Sequence Alignment and Genome Assembly

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Outline of the Talk:

- *Global and Local Alignment*
- *Statistical* significance of alignment
- *Alignment method*

Biological Motivation
Why We Need Sequence Alignment

- Inference of Homology
  - Two genes are homologous if they share a common evolutionary history.
  - Evolutionary history can tell us a lot about properties of a given gene
  - Homology can be inferred from similarity between the genes
- Searching for Proteins with same or similar functions

Sequence Alignment

Global Alignment:

Goal: How similar are two sequences S_1 and S_2

Input: two sequences S_1, S_2 over the same alphabet

Output: two sequences S'_1, S'_2 of equal length

(S'_1, S'_2 are S_1, S_2 with possibly additional gaps)

Example:

- S_1= GCGCATGGATTGAGCGA
- S_2= TGCGCCATTGATGACC
- A possible alignment:

```
S'1= -GCGC-ATGGATTGAGCGA
S'2= TGCGCCATTGAT-GACC--
```

Sequence Alignment (cont)

Local Alignment:

Goal: Find the pair of substrings in two input sequences which have the highest similarity

Input: two sequences S_1, S_2 over the same alphabet
Output: two sequences S'_1, S'_2 of equal length
(S'_1, S'_2 are substrings of S_1, S_2 with possibly additional gaps)

Example:

- S_1= GCGCATGGATTGAGCGA
- S_2= TGCGCCATTGATGACC
- A possible alignment:

S'_1= ATTGA-G
S'_2= ATTGATG

Global vs. Local Alignment

- The Global Alignment Problem tries to find the longest path between vertices *(0,0)* and (*n*,*m*) in the edit graph.

- The Local Alignment Problem tries to find the longest path among paths between **arbitrary vertices** (*i*,*j*) and (*i'*, *j'*) in the edit graph.

Global vs. Local Alignment (cont'd)

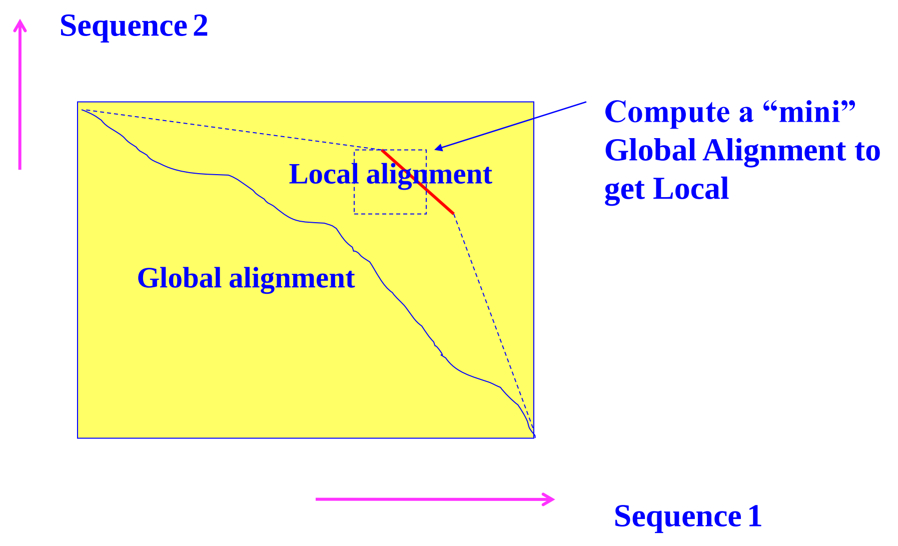
- **Global Alignment**

```
--T—-CC-C-AGT—-TATGT-CAGGGGACACG—A-GCATGCAGA-GAC
  |  || |  ||  | | | |||    || | | |  | ||||   |
AATTGCCGCC-GTCGT-T-TTCAG----CA-GTTATG—T-CAGAT--C
```

- **Local Alignment—betten alignment to find conserved segment**

```
                  tccCAGTTATGTCAGgggacacgagcatgcagagac
                     ||||||||||||
aattgccgccgtcgttttcagCAGTTATGTCAGatc
```

Local Alignment: Example



Statistic Significance of Alignment

We need to know how to evaluate the significance of the alignment. There are two scenarios:

First, the alignment indicates an evolutionary relationship between the sequences.

Second, the alignment is a chance occurrence. What answer is correct?

Here, the statistics are important to estimate of probability that the given alignment score might occur by chance.

E Value (E)

- **E value (E)** of an alignment score is the expected number of unrelated sequences in a database that would have a score at least as good.
- **Low E-values suggest that sequences are homologous.**

  If *E value* ≤ 0.02 sequence probably homologous

  If *E value* ≤ 1 homology cannot be ruled out

  If *E value* > 1 a match just by chance
- **Statistical significance depends on both the size of the alignments and the size of the sequence database**
  - Important consideration for comparing results across different searches
  - E-value increases as database gets bigger
  - E-value decreases as alignments get longer

E value Measuring Alignment Significance

P Value (P)

The *E-value* is not a probability; it's an expected value, i.e. the expected outcome.

Another criteria of the Alignment Significance is the probability that an alignment with this score could have arisen by chance - *p*-value:

$$E\text{-value}(S) = n \bullet p\text{-value}(S),$$

Here n is the number of sequences in the database, *S*.

The lower the p-value, the more likely it is that the alignment score is not by chance but was caused by alignment procedure.

For example, p = .01 means there is a 1 in 100 chance the result occurred by chance.

Methods of DNA Sequence Alignment

- Dot matrix analysis
- The dynamic programming (DP) algorithm
  - Needleman-Wunsch Algorithm
  - Smith-Waterman Algorithm
- Suffix tree
- Hash table based algorithm
- Short read alignment tools

Dot Matrix Analysis

• A dot matrix analysis is a method for comparing two sequences to look for possible alignment (Gibbs and McIntyre 1970)

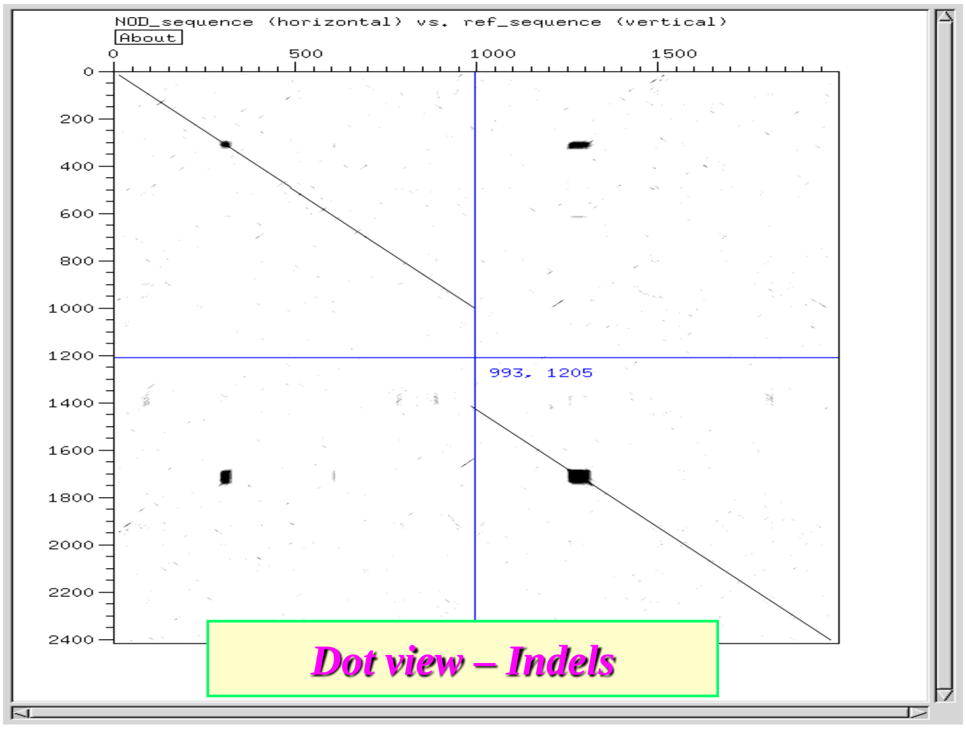
• One sequence (A) is listed across the top of the matrix and the other (B) is listed down the left side

• Starting from the first character in B, one moves across the page keeping in the first row and placing a dot in many column where the character in A is the same

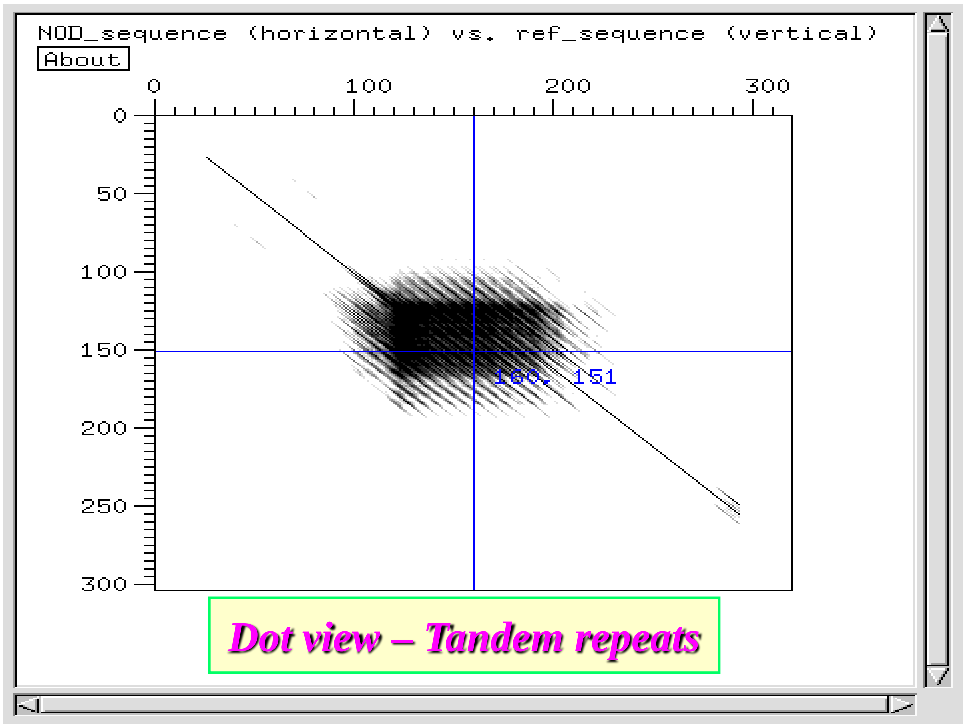
• The process is continued until all possible comparisons between A and B are made

• Any region of similarity is revealed by a diagonal row of dots

• Isolated dots not on diagonal represent random matches



Dot view – Indels

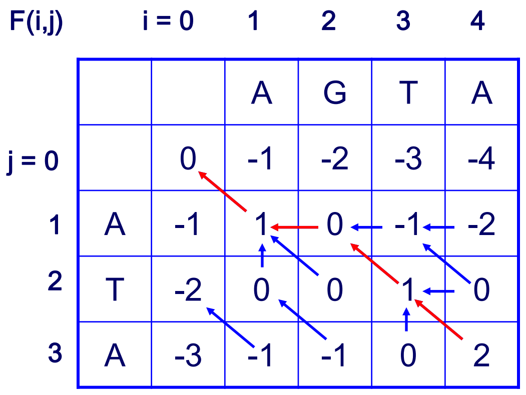


Dot view – Tandem repeats

The Needleman-Wunsch Algorithm

x = AGTA
y = ATA

m = 1
s = -1
d = -1



F(i,j)		i = 0	1	2	3	4
			A	G	T	A
j = 0		0	-1	-2	-3	-4
1	A	-1	1	0	-1	-2
2	T	-2	0	0	1	0
3	A	-3	-1	-1	0	2

Optimal Alignment:

F(4,3) = 2

AGTA
A - TA

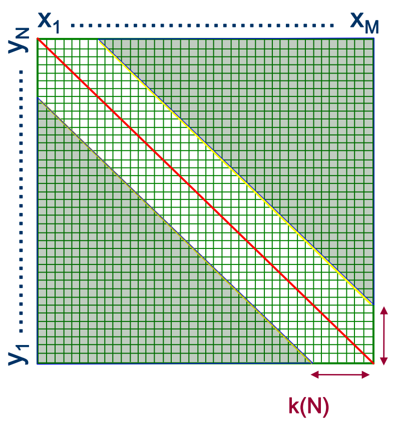
Smith-Waterman Algorithm

- Only works effectively when gap penalties are used
- In example shown
  - match = +1
  - mismatch = -1/3
  - gap = -1+1/3k (k=extent of gap)
- Start with all cell values = 0
- Looks in subcolumn and subrow shown and in direct diagonal for a score that is the highest when you take alignment score or gap penalty into account

	C	A	G	C	C	U	C	G	C	U	U	A	G
A	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
A	0.0	1.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.7
U	0.0	0.0	0.8	0.3	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.7
G	0.0	0.0	1.0	0.3	0.0	0.0	0.7	1.0	0.0	0.0	0.7	0.7	1.0
C	1.0	0.0	0.0	2.0	1.3	0.3	1.0	0.3	2.0	0.7	0.3	0.3	0.3
C	1.0	0.7	0.0	1.0	3.0	1.7	?						
A													
U													
U													
G													
A													
C													
G													
G													

$$H_{ij}=\max\{H_{i-1,\,j-1}+s(a_i,b_j),\ \max\{H_{i-k,j}-W_k\},\ \max\{H_{i,\,j-l}-W_l\},\ 0\}$$

Bounded Dynamic Programming



Initialization:

F(i,0), F(0,j) undefined for i, j > k

Iteration:

```
For i = 1…M
  For j = max(1, i – k)…min(N, i+k)
```

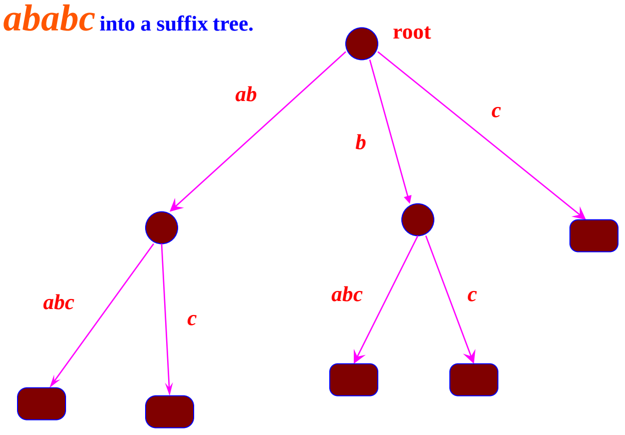
$$F(i,j)=\max\begin{cases}F(i-1,\,j-1)+s(x_i,y_j)\\ F(i,\,j-1)-d, \text{ if } j > i-k(N)\\ F(i-1,\,j)-d, \text{ if } j < i+k(N)\end{cases}$$

Termination: same

Easy to extend to the affine gap case

Suffix Tree Example

Mapping the string
ababc **into a suffix tree.**



Non-overlap Hashing v Overlap Hashing

ATGGCGTGCAGTCCATGTTCGGATCA
ATGGCGTGCAGT
TGGCGTGCAGTC
GGCGTGCAGTCC
GCGTGCAGTCCA
CGTGCAGTCCAT

Non-overlap hashing
W = N-k+1
(k = 12)

ATGGCGTGCAGTCCATGTTCGGATCATTACGTAAGC
ATGGGCAGATGT
CCATGTTCGGAT
CATTACGTAAGC

Overlap Hashing
W = N/k

Sequence Representation

Sequence S: $(s_1 s_2, \ldots, s_i, \ldots, s_m)$ $i = 1, 2, \ldots, m$

K-tuple: $(s_i s_{i+1} \ldots s_{i+k-1})$

Using two binary digits for each base, we may have the following representations:

"A" =00; "C" = 01; "G" = 10; "T" = 11

For any of the m/k no-overlapping k-tuples in the sequence, an integer may be used to represent the k-tuple in a unique way

$$E = \sum_{i=1}^{2k} \beta_i 2^{i-1} \quad \text{with } E_{\max} = 2^{2k} - 1$$

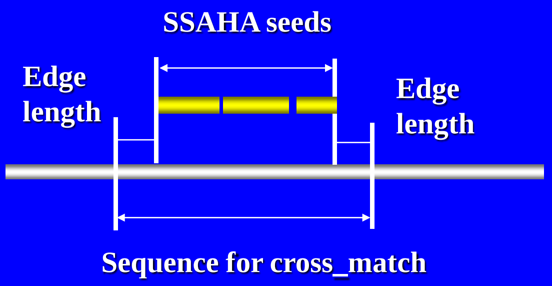
where β_i = 0 or 1, depending on the value of the sequence base and E_{max} is the maximum value of the possible E values.

Hash Table: *A 2-tuple hashing table of S1, S2 and S3*

E	*k*-tuple	N_i	Indices and Offsets						
0	AA	1	2, 19						
1	AC	3	1, 9	2, 5	2, 11				
2	AG	2	1, 15	2, 35					
3	AT	2	2, 13	3, 3					
4	CA	7	2, 3	2, 9	2, 21	2, 27	2, 33	3, 21	3, 23
5	CC	4	1, 21	2, 31	3, 5	3, 7			
6	CG	1	1, 5						
7	CT	6	1, 23	2, 39	2, 43	3, 13	3, 15	3, 17	
8	GA	4	1, 3	1, 17	2, 15	2, 25			
9	GC	0							
10	GG	5	1, 25	1, 31	2, 17	2, 29	3, 1		
11	GT	6	1, 1	1, 27	1, 29	2, 1	2, 37	3, 19	
12	TA	1	3, 25						
13	TC	6	1, 7	1, 11	1, 19	2, 23	2, 41	3, 11	
14	TG	3	1, 13	2, 7	3, 9				
15	TT								

S1=(GTGACGTCACTCTGAGGATCCCCTGGGTGTGG)
S2=(GTCAACTGCAACATGAGGAACATCGACAGGCCCAAGGTCTTCCT)
S3=(GGATCCCCTGTCCTCTCTGTCACATA)

SSAHA2 = SSAHA + Cross_Match



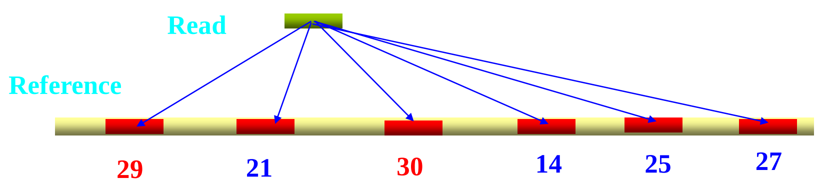
SSAHA for matching seeds, cross_match for sequence alignment.

Mapping Score in ssaha2

Read mapping score is used to assess the repetitive feature of the read in the genome. With a maximum mapping score 50, we have:

$$S_{map} = \begin{cases} 10*(30/R)(S_{\max} - S_{\max 2}) & if\,(S_{map} <= 50) \\ 50 & if\,(S_{map} > \ 50) \end{cases}$$

R = read length; S_{max} - maximum alignment score (smith-waterman) of the hits on genome; S_{max2} - second best alignment score of the hits on genome; Say you have one read of 30 bases which has a few hits on the genome: Best hit: exact match with S_{max} 30; Second best hit: one base mismatch with S_{max2} 29. The mapping score for this read is S_{map} = 10;

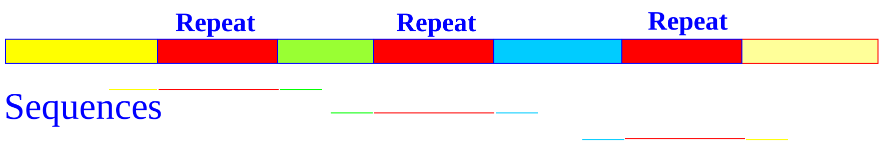


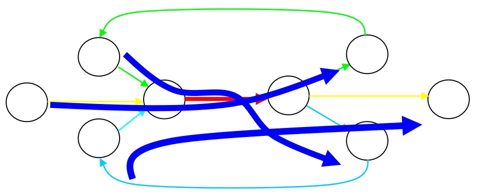
Genome Assembly using Solexa Short Reads Algorithms and Applications

Outline of the Talk:

- *Sequence Reconstruction and Euler Path*
- *Assembly strategy*
- *Sequence extension using read pairs, base qualities, fuzzy kmers or longer reads*
- *Repeat junctions*
- *Gap5 - visual inspection for mis-assembly errors*

Sequence Repeat Graph



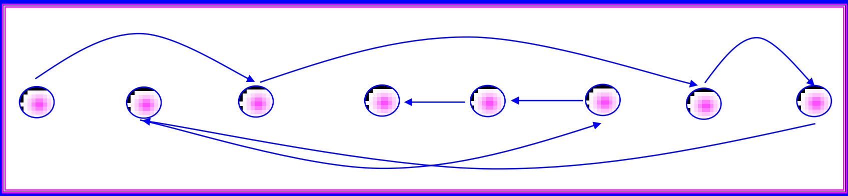


Sequence Reconstruction
- Hamiltonian path approach

S=(ATGCAGGTCC)

ATG -> TGC -> GCA -> CAG -> AGG -> GGT -> GTC -> TCC

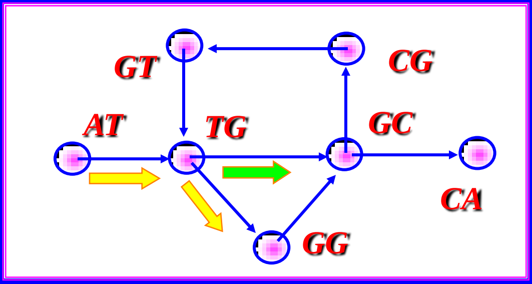
ATG AGG TGC TCC GTC GGT GCA CAG



<u>Vertices</u>: *k-tuples from the spectrum shown in red (8);*
<u>Edges</u>: *overlapping k-tuples (7);*
<u>Path</u>: *visiting all vertices corresponding to the sequence.*

Sequence Reconstruction - Euler path approach

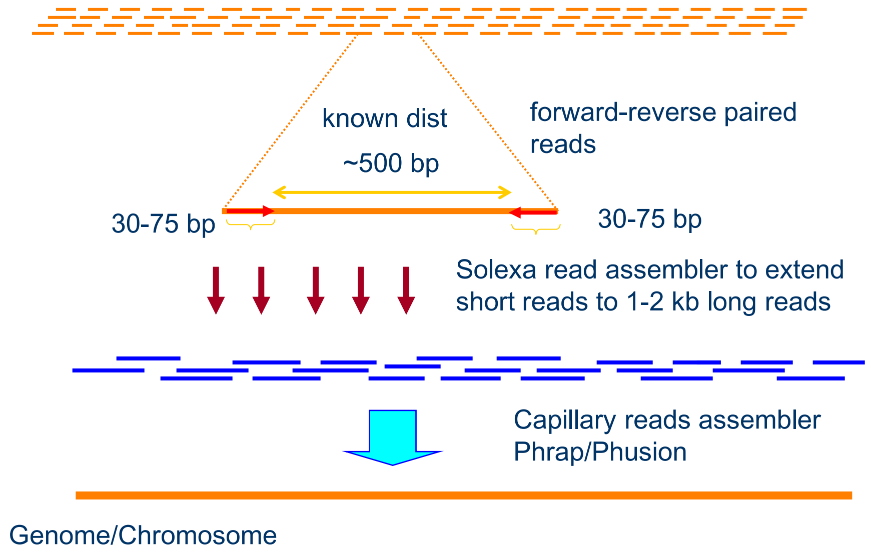
ATG -> TGG -> GGC -> GCG -> CGT -> GTG -> TGC -> GCA

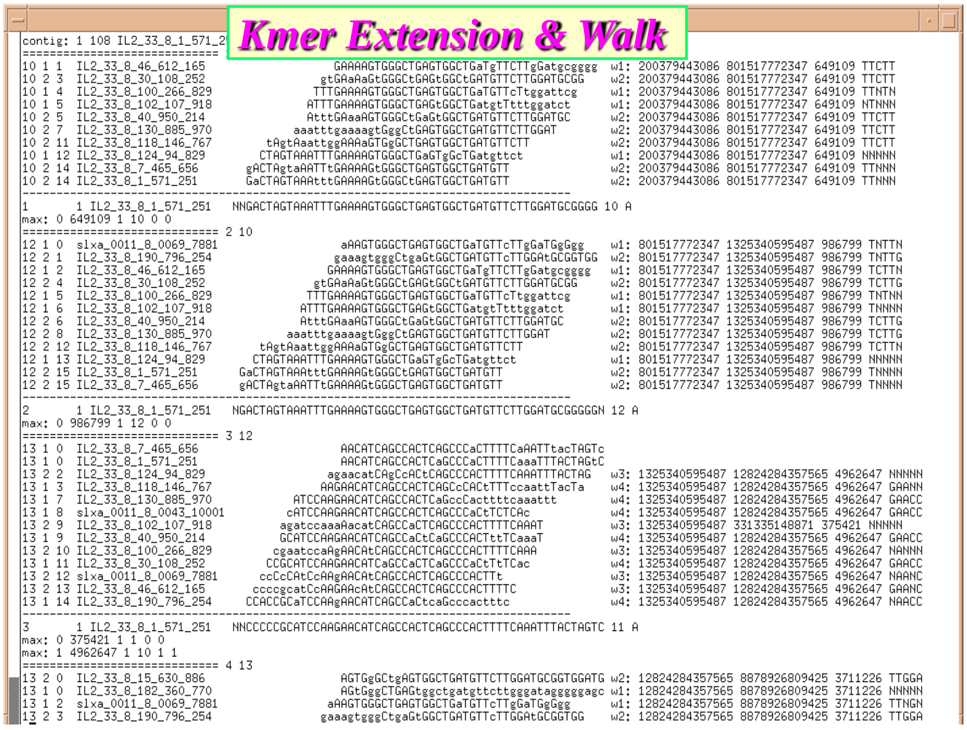


ATGGCGTGCA ATGCGTGGCA

Vertices:	correspond to (k-l)-tuples (7);
Edges:	correspond to k-tuples from the spectrum (8);
Path:	visiting all EDGES corresponding to the sequence.

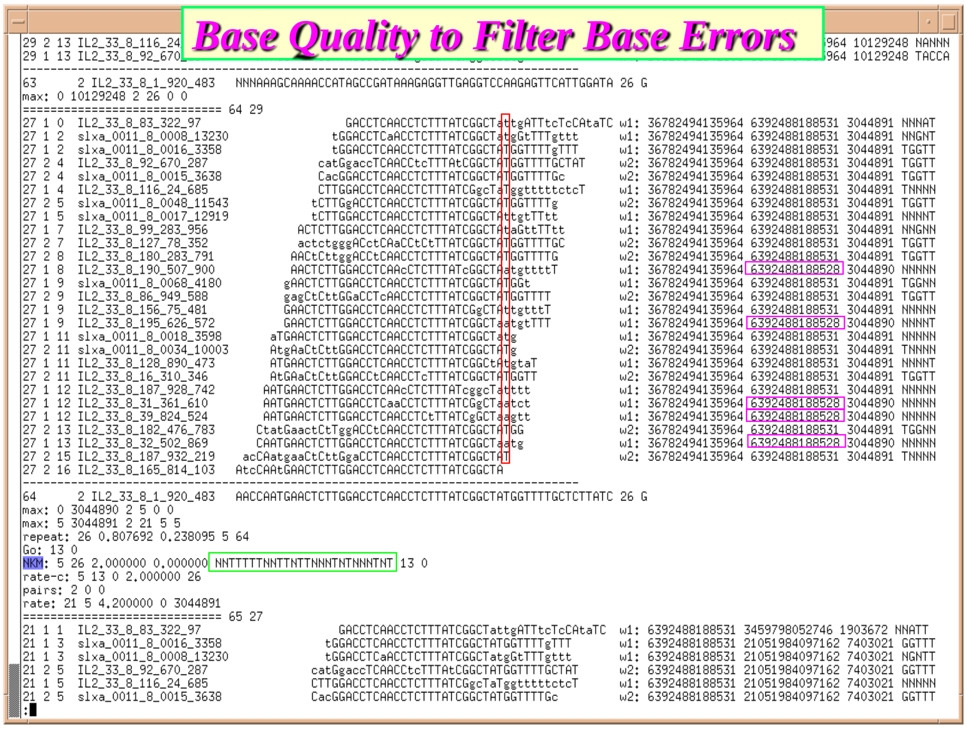
Assembly Strategy


known dist
~500 bp
forward-reverse paired reads
30-75 bp
30-75 bp
Solexa read assembler to extend short reads to 1-2 kb long reads
Capillary reads assembler Phrap/Phusion
Genome/Chromosome



Kmer Extension & Walk

```
contig: 1 108 IL2_33_8_1_571_2
=============================
10 1 1  IL2_33_8_46_612_165                  GAAAAGTGGGCTGAGTGGCTGaTgTTCTTgGatgcgggg  w1: 200379443086 801517772347 649109 TTCTT
10 2 3  IL2_33_8_30_108_252                gtGAaAaGtGGGCtGAGtGGCtGATGTTCTTGGATGCGG    w2: 200379443086 801517772347 649109 TTCTT
10 1 4  IL2_33_8_100_266_829              TTTGAAAAGTGGGCTGAGTGGCTGaTGTTcTtggattcg     w1: 200379443086 801517772347 649109 TTNTN
10 1 5  IL2_33_8_102_107_918             ATTTGAAAAGTGGGCTGAGtGGCTGatgtTtttggatct      w1: 200379443086 801517772347 649109 NTNNN
10 2 5  IL2_33_8_40_950_214             AtttGAaaAGTGGGCtGaGtGGCTGATGTTCTTGGATGC      w2: 200379443086 801517772347 649109 TTCTT
10 2 7  IL2_33_8_130_885_970          aaatttgaaaagtGggCtGAGTGGCTGATGTTCTTGGAT        w2: 200379443086 801517772347 649109 TTCTT
10 2 11 IL2_33_8_118_146_767       tAgtAaattggAAAaGTGgGCTGAGTGGCTGATGTTCTT            w2: 200379443086 801517772347 649109 TTCTT
10 1 12 IL2_33_8_124_94_829       CTAGTAAATTTGAAAAGTGGGCTGaGTgGcTGatgttct             w1: 200379443086 801517772347 649109 NNNNN
10 2 14 IL2_33_8_7_465_656      gACTAgtaAATTtGAAAAGtGGGCTGAGTGGCTGATGTT               w2: 200379443086 801517772347 649109 TTNNN
10 2 14 IL2_33_8_1_571_251      GaCTAGTAAAtttGAAAAGtGGGCtGAGTGGCTGATGTT               w2: 200379443086 801517772347 649109 TTNNN
-------------------------------------------------------------------------------
1       1 IL2_33_8_1_571_251   NNGACTAGTAAATTTGAAAAGTGGGCTGAGTGGCTGATGTTCTTGGATGCGGGG 10 A
max: 0 649109 1 10 0 0
============================= 2 10
12 1 0  slxa_0011_8_0069_7881                 aAAGTGGGCTGAGTGGCTGaTGTTcTTgGaTGgGgg    w1: 801517772347 1325340595487 986799 TNTTN
12 2 1  IL2_33_8_190_796_254                 gaaagtgggCtgaGtGGCTGATGTTcTTGGAtGCGGTGG  w2: 801517772347 1325340595487 986799 TNTTG
12 1 2  IL2_33_8_46_612_165                 GAAAAGTGGGCTGAGTGGCTGaTgTTCTTgGatgcgggg  w1: 801517772347 1325340595487 986799 TCTTN
12 2 4  IL2_33_8_30_108_252               gtGAaAaGtGGGCtGAGtGGCtGATGTTCTTGGATGCGG    w2: 801517772347 1325340595487 986799 TCTTG
12 1 5  IL2_33_8_100_266_829             TTTGAAAAGTGGGCTGAGTGGCTGaTGTTcTtggattcg     w1: 801517772347 1325340595487 986799 TNTNN
12 1 6  IL2_33_8_102_107_918            ATTTGAAAAGTGGGCTGAGtGGCTGatgtTtttggatct      w1: 801517772347 1325340595487 986799 TNNNN
12 2 6  IL2_33_8_40_950_214             AtttGAaaAGTGGGCtGaGtGGCTGATGTTCTTGGATGC      w2: 801517772347 1325340595487 986799 TCTTG
12 2 8  IL2_33_8_130_885_970          aaatttgaaaagtGggCtGAGTGGCTGATGTTCTTGGAT        w2: 801517772347 1325340595487 986799 TCTTG
12 2 12 IL2_33_8_118_146_767      tAgtAaattggAAAaGTGgGCTGAGTGGCTGATGTTCTT            w2: 801517772347 1325340595487 986799 TCTTN
12 1 13 IL2_33_8_124_94_829      CTAGTAAATTTGAAAAGTGGGCTGaGTgGcTGatgttct             w1: 801517772347 1325340595487 986799 NNNNN
12 2 15 IL2_33_8_1_571_251     GaCTAGTAAAtttGAAAAGtGGGCtGAGTGGCTGATGTT               w2: 801517772347 1325340595487 986799 TNNNN
12 2 15 IL2_33_8_7_465_656     gACTAgtaAATTtGAAAAGtGGGCTGAGTGGCTGATGTT               w2: 801517772347 1325340595487 986799 TNNNN
-------------------------------------------------------------------------------
2       1 IL2_33_8_1_571_251   NGACTAGTAAATTTGAAAAGTGGGCTGAGTGGCTGATGTTCTTGGATGCGGGGGN 12 A
max: 0 986799 1 12 0 0
============================= 3 12
13 1 0  IL2_33_8_7_465_656                    AACATCAGCCACTCAGCCCaCTTTTCaAATTtacTAGTc
13 1 0  IL2_33_8_1_571_251                    AACATCAGCCACTCaGCCCaCTTTTCaaaTTTACTAGtC
13 2 2  IL2_33_8_124_94_829                 agaacatCAgCcACtCAGCCCACTTTTCAAATTTACTAG   w3: 1325340595487 12824284357565 4962647 NNNNN
13 1 3  IL2_33_8_118_146_767               AAGAACATCAGCCACTCAGCcCACtTTTccaattTacTa    w4: 1325340595487 12824284357565 4962647 GAANN
13 1 7  IL2_33_8_130_885_970           ATCCAAGAACATCAGCCACTCaGccCacttttcaaattt        w4: 1325340595487 12824284357565 4962647 GAACC
13 1 8  slxa_0011_8_0043_10001        cATCCAAGAACATCAGCCACTCAGCCCaCtTCTCAc            w4: 1325340595487 12824284357565 4962647 GAACC
13 2 9  IL2_33_8_102_107_918          agatccaaaAacatCAGCCaCTCAGCCCACTTTTCAAAT         w3: 1325340595487 331335148871 375421 NNNNN
13 1 9  IL2_33_8_40_950_214           GCATCCAAGAACATCAGCCaCtCaGCCCACTttTCaaaT         w4: 1325340595487 12824284357565 4962647 GAACC
13 2 10 IL2_33_8_100_266_829         cgaatccaAgAACAtCAGCCACTCAGCCCACTTTTCAAA          w3: 1325340595487 12824284357565 4962647 NANNN
13 1 11 IL2_33_8_30_108_252         CCGCATCCAAGAACATCaGCCaCTCaGCCCaCtTtTCac           w4: 1325340595487 12824284357565 4962647 GAACC
13 2 12 slxa_0011_8_0069_7881      ccCcCAtCcAAgAACAtCAGCCACTCAGCCCACTTt              w3: 1325340595487 12824284357565 4962647 NAANC
13 2 13 IL2_33_8_46_612_165       ccccgcatCcAAGAAcAtCAGCCACTCAGCCCACTTTTC            w3: 1325340595487 12824284357565 4962647 GAANC
13 1 14 IL2_33_8_190_796_254     CCACCGCaTCCAAgAACATCAGCCaCtcaGcccactttc             w4: 1325340595487 12824284357565 4962647 NAACC
-------------------------------------------------------------------------------
3       1 IL2_33_8_1_571_251   NNCCCCCGCATCCAAGAACATCAGCCACTCAGCCCACTTTTCAAATTTACTAGTC 11 A
max: 0 375421 1 1 0 0
max: 1 4962647 1 10 1 1
============================= 4 13
13 2 0  IL2_33_8_15_630_886                   AGTGgGCtgAGTGGCTGATGTTCTTGGATGCGGTGGATG w2: 12824284357565 8878926809425 3711226 TTGGA
13 1 0  IL2_33_8_182_360_770                  AGtGggCTGAGtggctgatgttcttgggatagggggagc w1: 12824284357565 8878926809425 3711226 NNNNN
13 1 2  slxa_0011_8_0069_7881               aAAGTGGGCTGAGTGGCTGaTGTTcTTgGaTGgGgg      w1: 12824284357565 8878926809425 3711226 TTNGN
13 2 3  IL2_33_8_190_796_254               gaaagtgggCtgaGtGGCTGATGTTcTTGGAtGCGGTGG    w2: 12824284357565 8878926809425 3711226 TTGGA
```



Base Quality to Filter Base Errors

```
29 2 13 IL2_33_8_116_24                                                                          964 10129248 NANNN
29 1 13 IL2_33_8_92_670                                                                          964 10129248 TACCA
-------------------------------------------------------------------------------
63      2 IL2_33_8_1_920_483   NNNAAAGCAAAACCATAGCCGATAAAGAGGTTGAGGTCCAAGAGTTCATTGGATA 26 G
max: 0 10129248 2 26 0 0
============================= 64 29
27 1 0  IL2_33_8_83_322_97                     GACCTCAACCTCTTTATCGGCTattgATTtcTcCAtaTC w1: 36782494135964 6392488188531 3044891 NNNAT
27 1 2  slxa_0011_8_0008_13230               tGGACCTCaACCTCTTTATCGGCTatgGtTTTgttt        w1: 36782494135964 6392488188531 3044891 NNGNT
27 1 2  slxa_0011_8_0016_3358                tGGACCTCAACCTCTTTATCGGCTATGGTTTTgTTT        w1: 36782494135964 6392488188531 3044891 TGGTT
27 2 4  IL2_33_8_92_670_287                catGgaccTCAACCtcTTTAtCGGCTATGGTTTTGCTAT      w2: 36782494135964 6392488188531 3044891 TGGTT
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27 1 4  IL2_33_8_116_24_685                CTTGGACCTCAACCTCTTTATCGgcTaTggtttttctcT      w1: 36782494135964 6392488188531 3044891 TNNNN
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27 1 5  slxa_0011_8_0017_12919            tCTTGGACCTCAACCTCTTTATCGGCTAttgtTTtt           w1: 36782494135964 6392488188531 3044891 NNNNT
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27 1 8  IL2_33_8_190_507_900            AACTCTTGGACCTCAAcCTCTTTATcGGCTAatgttttT          w1: 36782494135964 6392488188528 3044890 NNNNN
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27 2 16 IL2_33_8_165_814_103     AtcCAAtGAACTCTTGGACCTCAACCTCTTTATCGGCTA
-------------------------------------------------------------------------------
64      2 IL2_33_8_1_920_483   AACCAATGAACTCTTGGACCTCAACCTCTTTATCGGCTATGGTTTTGCTCTTATC 26 G
max: 0 3044890 2 5 0 0
max: 5 3044891 2 21 5 5
repeat: 26 0.807692 0.238095 5 64
Go: 13 0
NKM: 5 26 2.000000 0.000000 NNTTTTTNNTTNTTNNNTNTNNNTNT 13 0
rate-c: 5 13 0 2.000000 26
pairs: 2 0 0
rate: 21 5 4.200000 0 3044891
============================= 65 27
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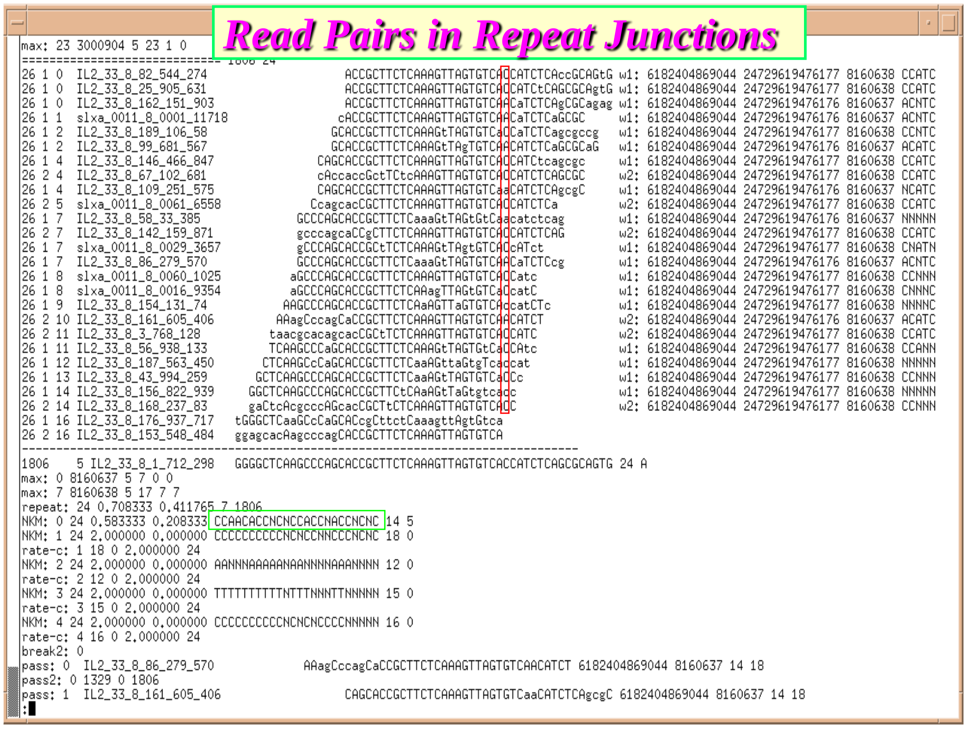
Read Pairs in Repeat Junctions



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26 1 0  IL2_33_8_25_905_631                    ACCGCTTCTCAAAGTTAGTGTCACCATCtCAGCGCAgtG w1: 6182404869044 24729619476177 8160638 CCATC
26 1 0  IL2_33_8_162_151_903                   ACCGCTTCTCAAAGTTAGTGTCAACaTCTCAgCGCagag w1: 6182404869044 24729619476176 8160637 ACNTC
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26 1 4  IL2_33_8_109_251_575               CAGCACCGCTTCTCAAAGTTAGTGTCaaCATCTCAgcgC     w1: 6182404869044 24729619476176 8160637 NCATC
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26 1 7  IL2_33_8_86_279_570             GCCCAGCACCGCTTCTCaaaGtTAGTGTCAACaTCTCcg        w1: 6182404869044 24729619476176 8160637 ACNTC
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26 2 11 IL2_33_8_3_768_128          taacgcacagcacCGCtTCTCAAAGTTAGTGTCACCATC            w2: 6182404869044 24729619476177 8160638 CCATC
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26 1 12 IL2_33_8_187_563_450       CTCAAGCcCaGCACCGCTTCTCaaAGttaGtgTcaccat             w1: 6182404869044 24729619476177 8160638 NNNNN
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26 1 16 IL2_33_8_176_937_717   tGGGCTCaaGCcCaGCACcgCttctCaaagttAgtGtca
26 2 16 IL2_33_8_153_548_484   ggagcacAagcccagCACCGCTTCTCAAAGTTAGTGTCA
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1806    5 IL2_33_8_1_712_298   GGGGCTCAAGCCCAGCACCGCTTCTCAAAGTTAGTGTCACCATCTCAGCGCAGTG 24 A
max: 0 8160637 5 7 0 0
max: 7 8160638 5 17 7 7
repeat: 24 0.708333 0.411765 7 1806
NKM: 0 24 0.583333 0.208333 CCAACACCNCNCCACCNACCNCNC 14 5
NKM: 1 24 2.000000 0.000000 CCCCCCCCCCNCNCCNNCCCNCNC 18 0
rate-c: 1 18 0 2.000000 24
NKM: 2 24 2.000000 0.000000 AANNNAAAAANAANNNNAAANNNN 12 0
rate-c: 2 12 0 2.000000 24
NKM: 3 24 2.000000 0.000000 TTTTTTTTTTNTTTNNNTTNNNNN 15 0
rate-c: 3 15 0 2.000000 24
NKM: 4 24 2.000000 0.000000 CCCCCCCCCCNCNCNCCCCNNNNN 16 0
rate-c: 4 16 0 2.000000 24
break2: 0
pass: 0  IL2_33_8_86_279_570             AAagCccagCaCCGCTTCTCAAAGTTAGTGTCAACATCT 6182404869044 8160637 14 18
pass2: 0 1329 0 1806
pass: 1  IL2_33_8_161_605_406                  CAGCACCGCTTCTCAAAGTTAGTGTCaaCATCTCAgcgC 6182404869044 8160637 14 18
:
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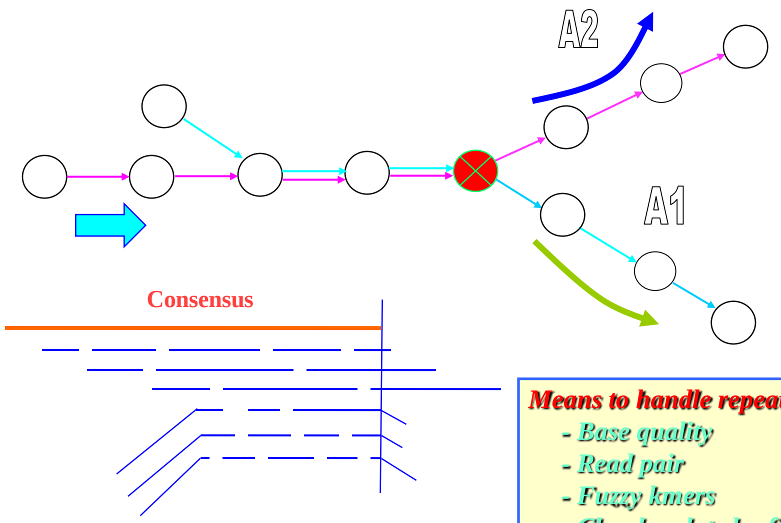
Kmer Extension & Repeat Junctions

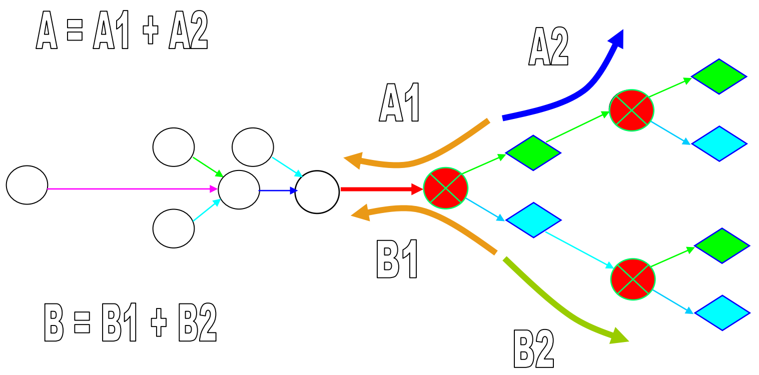


Pileup of other reads like 454, Sanger etc at a repeat junction

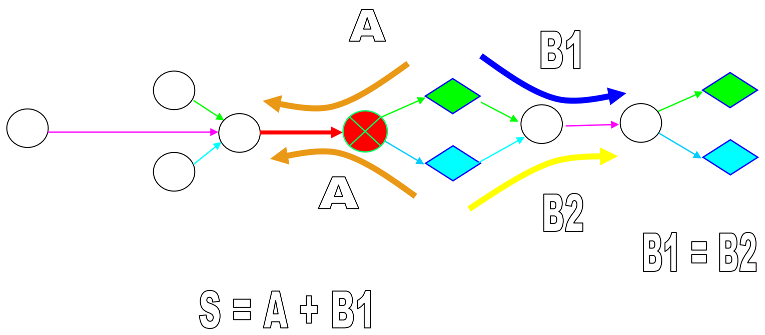
Means to handle repeats:

- *Base quality*
- *Read pair*
- *Fuzzy kmers*
- *Closely related reference*
- *454 or Sanger reads*

Handling of Repeat Junctions



Handling of Single Base Variations



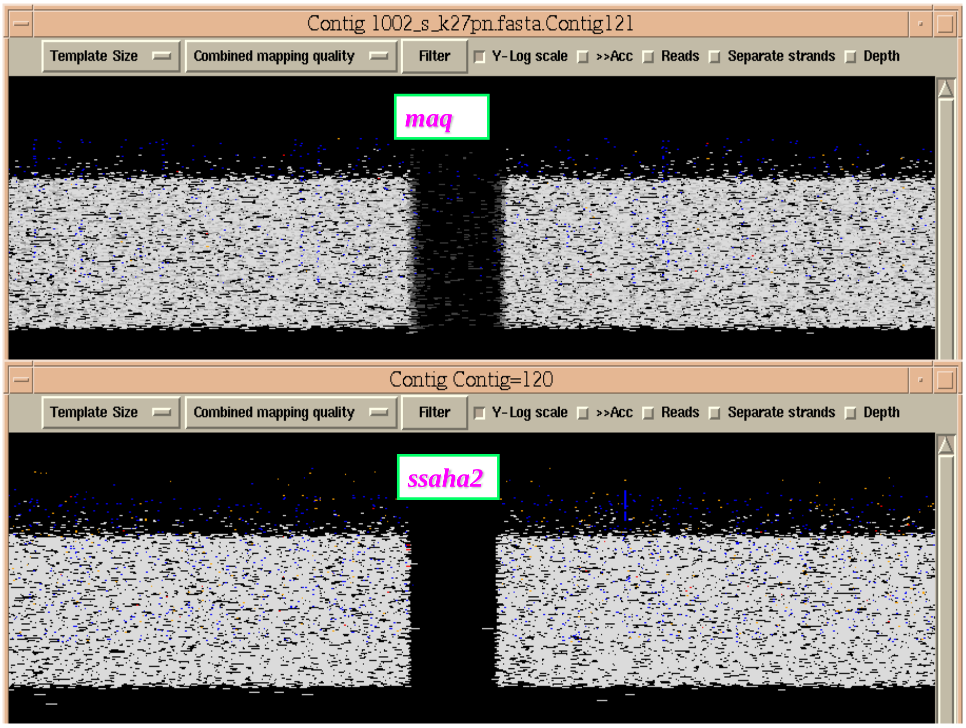
Salmonella seftenberg Solexa Assembly from Pair-End Reads

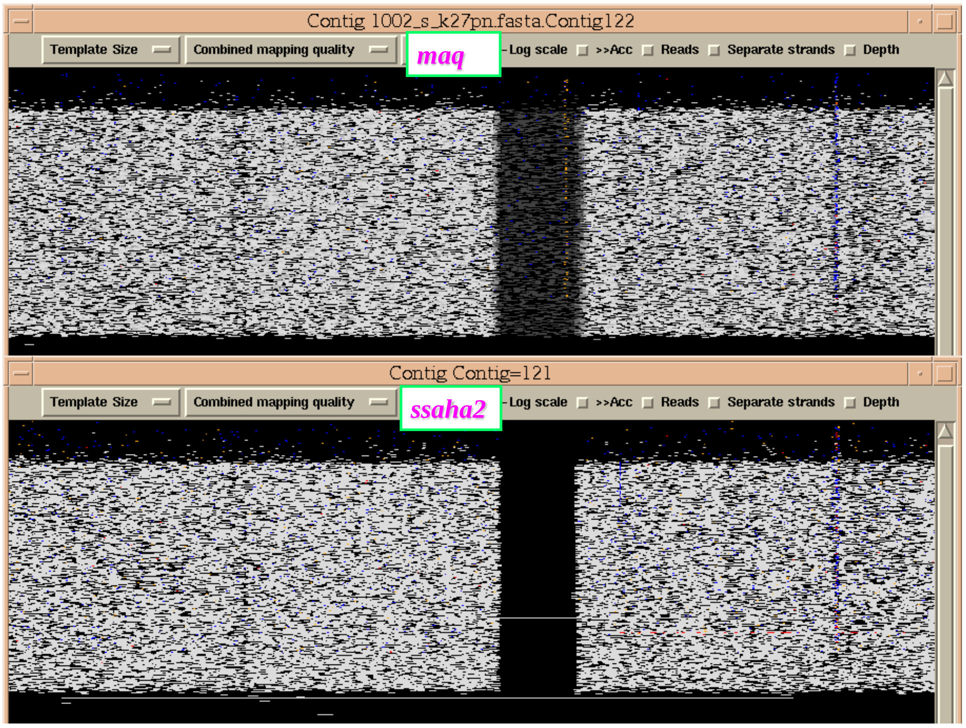
Solexa reads:

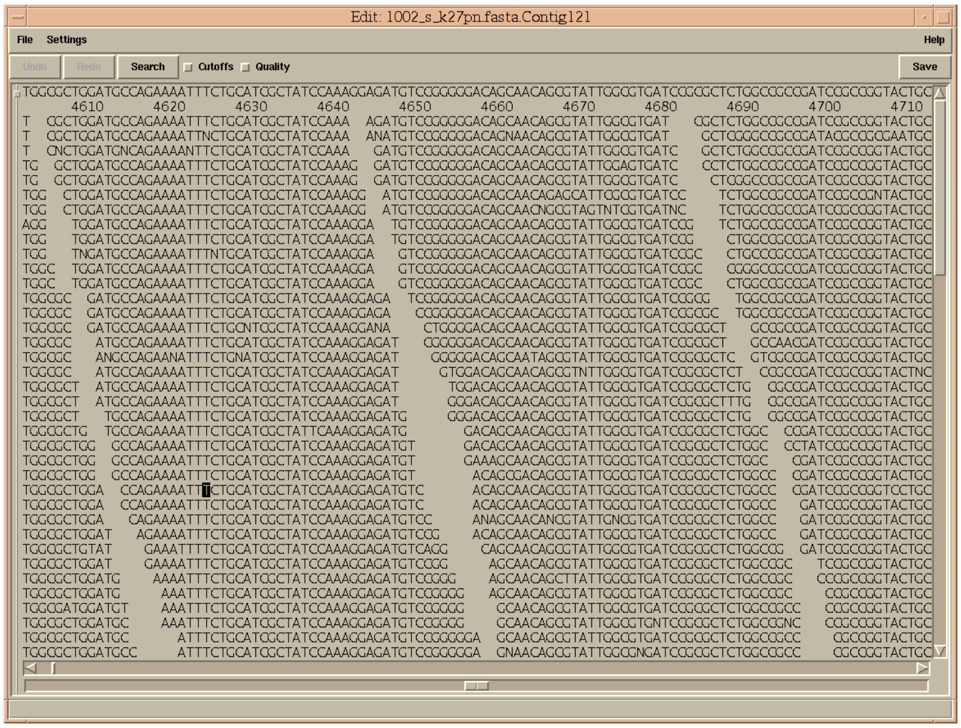
Number of reads:	6,000,000;
Finished genome size:	~4.8 Mbp;
Read length:	2x37 bp;
Estimated read coverage:	~92.5 X;
Insert size:	170/50-300 bp;

Assembly features: - contig stats

	Solexa	454
Total number of contigs:	75;	390
Total bases of contigs:	4.80 Mbp	4.77 Mb
N50 contig size:	139,353	25,702
Largest contig:	395,600	62,040
Averaged contig size:	63,969	12,224
Contig coverage on genome:	~99.8 %	99.4%
Contig extension errors:	0	
Mis-assembly errors:	0	4







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T  CGCTGGATGCCAGAAAATTTCTGCATCGCTATCCAAA  AGATGTCCGGGGGACAGCAACAGCGTATTGGCGTGAT   CGCTCTGGCCGCCGATCCGCCGGTACTGC
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T  CNCTGGATGNCAGAAAANTTCTGCATCGCTATCCAAA   GATGTCCGGGGGACAGCAACAGCGTATTGGCGTGATC   GCTCTGGCCGCCGATCCGCCGGTACTGC
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TG  GCTGGATGCCAGAAAATTTCTGCATCGCTATCCAAAG  GATGTCCGGGGGACAGCAACAGCGTATTGGCGTGATC    CTCGGCCCGCCGATCCGCCGGTACTGC
TGG  CTGGATGCCAGAAAATTTCTGCATCGCTATCCAAAGG  ATGTCCGGGGGACAGCAACAGAGCATTCGCGTGATCC    TCTGGCCGCCGATCCGCCGNTACTGC
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TGGC  TGGATGCCAGAAAATTTCTGCATCGCTATCCAAAGGA   GTCCGGGGGACAGCAACAGCGTATTGGCGTGATCCGC   CGGGCCGCCGATCCGCCGGTACTGC
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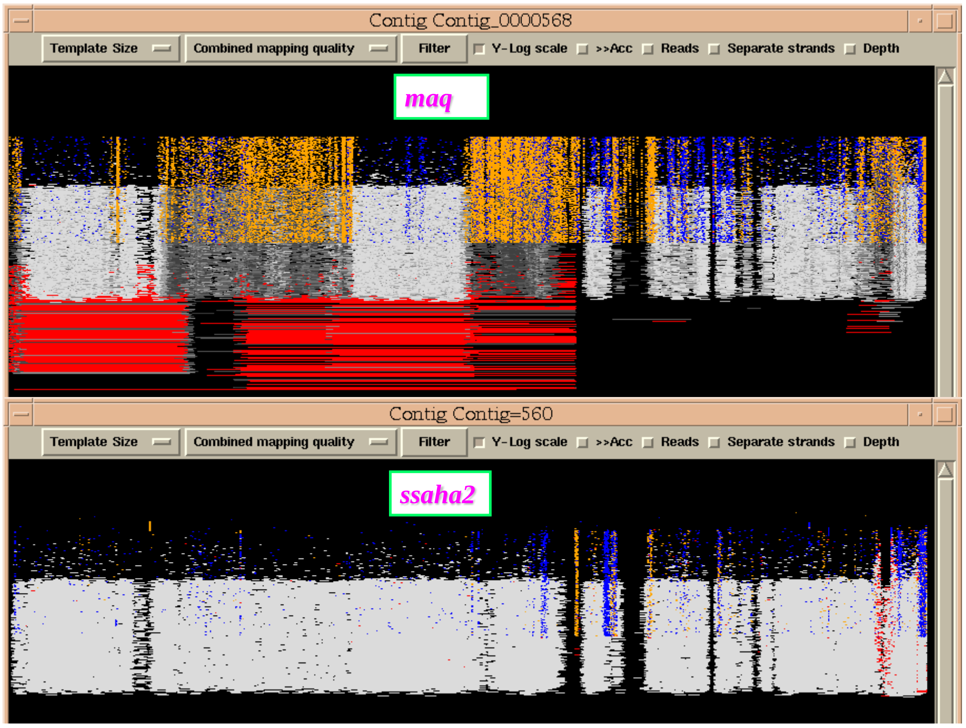
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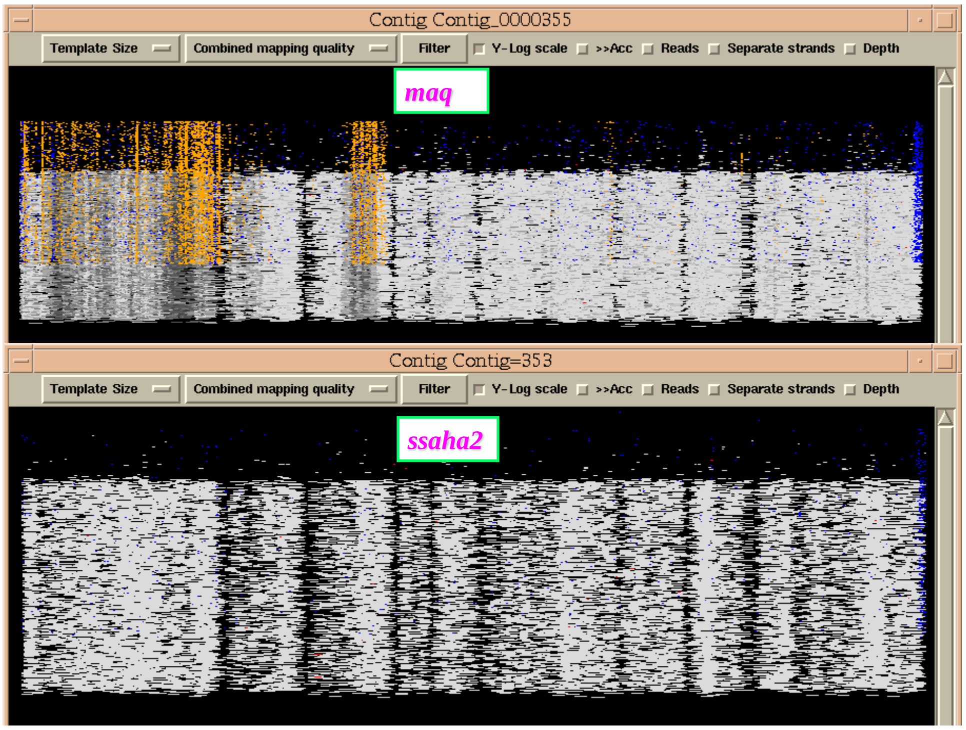
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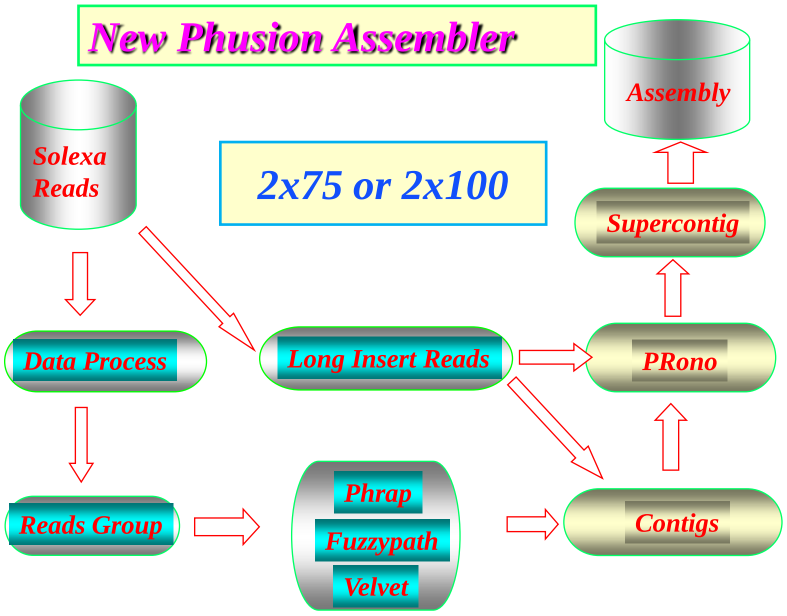

Genome Assembly - Normal Cell

Solexa reads:

Number of reads:	557 Million;
Finished genome size:	3.0 GB;
Read length:	2x75bp;
Estimated read coverage:	~25X;
Insert size:	190/50-300 bp;
Number of reads clustered:	458 Million

Assembly features: - contig stats

Total number of contigs:	1,020,346;
Total bases of contigs:	2.713 Gb
N50 contig size:	8,344;
Largest contig:	107,613
Averaged contig size:	2,659;
Contig coverage over the genome:	~90 %;
Mis-assembly errors:	?

Acknowledgements:

- *Jim Mullikin*
- *Yong Gu*
- *Hannes Ponstingl*
- *James Bonfield*
- *Heng Li*
- *Daniel Zerbino (EBI)*
- *Tony Cox*
- *Richard Durbin*

Solexa