

APPLICATION OF K-SEQ GENOTYPING PROTOCOL IN TWO LARGE GENOMES ORNAMENTAL SPECIES

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Reduced-representation sequencing (RRS) techniques generate genome-wide high-throughput sequencing data, making it possible to obtain a large number of genetic polymorphisms. Thus, they find application in molecular marker development, population genetic studies, linkage map development and QTL mapping. The most frequently applied are restriction-site associated DNA (RADseq) and GBS (genotyping by sequencing), which are in principle applicable to any genetic background, suitable also for non-model species. However, being patented (SBG, Keygene; EP 2292788), they require a license for being used, in particular for non-academic purposes.

The first example of a RRS methodology not based on enzyme restriction is the repeat Amplification Sequencing (rAmpSeq, Buckler et al. 2016, www.biorxiv.org/content/early/2016/12/24/096628), which consists in the use of primers designed on repetitive sequences to amplify middle repetitive regions. Starting from this concept, the K-seq protocol (Ziarsolo et al. 2021, Plant Methods 17, 30) was recently developed, based on Klenow polymerase-based PCR using sets of primers designed by analyzing k-mer sets in nonrepetitive regions. This methodology can find wide application in both genome-orphan and well-studied species, with a high level of repeatability. Furthermore, the license-free nature of the techniques, combined with potentially low library preparation costs, makes K-seq a valuable option as a genotyping by sequencing technique.

Persian buttercup (*Ranunculus asiaticus* L.; $2n=2x=16$; estimated genome size: 7.6Gb) and poppy anemone (*Anemone coronaria* L.; $2n=2x=16$; estimated genome size: 9.08 Gb) are ornamental, outcrossing, perennial crops marketed

both as cut flower or potted plant. To date, the application of DNA-based techniques in varietal development of these species is very limited, and reference genomes have not been developed. Here we investigated the K-seq protocol application in these two species through the development of a set of primers based on the reference genome of the closely-related *Aquilegia kansuensis* (Brühl). K-seq protocol was applied on 10 commercial genotypes for *R. asiaticus* and 6 genotypes for *A. coronaria*. The pipeline was then investigated for optimization purposes. The results for *A. coronaria* were compared with the SSR-base fingerprinting published by Martina et al., 2021. Our findings make it possible to optimize genotyping protocol in such a complex species, potentially lowering wet-lab and sequencing costs for wide genotyping projects.