**Generation of temp\_file1**

VCF file

VariantFiltration

(*--clusterSize 2* and *--clusterWindowSize 3*)

SelectVariants

(FILTER SnpCluster)

Annotation by SnpEff

Selecting variants records with SILENT, MISSENSE and NONSENSE SnpEff’s Functional Classes

**Generation of temp\_file2**

Merging ALT2 Codons to produce ALT\_merge-2VAR,

Correcting Amino Acid Change (AA\_change-2VAR)

Merging ALT3 Codons to produce ALT\_merge-3VAR,

Correcting Amino Acid Change (AA\_change-3VAR)

**Generation of temp\_file3**

**Generation of temp\_file4**

List of clustered SNVs if new AA change is not matched with any of the individual SNV AA change

**Generation of OUTPUT file**

List of clustered SNVs if any of the sample has no reference homozygous

Read counts extraction of SNVs codons form BAM file by an in-house Accessory Script\*

Figure S1. MACARON workflow showing the generation of temporary files (temp\_fileN) and the final OUTPUT txt file. \*The input of Accessory Script is a subset of BAM file covering 50 bps around candidate clustered SNVs.

Selection of clustered SNVs affecting their respective codons