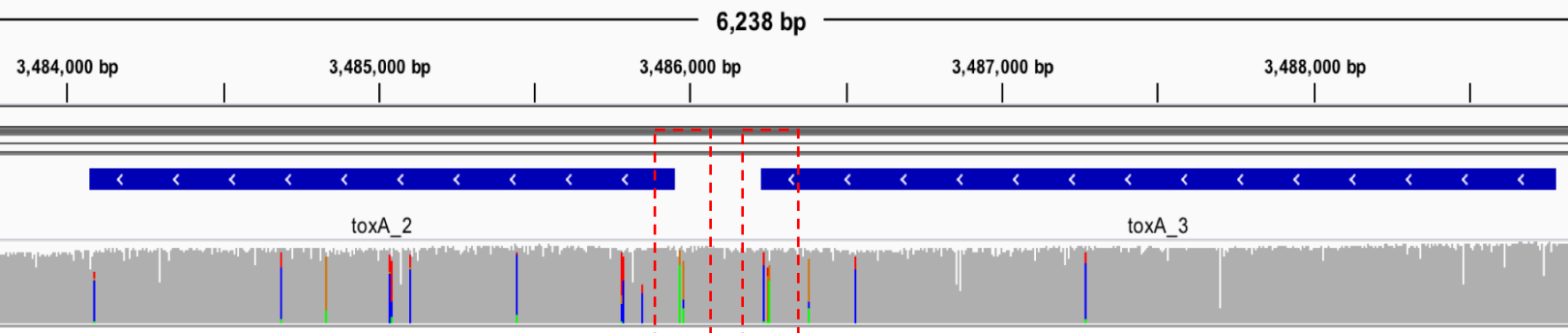


We take toxA_2 and toxA_3 as an example:

deletion in the short reads



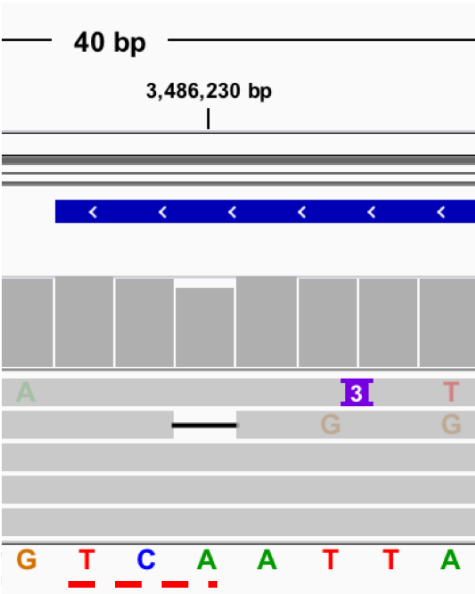
Issue: the “stop codon” so happens at the end of toxA_3

Method:, blastn toxA_3 to tcdA genes: AA causing the frame shift

```
> NC_009089.1:795843-803975 Clostridioides difficile 630, complete genome
Length=8133

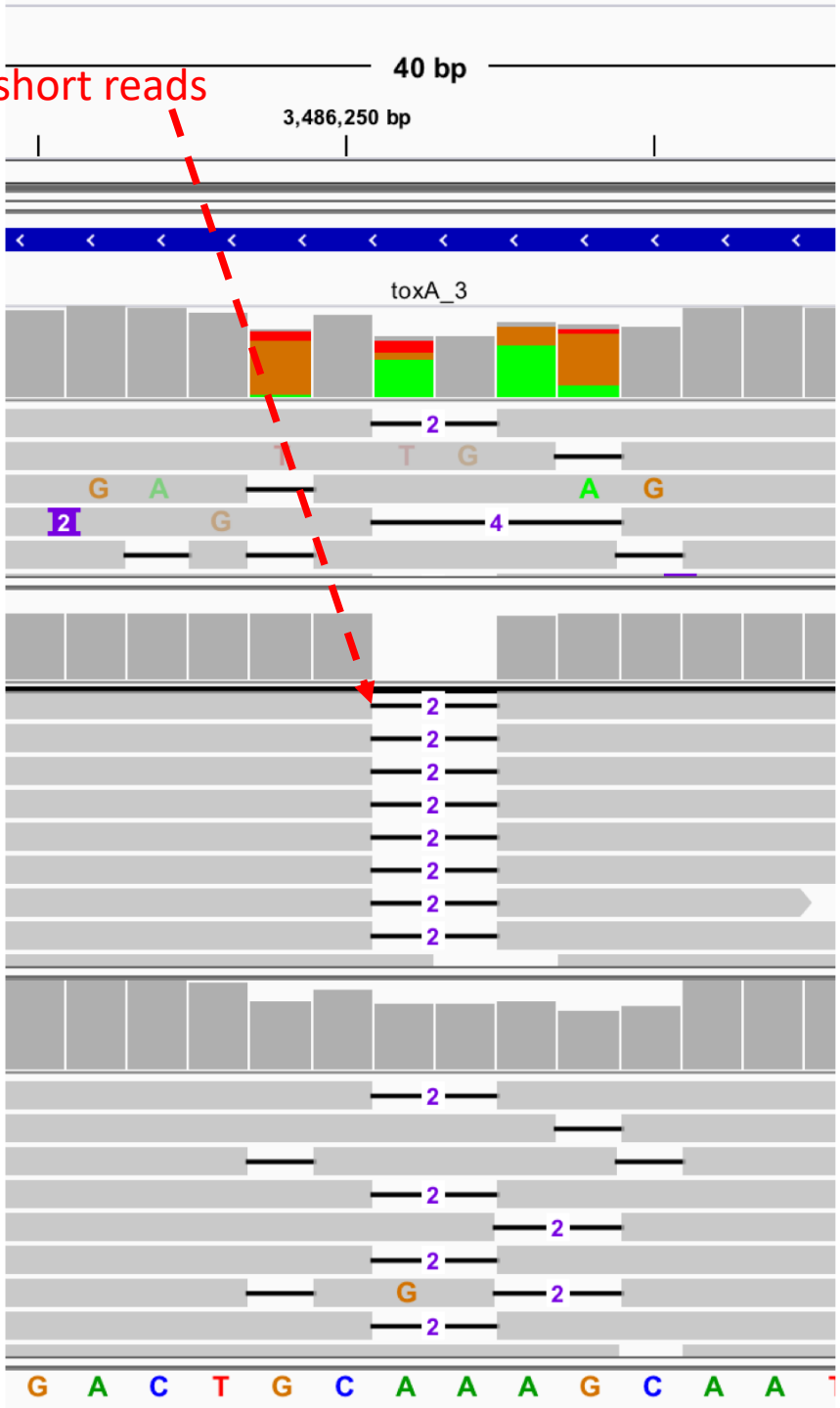
Score = 4680 bits (2534), Expect = 0.0
Identities = 2546/2551 (99%), Gaps = 3/2551 (0%)
Strand=Plus/Minus

Query 1      TCAATTACTTGCAATCCGACTGCAAAGCAATAGCATTATTAGGATTAAAGTAATA
            |||
Sbjct 5912   TCAATTACTTGCAATCCGACTGCA--GCAATAGCATTATTAGGATTAAAGTAATA
```



- strand: **AUG**

Conclusion: the assembly error in the consensus sequences caused the **frame shift**, which causing “stop codon” breaks the gene. Multiple rounds of Pilon should be able to polish the (hopefully).



barcode01 : draft2

