Self-incompatibility in Rosaceae is determined by a Gametophytic Self-Incompatibility System (GSI) that is mainly controlled by the multiallelic locus S. In apricot, the determination of self- and inter-(in)compatibility relationships is increasingly important, since the release of an important number of new cultivars has resulted in the increase of cultivars with unknown pollination requirements. Here, we describe a methodology that combines the determination of self-(in)compatibility by hand-pollinations and microscopy with the identification of the S-genotype by PCR analysis. For self(in)compatibility determination, flowers at balloon stage from each cultivar were collected in the field, hand-pollinated in the laboratory, fixed, and stained with aniline blue for the observation of pollen tube behavior under the fluorescence microscopy. For the establishment of incompatibility relationships between cultivars, DNA from each cultivar was extracted from young leaves and S-alleles were identified by PCR. This approach allows establishing incompatibility groups and elucidate incompatibility relationships between cultivars, which provides a valuable information to choose suitable pollinizers in the design of new orchards and to select appropriate parents in breeding programs.