Current status of nanopore sequencing using the MinION device – from full length cDNA sequencing to genome assembly improvements

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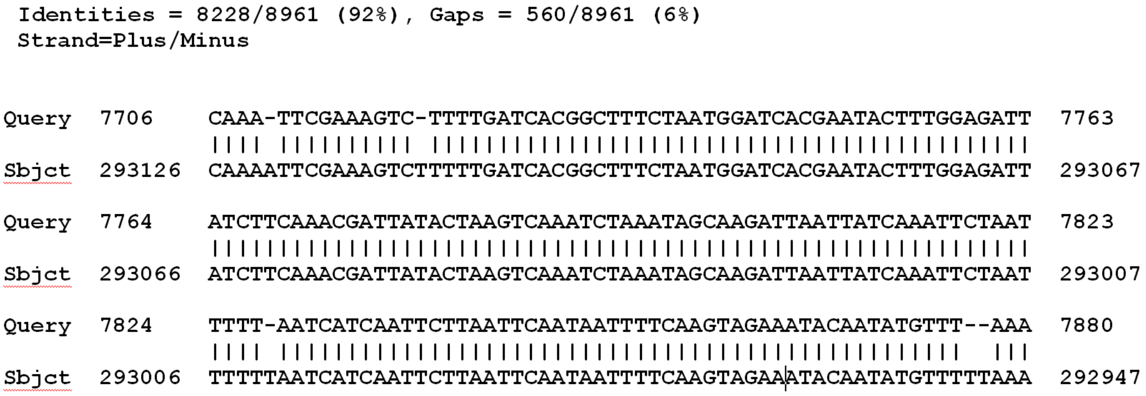
The MinION is a small device that may transform the way sequencing is done by taking the sequencer out of the core labs. It detects the current through nanopores as single DNA strands passes through in order to decode the sequence and is capable of generating sequences longer than any other platform currently on the market. The MinION is under active development in an early-access program (MAP). Like other single molecule sequencers, the accuracy of one-pass reads is lower than that of the amplification-based sequencers but through recent improvements to chemistry and basecalling it is now possible to generate long reads with over 90% alignment accuracy.

We are studing the transcription factor ZBED6, which is a repressor of IGF2 in muscle tissue. It is co-expressed with ZC3H11A through intron retention in a 13 kb transcript. From Illumina RNA-seq after knockout of ZBED6 we observe a drastic change of IGF2 expression, and also a potential shift in promotor usage. As a member of MAP I plan use the MinION to analyse isoforms of these transcripts from full-length cDNA sequencing, but given the nature of the reads it has a great potential also to be used for improvement of genome assemblies. As a first experiment to get a better understanding of the system and its reads I generated long genomic reads from the honeybee Apis Mellifera, which is used as a model organism in the lab and here I will describe these results and discuss the current status and future perspectives of nanopre sequencing in terms of data quality and throughput.



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 Identities = 8228/8961 (92%), Gaps = 560/8961 (6%)
 Strand=Plus/Minus


Query  7706    CAAA-TTCGAAAGTC-TTTTGATCACGGCTTTCTAATGGATCACGAATACTTTGGAGATT  7763
               |||| |||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  293126  CAAAATTCGAAAGTCTTTTTGATCACGGCTTTCTAATGGATCACGAATACTTTGGAGATT  293067

Query  7764    ATCTTCAAACGATTATACTAAGTCAAATCTAAATAGCAAGATTAATTATCAAATTCTAAT  7823
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  293066  ATCTTCAAACGATTATACTAAGTCAAATCTAAATAGCAAGATTAATTATCAAATTCTAAT  293007

Query  7824    TTTT-AATCATCAATTCTTAATTCAATAATTTTCAAGTAGAAATACAATATGTTT--AAA  7880
               |||| ||||||||||||||||||||||||||||||||||||||||||||||||||  |||
Sbjct  293006  TTTTTAATCATCAATTCTTAATTCAATAATTTTCAAGTAGAAATACAATATGTTTTTAAA  292947
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Figure 1. The MinION device is capable of sequencing long unamplified genomic samples with high accuracy. BLAST alignment of an 8.2 kb read shows that most errors are due to gaps from skipped bases.