Nonsense or Frameshift

- Predicted to undergo NMD: Stop codon 5’ of p.Pro1850 → Exon is present in biologically-relevant transcript NM_177438.2 → PVS1
- Not predicted to undergo NMD: Stop codon 3’ of p.Pro1850 → Role of region in protein function is unknown → LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript NM_177438.2 → PVS1_Moderate

GT--AG 1,2 splice sites
(If applied, PP3 not to be used in combination)

- Exon skipping or use of a cryptic splice site disrupts reading frame and is predicted to undergo NMD: Stop codon 5’ of p.Pro1850 → Exon is present in biologically-relevant transcript NM_177438.2 → PVS1
- Exon skipping or use of a cryptic splice site disrupts reading frame and is **NOT** predicted to undergo NMD: Stop codon 3’ of p.Pro1850 → Truncated/altered region is critical to protein function: includes any residue that merits PM1 (p.S1344, p.E1705, p.D1709, p.D1713, p.G1809, p.D1810, p.E1813) → PVS1_Strong
  - Role of region in protein function is unknown → LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript NM_177438.2
    - Variant removes >10% of protein (i.e. ≥193 AAs) → PVS1_Strong
    - Variant removes <10% of protein: exon 27 (final exon) SAS → PVS1_Moderate
- Exon skipping or use of a cryptic splice site preserves reading frame (exons 5, 10, 15, 18, 22) → Role of region in protein function is unknown → LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript NM_177438.2
  - Variant removes >10% of protein (exon 10 SDS/SAS) → PVS1_Strong
  - Variant removes <10% of protein (exons 5, 15, 18, 22 SDS/SAS) → PVS1_Moderate

Deletion
(Single exon to full gene)

- Full gene deletion → PVS1
- Single to multi exon deletion – Disrupts reading frame and is predicted to undergo NMD: Stop codon 5’ of p.Pro1850 → Exon is present in biologically-relevant transcript NM_177438.2 → PVS1
- Single to multi exon deletion – Disrupts reading frame and is **NOT** predicted to undergo NMD: Stop codon 3’ of p.Pro1850 → Truncated/altered region is critical to protein function: includes any residue that merits PM1 (p.S1344, p.E1705, p.D1709, p.D1713, p.G1809, p.D1810, p.E1813) → PVS1_Strong
  - Role of region in protein function is unknown → LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript NM_177438.2
    - Variant removes >10% of protein (i.e. ≥193 AAs) → PVS1_Strong
    - Variant removes <10% of protein (i.e. <193 AAs) → PVS1_Moderate
- Single to multi exon deletion – Preserves reading frame → Truncated/altered region is critical to protein function: includes any residue that merits PM1 (p.S1344, p.E1705, p.D1709, p.D1713, p.G1809, p.D1810, p.E1813) → PVS1_Strong

Duplication
(≥1 exon in size and must be completely contained within gene)

- Proven in tandem → Reading frame disrupted and NMD predicted to occur (Stop codon 5’ of p.Pro1850) → PVS1
- Presumed in tandem → No or unknown impact on reading frame and NMD → N/A
- Presumed in tandem → Reading frame presumed disrupted and NMD predicted to occur (Stop codon 5’ of p.Pro1850) → PVS1_Strong
- Proven not in tandem → N/A

Initiation Codon

- Different functional transcript uses alternative start codon → N/A