

Contaminating DNA in Human Saliva Alters the Detection of Variants from Whole Genome Sequencing.

Samson CA, School of Biological Sciences, The University of Auckland, Auckland, New Zealand. csam488@aucklanduni.ac.nz

Whitford W, School of Biological Sciences, The University of Auckland, Auckland, New Zealand.

Snell RG, School of Biological Sciences, The University of Auckland, Auckland, New Zealand.

Jacobsen JC, School of Biological Sciences and Centre for Brain Research, The University of Auckland, Auckland, New

Zealand. j.jacobsen@auckland.ac.nz *

Lehnert K, School of Biological Sciences, The University of Auckland, Auckland, New Zealand.

*Corresponding author

Supplementary Table 1- Examples of anomalous variant calls which lead to the identification of atypically aligning reads. Variants displayed are derived from the alignment of internal sample A

| Chromosome | Position | Reference allele | Alternate Allele | Genotype | Allelic Depth Ratio (Ref,Alt) | GQ Score |
|------------|----------|------------------|--|----------|-------------------------------|----------|
| 1 | 1443328 | C | CCGCGTGCGCCGTGAGCGCATGGGTCACATCGACCTGGCCG | 0/1 | 30,3 | 78 |
| 1 | 3019209 | T | TCGTTTCGCCATGCACTCTGCCTATTTGCGCCATCTTATCAAAGGCGA GAGCGTCTTCTTGGTCAAAGACGGCGTTATCAAATTTAAGAACTTCAA GCGTA | 0/1 | 24,3 | 53 |
| 1 | 3431499 | G | GTACGCGCCTTCCTTAGCGAAAACCGCACGGACAGTACGCAGAAGAA TAATGAAGTCACCGGTAAGCGACCAGTTCTCAACATAGTAGAGATCC AGACGAACCGACTCTTCCAAGAGAGATCA | 0/1 | 25,3 | 49 |
| 1 | 4213486 | T | TCCGCACTGGCTCAATTTTTGGGCACAACGTTTGAATATCGCGATTAA AAAATCAGTGTGTTGAACCGGATATAAAAAGGCCGTCTGAAATCAAGG TTTCAGACGGCCTGCTATTTCAAATCAAGAAGGA | 0/1 | 29,11 | 99 |

| | | | | | | |
|---|---------|---|---|-----|-------|----|
| 1 | 4282539 | G | GCGCTACTGAGGGCAGCGTTTTTGCCGCCGTACGGATAATGCCGAG CACGTAGGCTCGCA | 0/1 | 31,6 | 99 |
| 1 | 4282543 | C | CGTTGA | 0/1 | 31,6 | 99 |
| 1 | 4282545 | C | CTCAATCATGTTCCGCCCGGGCTCAGTACTGACCCCTAGCTCTTTCGC G | 0/1 | 29,6 | 99 |
| 1 | 5509136 | A | ATCGCAGCCGCACCGGCTACGATTTCA | 0/1 | 24,3 | 53 |
| 1 | 5509138 | A | ATTCATTTGTGATACTTGCTTGACGAGCTTTGTTATACACCAACCGCA GATCATTAAATTAACCTGCAT | 0/1 | 23,3 | 53 |
| 1 | 6392150 | C | CATCGCCTCCCTCTTCGTTGAG | 0/1 | 18,3 | 67 |
| 1 | 6392152 | A | AGG | 0/1 | 19,3 | 69 |
| 1 | 6392155 | G | GCATTCCTGGTGGATGCGGACGCTATCTCCCGCTCCCTCATGGAGCCC GGCGAGGCGGTGCTCGCCCGCACGGTCGCGGAGTTCGGTGAGCA | 0/1 | 19,3 | 69 |
| 1 | 7830320 | T | TCGCGCTGGCTG | 0/1 | 28,12 | 99 |
| 1 | 7830322 | C | CCGTCGTTGACCTTGCCCGGCGCAATGGAGGG | 0/1 | 29,12 | 99 |
| 1 | 7830325 | T | TGGCATTTTTGTTGAAACCGGATTGGTCGTATTTGAAACCGGGTACG ATGCTGACTG | 0/1 | 31,12 | 99 |
| 1 | 8579657 | A | C | 0/1 | 24,3 | 54 |
| 1 | 8579660 | C | CAGCCTGGTTCAACAACACGAAGTCCGCTGGACTTGGGTAAAAGGCC ATGCCGGACACCCTGAAAACGAAAAGCCGACGAGCTCGCCAACCG AGGCGCAGCAAATTTTT | 0/1 | 24,3 | 54 |
| 1 | 8658064 | G | GTTTTTA | 0/1 | 36,11 | 99 |
| 1 | 8658066 | C | CGTGTTATTGCCCTA | 0/1 | 37,11 | 99 |
| 1 | 8658069 | T | TGAAAATCTTTTTACCATATTGGATATAATCCAA | 0/1 | 37,10 | 99 |
| 1 | 8658073 | C | A | 0/1 | 39,9 | 99 |
| 1 | 8658076 | C | CTTAA | 0/1 | 38,9 | 99 |
| 1 | 8658078 | C | CGCACTGCAAAATTACGAAATATATATGGCAACAACCAACAATTTCAA AATATT | 0/1 | 38,9 | 99 |

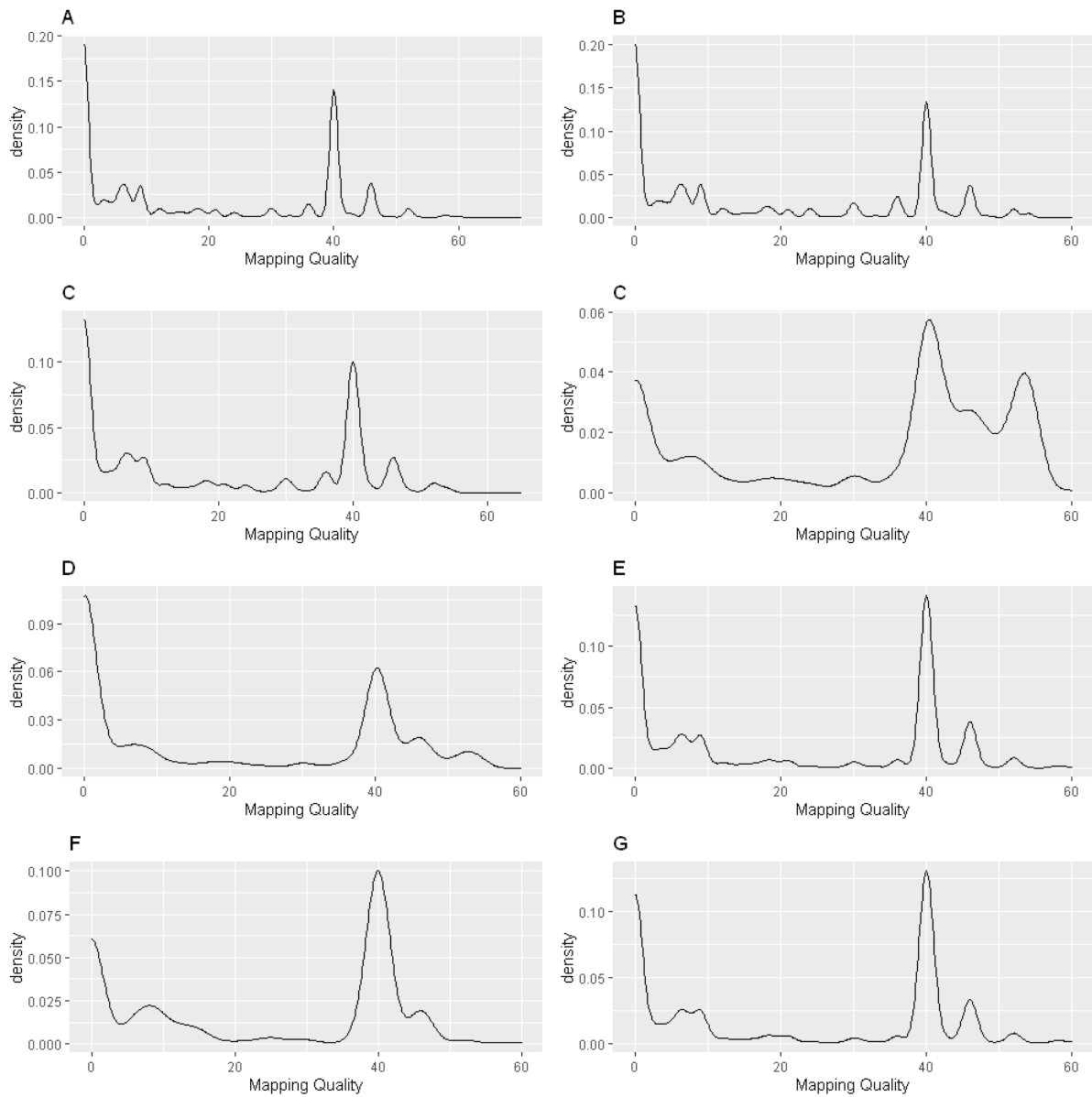
Supplementary Table 2- Top 77 genera used for decoy development

| Species | Number of Sequences Returning with Match | % of Submitted Sequences | Accession of Sequence(s) Used |
|--------------------|--|--------------------------|-------------------------------|
| Prevotella | 56417122 | 22.78228 | NZ_CP013195.1 |
| Neisseria | 24623277 | 9.943334 | NC_003112.2 |
| Streptococcus | 17799602 | 7.187808 | NC_015678.1 |
| Haemophilus | 11684583 | 4.718451 | NC_000907.1 |
| Veillonella | 8964212 | 3.619915 | ACIK02000001.2-ACIK02000025.2 |
| Rothia | 5967605 | 2.409829 | NC_013715.1 |
| Actinomyces | 5504468 | 2.222806 | NZ_CP012590.1 |
| Porphyromonas | 5503687 | 2.222491 | NC_010729.1 |
| Eubacterium | 3554132 | 1.435224 | NZ_CP012068.1 |
| Fusobacterium | 3470611 | 1.401497 | NC_003454.1 |
| Campylobacter | 3267852 | 1.319619 | NZ_CP012541.1 |
| Granulicatella | 2538844 | 1.025232 | NZ_KI391971 |
| Atopobium | 2138896 | 0.863726 | NC_013203.1 |
| Gemella | 2052068 | 0.828663 | NZ_CP014233.1 |
| Leptotrichia | 1945440 | 0.785605 | CP014231.1 |
| Oribacterium | 1515533 | 0.612 | ACKX01000001.1-ACKX01000254.1 |
| Capnocytophaga | 1515252 | 0.611887 | NZ_CP012589.1 |
| Solobacterium | 1506910 | 0.608518 | AECQ01000001.1-AECQ01000066.1 |
| Selenomonas | 1380182 | 0.557343 | NZ_CP012071.1 |
| Lautropia | 1010801 | 0.40818 | AEQP01000001.1-AEQP01000030.1 |
| Megasphaera | 875359 | 0.353486 | AECS01000001.1-AECS01000049.1 |
| Peptoniphilus | 812443 | 0.32808 | PNGX01000001.1-PNGX01000004.1 |
| Achromobacter | 481071 | 0.194265 | NZ_LN831029.1 |
| Aggregatibacter | 462896 | 0.186926 | NZ_CP012067.1 |
| Corynebacterium | 454905 | 0.183699 | NZ_LN831026.1 |
| Candidatus | 363746 | 0.146887 | NZ_CP007496.1 |
| Lachnospiraceae | 264750 | 0.106911 | NBBL01000001.1-NBBL01000017.1 |
| Catonella | 236983 | 0.095698 | ACIL03000001.3-ACIL03000026.3 |
| Eikenella | 152940 | 0.06176 | NHRS01000001.1-NHRS01000006.1 |
| Treponema | 139123 | 0.05618 | NZ_CP009228.1 |
| Cardiobacterium | 139052 | 0.056152 | FKLO01000001.1-FKLO01000088.1 |
| Kingella | 125553 | 0.050701 | AEWV01000001.1-AEWV01000057.1 |
| Tannerella | 108155 | 0.043675 | CP017038.1 |
| Lactobacillus | 55357 | 0.022354 | NC_013198.1 |
| Abiotrophia | 51159 | 0.020659 | ACIN03000001.3-ACIN03000020.3 |
| Peptostreptococcus | 46552 | 0.018799 | FOVA01000001.1-FOVA01000060.1 |
| Dialister | 45854 | 0.018517 | GG698602.1 |

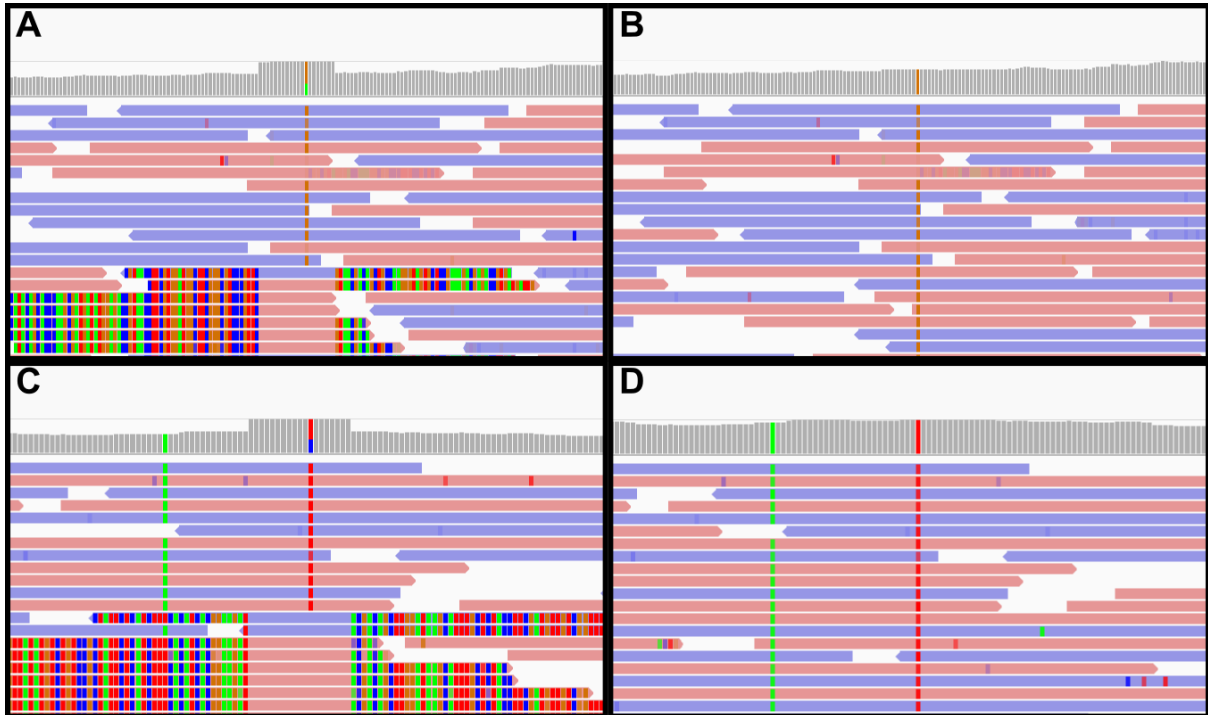
| | | | |
|---------------------|-------|----------|-----------------------------------|
| Lachnoanaerobaculum | 35132 | 0.014187 | AEPW01000001.1- AEPW01000150.1 |
| Propionibacterium | 33231 | 0.013419 | NC_006085.1 |
| Parvimonas | 19673 | 0.007944 | NZ_CP009761.1 |
| Bacteroides | 17869 | 0.007216 | BAIU01000001.1-BAIU01000095.1 |
| Mogibacterium | 17277 | 0.006977 | JALU01000001.1-JALU01000030.1 |
| Centipeda | 16449 | 0.006642 | AFHQ01000001.1-AFHQ01000072.1 |
| Pseudomonas | 16277 | 0.006573 | NC_002516.2 |
| Bacteroidetes | 15771 | 0.006369 | NVWV01000001.1- NVWV01000022.1 |
| Mycoplasma | 15486 | 0.006254 | NC_000912.1 |
| Simonsiella | 14083 | 0.005687 | ADCY02000001.2-ADCY02000082.2 |
| Klebsiella | 12982 | 0.005242 | NC_016845.1 |
| Enterococcus | 11871 | 0.004794 | NC_004668.1 |
| Actinobaculum | 10155 | 0.004101 | AWSB01000001.1- AWSB01000117.1 |
| Staphylococcus | 8968 | 0.003621 | NC_004461.1 |
| Finegoldia | 8754 | 0.003535 | NC_010376.1 |
| Mitsuokella | 7349 | 0.002968 | ABWK02000001.2- ABWK02000031.2 |
| Johnsonella | 7300 | 0.002948 | ACZL01000001.1-ACZL01000080.1 |
| Eggerthia | 7203 | 0.002909 | ACZL01000001.1-ACZL01000080.1 |
| Bifidobacterium | 7003 | 0.002828 | NZ_AP012324.1 |
| Bulleidia | 6923 | 0.002796 | ADFR01000001.1-ADFR01000016.1 |
| Olsenella | 6791 | 0.002742 | CP012069.2 |
| Peptostreptococceae | 6427 | 0.002595 | AWVB01000001.1- AWVB01000160.1 |
| Filifactor | 6208 | 0.002507 | NC_016630.1 |
| Escherichia | 5763 | 0.002327 | NC_002695.1 |
| Bacillus | 4668 | 0.001885 | NC_003997.3 |
| Shuttleworthia | 4517 | 0.001824 | ACIP02000001.2-ACIP02000010.2 |
| Gardnerella | 4261 | 0.001721 | NC_014644.1 |
| Lactococcus | 4257 | 0.001719 | NC_002662.1 |
| Eggerthella | 4138 | 0.001671 | AGEJ01000001.1 |
| Stenotrophomonas | 3691 | 0.00149 | NC_010943.1 |
| Listeria | 3284 | 0.001326 | NC_003210.1 |
| Scardovia | 3261 | 0.001317 | AKCI00000000.1 |
| Anaerococcus | 3033 | 0.001225 | NC_013171.1 |
| Lysinibacillus | 2993 | 0.001209 | NZ_CP010820.1 |
| Anaeroglobus | 2816 | 0.001137 | AGCJ01000001.1-AGCJ01000103.1 |
| Gracilibacteria | 2812 | 0.001136 | PDRB01000001.1-PDRB01000010.1 |
| Moraxella | 2745 | 0.001108 | NZ_CP014234.1 |
| Enterobacter | 2597 | 0.001049 | NZ_CP025225.1 |
| Desulfobulbus | 2594 | 0.001048 | NZ_CP021255.1 |
| Bordetella | 2586 | 0.001044 | NC_002929.2 |

Supplementary Table 3- Species representing at least 2% of BLAST results for atypically aligning reads

| Species | Number of Sequences Returning with Match | % of Submitted Sequences | Accession of Sequence(s) Used |
|---------------------------------|---|---------------------------------|--------------------------------------|
| <i>Prevotella jejuni</i> | 69100 | 25.22634 | NZ_CP023863.1- NZ_CP023864.1 |
| <i>Prevotella intermedia</i> | 35311 | 12.89099 | NZ_CP019300.1- NZ_CP019301.1 |
| <i>Haemophilus influenzae</i> | 15001 | 5.476416 | NC_000907.1 |
| <i>Capnocytophaga sputigena</i> | 9521 | 3.475832 | NZ_CP022385.1 |
| <i>Prevotella scopos</i> | 8342 | 3.045415 | NZ_CP016204.1- NZ_CP016206.1 |
| <i>Rothia aeria</i> | 6759 | 2.467509 | AP017895.1 |
| <i>Neisseria elongata</i> | 5914 | 2.159025 | NZ_CP007726 |



Supplementary Figure 1 – The distribution of mapping qualities of atypically aligning reads. For all samples density peaks are seen in the distributions as 0 and 40. The distributions of internal sample A (A), internal sample B (B), internal sample C (C) ERX1462737 (D), ERX1462740 (E), SRX2830689 (F), SRX2830684 (G), and SRX2830689 (H) are shown.



Supplementary Figure 2 – Examples of SNVs altered by the presence of atypical read pairs. A heterozygous, non-coding, SNV (A>G) in internal sample B (A) is called as homozygous when the atypical pairs are removed (B). The same effect is observed in SRX2830689 for a coding SNV (C-D).