



Figure S10: DENT-seq signal at the single on-target site of D10A spCas9 nickase paired with a guide RNA targeting the *VEGFA* gene. This guide RNA was chosen as it has been tested with wild-type Cas9 in the development of genome-wide DSB detection methods (Digenome-seq, Kim et al. 2015; GUIDE-seq, Tsai et al. 2015; BLESS, Slaymaker et al. 2016; BLISS, Yan et al. 2017). We could not identify off-target nick sites using the same methods applied for the *AAVS1* gene without using *a priori* knowledge of expected Cas9 off-target sites for this guide RNA. This may be in part due to the repetitive nature of the genome at potential off-target *VEGFA* nick sites and previously documented off-target DSB sites. Cas-OFFinder was used to identify potential *VEGFA* off-targets by searching for all locations genome-wide with up to four mismatches to the guide RNA. Of these sites, 92.4% are determined to be in repetitive regions of the genome by RepeatMasker and Tandem Repeats Finder. For comparison, only 31.2% of sites are determined to be in repetitive regions when the same analysis is performed for the *AAVS1* guide RNA for which data is displayed in Fig. 6. Off-target nicking activity that occurs in a repetitive region of the genome may be more challenging to detect due to the difficulty of accurate mapping of short sequence reads in such regions, and could potentially limit some applications of DENT-seq. Nonetheless, the DENT-seq data showed some mutational signal at 10/49 published BLISS off-targets and 11/58 GUIDE-seq off-targets (5 of which are identified by both methods). 12 additional sites with four or fewer mismatches to the guide RNA also show DENT-seq mutational signal. **A)** The on-target site displays enrichment of sequence reads. Black dashed line represents nick site, gray-dashed lines represent the bounds of a MACS2 peak. **B)** Mutational signal within the MACS2 peak allows for detection of the on-target nick. Comparison of the mutational signal's location with the enrichment peak allows for proper strand identification. **C)** Close up of base calling just around the on-target nick, within the guide RNA binding region. The genomic sequence of the guide RNA binding region is shown in blue with the PAM in green at bottom.