Common problems in Influenza bioinformatics

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Common issues

- Failed Mira QC
  - Low-coverage / Incomplete segment coverage
    - Not enough reads in your library → Back to the lab
      - Ct <= 28?
      - Gel image resolves segment bands cleanly?
      - Proper sample number on your flow cell?
    - MIRA <= v2.0.0 ??
      - Reads are being subsampled

Common issues

- Failed Mira QC
  - Minor variant count >10
    - In standard *clinical* samples, we have never observed >6 minor alleles (>=5% frequency) with the majority having 1-3, per segment
    - Cell cultures regularly have high genetic variability which can result in high counts
    - Could be a co-infection. Unlucky person got both H1N1 and H3N2 infection *at the same time*
    - **Most likely contamination!**

sample_id	total_reads	pass_qc	reads_mapped	reference	percent_reference_coverage	median_coverage	count_minor_snv_at_or_over_5_pct	pass_fail_reason
95f48e8a	95828	95828	10388	A_HA_H1	99.82	735	2	Pass
95f48e8a	95828	95828	5818	A_MP	100	686	148	Count of minor variants at or over 5% > 10
95f48e8a	95828	95828	9528	A_NA_N1	99.79	814	3	Pass
95f48e8a	95828	95828	9146	A_NP	100	722	274	Count of minor variants at or over 5% > 10
95f48e8a	95828	95828	5704	A_NS	97.1	777	147	Count of minor variants at or over 5% > 10
95f48e8a	95828	95828	11790	A_PA	100	607	361	Count of minor variants at or over 5% > 10
95f48e8a	95828	95828	13230	A_PB1	100	687	246	Count of minor variants at or over 5% > 10
95f48e8a	95828	95828	12175	A_PB2	100	590	391	Count of minor variants at or over 5% > 10
c282a097	16170	16170	1992	A_HA_H3	99.82	158	35	Count of minor variants at or over 5% > 10
c282a097	16170	16170	1382	A_MP	100	187	12	Count of minor variants at or over 5% > 10
c282a097	16170	16170	1852	A_NA_N2	100	178	25	Count of minor variants at or over 5% > 10
c282a097	16170	16170	1720	A_NP	100	157	18	Count of minor variants at or over 5% > 10
c282a097	16170	16170	1336	A_NS	97.1	206	10	Pass
c282a097	16170	16170	2516	A_PA	100	148	22	Count of minor variants at or over 5% > 10
c282a097	16170	16170	2712	A_PB1	100	161	23	Count of minor variants at or over 5% > 10
c282a097	16170	16170	2660	A_PB2	100	160	28	Count of minor variants at or over 5% > 10

Two populations are present.

One matching reference (gray) one sharing all the colored SNVs

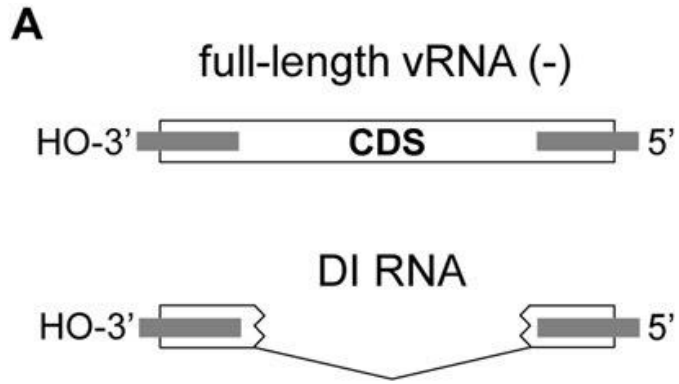
2+ mutations on one molecule are “phased” or “linked”

Common issues

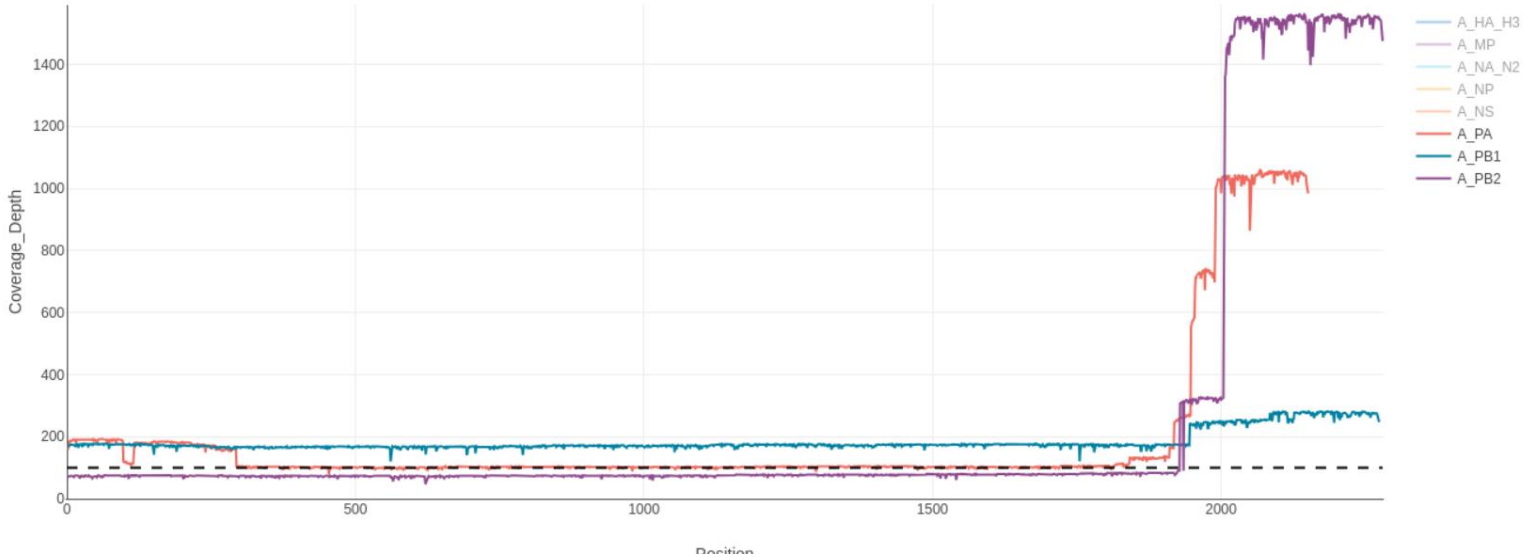
- Failed Mira QC
  - Premature stop-codon?
    - ONT homopolymer issue may require manual correction
    - DI particle induced alignment

DI Particles

- DI Particles can interfere with NGS
- Common in polymerase segments
- Coverage shape can spike at end or “bat ears”
- Can create erroneous indel mutations at coverage dropoff points



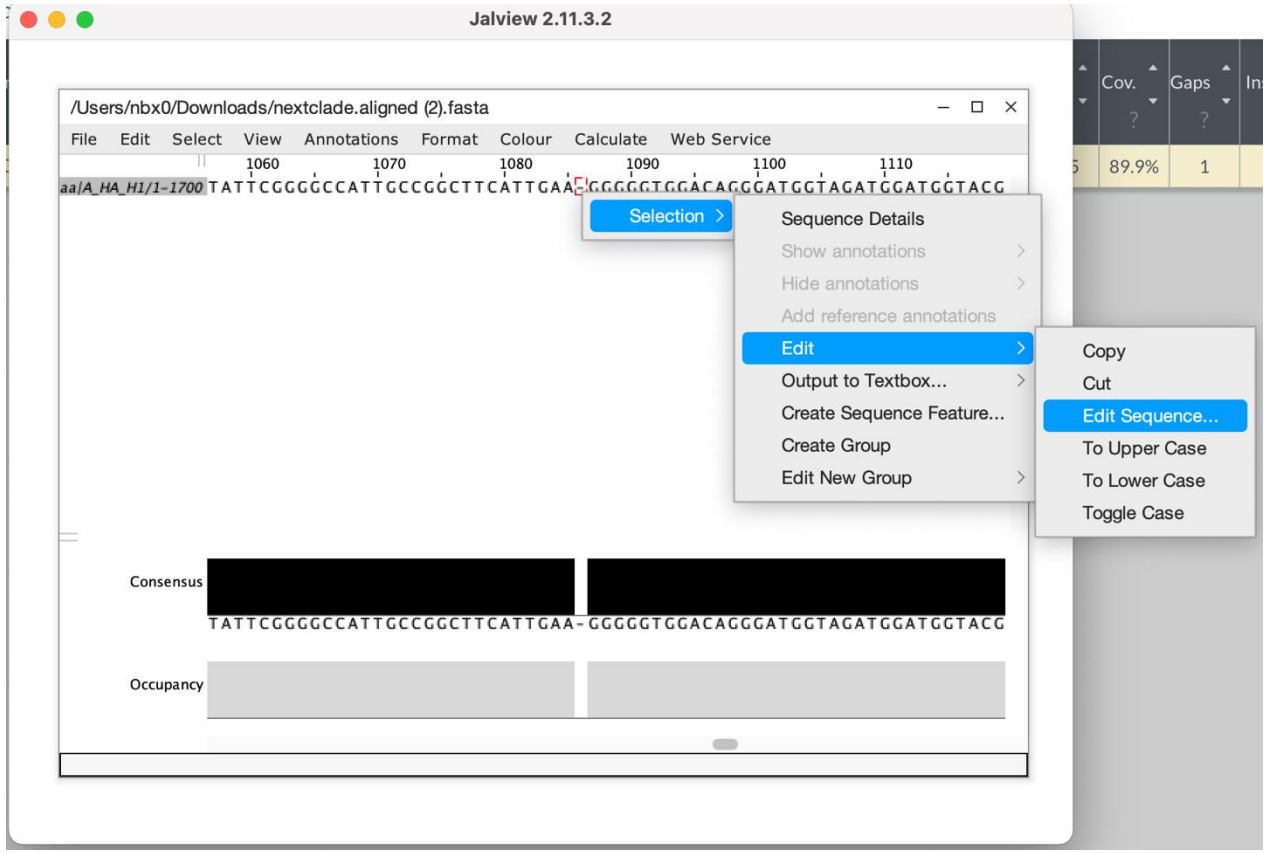
https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/biot.201400429



DI Particles

Frameshifts

- Prevalent in homopolymer regions of Oxford Nanopore Sequencing
- DAIS-Ribosome is frameshift-tolerant
  - Shows as (~) mutation
- Convert back to nucleotide space and add or remove base to fix



Mira demands high quality results

- Together we can stop the “Garbage In” side of the data analysis mantra: “Garbage In, Garbage Out”
- Built-in thresholds are for standardizing QC
- Amended consensus gives us extra information from a single sequence.
  - A mixed site may be under active or balancing selection in the host
- Consider each sample as a whole: if HA and NA pass but all other segments fail, consider why.
- High priority samples can be useful even when QC thresholds are not met

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