Is variant present in amplicon replicate?

- **I**
  - **Filtered reads**
  - Cluster identical reads
    - yes | no
    - Clusters
    - Singletons
    - sort Clusters according to frequency
    - Cluster1, Cluster2, Cluster3, ..., (end)
    - putative artifact
    - Run step I for all amplicons before step II

- **IIa**
  - 1-2 bp diff
  - Is variant a chimera?
    - yes | no
    - find most similar within more frequent clusters
    - Chimera
    - 1-2 bp diff
    - >2 bp diff
    - putative allele
    - unclassified
    - variant
    - Start each analysis from Cluster2. If needed information is missing, finish step III and re-run until all clusters are classified

- **IIb**
  - >2 bp diff
  - Is variant frequency lower than any putative artifact?
    - yes | no
    - putative artifact
    - Chimera
    - run step II a,b,c for all amplicons before step III

- **IIc**
  - Chimera
  - Is variant present in other replicates?
    - yes | no
    - putative allele
    - unclassified
    - variant

- **IIIa**
  - 1-2 bp diff
  - Is variant present in amplicon replicate?
    - yes | no
    - Is variant frequency lower than any putative artifact?
      - yes | no
      - putative artifact
      - Chimera
      - 1-2 bp diff
      - >2 bp diff
      - putative allele
      - unclassified
      - variant
      - Start each analysis from Cluster2. If needed information is missing, finish step III and re-run until all clusters are classified

- **IIIb**
  - >2 bp diff
  - Is variant present in amplicon replicate?
    - yes | no
    - Is variant frequency lower than any putative artifact or unclassified in other individuals?
      - yes | no
      - putative artifact
      - Chimera
      - run step II a,b,c for all amplicons before step III

- **IIIc**
  - Chimera
  - Is variant present in other replicates?
    - yes | no
    - putative allele
    - unclassified
    - variant
    - Start each analysis from Cluster2. If needed information is missing, finish step III and re-run until all clusters are classified