

Detection and analysis of Cocoa Swollen Shoot Virus by Capture Sequencing – a universal method



Research and Development

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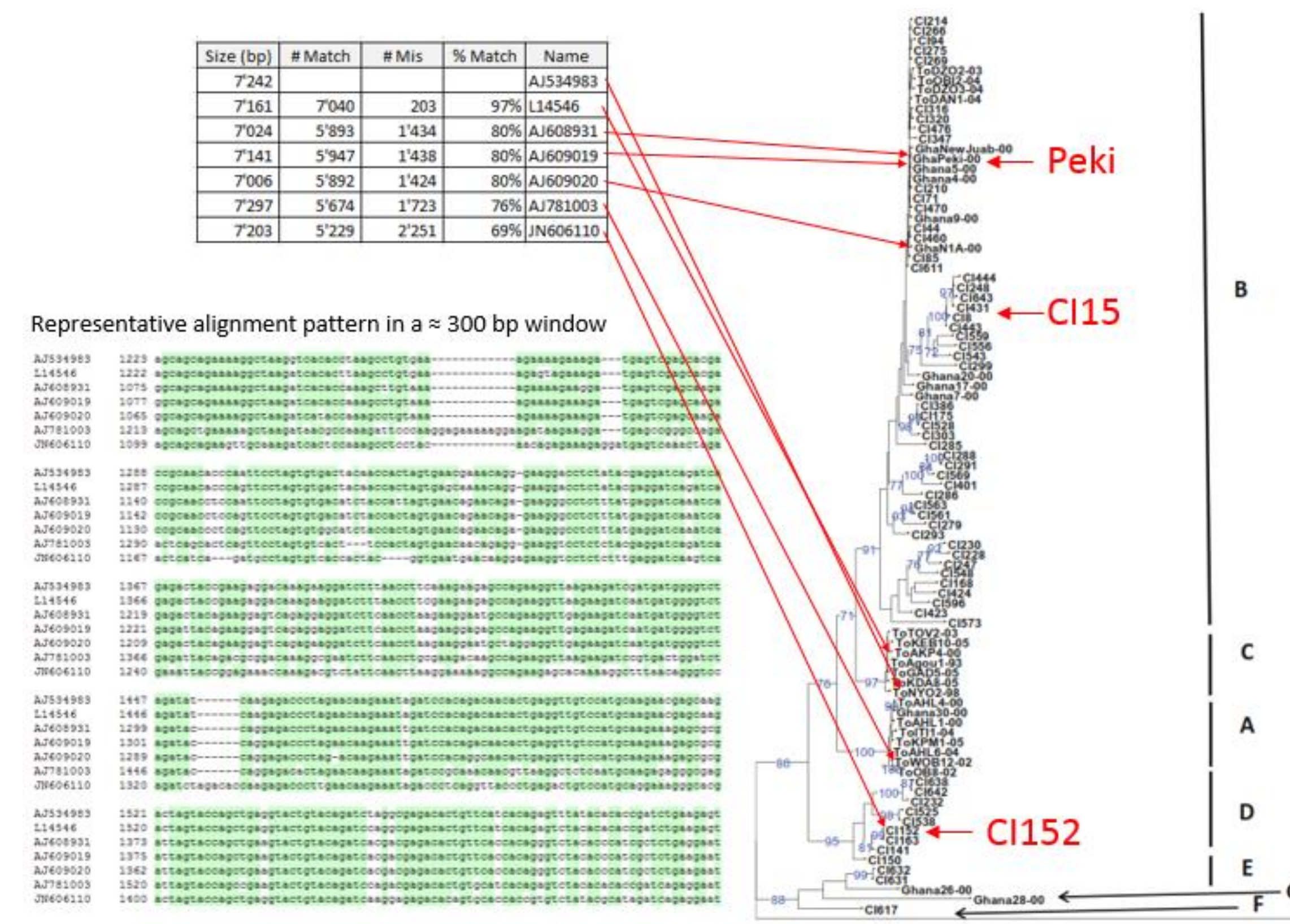
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Introduction

- Cocoa Swollen Shoot disease (CSSD)
 - Major viral disease on cocoa
 - Impacting cocoa production
 - Endemic in West Africa
 - Virus from the family Caulimoviridae, genus Badnavirus (7 kbp DNA genome)
- Current methods for CSSV diagnosis:
 - Visual inspection of plant
 - Inadequate for detecting latent infection in asymptomatic trees
- Molecular based methods: PCR, qPCR, immuno-capture methods:
 - Difficulties to design universal/specific primers
 - Not universal assay



Alternative detection strategies



Full genome pairwise alignment within the seven CSSV isolates reveals low conservation within the entire family. The phylogenetic tree has been generated from a ≈ 400 bp region only. A representative 300 bp region alignment highlights the difficulty to identify even short stretches of conserved sequences.

Difficulty for developing a universal CSSV diagnostic tool:

- Low sequence conservation within the CSSV family
- Poorly characterized at the genetic level
- Complete genome for only 7 isolates
- CSSV qPCR assays not used as a large spectrum diagnostic tool

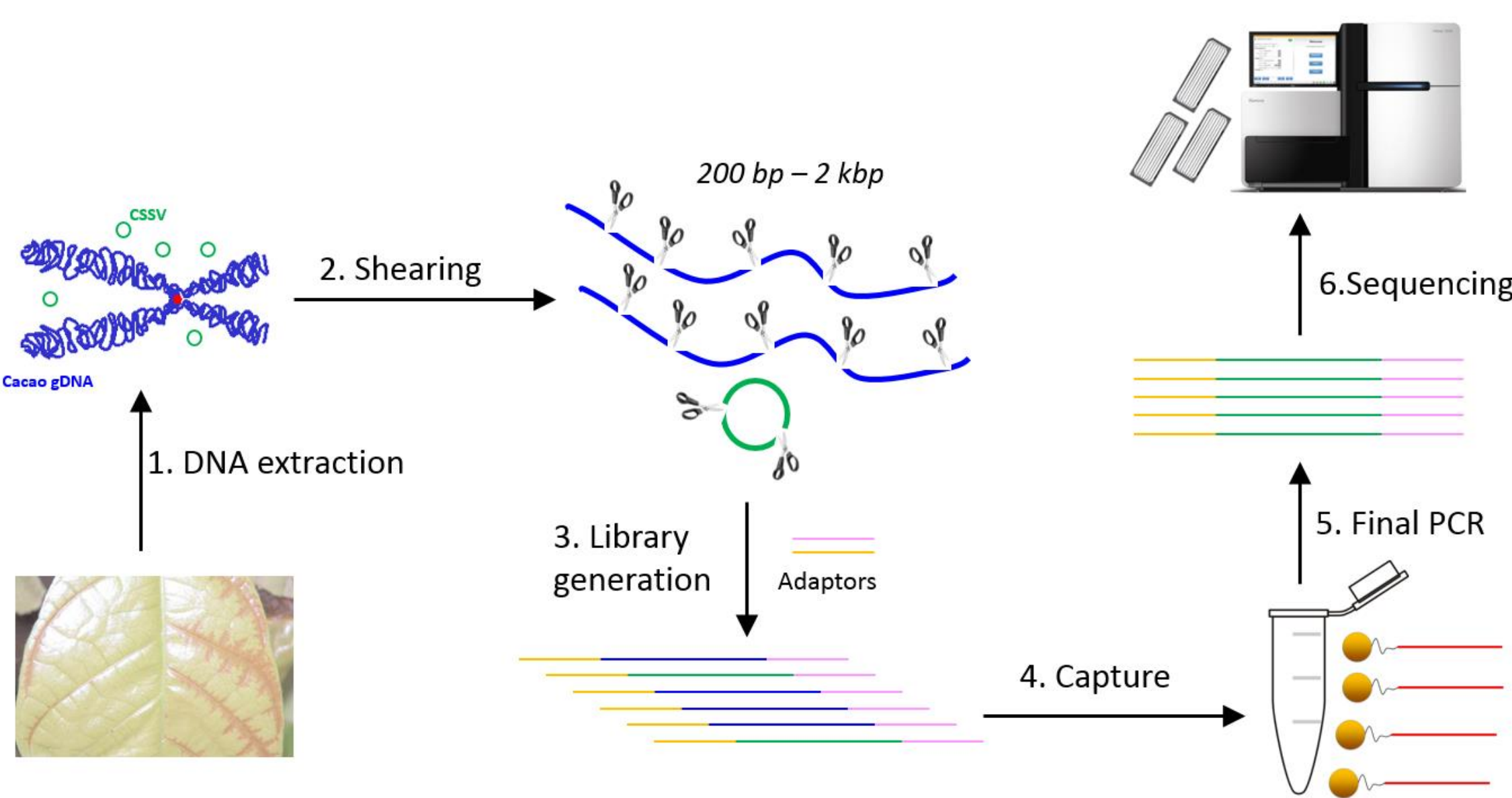


Capture-based approach

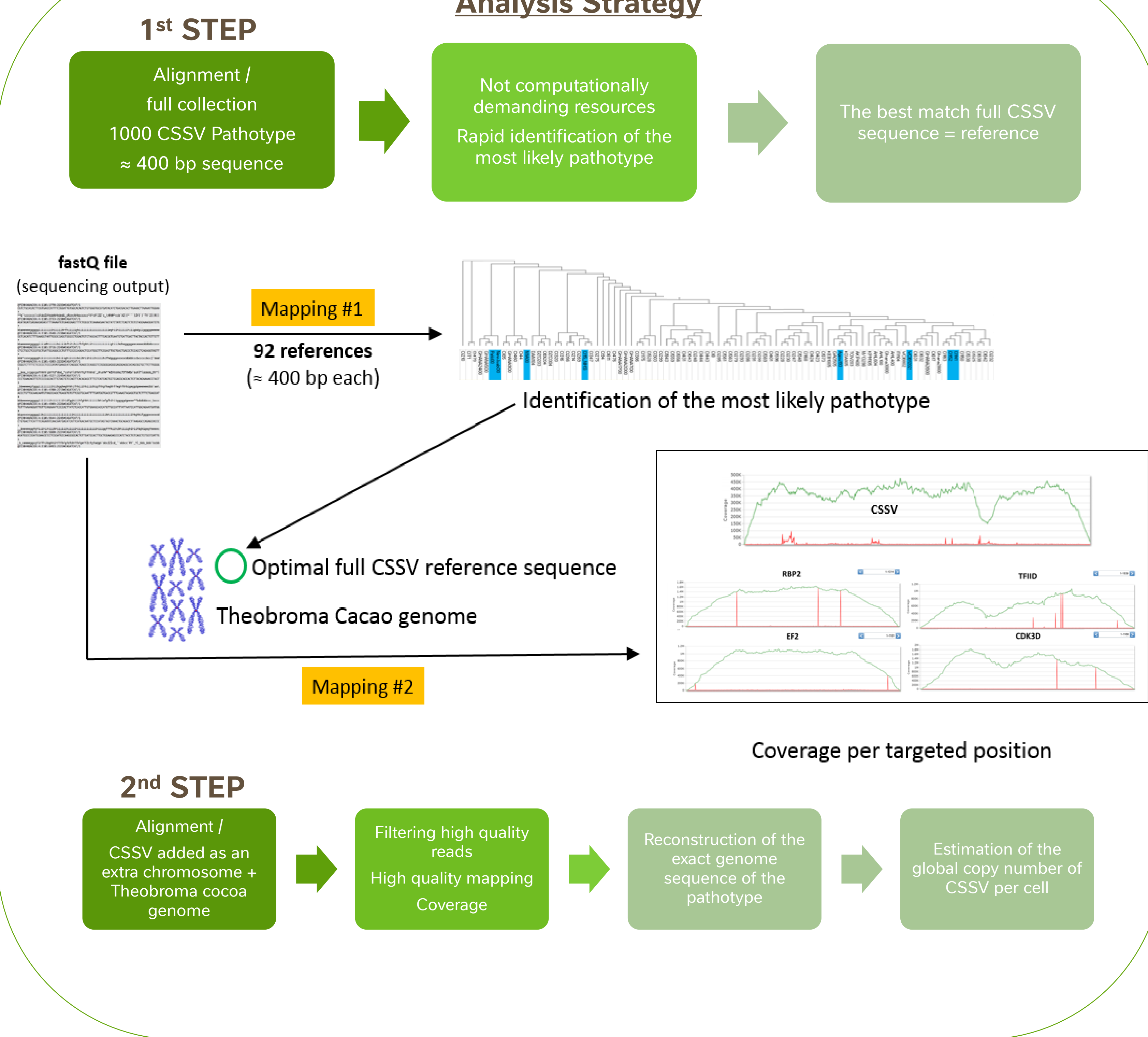
- Enriching DNA of interest: CSSV genome / cocoa genome.
- Flexible Capture Design
- High tolerance to mismatches
- Universal approach/protocol any CSSV pathotype

Materials & Methods

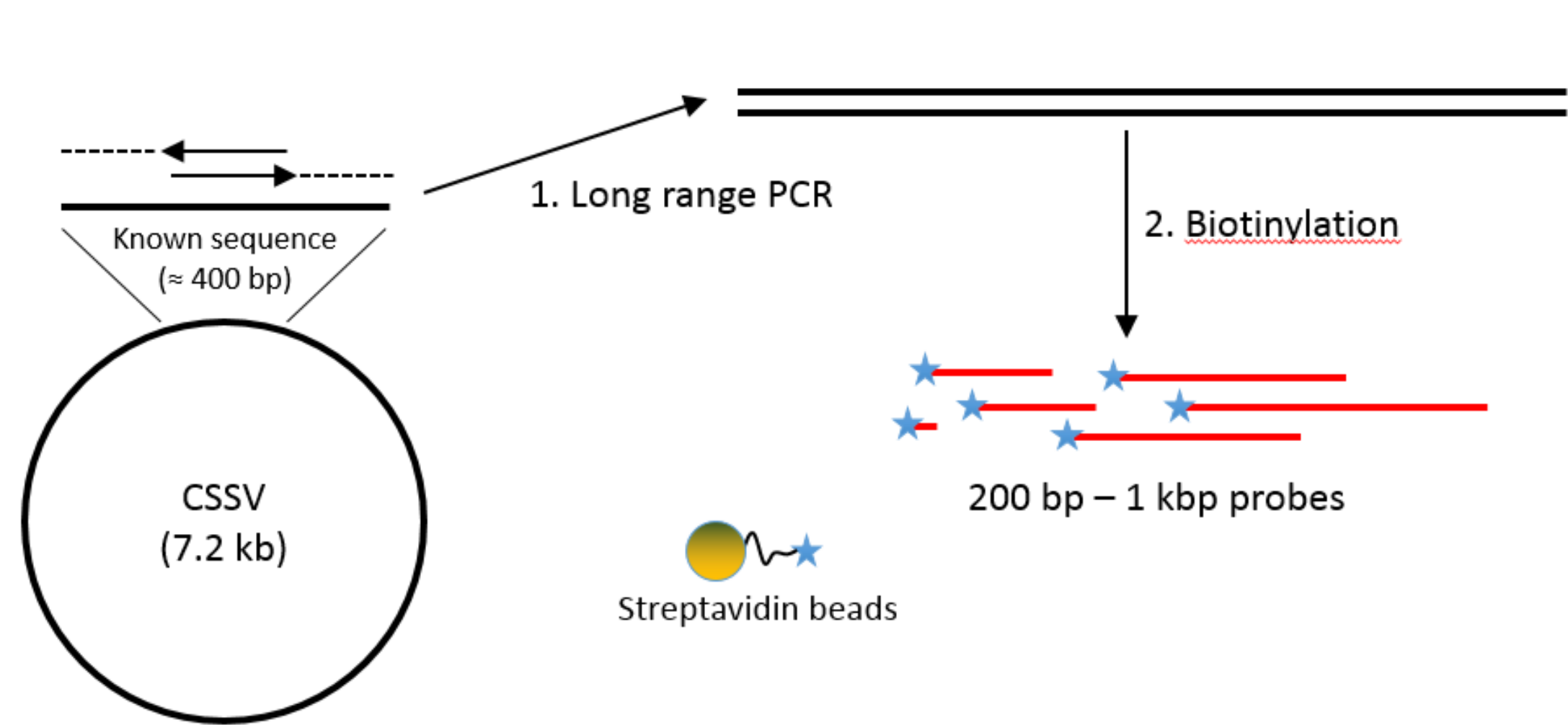
Workflow of Capture and Sequencing



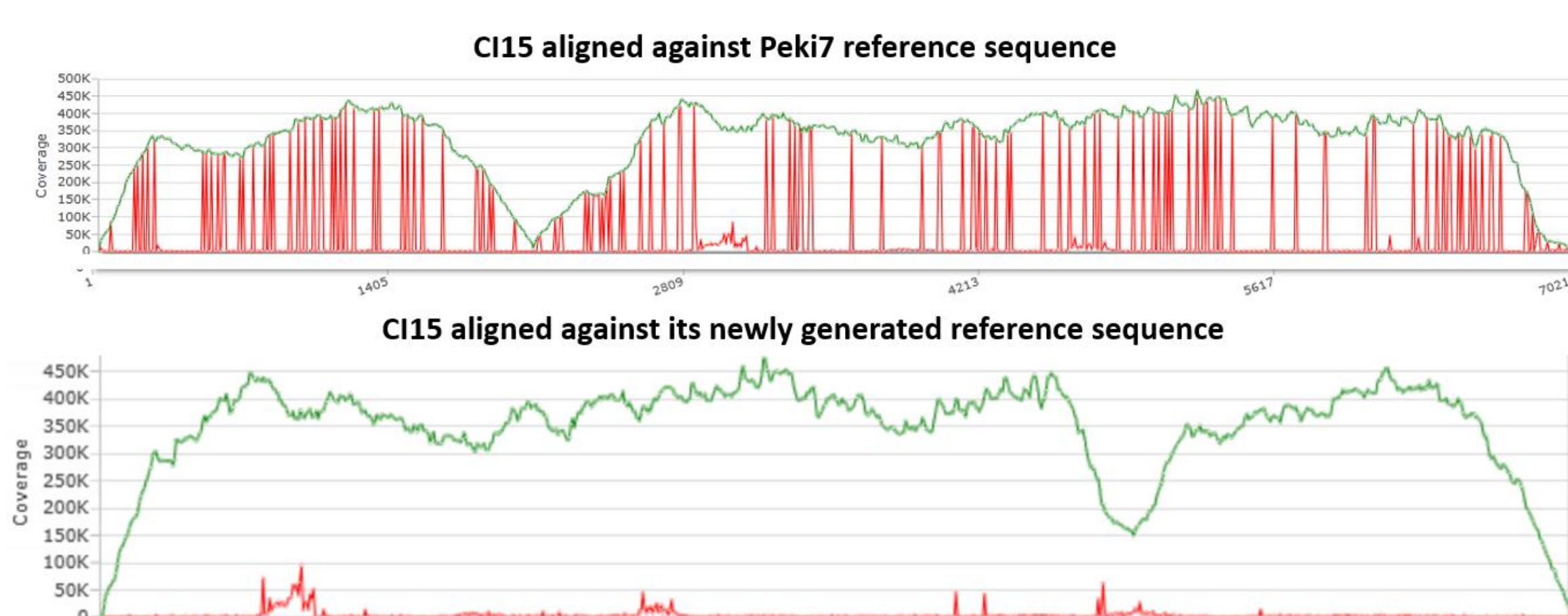
Analysis Strategy



Principle for the generation of CSSV capture probes

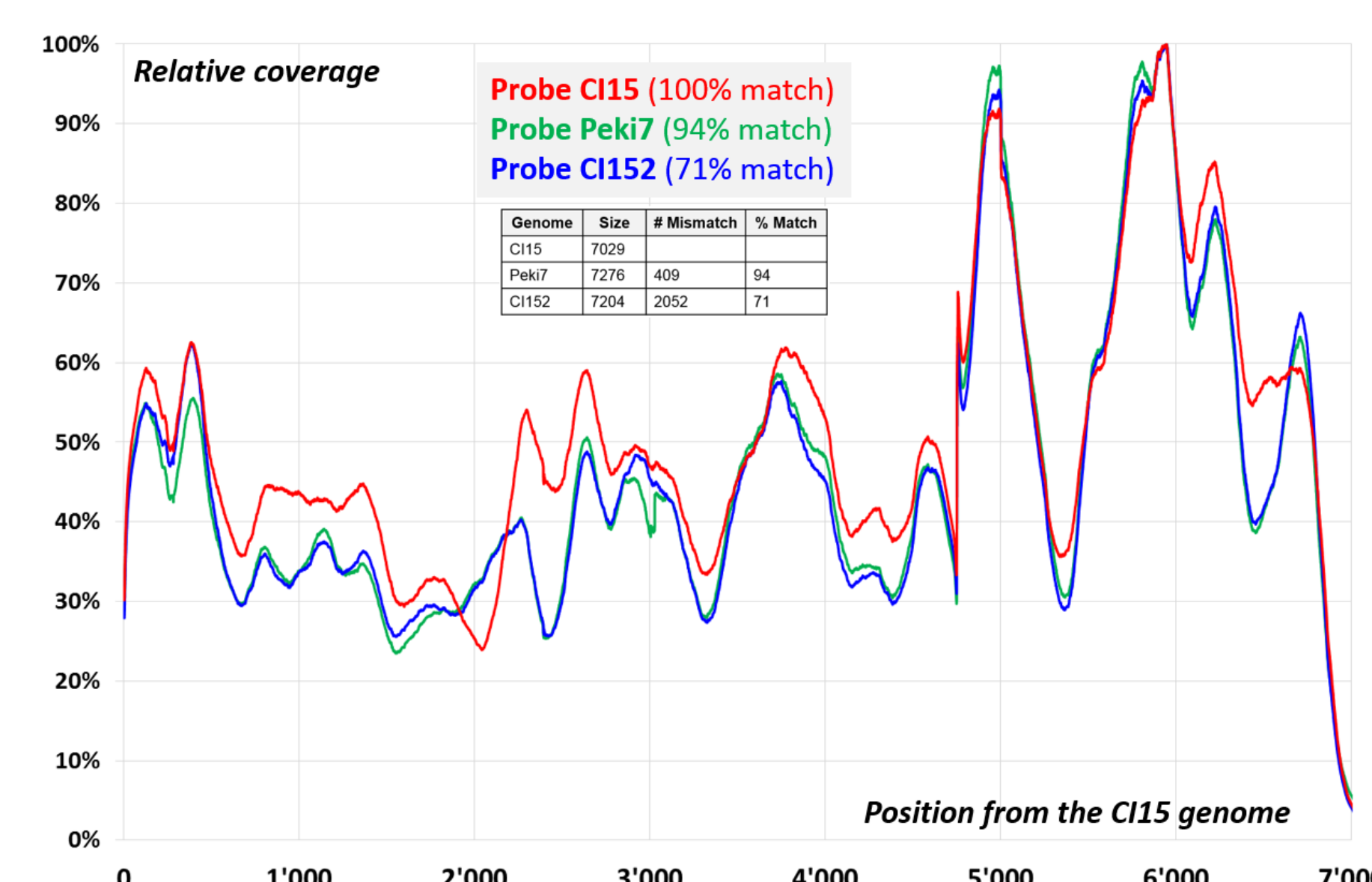


Results



CI15 coverage plot.

Alignment of CI15 sequencing reads against the Peki7 reference sequence reveals a large number of mismatch positions



CSSV coverage plot obtained following the capture with probes with various degree of homology to the target sequence (a CI15 pathotype). The capture method is tolerant to probe / target mismatches.

Conclusion

Our method is

- Universal
- No need to know a priori the pathotype
- Tolerant to divergence up to 30% from virus's genome / probes.

Our approach allows to

- Establish a compendium of the CSSV genomic sequences and identify mutations.
- Can be used as a control tool to check whether a cacao tree is infected or not (symptomatic or not)
- Specify which pathotype is present when a cacao leaf sample is infected
- Can be used as a detection tool in the nurseries and in the fields
- High support combined to epidemiology studies