# dCAPS method: advantages, troubles and solution

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**ABSTRACT**

In our work, we focus on the evolutionary studies of sex chromosomes. As model organisms we use several species of the plant genus *Silene*. An important part of our research is represented by genetic mapping based on the assays of DNA length or sequence polymorphisms. Apart from the other methods we also use the dCAPS method, which is very useful for detection of the sequence polymorphisms (SNPs). This method is unique as it is able to detect SNPs that are not situated in any restriction site; a fundamental principle of this method is usage of primer designed with one or two mismatches that bring into the target sequence the mutation in vicinity of SNP. Using this method, we found out some improvements that can make analyses more cost-effective.

**Keywords:** detection of polymorphism; SNP; restriction endonuclease; semi-nested PCR; DNA agar; genetic mapping; *Silene*

Many types of genetic studies (e.g. recombination mapping) are based on the analysis of DNA polymorphisms (in length or in sequence). Various methods for detection of any kind of polymorphisms have been developed (e.g. RFLP, AFLP, Minisequencing, SSLP, DFLP). Methods based on utilization of polymerase chain reaction (PCR) are commonly used. Very popular methods based on PCR are DFLP (DNA Fragment Length Polymorphism; Hongtrakul et al. 1998) for detection of the length polymorphism of amplified products, and CAPS (Cleaved Amplified Polymorphic Sequences; Konieczny and Ausubel 1993) for detection of differences in presence or absence of target sequences for restriction endonucleases. The CAPS method is analogy of RFLP, but the cleavage is performed on amplified products instead of the original genomic DNA. The usage of this method is restricted only to detection of single nucleotide polymorphisms (SNPs) that create or disrupt the target site for the respective restriction endonuclease. This is the reason why a modification of this method, so-called dCAPS (derived Cleaved Amplified Polymorphic Sequence; Michaels and Amasino 1998, Neff et al. 1998), has been developed. dCAPS differs from CAPS especially in using specific primers, which are designed with one or two mismatches. Through these mismatches, primers bring mutations into the target sequences during amplification and in conjunction with the discovered SNP it results in creation of a unique restriction site, solely in one of the alleles studied in genetic assays. The scheme of this method is described in Figure 1.

## PROTOCOL AND TROUBLESHOOTING

In our laboratory we usually start genetic studies with specific primers and amplify the segment from the gene of interest. Afterwards, we separate obtained products by agarose gel electrophoresis. If there is a specific length polymorphism among products, we use the DFLP method; otherwise we have to search for sequence polymorphisms, especially for SNPs. For this purpose, purified PCR products are either directly sequenced or they are cloned into a suitable plasmid and subsequently

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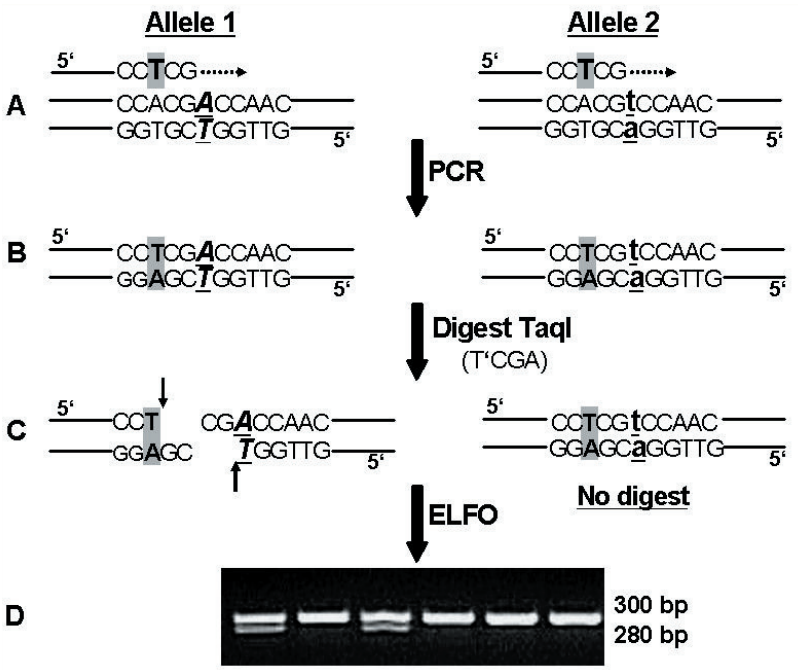


Figure 1. Scheme of the dCAPS method. (A) Segments of two different alleles (difference is highlighted by bold and underlined letters) of the studied gene with specific dCAPS primer with one mismatch (letters in grey box). (B) Products of PCR performed with dCAPS primer (originated mutations are marked by grey boxes). (C) Results of digestion of PCR products by restriction endonuclease. (D) Products of digestion separated by gel electrophoresis

sequenced. The sequences obtained from PCR products can be analyzed by PC software to visualize the chromatograms of sequences – e.g. BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html). Then, we look for two overlapping peaks in the sequence, which might indicate a polymorphism (SNP) suitable for mapping. If sequences are derived from a number of plasmid clones, we align all the sequences using the program ClustalW 1.8 (Thompson et al. 1994; http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html) and visualize the alignment using the program BOXSHADE 3.21 (www.ch.embnet.org/software/BOX_form.html). Finally, we search for the differences (SNPs) among sequences. If the found SNP can be distinguished by specific restriction endonuclease, we use the CAPS method; on the contrary, if the SNP does not affect any restriction site, we utilize the dCAPS method.

The first step in the dCAPS method is designing the primer with a specific mismatch. This procedure can be performed manually, however, a useful program is available – dCAPS Finder 2.0 (Neff et al. 2002; http://helix.wustl.edu/dcaps/dcaps.html). If we put two segments of sequences containing SNP in the input form and determine the number of desired mismatches in primers, the program calculates several suitable primers. The output of this program provides a set of suitable primer sequences along with appropriate restriction endonucleases.

We perform PCR with a combination of dCAPS primer in pair with a primer specific for the target sequence. We test suitability of this primer pair by the program Primer3 (Rozen and Skaletsky 2000; http://frodo.wi.mit.edu/). As the position of primer is fixed to the surrounding of the SNP, it is often impossible to obtain a primer of optimal PCR performance. Relatively often, PCR with this pair of primers gives either non-specific products or, sometimes, no product. We solved this problem by two-step amplification – semi-nested PCR. In the first reaction we apply specific primer pair on genomic DNA template. In the second amplification reaction, we use the dCAPS primer in pair with one of the specific primers and a small amount of PCR products (in principle 0.2 ng) from previous reaction instead of the genomic DNA template. Recently, a similar approach has been published by Gale et al. (2005), where the authors described their good experience with the same solution of this problem; they did an extra step of PCR products purification before the second amplification reaction. We think this purification is not necessary, as we obtained very good results even if this additional step was omitted.

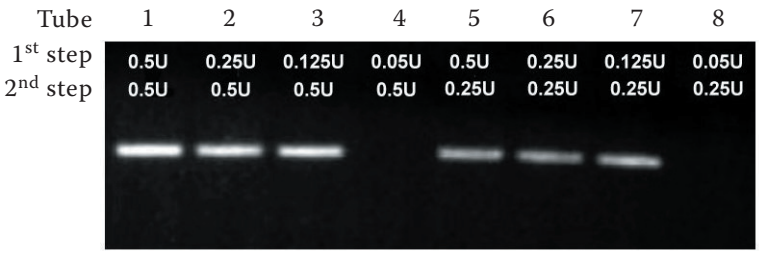


Figure 2. Gel separation of products obtained from semi-nested PCR with dCAPS primer. Varying amounts of Taq polymerase (TopBio) in both steps are assigned above each line

For the economization of the protocol we tested the minimum amount of Taq polymerase (TopBio) in both steps of semi-nested PCR to obtain sufficient amount of PCR products. In the first step we used 0.5U, 0.25U, 0.125U or 0.05U of Taq polymerase for one reaction (25 µl). The remaining components of the reaction were as follows: 1/10 of total reaction volume of 10 × PCR Blue Buffer (TopBio); 200µM of each dNTP (Promega); 200nM forward primer; 200nM reverse primer; 30 ng of genomic DNA; and deionized water up to total volume 25 µl. We performed PCR on the PTC 200 MJ Research Thermocycler under the following conditions: primary denaturation 94°C/3 min; next 35 cycles: 94°C/30 s, $T_m$ (according to the used primers)/1 min, 72°C/$t_e$ (according to the length of products); final extension 72°C/5–10 min. At the end of the PCR process, we took a small fraction (about 0.2 ng) from each reaction tube and we used it as a template in the second step. The reaction components and conditions in the second step are similar as in the first step, except for template and one primer (dCAPS primer). The amount of Taq polymerase was used according to Figure 2. Finally, the PCR products of the second step were separated on 1% agarose gel (stained with ethidium bromide). From the results we detected the combination of minimum amount of Taq polymerase – for the first step it was 0.125U, for the second step it was 0.25U (Figure 2).

After the amplification we digested 10–15 µl of the obtained PCR products by restriction endonuclease specific for the target site created by SNP along with dCAPS primer. The choice of buffer and temperature depends on the used restriction endonuclease. We specified the incubation time for all of digestion reactions to 2–3 hours. After the termination of digestion we have to prepare agarose gel in a high concentration (3–3.5%), because the length difference between digested and non-digested products is usually very small (about 20 base pairs, according to the length of dCAPS primer). We have also a positive experience with the usage of DNA agar (Marine Bioproducts), instead of agarose for preparation of the high-concentrated gels (Žlůvová et al. 2005). The advantage of this improvement is that in comparison with agarose, there is only a half consumption of agar necessary to obtain the gel with the same differentiation ability. It is therefore much easier to prepare highly concentrated gels. We prepared the gels in TAE buffer and the electrophoresis was run in the same buffer. Before pouring the melted gel into tray, we added ethidium bromide to the final concentration 0.5 µg/ml. After the electrophoresis, we observed the gel under UV light. We can usually distinguish three types of patterns according to the presence or absence of the restriction site – two bands (heterozygote), only the shorter one (homozygote type I), or only the longer one (homozygote type II).

We successfully used the CAPS and dCAPS approaches for detection of SNP for genetic mapping of sex chromosomes of the plant *Silene latifolia* (Nicolas et al. 2004; unpublished data) and *S. otites* (unpublished data). During our work we discovered some improvements for performing the dCAPS method. These improvements can resolve some possible problems during process and they economize the protocol.

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