

Supplementary Material

MitoH3: Mitochondrial Haplogroup and Homoplasmic/Heteroplasmic Variant Calling Pipeline for Alzheimer's Disease Sequencing Project

Supplementary Table 1. Sum of inconsistent ratios of variants among technical replicates of each subject at different variant allele fraction (VAF) thresholds.

| VAF 1_99% | Number of homoplasmic variants | | | | | | | | | | | | | | | Sum of inconsistent ratios |
|--------------|--------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----------------------------------|
| | 11 | 12 | 16 | 22 | 23 | 24 | 29 | 36 | 37 | 38 | 41 | 42 | 78 | 79 | 80 | |
| Subject 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 1.6 |
| Subject 2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 3.8 |
| Subject 3 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 4.4 |
| Subject 4 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 6.8 |
| Subject 5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 4.7 |
| Subject 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 1 | 0.5 |

| VAF 1_99% | Number of heteroplasmic variants | | | | | | | | | | | | | Sum of inconsistent ratios |
|--------------|----------------------------------|---|---|---|---|---|---|----|----|----|----|----|----|----------------------------------|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 10 | 15 | 17 | 25 | 28 | 29 | |
| Subject 1 | 1 | 4 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 |
| Subject 2 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 3.8 |
| Subject 3 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 4.6 |
| Subject 4 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 7 |
| Subject 5 | 0 | 0 | 0 | 1 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 5.3 |
| Subject 6 | 0 | 1 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.3 |

| VAF 2_98% | Number of homoplasmic variants | | | | | | | | | Sum of inconsistent ratios |
|--------------|--------------------------------|----|----|----|----|----|----|----|----|----------------------------------|
| | 14 | 17 | 19 | 35 | 37 | 38 | 42 | 79 | 80 | |
| Subject 1 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Subject 2 | 0 | 1 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 2.2 |
| Subject 3 | 1 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 2.6 |
| Subject 4 | 0 | 0 | 1 | 1 | 0 | 4 | 0 | 0 | 0 | 3.7 |
| Subject 5 | 0 | 0 | 0 | 0 | 1 | 0 | 5 | 0 | 0 | 0.8 |
| Subject 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0.2 |

| VAF 2_98% | Number of heteroplasmic variants | | | | | | | | | | Sum of inconsistent ratios |
|--------------|----------------------------------|---|---|---|---|---|---|----|----|----|----------------------------------|
| | 0 | 1 | 2 | 3 | 4 | 5 | 8 | 21 | 22 | 23 | |
| Subject 1 | 1 | 4 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 1.4 |
| Subject 2 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2.2 |
| Subject 3 | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2.7 |
| Subject 4 | 0 | 1 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 3.8 |
| Subject 5 | 0 | 0 | 0 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 1.5 |
| Subject 6 | 0 | 1 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |

| VAF 3_97% | Number of homoplasmic variants | | | | | | | Sum of inconsistent ratios |
|--------------|--------------------------------|----|----|----|----|----|----|----------------------------------|
| | 23 | 36 | 37 | 38 | 42 | 79 | 80 | |
| Subject 1 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Subject 2 | 0 | 1 | 8 | 0 | 0 | 0 | 0 | 0.1 |
| Subject 3 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Subject 4 | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 2.5 |
| Subject 5 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| Subject 6 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0.2 |

| VAF 3_97% | Number of heteroplasmic variants | | | | | | | Sum of inconsistent ratios |
|--------------|----------------------------------|---|---|---|---|---|----|----------------------------------|
| | 0 | 1 | 2 | 3 | 4 | 5 | 17 | |
| Subject 1 | 1 | 4 | 0 | 4 | 0 | 0 | 0 | 1 |
| Subject 2 | 0 | 0 | 8 | 1 | 0 | 0 | 0 | 0.1 |
| Subject 3 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subject 4 | 0 | 1 | 4 | 0 | 0 | 0 | 1 | 2.7 |
| Subject 5 | 0 | 0 | 0 | 3 | 2 | 1 | 0 | 0.7 |
| Subject 6 | 0 | 1 | 5 | 0 | 0 | 0 | 0 | 0.2 |

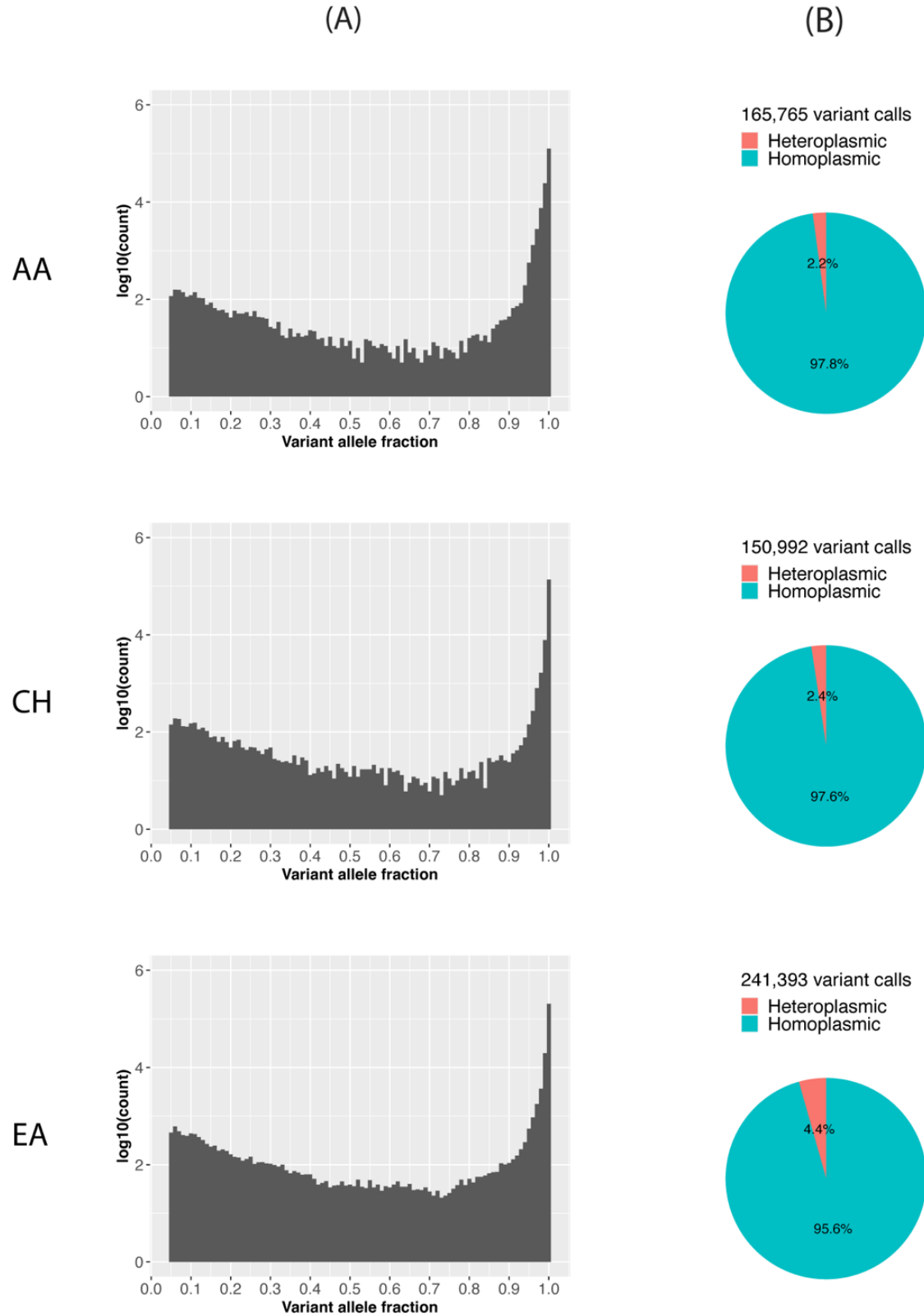
| VAF 4_96% | Number of homoplasmic variants | | | | | | Sum of inconsistent ratios |
|--------------|--------------------------------|----|----|----|----|----|----------------------------------|
| | 29 | 37 | 38 | 42 | 79 | 80 | |
| Subject 1 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Subject 2 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Subject 3 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Subject 4 | 1 | 0 | 5 | 0 | 0 | 0 | 1.5 |
| Subject 5 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| Subject 6 | 0 | 0 | 0 | 0 | 5 | 1 | 0.2 |

| VAF 4_96% | Number of heteroplasmic variants | | | | | | | Sum of inconsistent ratios |
|--------------|----------------------------------|---|---|---|---|---|----|----------------------------------|
| | 0 | 1 | 2 | 3 | 4 | 5 | 11 | |
| Subject 1 | 1 | 4 | 0 | 4 | 0 | 0 | 0 | 1 |
| Subject 2 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Subject 3 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subject 4 | 0 | 1 | 4 | 0 | 0 | 0 | 1 | 1.7 |
| Subject 5 | 0 | 0 | 0 | 3 | 2 | 1 | 0 | 0.7 |
| Subject 6 | 0 | 1 | 5 | 0 | 0 | 0 | 0 | 0.2 |

| VAF 5_95% | Number of homoplasmic variants | | | | Sum of inconsistent ratios |
|--------------|--------------------------------|----|----|----|----------------------------------|
| | 37 | 38 | 42 | 80 | |
| Subject 1 | 9 | 0 | 0 | 0 | 0 |
| Subject 2 | 9 | 0 | 0 | 0 | 0 |
| Subject 3 | 9 | 0 | 0 | 0 | 0 |
| Subject 4 | 1 | 5 | 0 | 0 | 0.2 |
| Subject 5 | 0 | 0 | 6 | 0 | 0 |
| Subject 6 | 0 | 0 | 0 | 6 | 0 |

| VAF 5_95% | Number of heteroplasmic variants | | | | | | Sum of inconsistent ratios |
|--------------|----------------------------------|---|---|---|---|---|----------------------------------|
| | 0 | 1 | 2 | 3 | 4 | 5 | |
| Subject 1 | 1 | 4 | 4 | 0 | 0 | 0 | 0.6 |
| Subject 2 | 0 | 6 | 3 | 0 | 0 | 0 | 0.6 |
| Subject 3 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subject 4 | 0 | 5 | 1 | 0 | 0 | 0 | 0.2 |
| Subject 5 | 0 | 0 | 0 | 3 | 2 | 1 | 0.7 |
| Subject 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |

Supplementary Figure 1. A) Variant allele fraction (VAF) distribution of each ethnic group. B) Summary of the proportion of heteroplasmic calls and homoplasmic calls by ethnic group.



Supplementary Figure 2. Stratified by ethnic group. A) The proportion of variants of different plasmity status. B) The number of variants detected in different allele frequencies strata. C) The proportion of variants of different plasmity status in different allele frequencies strata.

