Single-Pollen Genotyping Using the Next-Generation Sequencing

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Due to the limited amounts of DNA in a single-pollen grain, a regular use of the next-generation sequencing (NGS) has limited their adoption to pollen DNA analysis. However, recent development of PCR-based method to construct NGS libraries has opened new opportunities in NGS analysis of pollen DNA. Recently we have developed an effective method for constructing NGS libraries and genotyping of genome-wide single-nucleotide polymorphism (SNP) using NGS platform that termed "multiplexed ISSR genotyping by sequencing" (MIG-seq). Using the MIG-seq technique, thousands of genome-wide regions can be effectively amplified from a wide variety of genomes without prior genetic information. Unlike standard NGS methods based on restriction enzyme steps that require relatively large amounts of high-quality DNA, the MIG-seq procedure is based on PCR that can be applied to small amounts of DNA materials. We applied this technique to modern *Hemerocallis* pollen and successfully detected the pollen DNA data. In addition, whole-genome amplification (WGA) technique is another choice to analyze the limited amounts of pollen DNA. Once enough amounts of pollen DNA are supplied by WGA, they can be used for NGS analysis. We applied the WGA method to *Pinus* pollen grains found in a glacier, and successfully amplified pollen DNA. Combined the WGA with MIG-seq techniques, we also detected numbers of SNPs from the *Pinus* pollen grains which can be used for population genetic analysis in the past populations. These approaches would be suitable to only well-preserved pollen materials; however, we should take on challenges for the possible materials.