

Putative modification detection in Direct RNA nanopore reads

We also noted a consistent U-to-C miscall in the ADGRE1 stop codon of the high confidence scaffolds (Supplementary Fig. 8b) and in the stop codon of all other reads from this study that aligned to ADGRE1 (Supplementary Fig. 8c and d). The same pattern was observed for direct nanopore reads from a prior study that aligned to longer GENCODE annotated ADGRE1 isoforms (Supplementary Fig. 8e)¹. This pattern is consistent with nanopore miscalls caused by the conversion of uridine to pseudouridine (ψ) at U516 in *E. coli* 16S rRNA². Base miscalls relative to canonical training data in our ADGRE1 aligned reads (Supplementary Fig. 8g) strongly suggest an unannotated pseudouridine in the stop codon, which is known to cause translation read through³. Importantly, this miscall was absent from *in vitro* transcribed nanopore direct RNA sequence data¹. Further experiments would be required to confirm this putative pseudouridine.

1. Workman, R. E. *et al.* Nanopore native RNA sequencing of a human poly(A) transcriptome. *Nat. Methods* **16**, 1297–1305 (2019).
2. Smith, A. M., Jain, M., Mulroney, L., Garalde, D. R. & Akeson, M. Reading canonical and modified nucleobases in 16S ribosomal RNA using nanopore native RNA sequencing. *PLoS One* **14**, e0216709 (2019).
3. Adachi, H. & Yu, Y.-T. Pseudouridine-mediated stop codon readthrough in *S. cerevisiae* is sequence context-independent. *RNA* vol. 26 1247–1256 (2020).