

To illustrate the application of VEST, a subset of mutations individuals with Freeman-Sheldon Syndrome published by Shendure *et al.* are pre-formatted in transcript coordinates and made available for download at http://www.karchinlab.org/VEST_example.tmps. The subset of mutations are derived from the top scoring mutations and represent mutations observed in 3 individuals; since some mutations were observed in multiple individuals, they appear more than once in the list.

The following command line will score the mutations in the supplied dataset:
`cd $VESTDIR
./RunVest VEST_example.tmps`

VEST scores for the example dataset are shown in the Table below. An extra column with gene symbol has been added for illustrative purposes. In this example, we want to identify candidate mutations that might underlie Freeman Sheldon Syndrome based on 3 afflicted individuals. Here VEST provides a ranking of mutations based on predicted functional consequences for protein activity. The example mutations are ranked here based on VEST score. Genes harboring strong functional mutations in all 3 individuals may be good candidates for causal genes. In this case, mutations in MYH3 have the highest VEST scores across all individuals and in this case, MYH3 is indeed the causal gene.

Table 1 VEST scores for selected mutations from 3 disease exomes

HUGO Symbol	Mutation ID	Mutation	VEST score	p-value	BHFDR
AGPHD1	14331	NM_001013619.2_R312L	0.997	0.0000	0.05
MYH3	15671	NM_002470.3_R672C	0.994	0.0001	0.05
HAO1	17539	NM_017545.2_A244D	0.983	0.0008	0.05
PYGM	8566	NM_001164716.1_G361R	0.981	0.0012	0.05
BLVRB	13316	NM_000713.2_S111L	0.981	0.0012	0.05
MYH3	14828	NM_002470.3_R672H	0.977	0.0018	0.05
MYH3	11842	NM_002470.3_R672H	0.977	0.0018	0.05
NDST2	7573	NM_003635.3_L702F	0.976	0.0021	0.05
CDC27	16096	NM_001256.3_L27P	0.971	0.0030	0.05
ARHGAP5	13489	NM_001173.2_M901T	0.971	0.0030	0.05
RAB27B	15847	NM_004163.4_R80W	0.963	0.0042	0.05
GCDH	17293	NM_013976.3_R402W	0.957	0.0053	0.05
ABCC8	10391	NM_000352.3_T1508P	0.955	0.0059	0.05
MTHFR	110	NM_005957.4_A222V	0.955	0.0059	0.05
CDC27	16097	NM_001256.3_F26S	0.954	0.0059	0.05
CFTR	8030	NM_000492.3_M952T	0.953	0.0063	0.05
WDFY3	3809	NM_014991.4_C1751G	0.952	0.0064	0.05
SPG7	15338	NM_003119.2_A510V	0.952	0.0064	0.05
PDE4DIP	1104	NM_001002810.3_R25L	0.951	0.0065	0.05
PDE4DIP	1057	NM_001002810.3_R25L	0.951	0.0065	0.05
PDE4DIP	880	NM_001002810.3_R25L	0.951	0.0065	0.05
MAP2K3	15761	NM_002756.4_R65L	0.948	0.0070	0.05
SYNJ2	7324	NM_003898.3_R208H	0.946	0.0075	0.05
DHRS9	2625	NM_001142271.1_D286H	0.946	0.0075	0.05
POLG	14416	NM_001126131.1_G268A	0.942	0.0083	0.05
ACADS	12859	NM_000017.2_G209S	0.940	0.0086	0.05
ACADS	12141	NM_000017.2_G209S	0.940	0.0086	0.05

ACADS	9620	NM_000017.2_G209S	0.940	0.0086	0.05
OR51L1	10715	NM_001004755.1_G18V	0.938	0.0091	0.05
KRT75	11793	NM_004693.2_A161T	0.936	0.0097	0.05
SPAG5	15805	NM_006461.3_M1V	0.936	0.0097	0.05
EIF2B1	12191	NM_001414.3_N208Y	0.922	0.0126	0.05
PDE11A	2667	NM_001077196.1_Y283C	0.762	0.0569	0.10
SLC5A10	11886	NM_001270649.1_G435E	0.687	0.0815	0.10
PDE7A	8579	NM_002603.3_G50E	0.487	0.1681	0.20
PHKB	14929	NM_001031835.2_Y763C	0.452	0.1875	0.25
MME	4255	NM_007287.2_N689K	0.449	0.1889	0.25
FMO2	1472	NM_001460.2_S195L	0.439	0.1943	0.25
LRP2	2187	NM_004525.2_G669D	0.383	0.2281	0.25
FBN1	14115	NM_000138.4_C472Y	0.299	0.2995	0.30
FBN1	13375	NM_000138.4_C472Y	0.299	0.2995	0.30
FBN1	10671	NM_000138.4_C472Y	0.299	0.2995	0.30