?

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


Viewer *LACT_4XSSVC_12637*
'3X Combo' assembly: example of consensus generated by CAP3 using CLC, Velvet and Sanger EST assemblies as input
- CLC
- Velvet
- L. sativa ESTs
- L. serriola ESTs
total contig length - 11,688 nt
BLAST-X alignment:
11,085 nt
from 256 to 11,340 nt
GC% content
- protein length - 3754 aa
Query start
Query end
Done


de novo transcriptome assembly
de novo gene space assemblies
Intron discovery by alignment of gene space to transcriptome - step toward GBrowse data display
detection of intron-exon boundaries
Arabidopsis gene model
putative DNA-binding protein, Myb
3162k
3163k
3164k
3165k
Protein Coding Gene Models
AT1G09770.1
% Identity
100 95 90 85 80 <80
SBOX_VLV10_1429.CDS
SBPX_V1_37148
SBPC_140990_401_4
SBPX_V1_23544
SBPX_V1_17591
SBPA_11513271_424_5
SBPC_5241561_218_3
SBPB_8851046_212_4
SBPC_8873522_185_2
SBPC_2047735_182_2
SBPA_5307981_565_2
SBPB_12889813_301_1
SBPB_8460432_181_3
SBPC_5053359_284_3
SBPX_V1_9511



de novo transcriptome assembly

de novo gene space assemblies

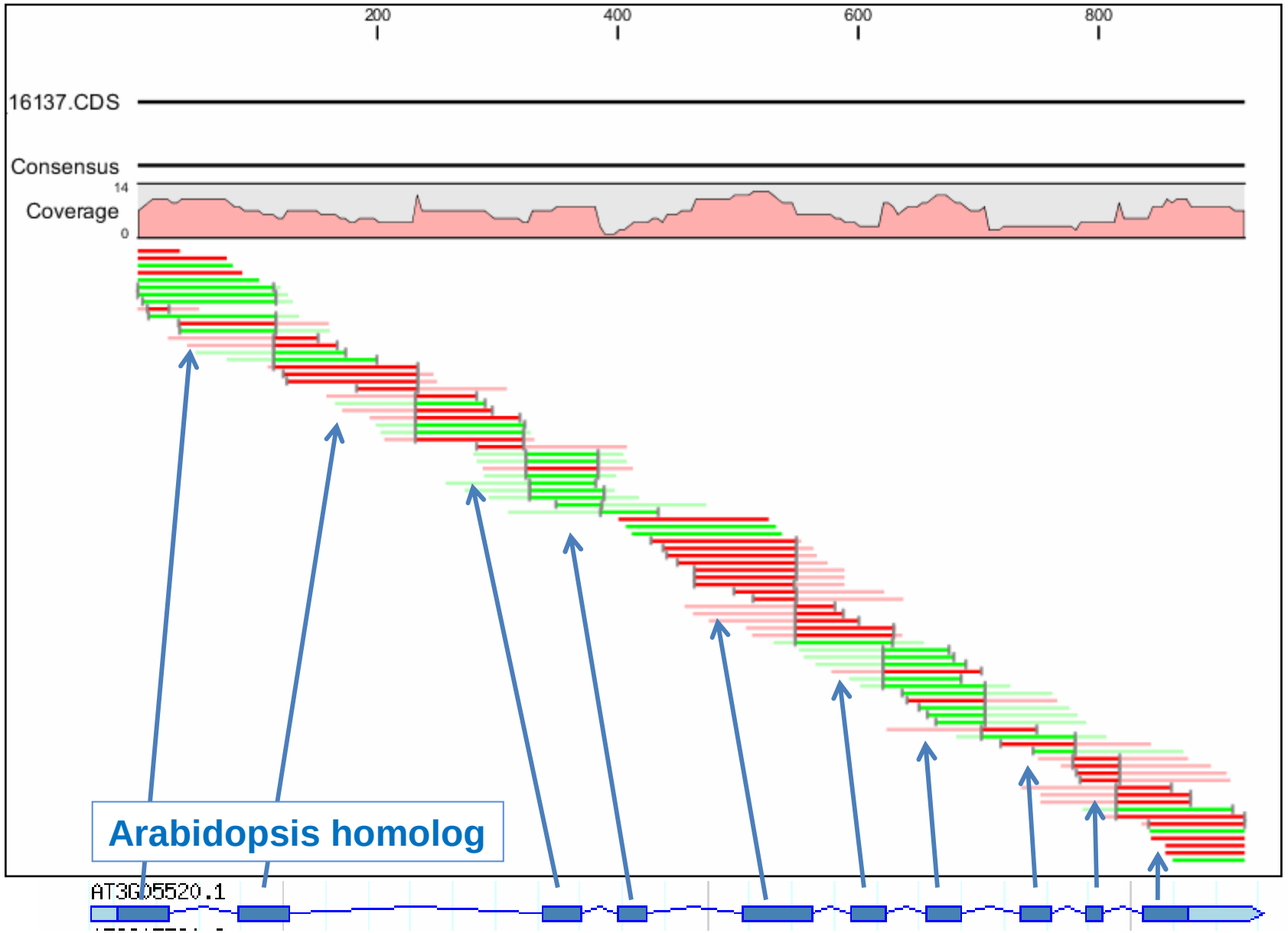
Intron discovery by alignment of gene space to transcriptome - step toward GBrowse data display

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

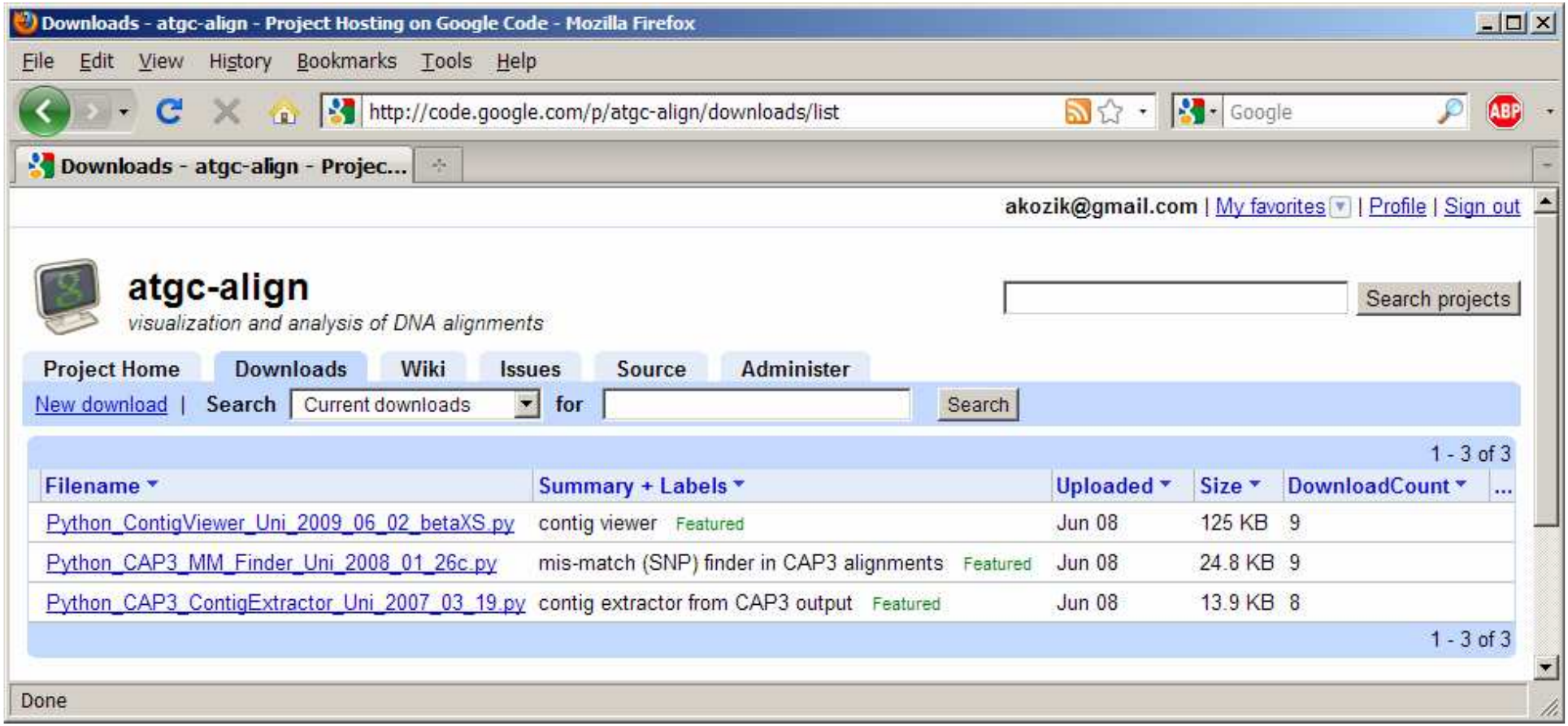
filtering for high quality reads

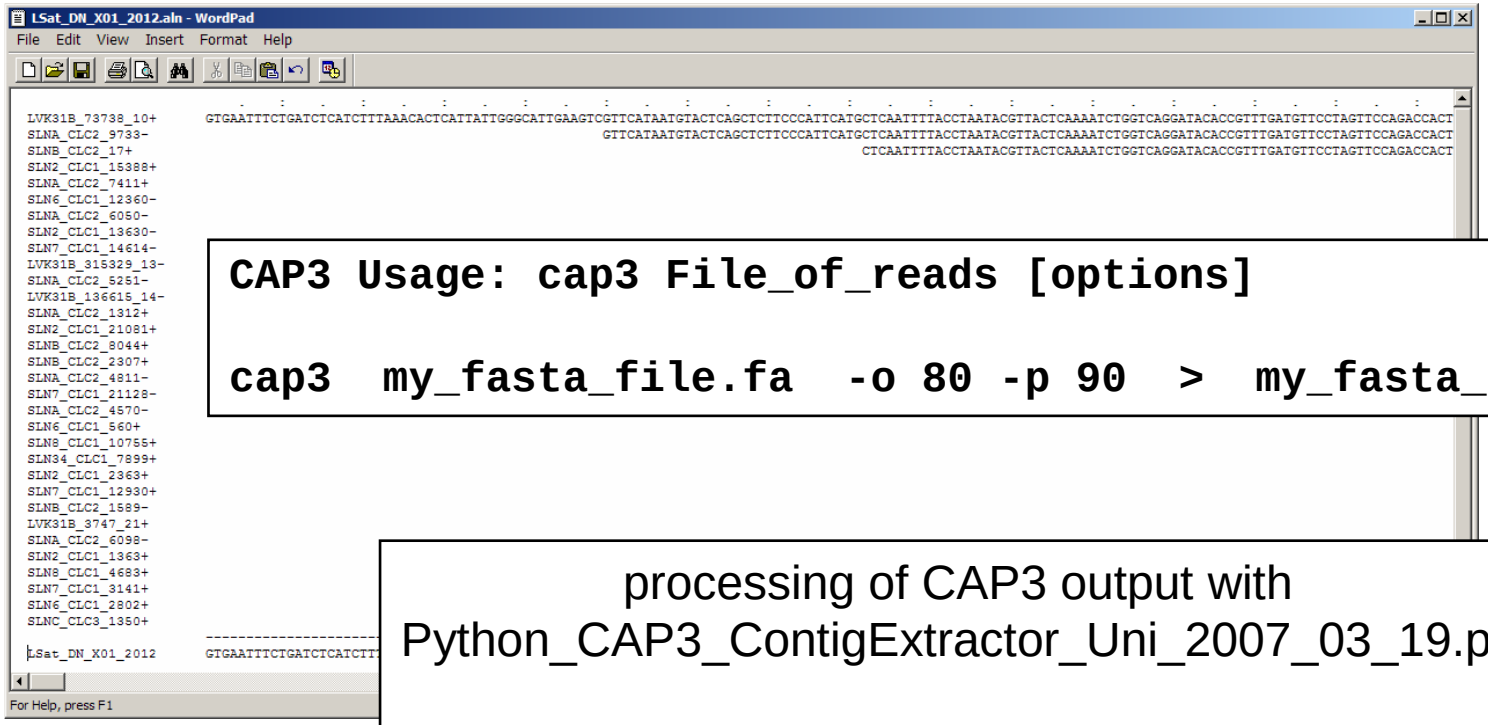
assembly parameters

visualization with Python Contig Viewer

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

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



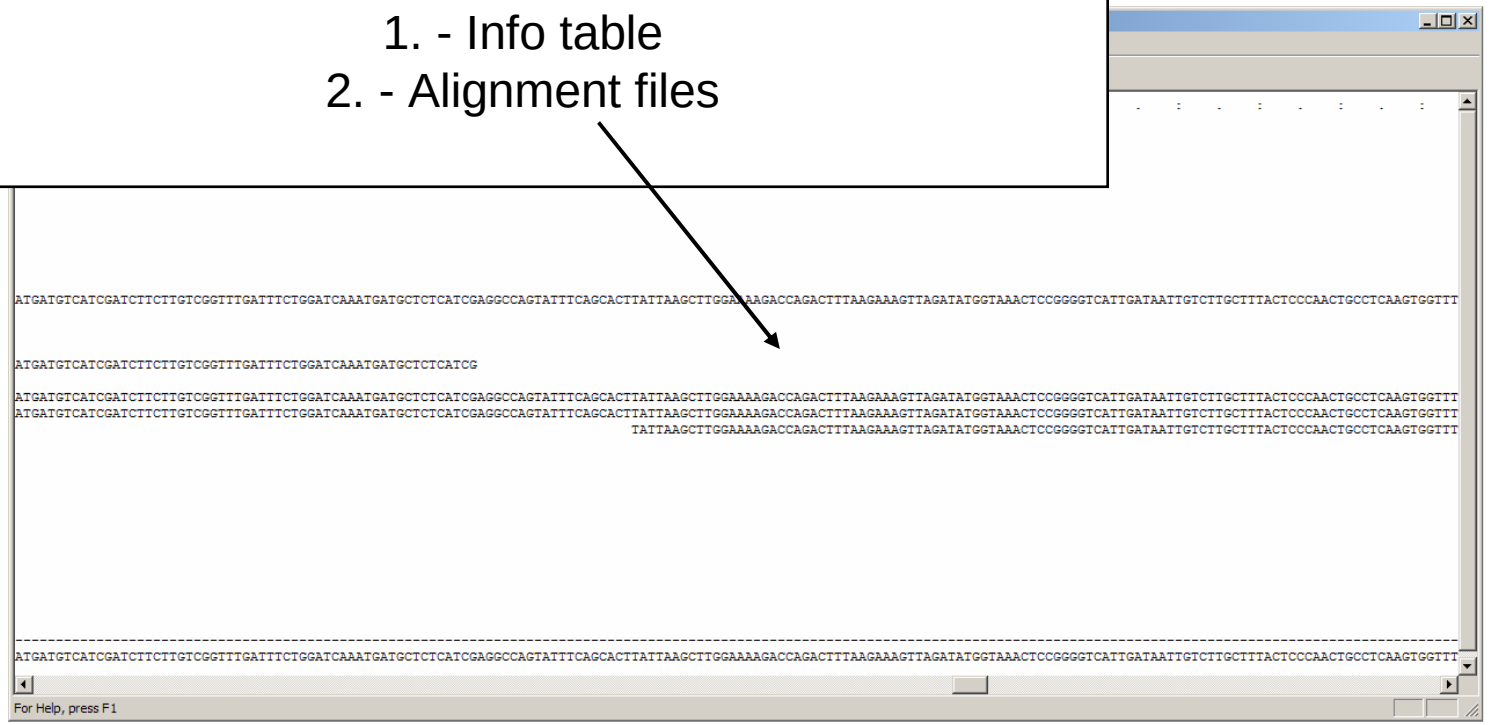


CAP3 Usage: cap3 File_of_reads [options]

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

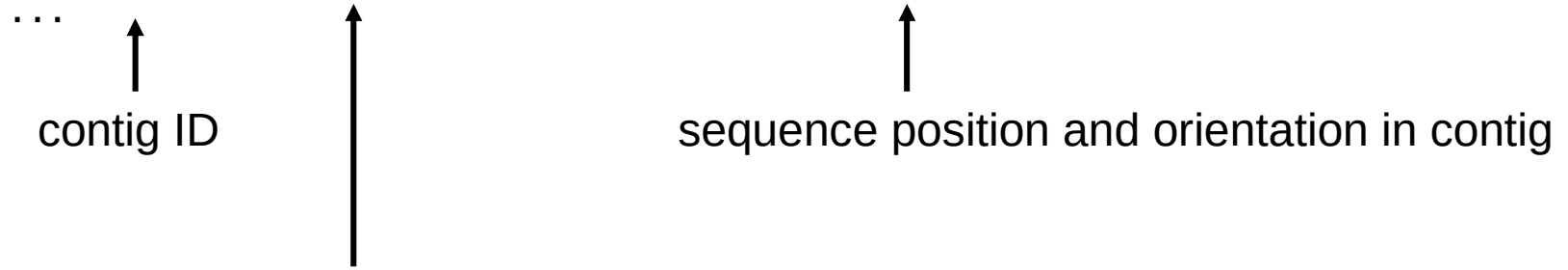
processing of CAP3 output with Python_CAP3_ContigExtractor_Uni_2007_03_19.py:

1. - Info table
2. - Alignment files



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)|...
...
```



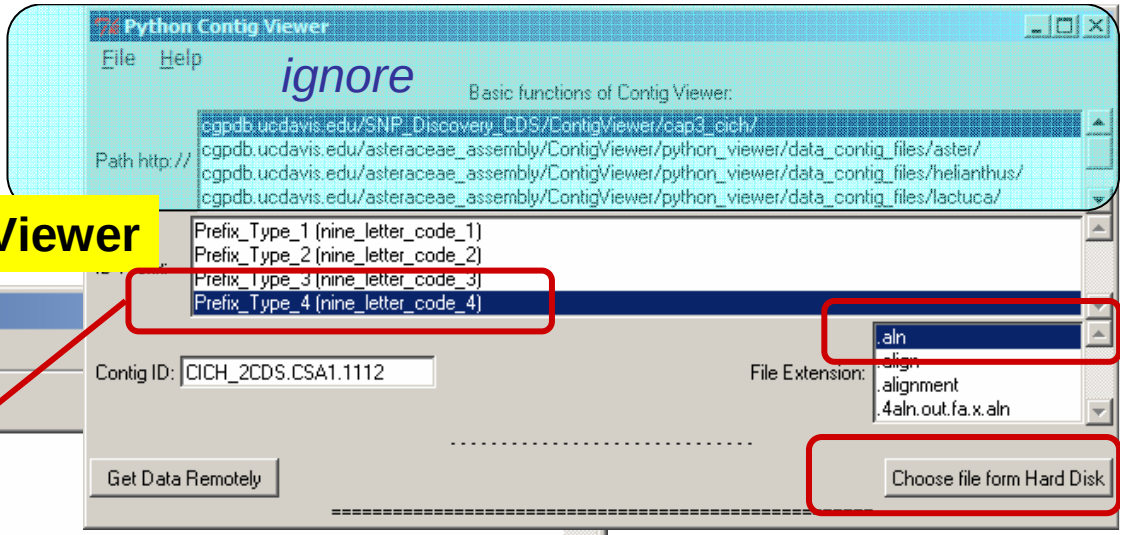
Python_CAP3_ContigExtractor_Uni_2007_03_19.py usage:

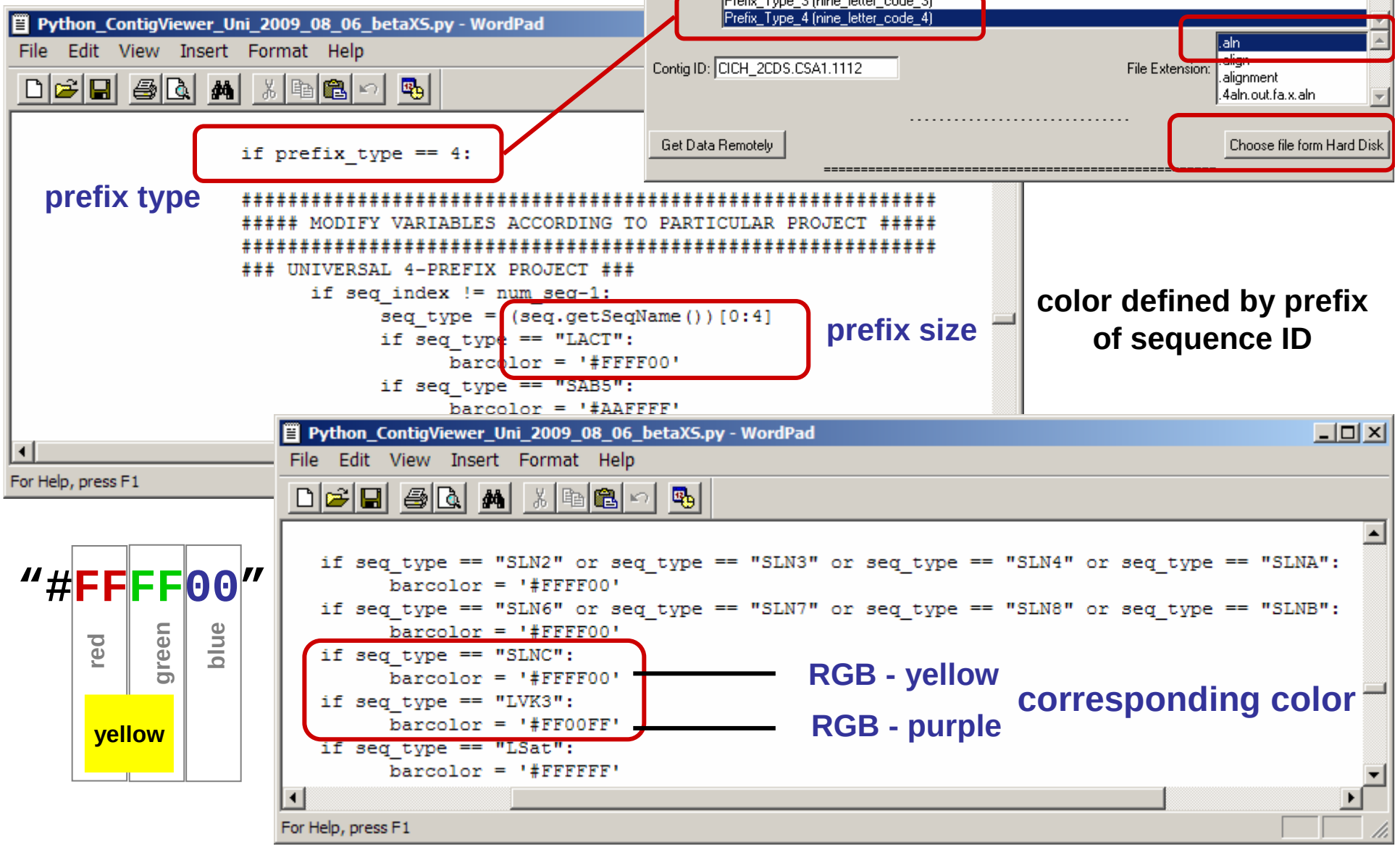
```
bash-2.03$
bash-2.03$ python Python_CAP3_ContigExtractor_Uni_2007_03_19.py
What type of output do you want? (1/2): 1
Enter the SOURCE file name: my_fasta_file.cap3.out
Enter the DESTINATION file name: my_fasta_file.cap3.out.Info
Default contig file name prefix is Contig
Enter the contig file name 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
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 ...
```

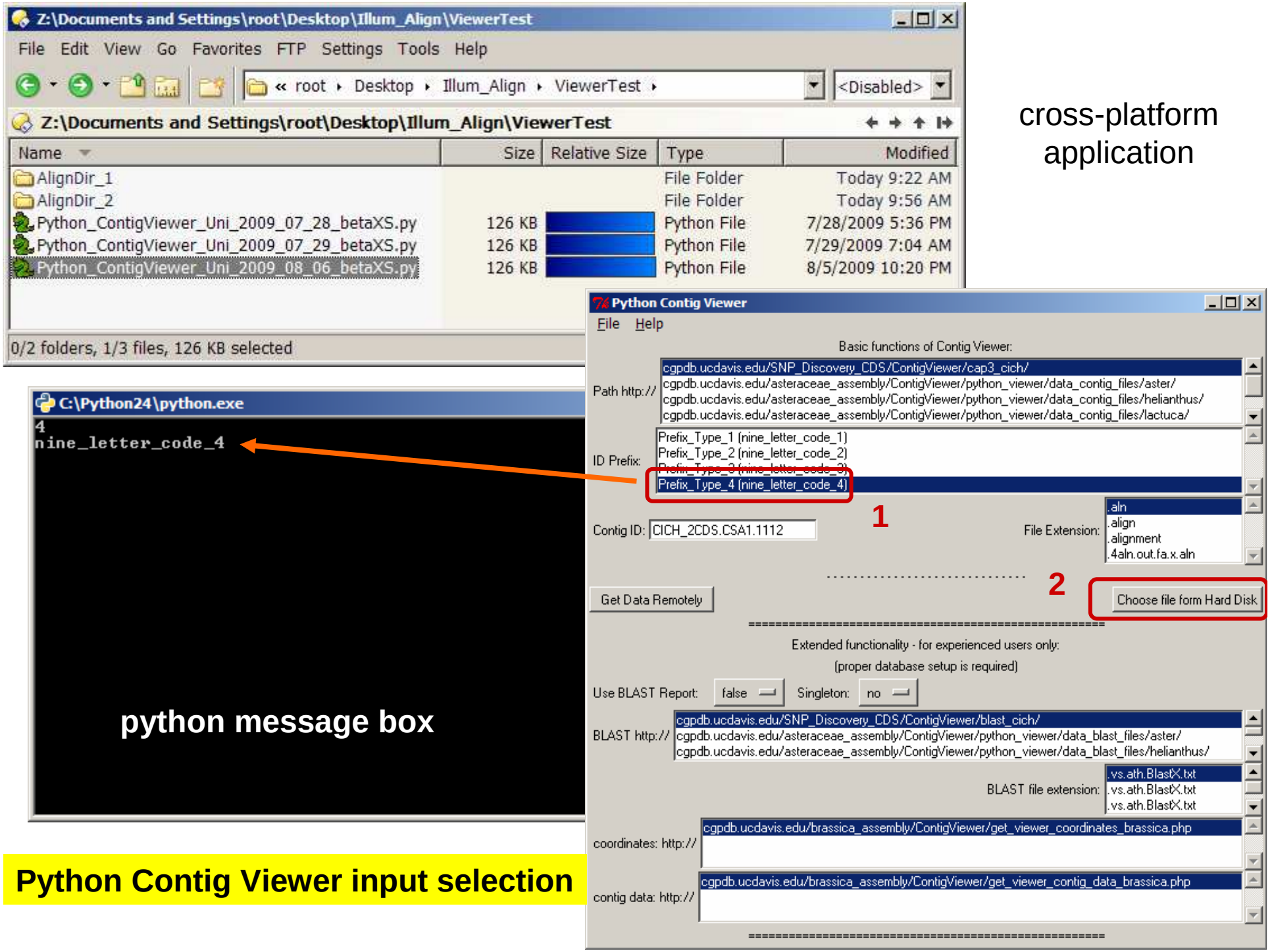
option ‘1’ to extract Info table

option ‘2’ to extract alignments

Color selection in Python Contig Viewer

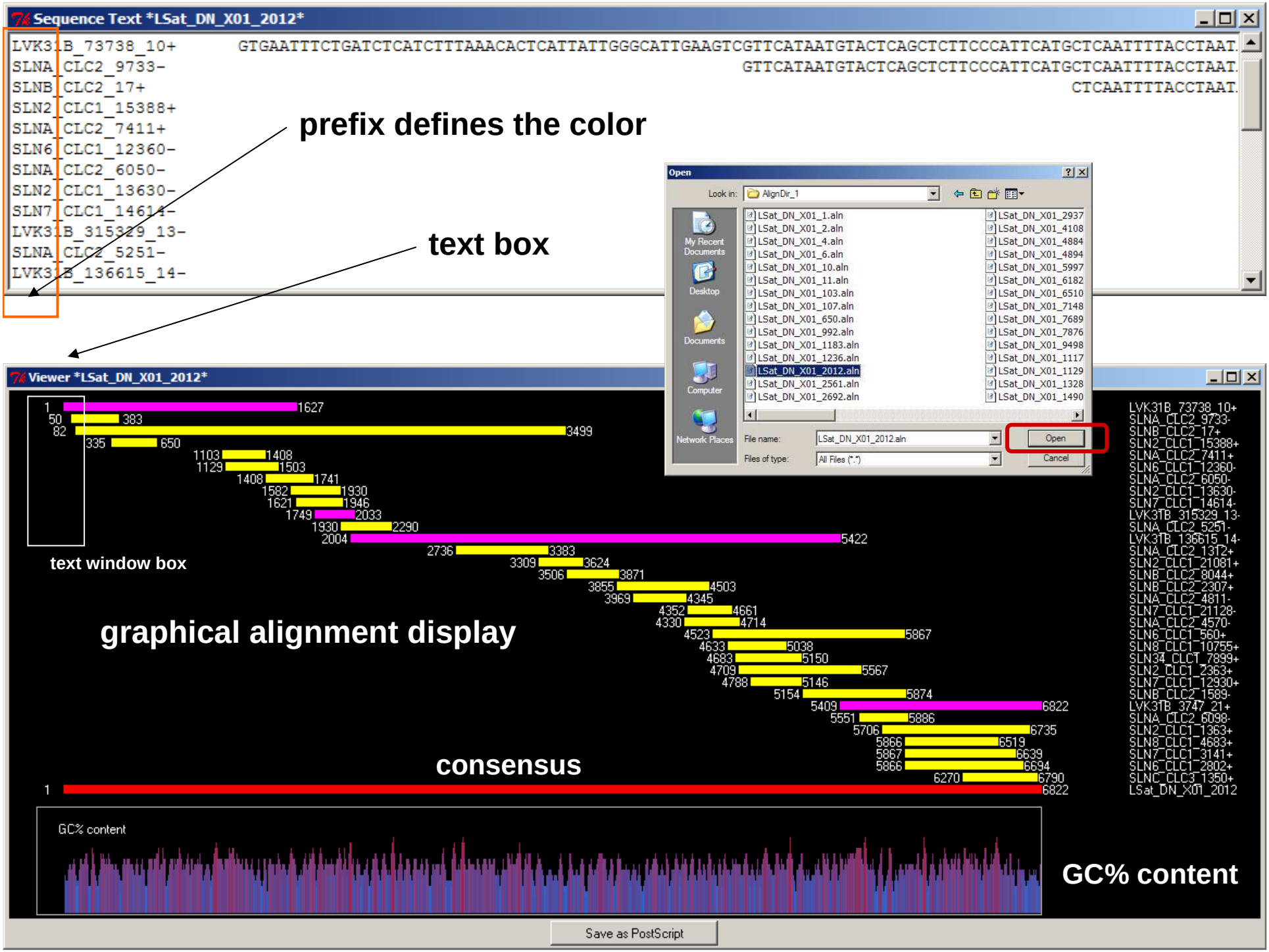






cross-platform application

Python Contig Viewer input selection


Sequence Text *LSat_DN_X01_2012*
LVK31B_73738_10+ GTGAATTTCTGATCTCATCTTTAAACACTCATTATTGGGCATTGAAGTCGTTCATAATGTACTCAGCTCTTCCCATTCATGCTCAATTTTACCTAAT.
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
Open
Look in: AlignDir_1
LSat_DN_X01_1.aln
LSat_DN_X01_2.aln
LSat_DN_X01_4.aln
LSat_DN_X01_6.aln
LSat_DN_X01_10.aln
LSat_DN_X01_11.aln
LSat_DN_X01_103.aln
LSat_DN_X01_107.aln
LSat_DN_X01_650.aln
LSat_DN_X01_992.aln
LSat_DN_X01_1183.aln
LSat_DN_X01_1236.aln
LSat_DN_X01_2012.aln
LSat_DN_X01_2561.aln
LSat_DN_X01_2692.aln
LSat_DN_X01_2937
LSat_DN_X01_4108
LSat_DN_X01_4884
LSat_DN_X01_4894
LSat_DN_X01_5997
LSat_DN_X01_6182
LSat_DN_X01_6510
LSat_DN_X01_7148
LSat_DN_X01_7689
LSat_DN_X01_7876
LSat_DN_X01_9498
LSat_DN_X01_1117
LSat_DN_X01_1129
LSat_DN_X01_1328
LSat_DN_X01_1490
My Recent Documents
Desktop
Documents
Computer
Network Places
File name: LSat_DN_X01_2012.aln
Files of type: All Files (*.*)
Open
Cancel
Viewer *LSat_DN_X01_2012*
text window box
graphical alignment display
consensus
GC% content
GC% content
Save as PostScript
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+
SLNB_CLC2_2307+
SLNA_CLC2_4811-
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+
LSat_DN_X01_2012

last remarks

UNIX tricks

fasta header modifications:

regular expressions and perl /find/replace/ function

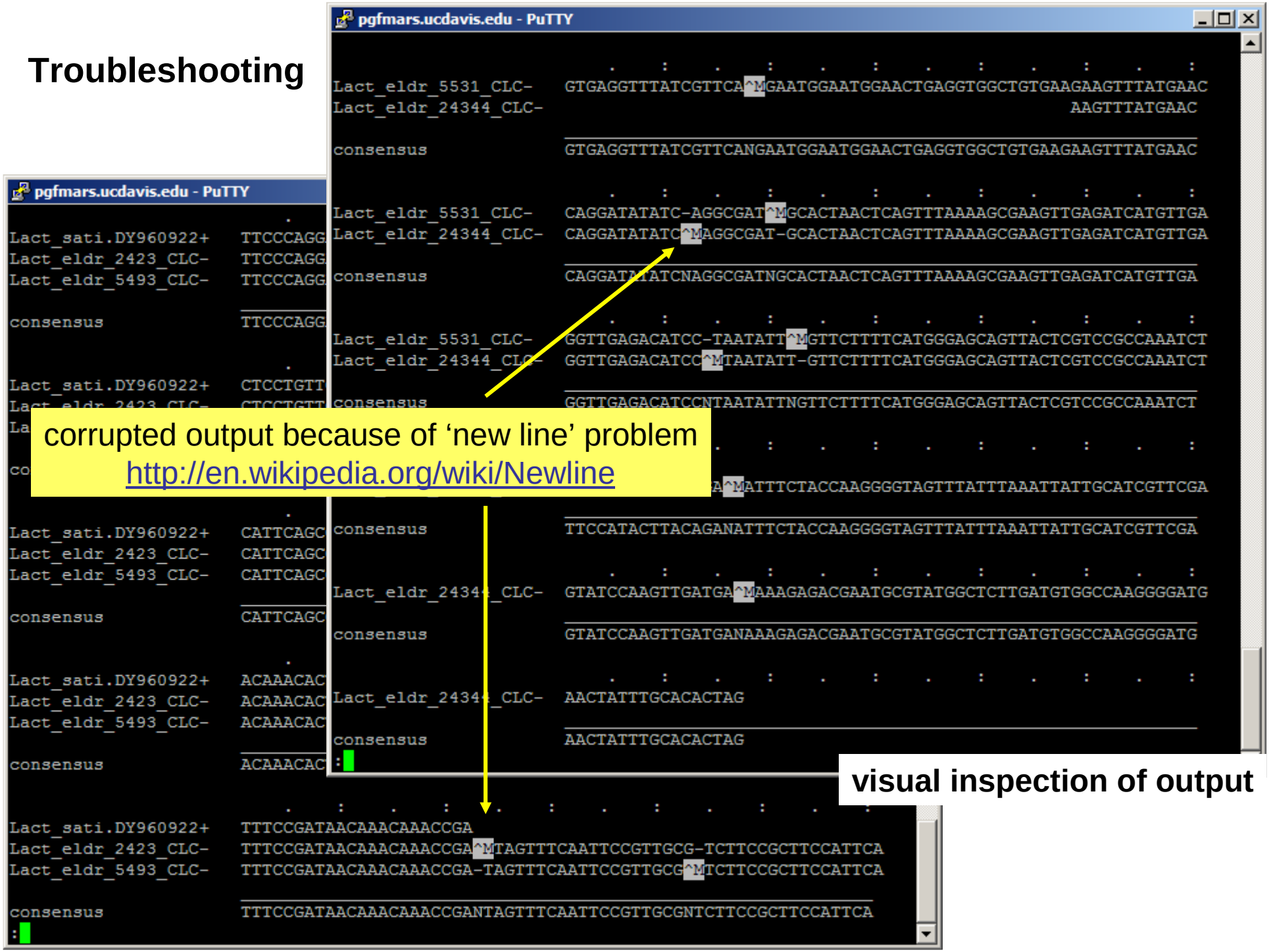
```
>NODE_24_length_783_cov_47.872288

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:

```
>SBO8_8_120_1640_1715
CCAAGAACGACATATAAACATTTTACATAATCTGGGGAACACTTGAAGAACATCAAAAGCTGGAAAAATGGATGAAAAACAGCTC

perl -p -i -e 's/\>SBO/XXXXXXXXXXXXXXX\>SBO/' my_fasta_file.orig.txt

XXXXXXXXXXXXXXX>SBO8_8_120_1640_1715
CCAAGAACGACATATAAACATTTTACATAATCTGGGGAACACTTGAAGAACATCAAAAGCTGGAAAAATGGATGAAAAACAGCTC

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

>SBO8_8_120_1640_1715
AAACATTTTACATAATCTGGGGAACACTTGAAGAACATCAAAAGCTGGAAAAATGGATGA
```

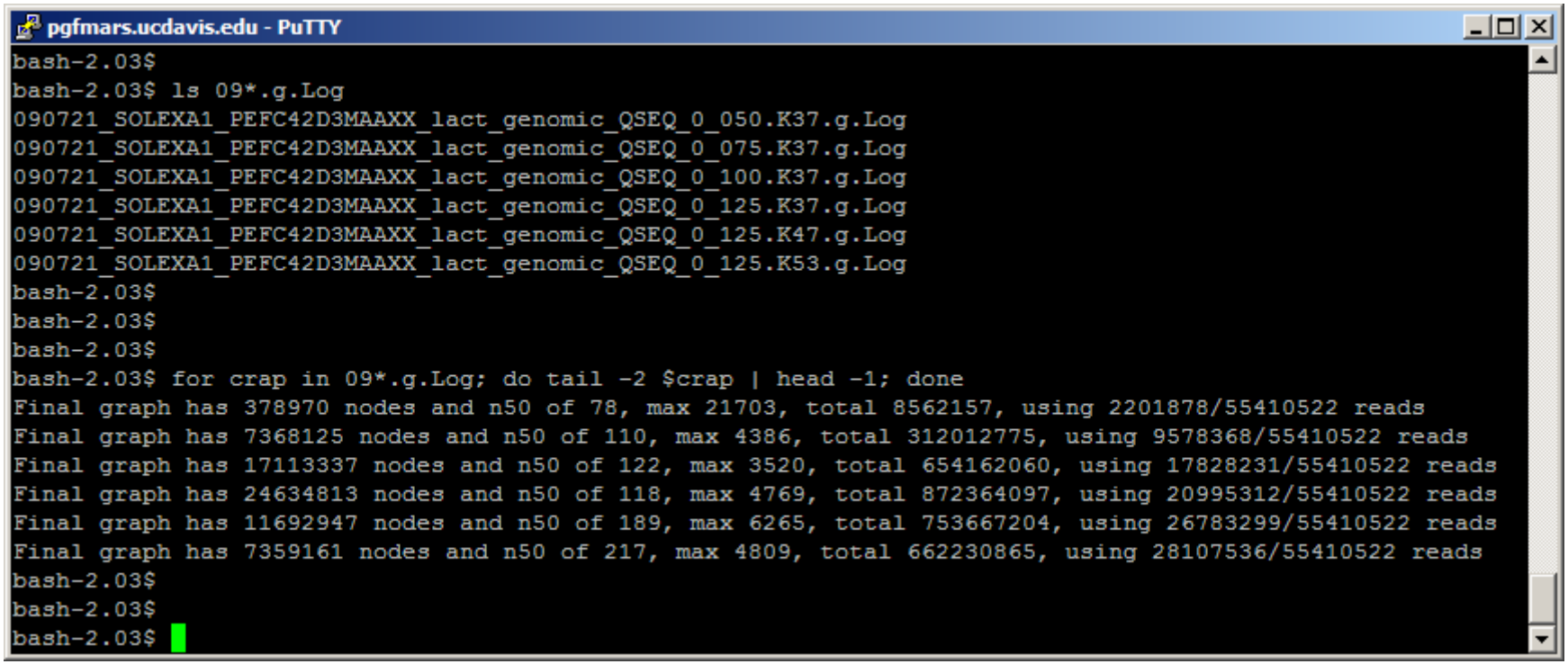
Unix ‘cut’ command

Troubleshooting



record and keep all Log files, run parameters

```
bash-2.03$ less 090721_SOLEXA1_PEFC42D3MAAXX_lact_genomic_QSEQ_0_125.K53/Log
Thu Sep  3 03:07:09 2009
 velveth.7.49.K53.exec 090721_SOLEXA1_PEFC42D3MAAXX_lact_genomic_QSEQ_0_125.K53 53 -fasta -short
090721_SOLEXA1_PEFC42D3MAAXX_lact_genomic_QSEQ_0_125.K37/Sequences
Thu Sep  3 09:02:46 2009
 velvetg.7.49.K53.exec 090721_SOLEXA1_PEFC42D3MAAXX_lact_genomic_QSEQ_0_125.K53 -min_contig_lgth 180
-unused_reads yes -amos_file yes -read_trkg yes
Final graph has 7359161 nodes and n50 of 217, max 4809, total 662230865, using 28107536/55410522 reads
```




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

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

Acknowledgements

Richard Michelmore

UCD Genome Center Bioinformatics Core for IT support

UCD DNA Technologies Core

special credits to Huaqin Xu - database and web programmer
Brian Chan - initial work with Python Contig Viewer

SEQanswers forum and Velvet newsgroup

Marta Matvienko