

Cas9 targeted nanopore sequencing with enhanced variant calling improves CYP2D6-CYP2D7 hybrid allele genotyping

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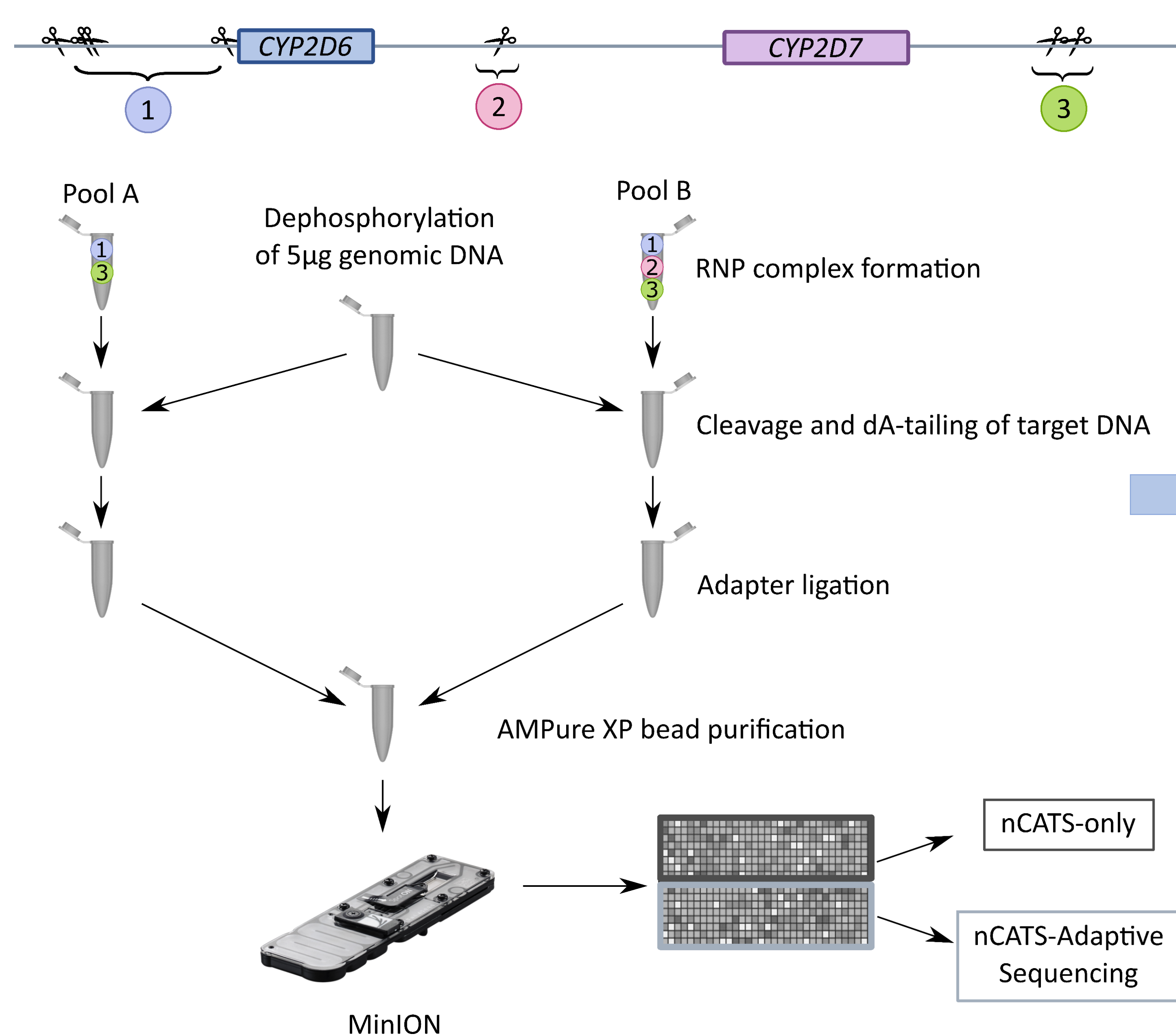
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INTRODUCTION

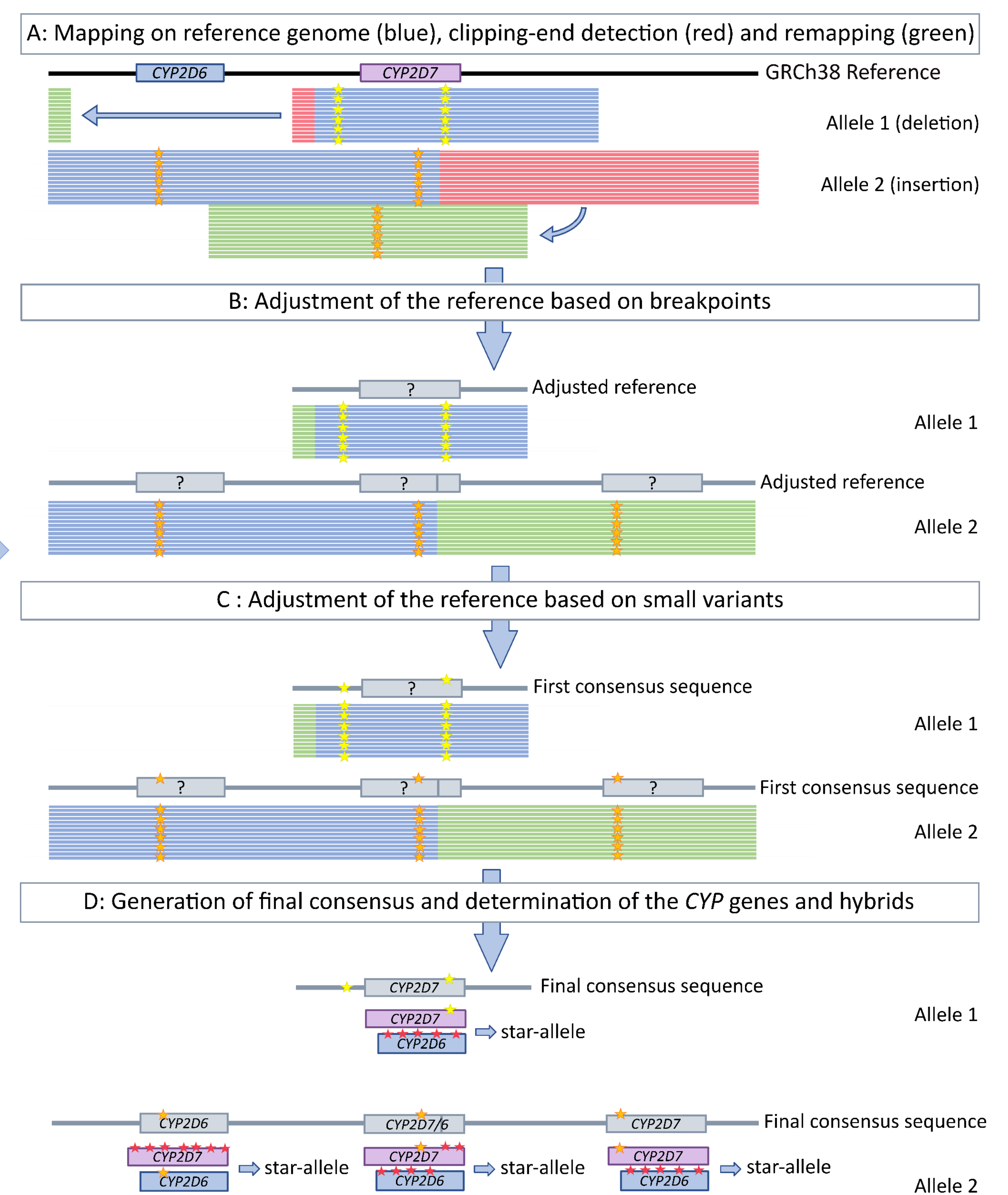
CYP2D6 is one of the most challenging pharmacogenes to genotype due to the high similarity with its neighboring pseudogenes and the frequent occurrence of CYP2D6-CYP2D7 hybrids. Unfortunately, most current genotyping methods are therefore unable to correctly determine the complete CYP2D6-CYP2D7 sequence. With this in mind, we developed a genotyping assay to generate complete allele-specific consensus sequences of complex regions, by optimizing the PCR-free nanopore Cas9-targeted sequencing (nCATS) method combined with adaptive sequencing, and developing a new comprehensive long read genotyping (CoLoRGen) pipeline. In contrast to state-of-the-art variant callers, CoLoRGen first generates consensus sequences of both alleles and subsequently determines both large structural and small variants to ultimately assign the correct star-alleles.

WORKFLOW

CRISPR-Cas9 targeted sequencing



CoLoRGen pipeline



RESULTS

Calling and phasing of small variants

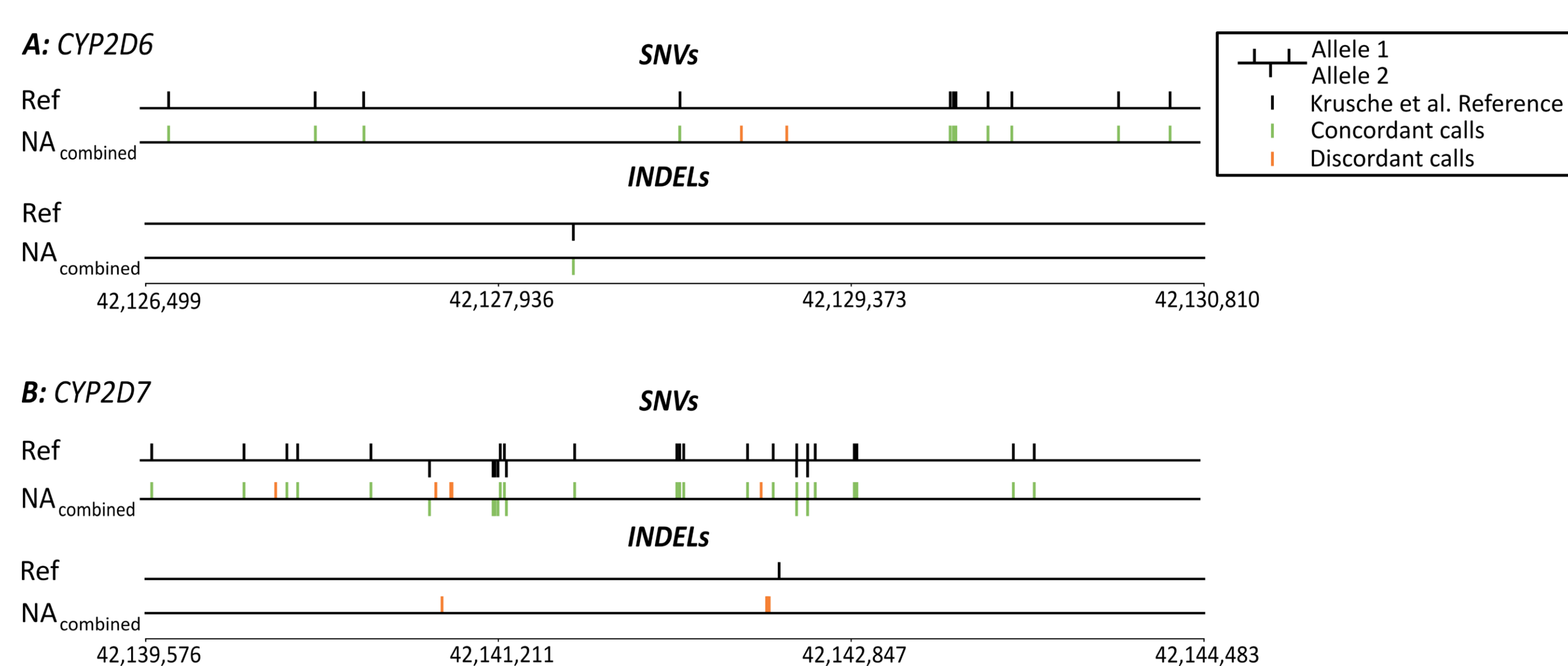


Fig.1. Representation of the called and phased small variants (SNVs and INDELS) in the CYP2D6 and CYP2D7 genes of the NA12878 library (combination of the nCATS-only and nCATS-Adaptive Sequencing libraries).

Star-allele assignment

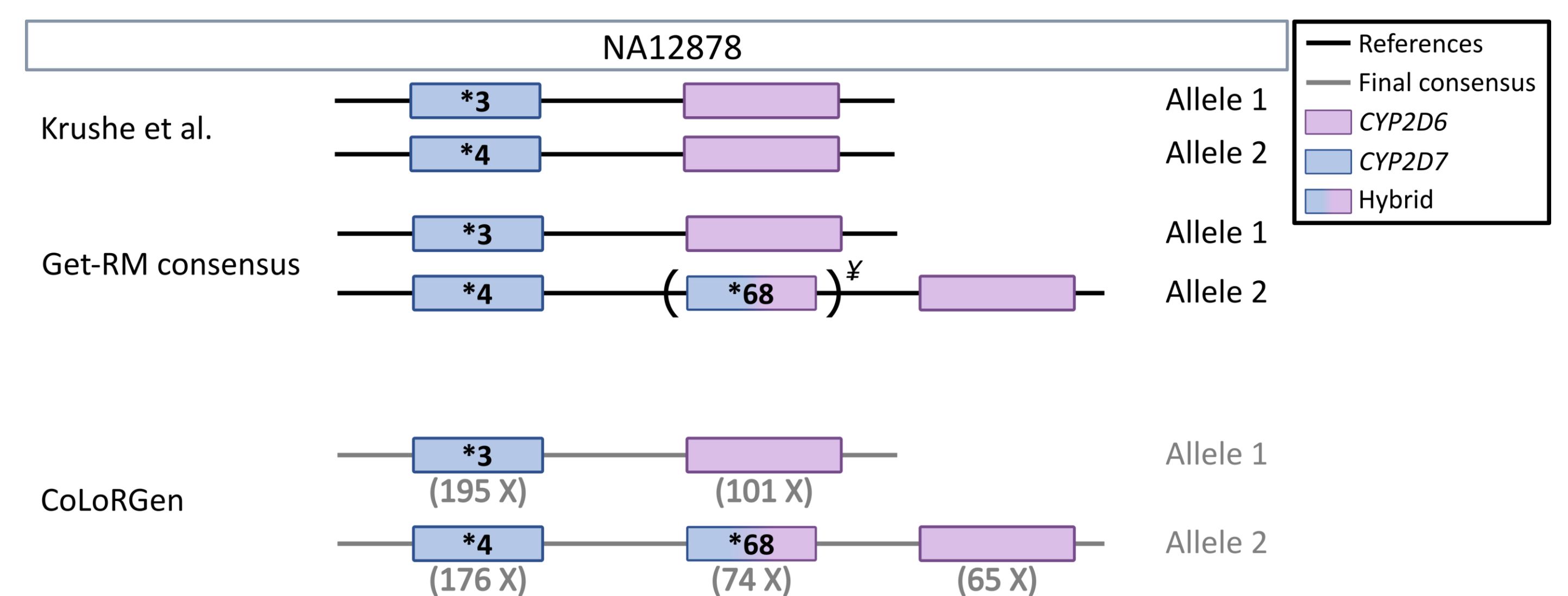


Fig.2. Star-alleles in literature references and star-alleles assigned by the CoLoRGen pipeline. The depths mentioned below the genes are the generated average depths on that position of the locus. †: The *68 allele was only detected when TaqMan-based genotyping was combined with CNV and structural variant detection using quantitative multiplex PCR and LR-PCR validation.

CONCLUSION

In 3 reference samples, including the NA12878 cell line as depicted here, the nCATS-CoLoRGen assay assigned the correct star-alleles to the CYP2D6 gene and CYP2D6-CYP2D7 hybrids. Moreover, our genotyping assay additionally confirmed the presence of SNVs, small INDELS, and large structural variants that go undetected by most current assays. Overall, the nCATS-CoLoRGen genotyping assay allows for more accurate gene function predictions by enabling the possibility to detect and phase *de novo* mutations in addition to known large structural and small variants.

References

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