Supplementary Material

Human Auditory Ossicles as an Alternative Optimal Source of Ancient DNA

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Supplementary Note 1

Pilot Work

Five archaeological samples representing a range of geographic locations were selected for a pilot project aimed at obtaining initial insight into use of the ossicles for ancient DNA analysis. We chose samples based on their age and depositional contexts to represent a range of molecular preservation (sample information provided in Supplementary Table 1). All specimens had at least two ossicles, and one petrous pyramid from the same individual was selected for comparative analysis. Skeletal material was processed in dedicated ancient DNA clean rooms at University College Dublin following standard anti-contamination protocols (Hofreiter et al. 2001; Poinar 2003; Llamas et al. 2017). Petrous bones were processed as described in Pinhasi et al. (2019) to create bone powder, and complete auditory ossicles were decontaminated through exposure to UV irradiation for 10 minutes on each side and milled to fine powder. DNA extraction and library preparation followed standard ancient DNA protocols, described in the following section. All extraction and library preparation took place in a separate clean room from that used for processing bones and also followed standard anti-contamination protocols.

We generated raw sequencing data for this pilot work using low-coverage whole-genome shotgun sequencing on the Illumina MiSeq and NextSeq platforms. Data were processed using a custom bioinformatics pipeline to enable a basic comparison of endogenous DNA yield from the cochlea and from the auditory ossicles (Supplementary Table 1). Our results suggested that the auditory ossicles were approximately equivalent to the cochlea for endogenous DNA preservation, with the difference in endogenous DNA content ranging between a 0.17-fold decrease and a 0.3-fold increase (Supplementary Table 1). The endogenous DNA yields ranged from 0.16 to 68.19%, with a median of 54.68%, and no substantial difference between the ossicles and cochlea was detected (Supplementary Table 1). We identified damage patterns consistent with expectations for ancient DNA in the sequencing data generated using both the ossicle and cochlea samples, with an average substitution frequency on the 5'-end of the DNA molecule of 14.50% for the ossicle samples and 14.40% for the petrous bone samples (Supplementary Table 1). Like endogenous yield, this difference is not substantial. Overall similarity in endogenous yield and damage frequencies between the auditory ossicle and cochlea samples from the same individual supported our hypothesis that auditory ossicles may also be an effective substrate for ancient DNA analysis.

Supplementary Figure S1: Comparative DNA damage patterns between cochlea (“Co”) and ossicles (“Oss”) for each tested individual, based on deamination frequencies of terminal bases. Data for the 30 terminal bases of the 5’ (left panels) and 3’ (right panels) ends of the reads is shown.

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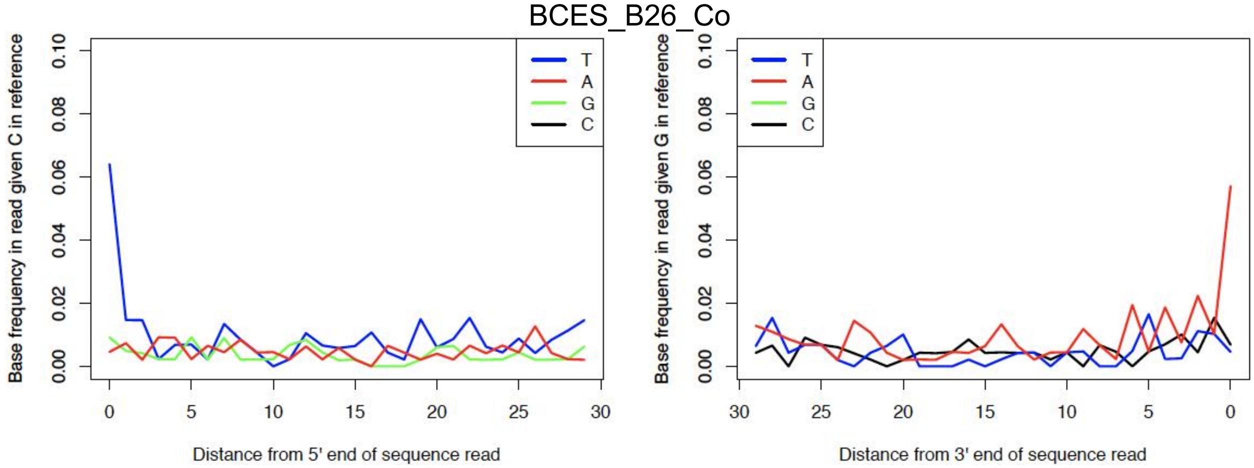

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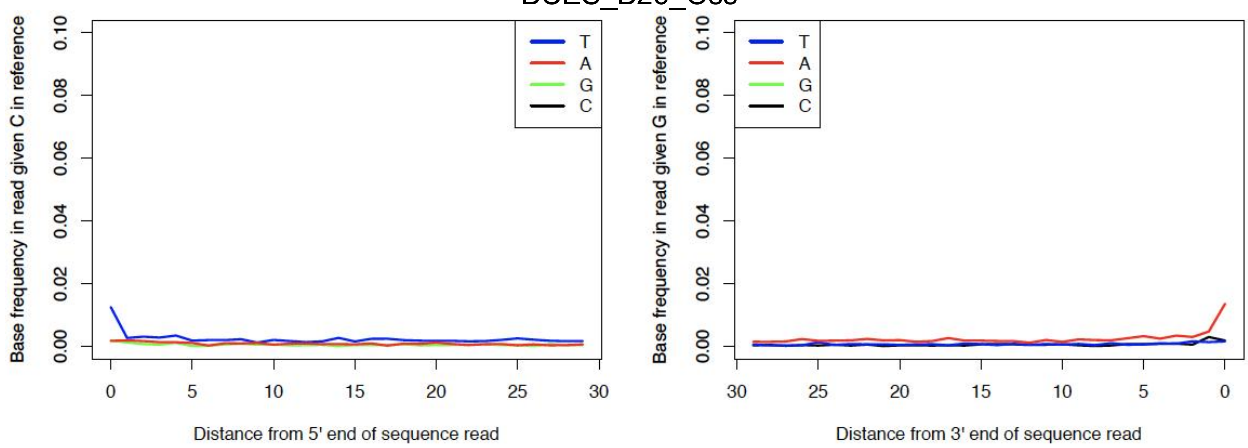

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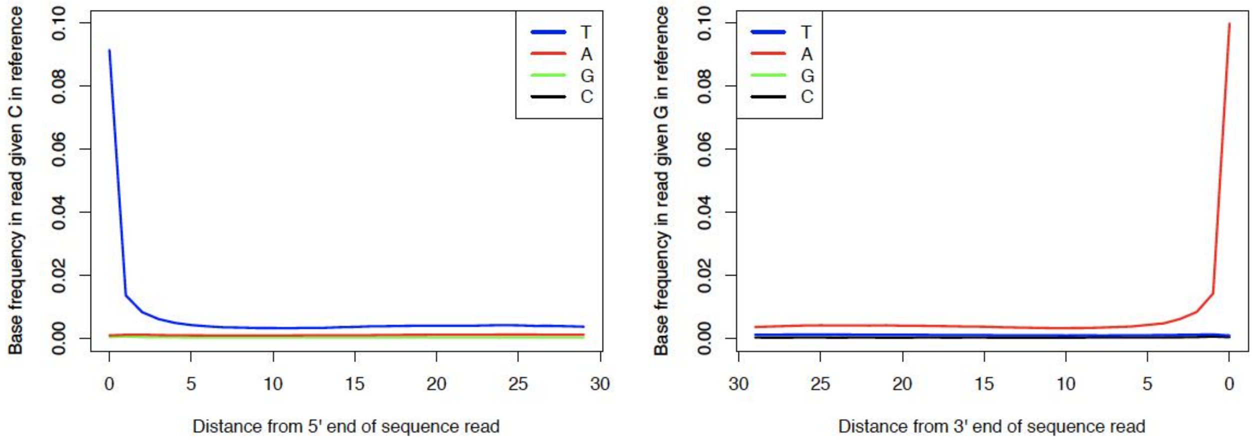

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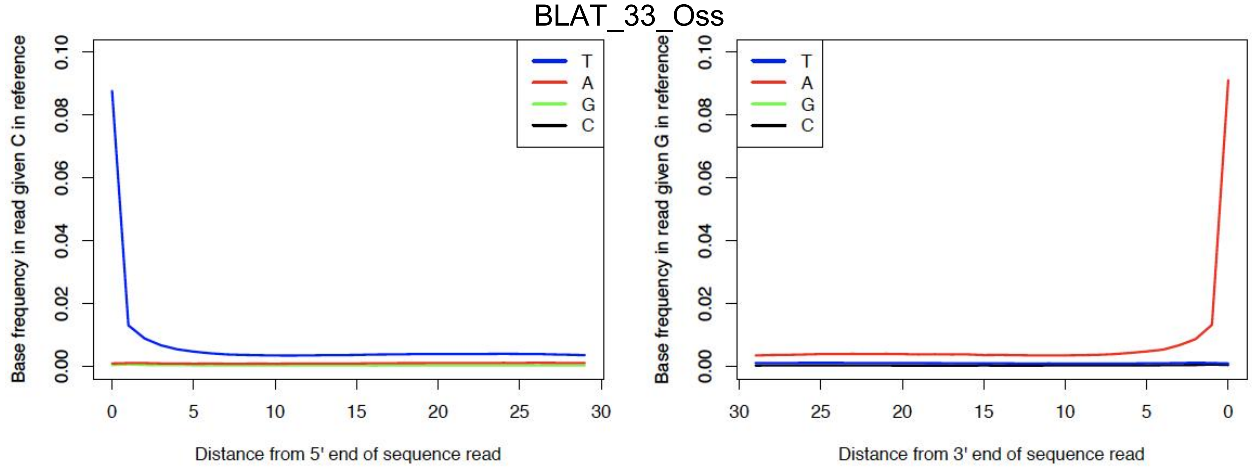

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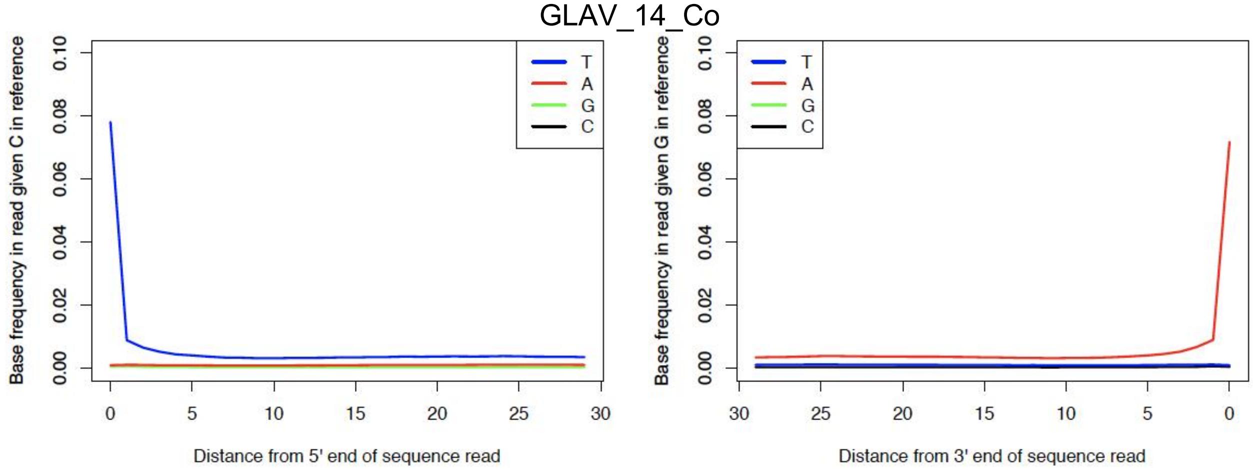

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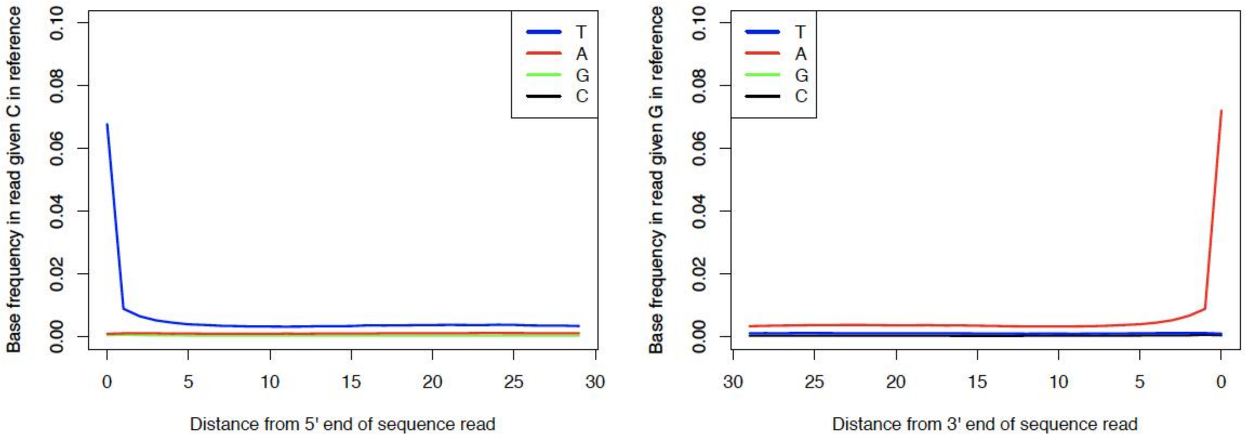

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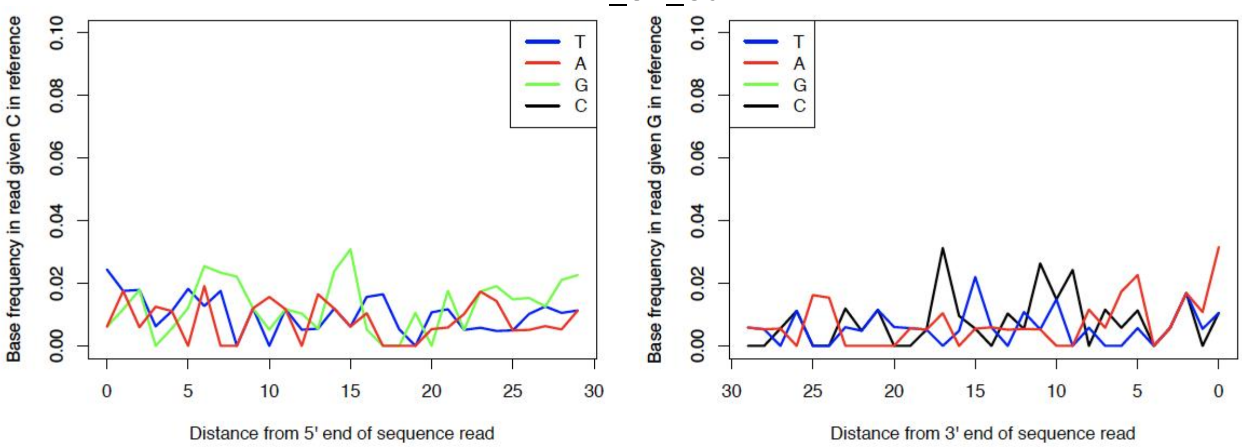

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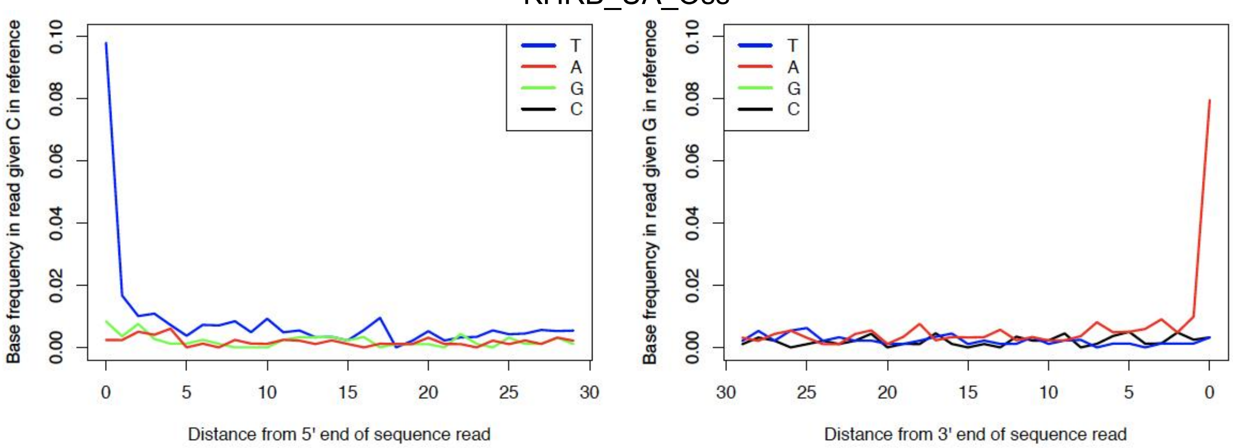

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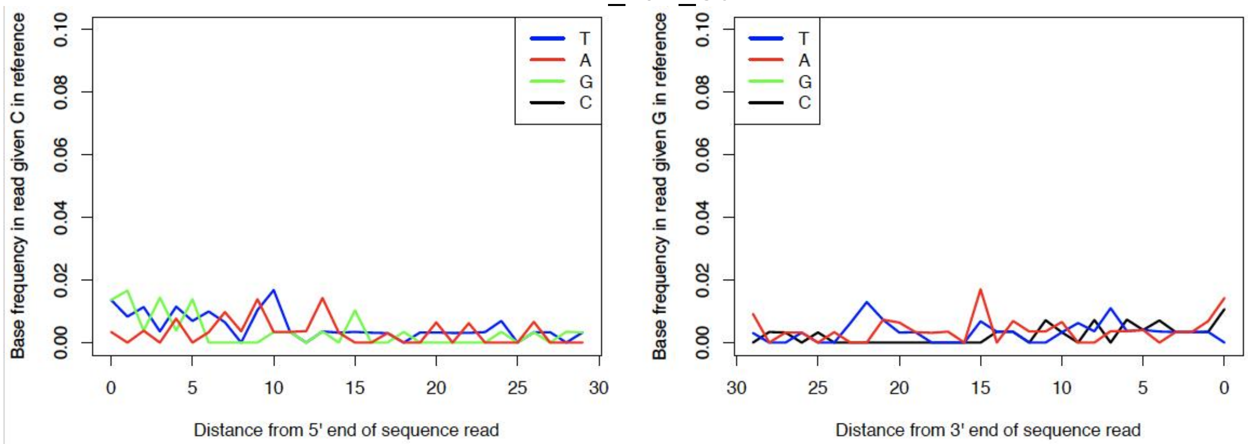

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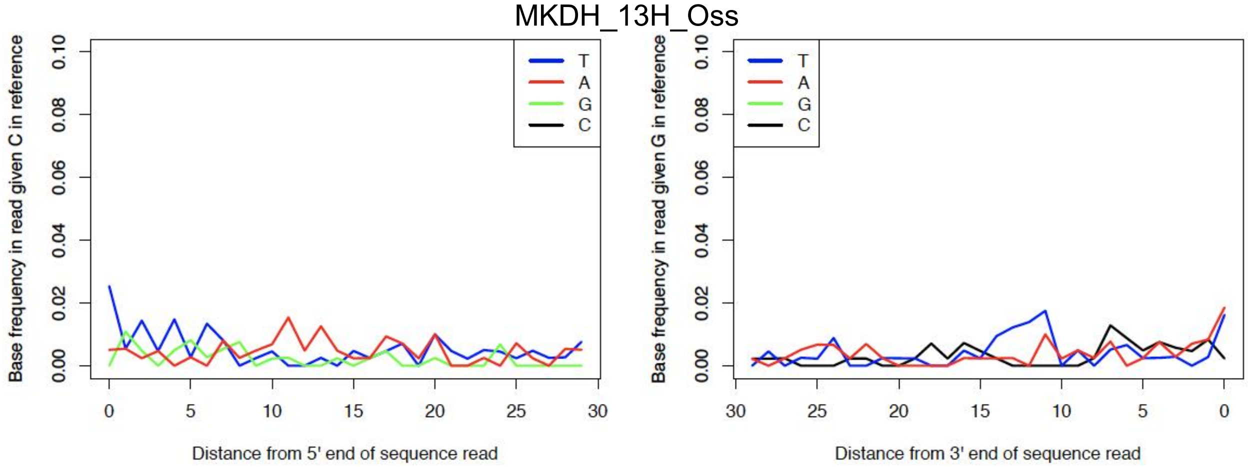

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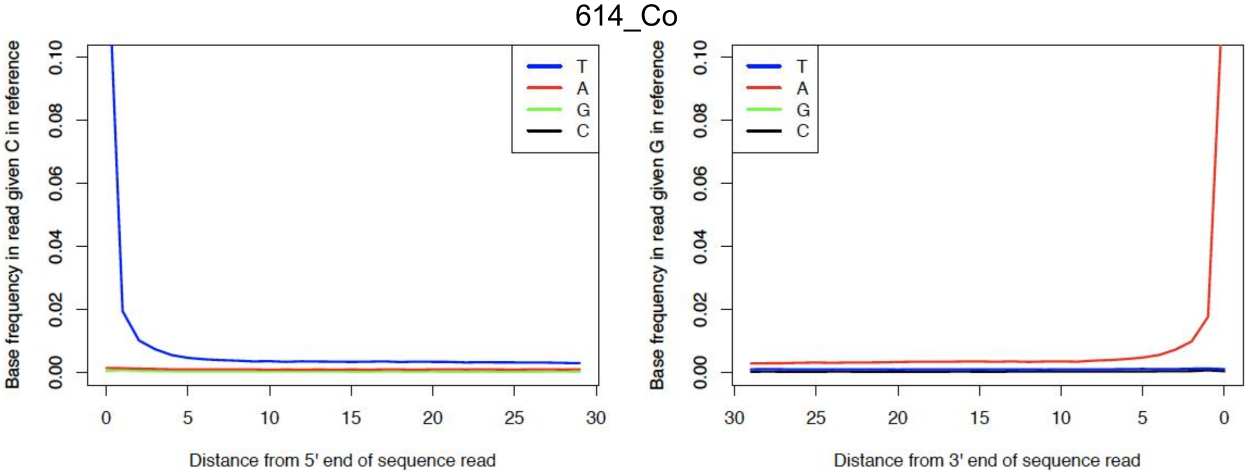

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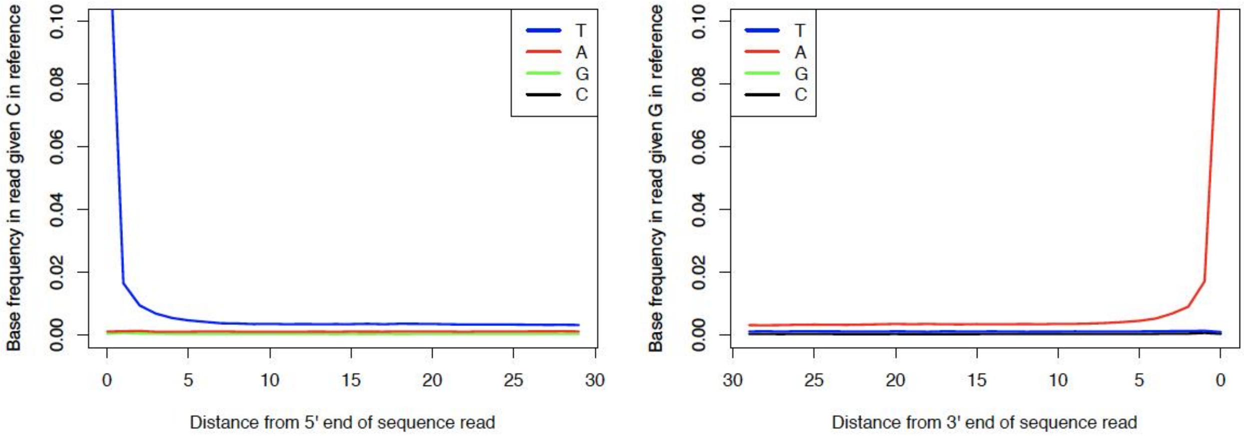

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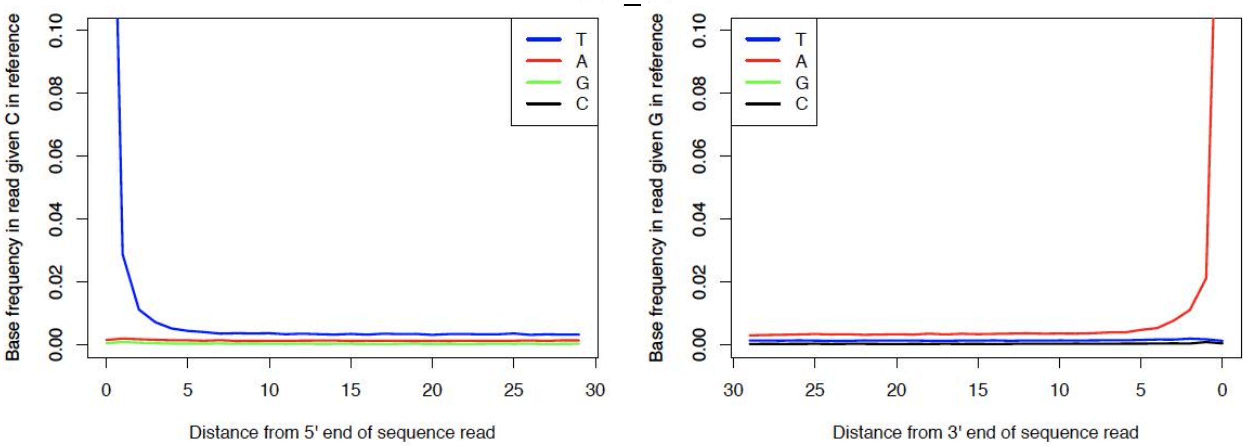

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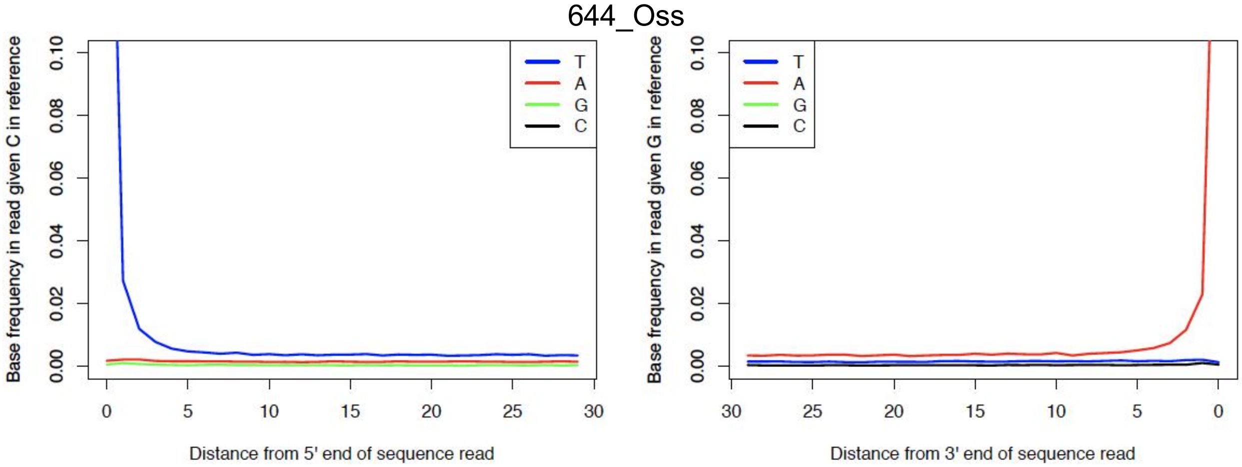

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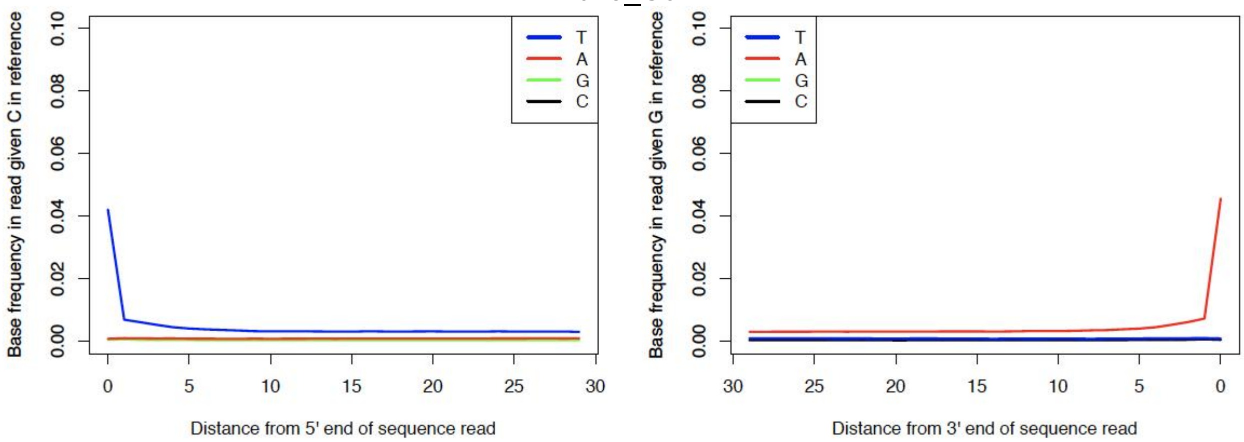

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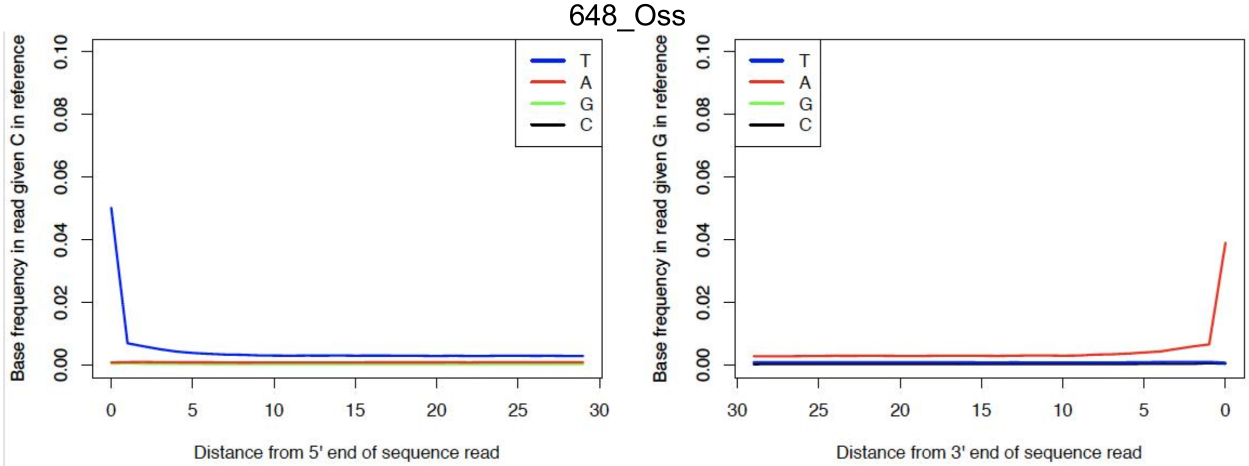

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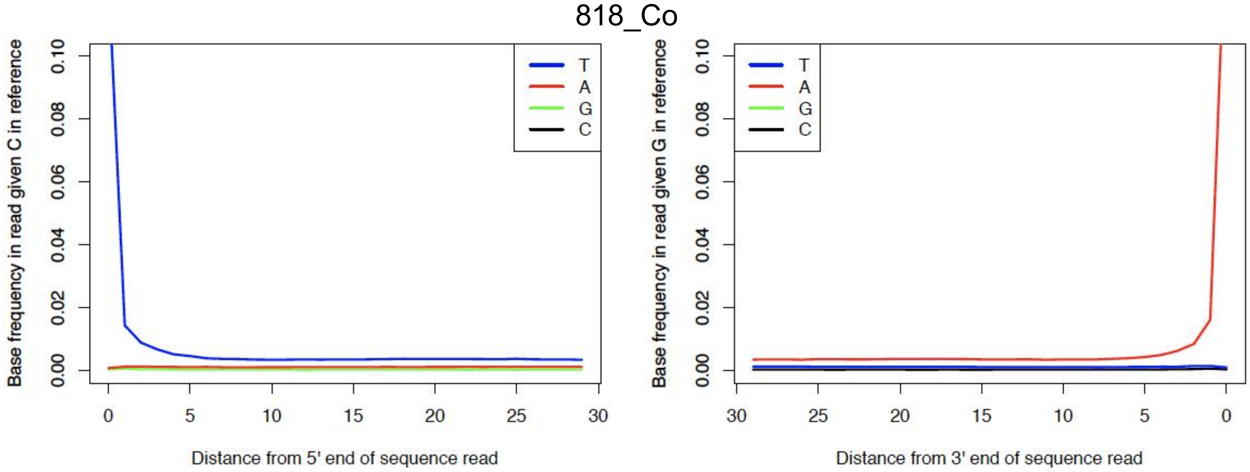

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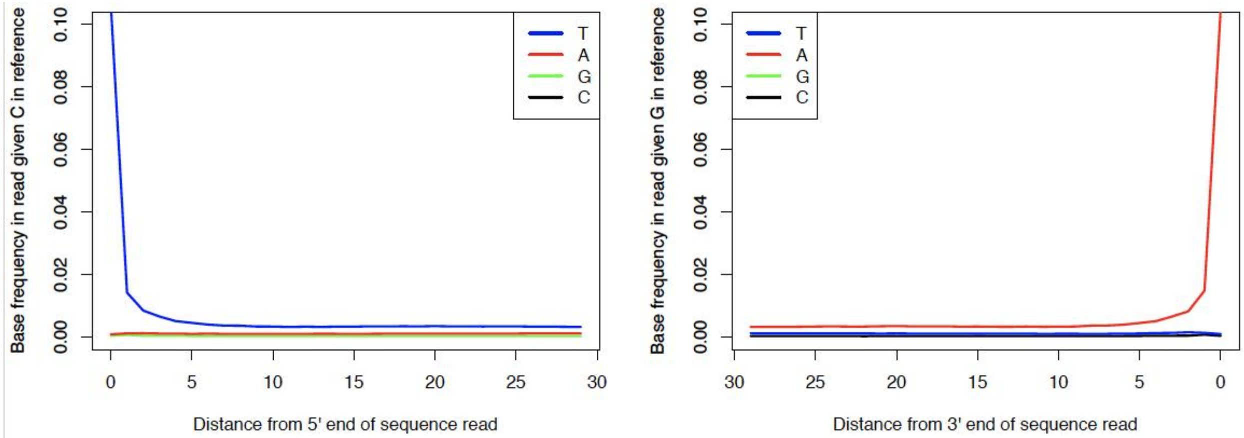

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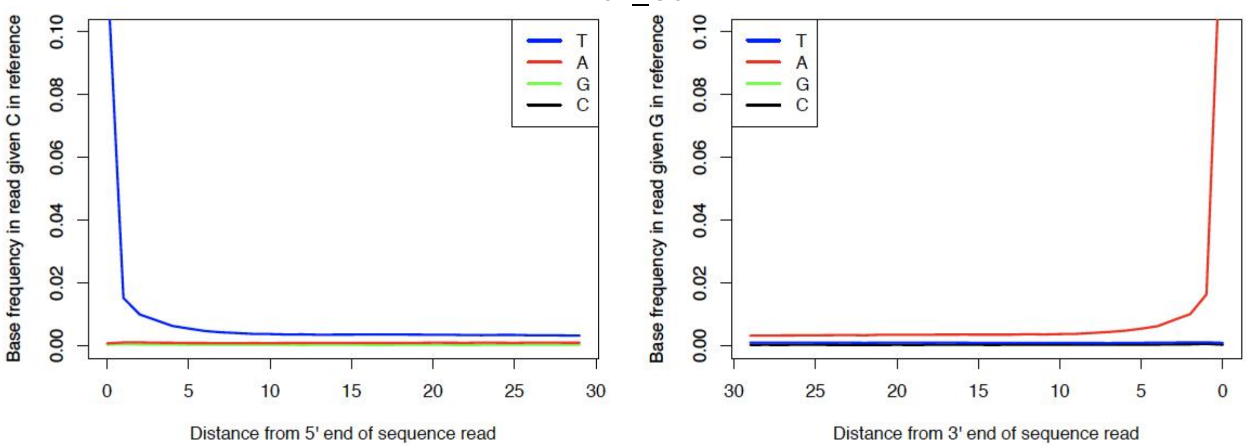

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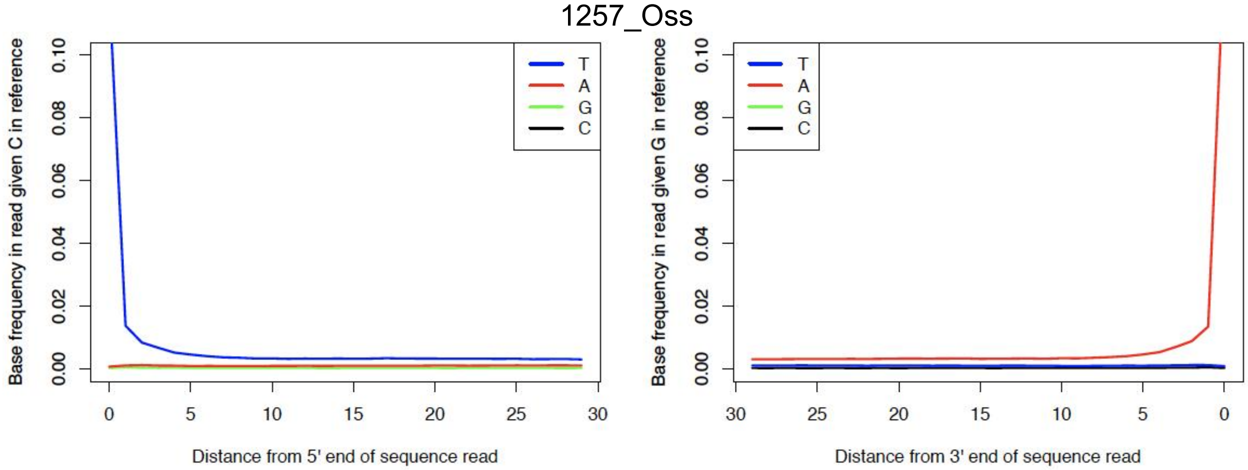

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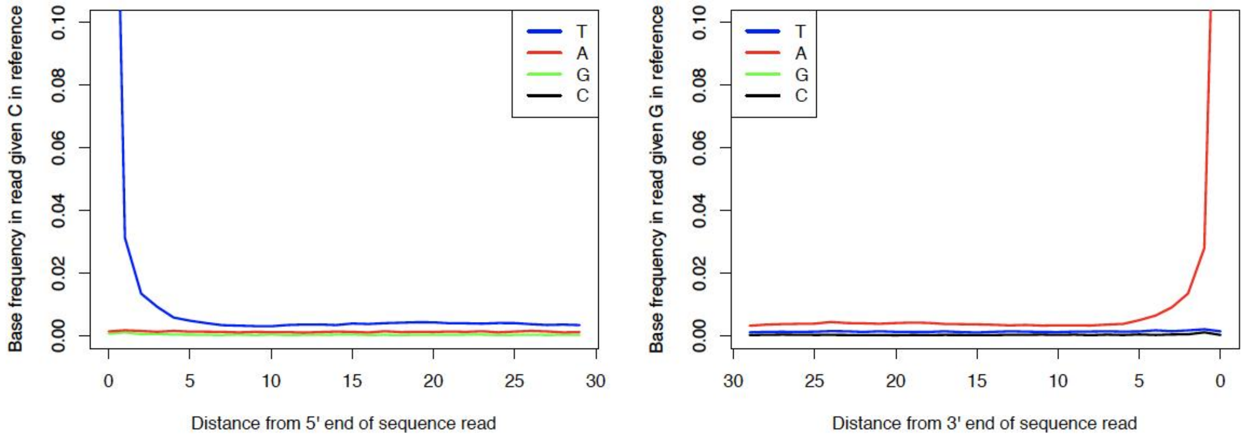

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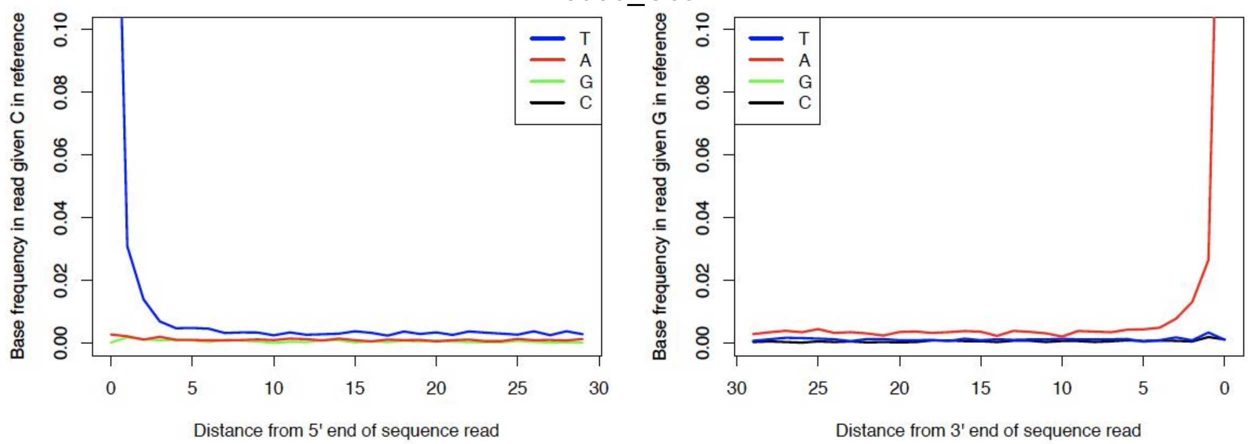

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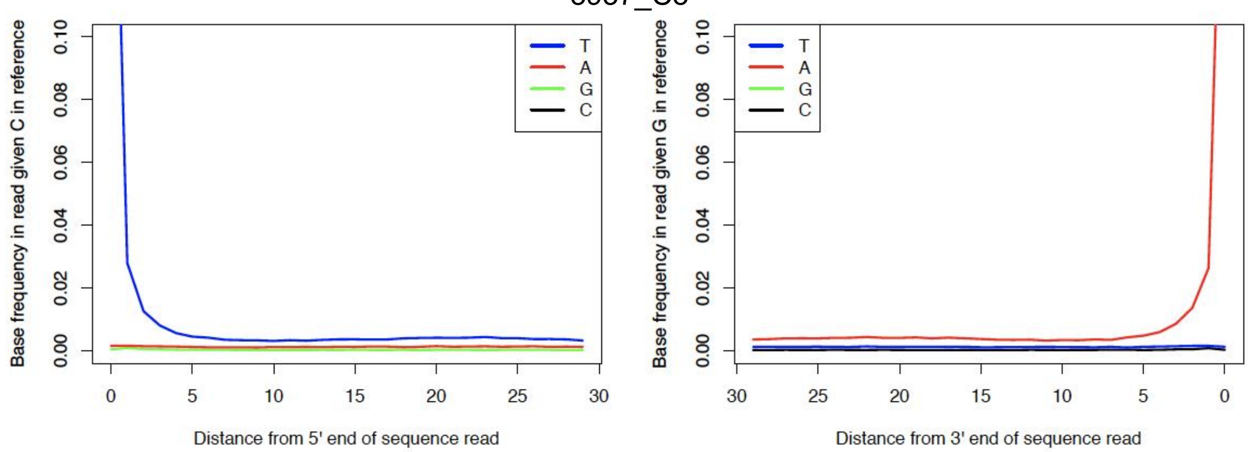

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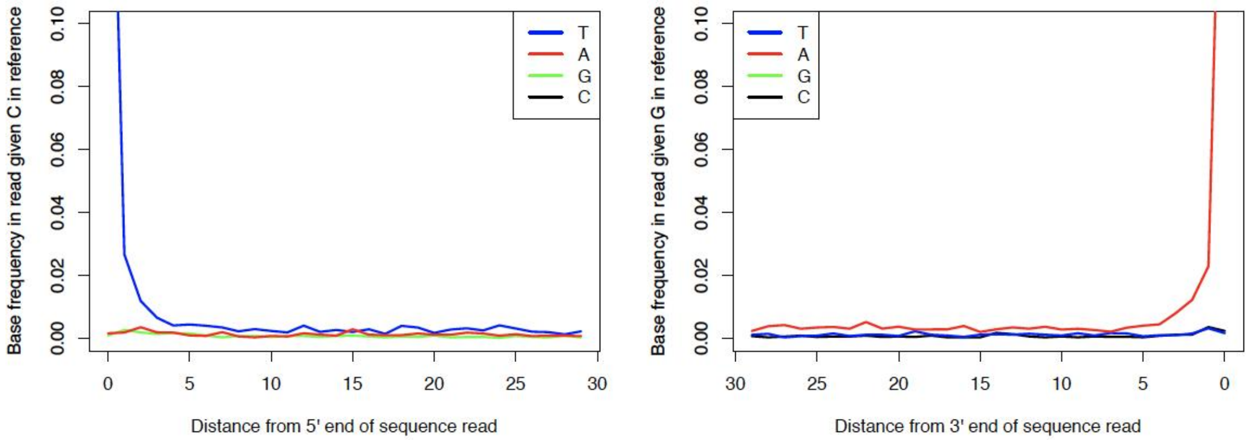

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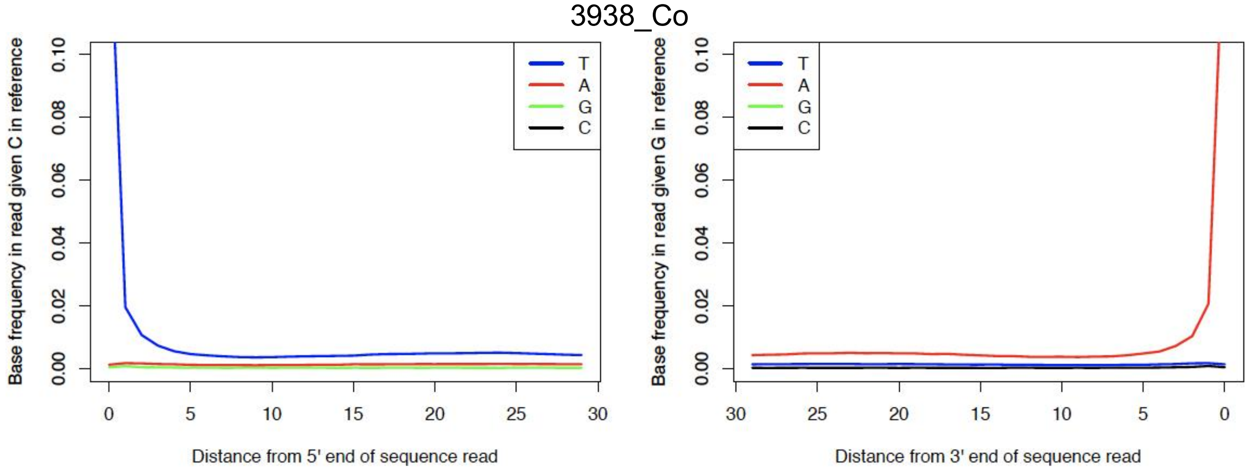

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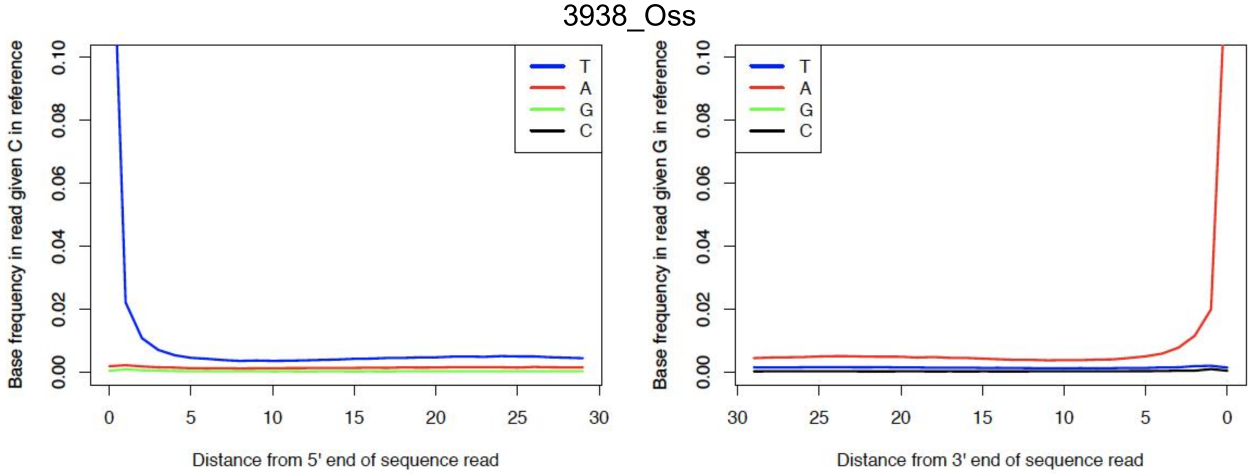

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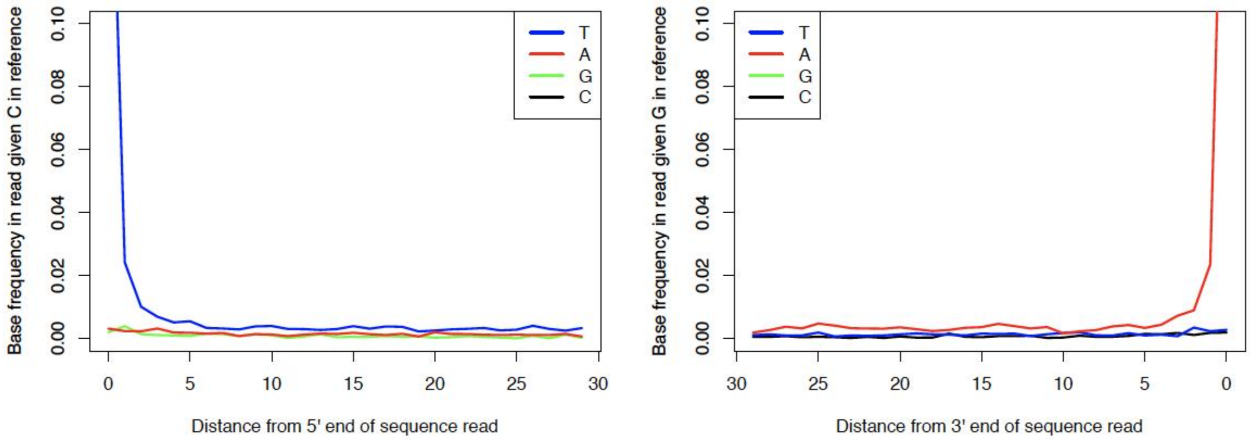

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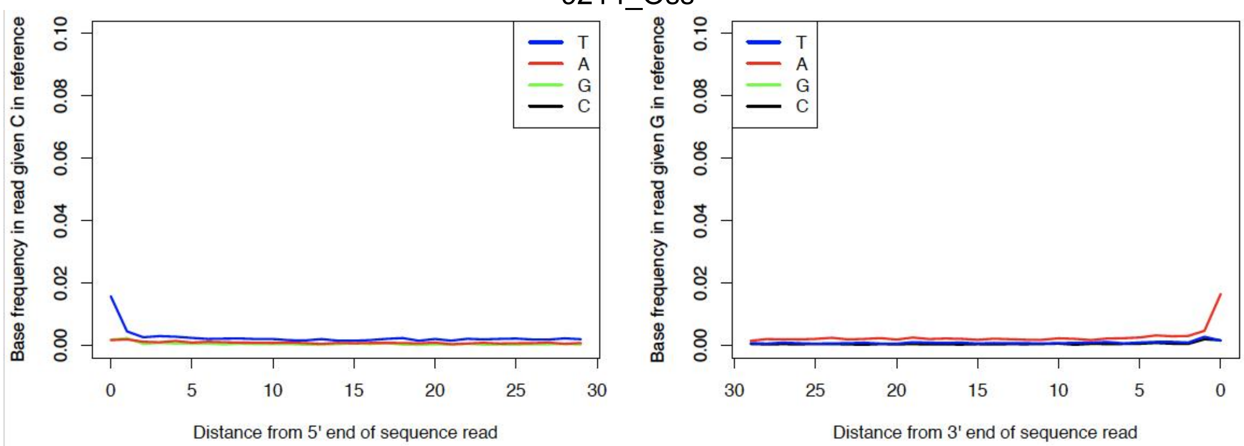

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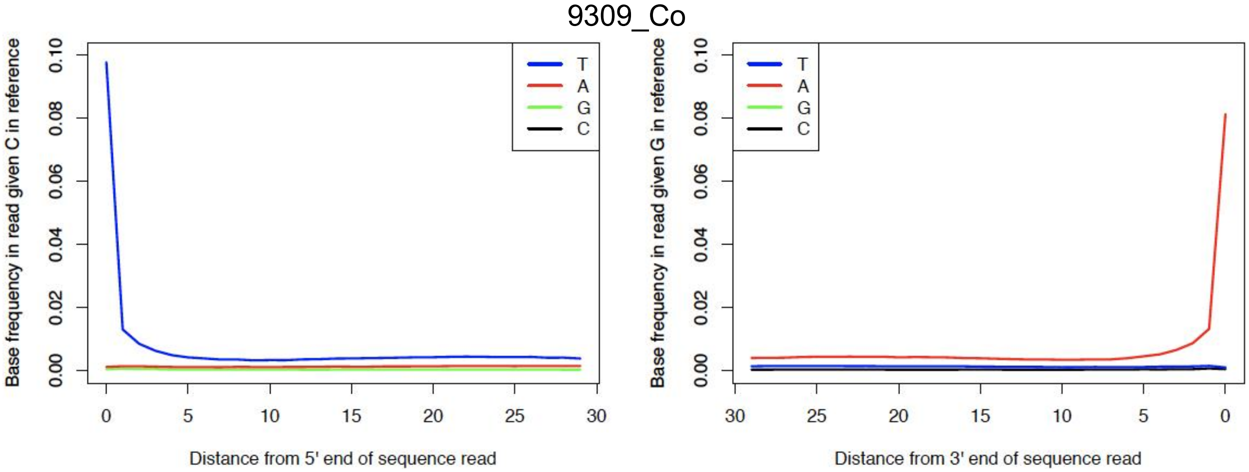

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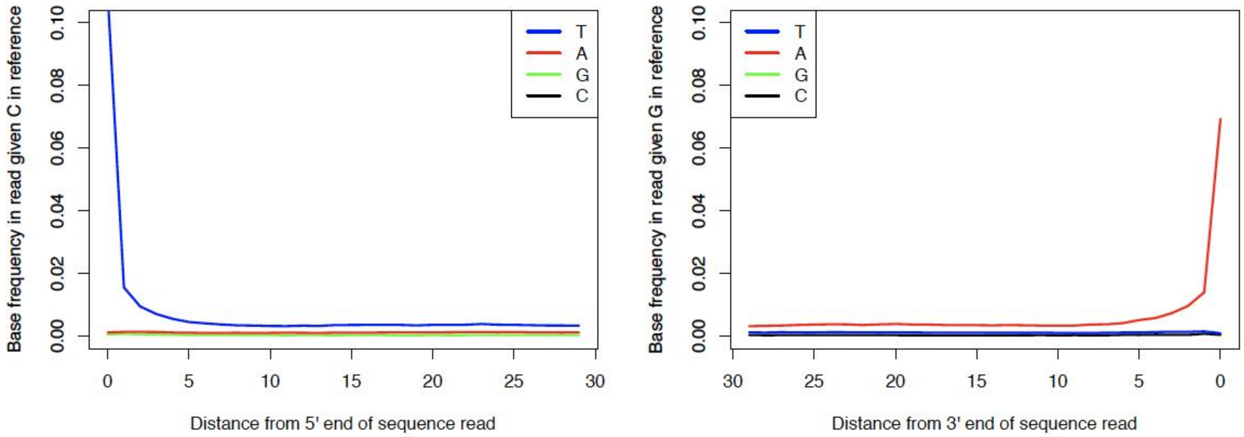

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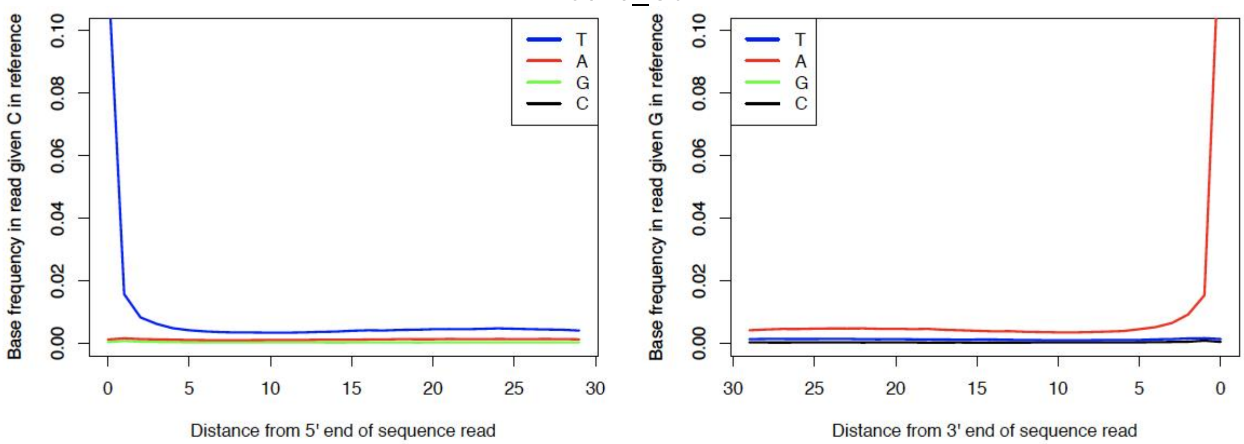

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