Assembly and Analysis of DNA Sequencing Data

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Raw DNA Sequencing Data are Huge and Short

- A huge number (up to a billion) of DNA sequences can be produced by a sequencing instrument in a day.
- DNA sequences are short (e.g. 100 to 200 base pairs in length).
- Their error rates are often below 1%.


Applications Places System
Tue Jul 22, 4:50 PM Xiaoqiu Huang
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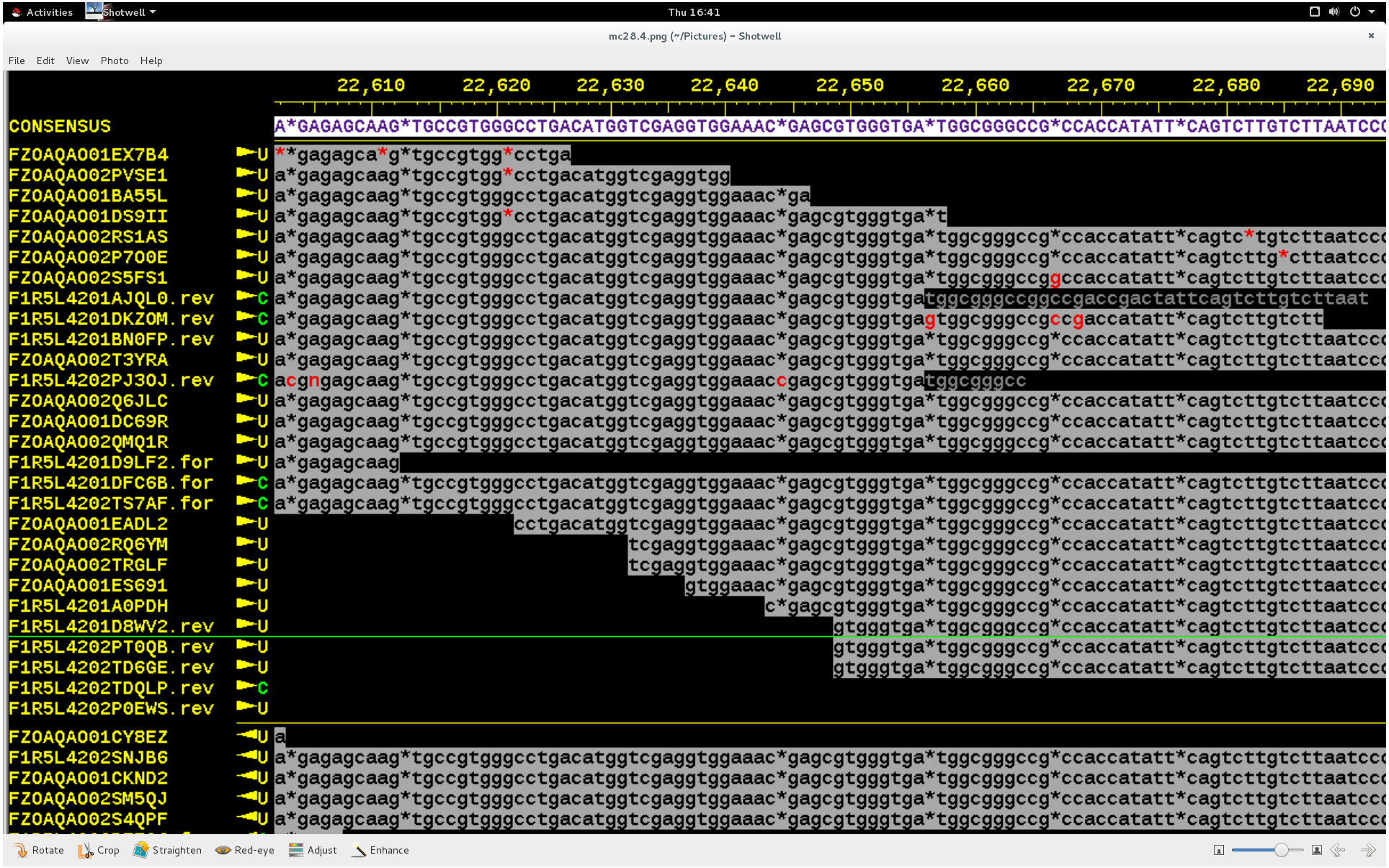
Assembly and Mapping

- Short redundant DNA sequences are put together to construct longer non-redundant genomic or RNA sequences (assembly).
- DNA sequences are aligned to a reference sequence (mapping) to find single nucleotide polymorphisms (SNPs).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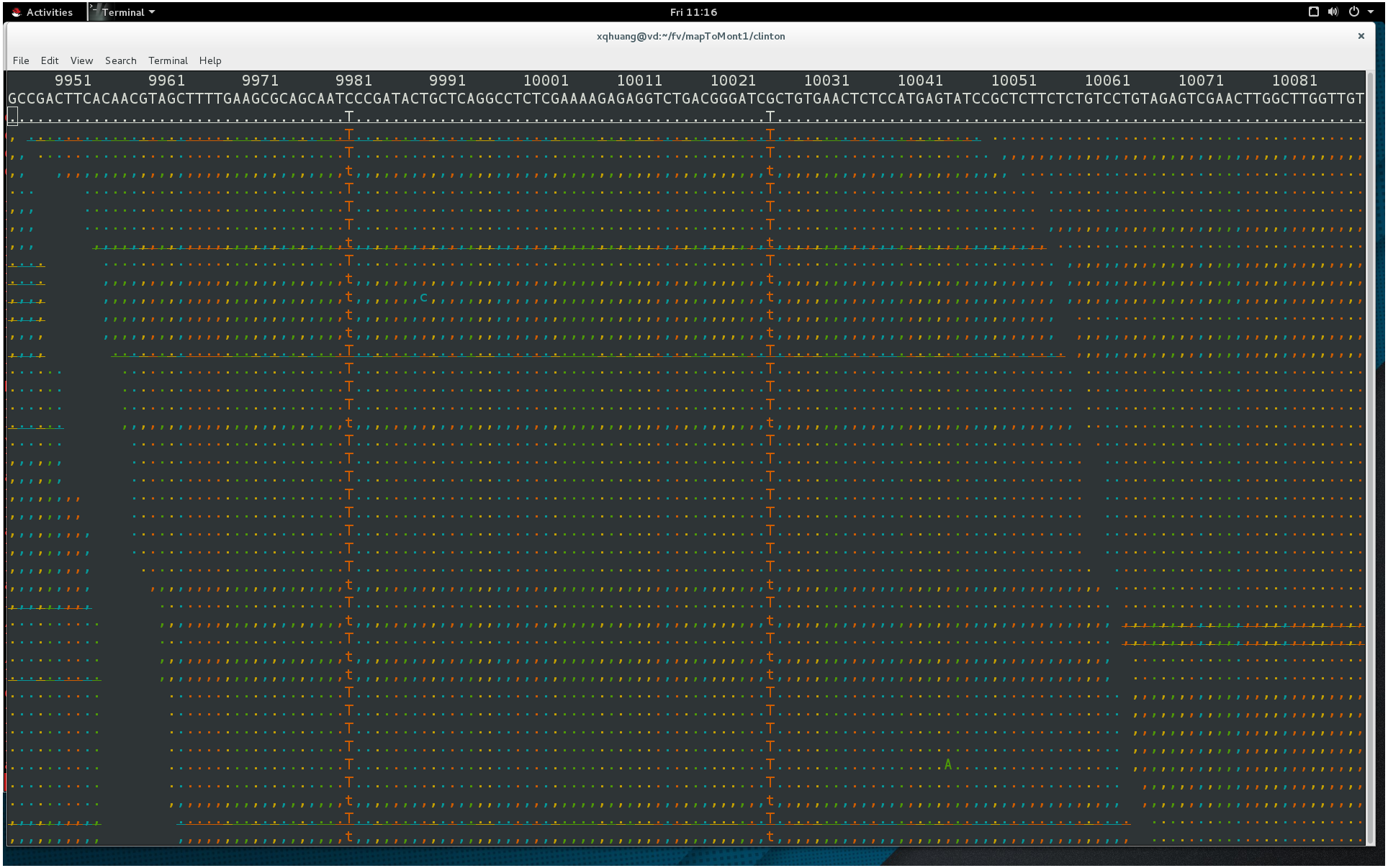
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Methods for Addressing the Challenges

- Dynamic programming
- Hashing
- Graph algorithms
- Parallel processing

Impact of Finding Genetic Variation

- Molecular evolution
- Association of genetic variation with traits
- Personalized lifestyle
- Precision agriculture

Genetic Variation and Reproduction

- Genetic variation is the ‘raw material’ for natural selection.
- Sexual reproduction generates genetic variation by recombination.
- Asexual reproduction lacks mechanisms to generate genetic variation.

Asexual reproduction in Fungi

- Many important plant pathogens have a long period of asexual reproduction.
- Two asexual plant pathogens:

  the fungus *Verticillium dahliae*

  the fungus *Fusarium virguliforme*

Fungus *Fusarium virguliforme*

- Sudden death syndrome (SDS) of soybean first appeared in Arkansas in 1971 and then spread in 30 years to all major soybean-producing regions in the U.S.
- SDS in North America is mostly caused by the fungus *Fusarium virguliforme (Fv)*.
- SDS is similar to Bean Root Rot (BRR).

Low Genetic Diversity but Variable Aggressiveness

- Low genetic diversity is detected in *Fv* populations based on small sequence data.
- But *Fv* isolates show variable aggressiveness on soybean.
- Paradox: *Fv* is subject to Muller's Ratchet but is highly adaptive.

Genome Sequence Data

- 454 sequences of up to 400 bp:
  3 Gb of data for *Fv* isolate Mont-1, single reads and paired reads with 3-kb and 20-kb inserts.
- Illumina sequences of 102 bp:
  390 Gb of data for 10 SDS/BRR isolates, 39 Gb of paired-end reads with a 300-bp insert per isolate.

Experimental Results

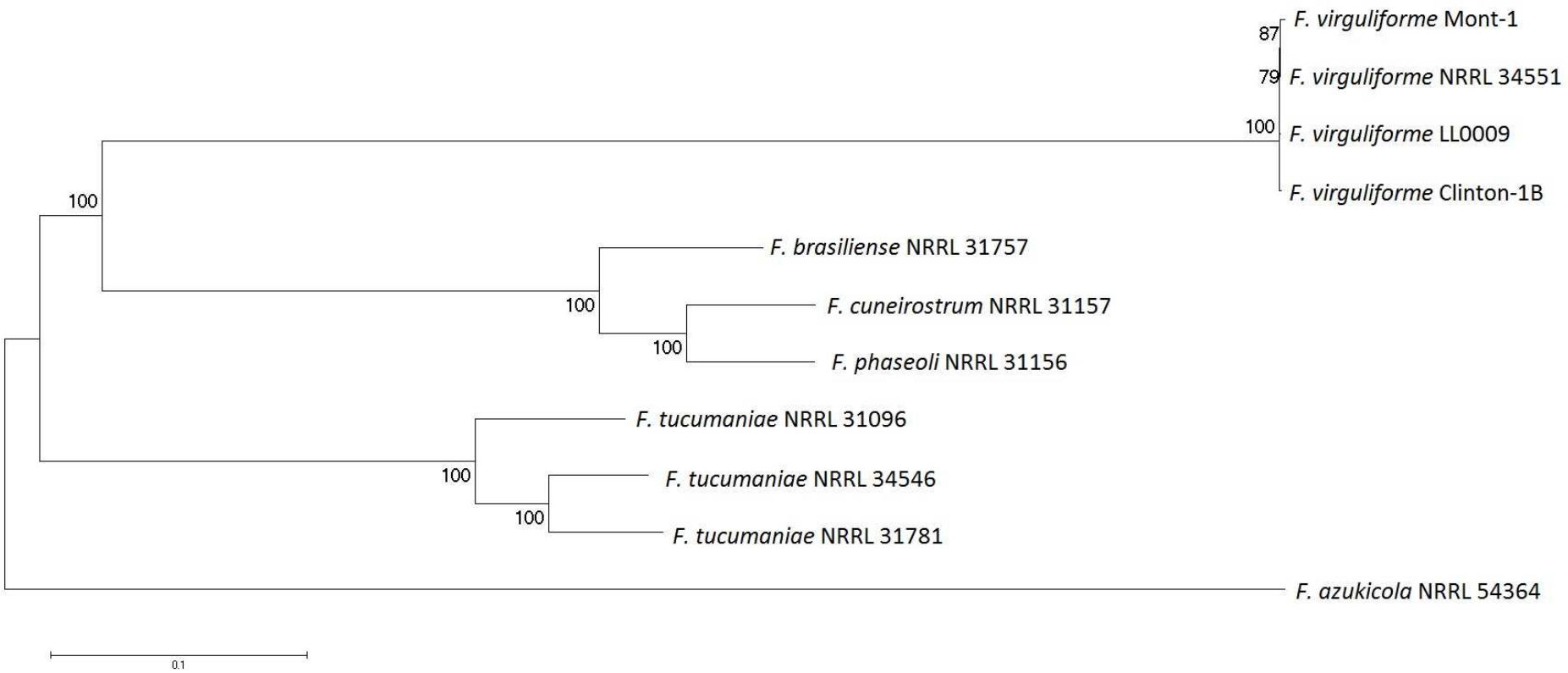
- PCAP was used to produce a chicken genome assembly on ABI 3730 reads (Hillier et al., Nature 432, 695-716).
- PCAP.454 was used to produce a genome assembly of *Fv* Mont-1 (Srivastava et al. PLoS One 9(1), e81832).
- PCAP.Illumina produced an assembly on each of the 10 SDS/BRR isolates.

Data Comparison

Type	No. of reads (in millions)	Size (Gb)
3730 Chicken	9.8	20
454 Fungus *Fv*	5	3
Illumina F. *Ft*	151	44

Computation Time Comparison

Type	#Processors	Time
3730 Chicken	100	1 week
454 Fungus *Fv*	8	2 days
Illumina F. *Ft*	1	2 days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F. virguliforme LL0009
F. virguliforme Clinton-1B
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F. phaseoli NRRL 31156
F. tucumaniae NRRL 31096
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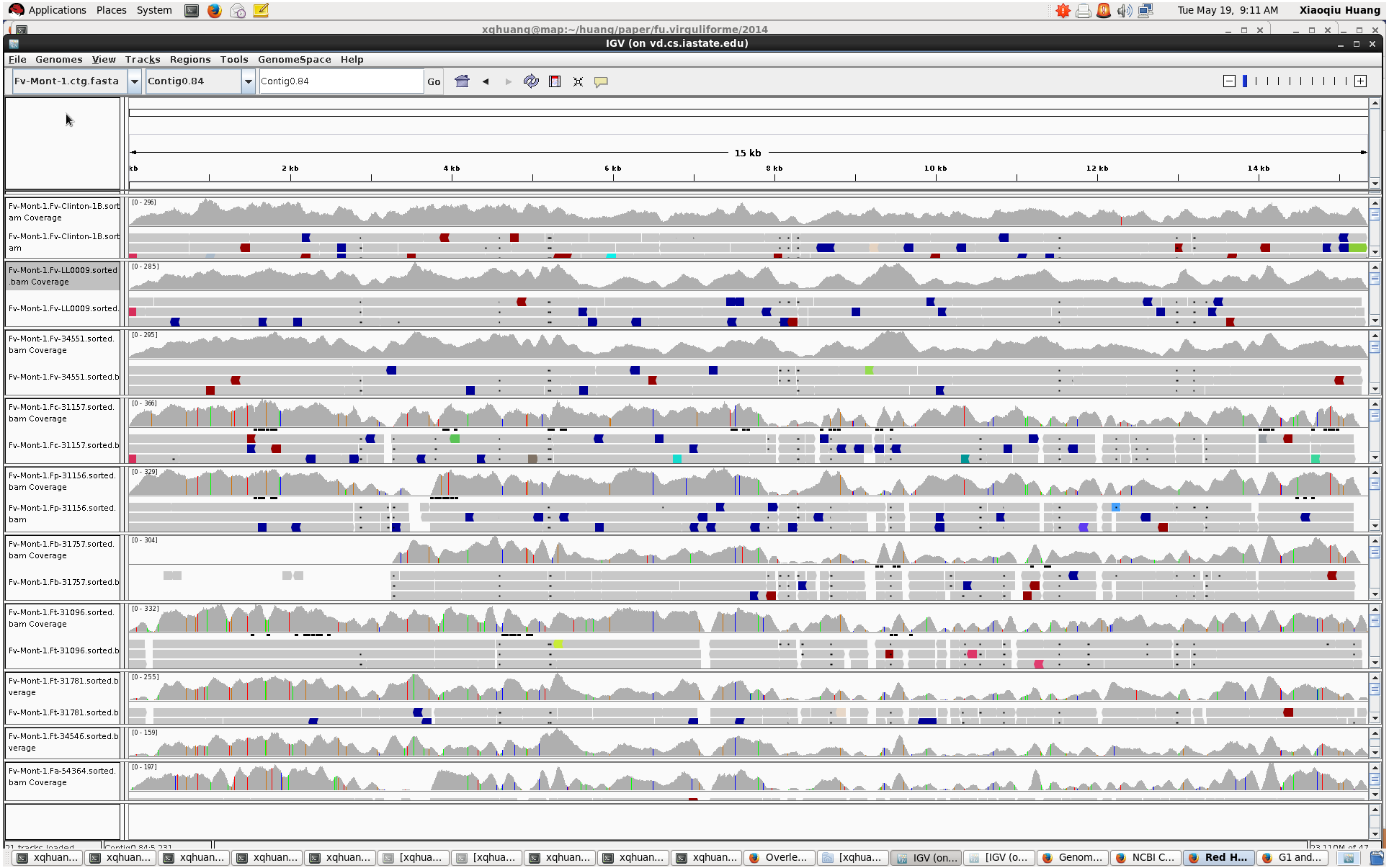
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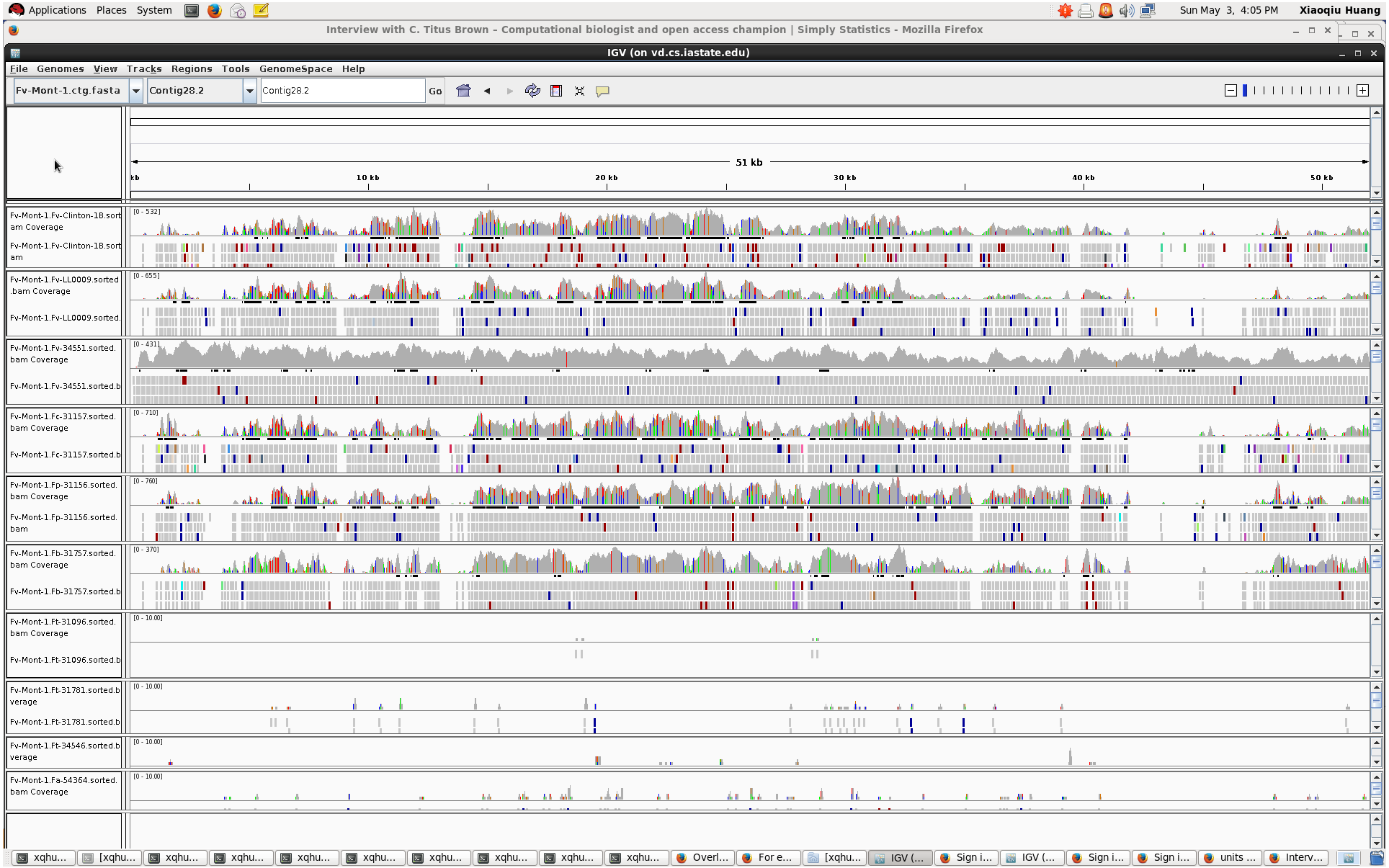
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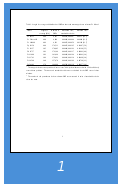
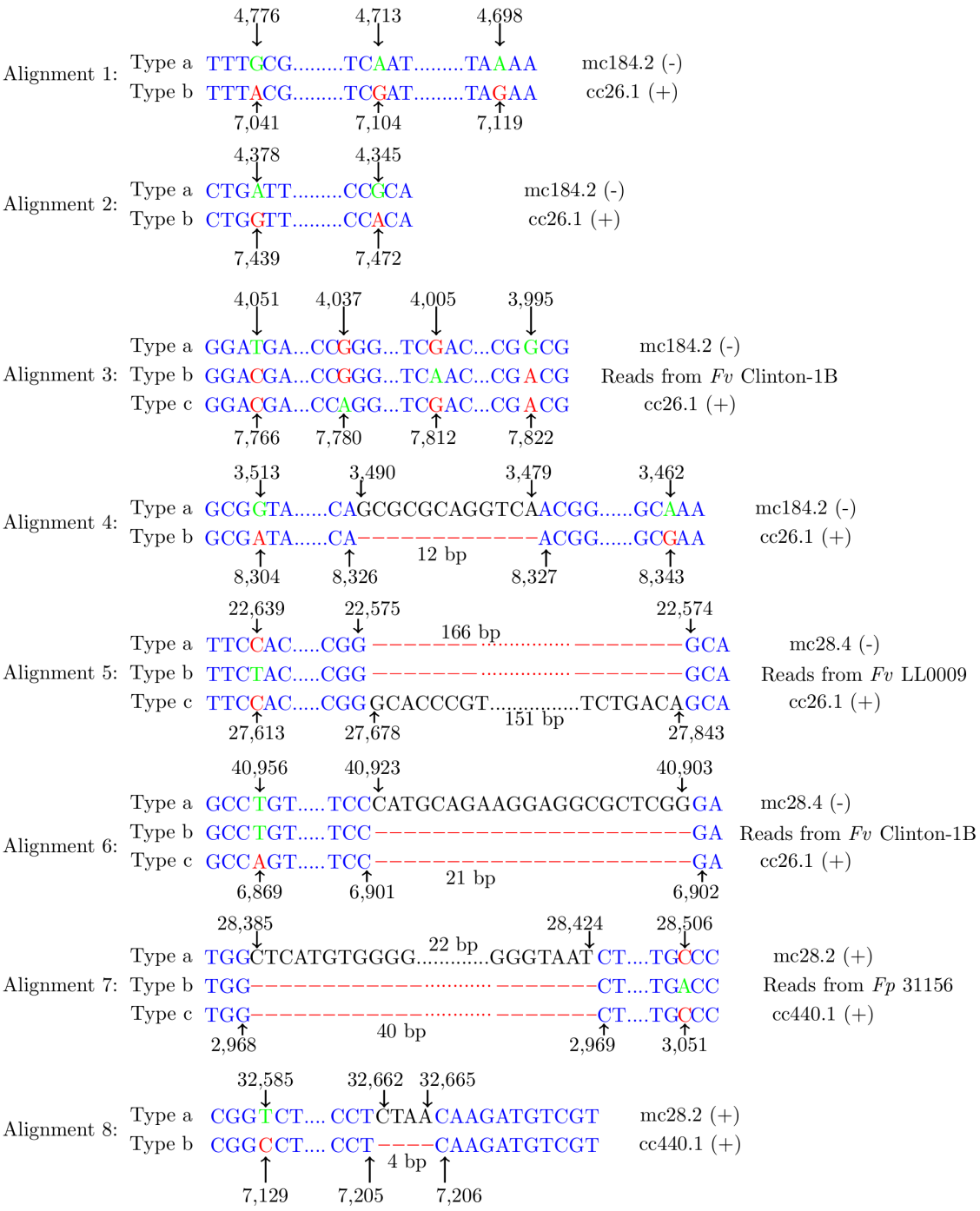


Table 1: Length of coverage and distribution of SNPs when reads were mapped onto reference *Fv* Mont-1

Isolate	Length of coverage (Mb)	Number of SNPs	Mean SNP rate/ standard deviation[a]	Max SNP rate[b]
Fv 34551	49.9	4,955	0.00003/0.00007	0.00177 (23.7)
Fv Clinton-1B	49.6	8,269	0.00006/0.00044	0.00960 (21.5)
Fv LL0009	49.2	8,541	0.00007/0.00052	0.01129 (21.7)
Fp 31156	40.0	178,511	0.00447/0.00125	0.01097 (5.2)
Fc 31157	39.5	176,065	0.00446/0.00123	0.01126 (5.5)
Fb 31757	39.3	172,100	0.00435/0.00117	0.00903 (4.0)
Ft 31096	39.3	181,420	0.00462/0.00128	0.00943 (3.8)
Ft 31781	39.2	172,823	0.00441/0.00114	0.00829 (3.4)
Ft 34546	38.9	157,076	0.00412/0.00102	0.00726 (3.1)
Fa 54363	37.9	188,209	0.00506/0.00119	0.00957 (3.8)

[a] The mapped reference was partitioned into at least 700 disjoint windows each with 35-kb sufficiently covered base positions. The mean and standard deviation were calculated for the SNP rates of these windows.

[b] The number in the parentheses is the maximum SNP rate measured in units of standard deviation above the mean.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7,104
7,119
Alignment 2:
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4,345
Type a CTGATT.....CCGCA mc184.2 (-)
Type b CTGGTT.....CCACA cc26.1 (+)
7,439
7,472
Alignment 3:
4,051
4,037
4,005
3,995
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Type b GGACGA...CCGGG...TCAAC...CGACG Reads from Fv Clinton-1B
Type c GGACGA...CCAGG...TCGAC...CGACG cc26.1 (+)
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7,780
7,812
7,822
Alignment 4:
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3,479
3,462
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22,574
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Type c TTCCAC.....CGGGCACCCGT.....TCTGACAGCA cc26.1 (+)
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7,205
7,206

Table 1: The number of reads from the isolate that link all alleles in the sequence

Sequence[a]	Number of reads from the isolate that cover the sequence[b]					
	Fv Clinton-1B	*Fv* LL0009	*Fv* 34551	*Fc* 31157	*Fp* 31156	*Fb* 31757
A1.Ta	16	16	16	0	0	0
A1.Tb	32	18	0	48	46	8
A2.Ta	88	149	147	0	0	9
A2.Tb	85	114	0	115	0	0
A3.Ta	52	41	61	0	0	0
A3.Tb	78	52	0	152	0	0
A3.Tc	33	0	0	34	46	0
A4.Ta	162	134	77	121	0	97
A4.Tb	54	0	0	127	242	0
A5.Ta	39	27	57	0	0	18
A5.Tb	0	8	0	39	0	0
A5.Tc	85	0	0	69	65	0
A6.Ta	0	0	46	0	0	0
A6.Tb	72	0	0	0	209	0
A6.Tc	116	121	35	554	244	74
A7.Ta	0	0	98	0	0	0
A7.Tb	0	31	0	0	54	0
A7.Tc	35	0	0	42	50	39
A8.Ta	0	35	34	40	42	0
A8.Tb	70	0	0	51	59	42

[a] Each sequence is denoted by its alignment number and type letter (Figure X): e.g., Types a and b in Alignment 1 are denoted by A1.Ta and A1.Tb, respectively.

[b] A read covers a sequence in a set of polymorphic sequences if the read and the sequence have the same allele at every occurrence of polymorphism.

Conclusion

- Low SNP rates show that the assembly consensus sequence is accurate.
- They confirm that *Fv* is asexual in reproduction mode.
- *Fv* genome has mutational hot regions.
- *Fv* has novel mechanisms for generating mutations.

Acknowledgements

- Madan Bhattacharyya at ISU
- Leonor Leandro at ISU
- Kerry O'Donnell at USDA ARS
- Subodh Srivastava at U. of Arkansa
- Shiaw-Pyng Yang at Monsanto
- Colleagues at WashU