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Detection of Single Nucleotide Changes

Single-Strand Conformational Polymorphism (SSCP)

Using the Sequi-Gen® II PAGE Apparatus

Introduction

SSCP is one of several methods that can be used to screen short genomic fragments for single base changes from multiple samples. This method is based on several observations: 1. Under nondenaturing conditions, denatured DNA runs as single strands. 2. Secondary structures form that are sequence dependent. 3. A single base change can alter the secondary structure in a way that affects electrophoretic mobility of the single strands.

The ability to detect single base changes rests on several factors which optimize band resolution. 1. Fragment size — estimated efficiency for detecting single base changes is 89–99% for 100–300 bp fragments, but drops to 67% for 300–450 bp fragments. 2. Gel temperature—some fragments give better results at room temperature, others at 4 °C. 3. Bis/acrylamide ratio—the bis/acrylamide ratio determines the percent of crosslinking. One to two percent crosslinking is optimal (i.e. 5% acrylamide with 0.05% bis = 1% crosslinking).

Additionally, glycerol (5–10%) can be added to the gels and run at 4 °C to improve resolution. Detection is accomplished by autoradiography if the PCR' products are radiolabelled (staining with silver or ethidium bromide is also possible depending on the DNA concentration). Optimal conditions must be determined for each fragment.

To test whether the chosen SSCP conditions and analysis are functional, start with a set of three DNA samples that have been previously characterized and are known to represent three different genotypes within the defined DNA region (AA [homozygote A], BB [homozygote B], AB [heterozygote]).

Methods

PCR Amplification

Amplify by PCR the DNA region to be screened, using DNA from the three samples described above as templates. Prepare ^{32}P labeled products in a 10 µl volume. After optimizing conditions (dilutions of DNA template, primer, Mg^{++} concentrations and PCR thermocycling settings), prepare reaction mixtures in the following way:

dNTP Mixture: (mixture volume is sufficient for three samples and a negative control) 1 µl 10 mM dGTP, 1 µl 10 mM dATP, 1 µl 10 mM dTTP, 1 µl 10 mM dCTP, and 10.3 µl deionized water, for a final volume of 14.3 µl.

PCR Mix: (mixture volumes sufficient for 3 samples and a negative control) 4 µl of 10x PCR buffer, 10 µl deionized H_2O, 8 µl of each primer [– 0.15 µM], 4 µl of the dNTP mixture, 1 µl of 5 U/µl AmpliTaq™ (Perkin Elmer), and 1 µl ^{32}P dCTP (3,000 Ci/mmole), for a total volume of 36 µl.

Add 9 µl of the PCR mix to 1 µl of each DNA template [50 ng] and overlay with 15 µl of mineral oil.

The remaining buffer is used for a non-template control in the PCR reaction; this negative sample identifies whether any contaminating DNA is present in the PCR reagents.

Thermocycling Procedure: Denature the DNA template with one cycle at 94 °C for 5 minutes. Denature, anneal, and extend the PCR amplified product for 30 cycles at the appropriate thermocycling temperature. Perform a final extension at 72 °C for 5 minutes. Annealing temperatures may vary based on primer-template T_m.

Use 1.5 µl of the PCR reaction for SSCP analysis on a 21 cm x 40 cm x 0.4 mm Sequi-Gen polyacrylamide gel.

SSCP Analysis

SSCP Loading Buffer: 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, 10 mM EDTA. Store at -20 °C.

Polyacrylamide Gel Preparation: Add 10 ml 5x TBE, 10 ml 50% glycerol (for gels run at 4 °C), 10 ml 49.5% acrylamide/0.5% bis, and 70 ml deionized H_2O for a final volume of 100 ml. Degas the gel mix for 5 minutes under vacuum. Add 200 µl 25% ammonium persulfate and 80 µl TEMED prior to casting the gel. Follow the instructions provided with the Sequi-Gen II 21 cm x 40 cm gel apparatus for the recommended gel casting techniques for 0.4 mm thick gels. The number of samples loaded on the gel will determine which type of sharkstooth comb (0.4 mm) should be used (24, 36, and 48 well sharkstooth combs are available).

Loading and Running the SSCP Gel: Prepare 1 liter of 0.5x TBE electrophoresis running buffer. Insert the Sequi-Gen IPC apparatus containing the gel into the lower buffer chamber base. Add the running buffer to both the lower buffer chamber and the IPC buffer chamber. Add 9 µl of SSCP loading buffer to 1.5 µl of each PCR reaction. Denature the strands by incubating the samples at 80 °C for 10 minutes, followed immediately by placing the samples on ice for 1 minute. Load 3 µl of each sample onto the gel. Store the remainder of each sample at

-20 °C. Run the gel at 40 watts (constant power) for 2–3 hours or until the bromophenyl blue dye front is 7–8 cm from the bottom of the gel. **Note:** It is important to keep the gel temperature below 25 °C so that single-strand secondary structures are maintained during the run. This can be accomplished by placing a fan as close to the glass plates as is safely possible. Alternatively, the gels can be run in a 4 °C cold room.

After electrophoresis is completed, remove the IPC from the base, separate the glass plates, and remove the gel with Whatman 3MM and dry the gel in a Bio-Rad Model 583 Gel Dryer for 1 hour at 80 °C. The dried gel is placed under film overnight (4 °C or -70 °C). Develop the film and interpret the results (see Figure 1 for example).

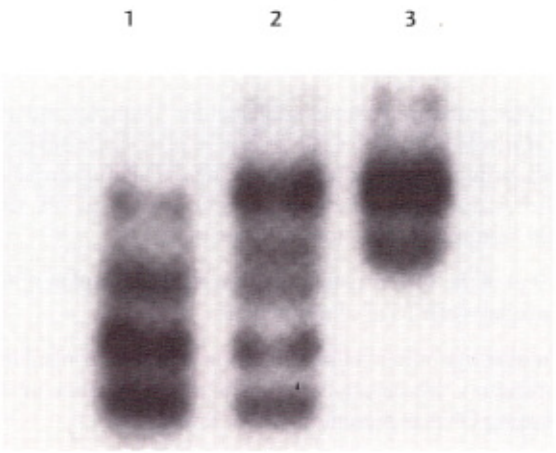

Fig. 1. Single base pair changes between the different genotypes are evident. The designations are as follows: Lane 1 AA (homozygote A); Lane 2 AB (heterozygote); Lane 3 BB (homozygote B). Photo contributed by Richard A. Gatti, M.D., Department of Pathology, UCLA School of Medicine, Los Angeles, California.

New Product Update

The Sequi-Gen II has been replaced with the new Sequi-Gen GT. All of the features that make the Sequi-Gen ideal for microsatellite and SSCP analysis have been kept in the new model. In addition, the new models offer a more convenient horizontal casting method and a size developed specifically for microsatellite and SSCP analysis (38 cm wide x 30 cm long). Please request bulletin 2006 for more information.

References

1. Screening of Multiple DNA Samples for Detection of Sequence Changes, Mashiyama, S., Sekiya, T., and Hayashi, K., *Technique*, **2**, 304 (1990).
2. PCR-SSCP: A Simple and Sensitive Method for Detection of Mutations in the Genomic DNA, Hayashi, K., *PCR Methods and Applications*, **1**, 34-38 (1991).
3. Protocols for an Improved Detection of Point Mutations by SSCP, Spinardi, L., Mazars, R., and Theillet, C., *Nucleic Acids Research*, **19**, 4009 (1991).
4. Helpful Hints for the Detection of Single-Stranded Conformation Polymorphisms, Dean, M., and Gerrard, B., *BioTechniques*, **10**, 3 (1991).
5. Detection of Polymorphisms of Human DNA by Gel Electrophoresis as Single-Strand Conformation Polymorphisms, Orita, M., Iwahana, H., Kanazawa, H.,Hayashi, K., and Sekiya,T., *PNAS*, **86**, 2766-2770 (1989).
6. Rapid and Sensitive Detection of Point Mutations and DNA Polymorphisms Using the Polymerase Chain Reaction, Orita, M., *et al., Genomics*, **5**, 874-879 (1989).
7. Diagnostic Single-Strand Conformational Polymorphism (SSCP): a Simplified Non-radioisotopic Method as Applied to a Tay Sachs B1 Variant, Ainsworth,P. J., Surh, L. C., and Coulter-Mackie, M. B., *Nucleic Acids Research*, **19**, 405 (1991).

Ordering Information

Catalog Number	Product Description
Sequi-Gen GT Sequencing Systems	
165-3860	**Sequi-Gen GT System,** 21 x 40 cm**
165-3861	**Sequi-Gen GT System,** 21 x 50 cm**
165-3862	**Sequi-Gen GT System,** 38 x 30 cm**
165-3863	**Sequi-Gen GT System,** 38 x 50 cm**
165-3802	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 40 cm, 100/120 V†
165-3805	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 40 cm, 220/240 V†
165-3803	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 50 cm, 100/120 V†
165-3806	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 50 cm, 220/240 V†
165-3810	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 30 cm, 100/120 V†
165-3811	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 30 cm, 220/240 V†
165-3804	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 50 cm, 100/120 V†
165-3807	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 50 cm, 220/240 V†

* PCR is covered by U.S. patent number 4,683,202 issued to Cetus Corp.

** Sequi-Gen GT systems include GT IPC assembly (IPC and bonded inner glass plate, outer glass plate, and clamp set), GT universal base, GT safety covers with cables, stabilizer bar, precision caster assembly (precision caster base, gasket, tubing, luer tapers, tubing, and syringe), 0.40 mm vinyl sharkstooth comb and spacers, gel temperature indicator, leveling bubble, drain port connector, and instruction manual.

† Sequi-Gen GT/PowerPac 3000 systems include the appropriate Sequi-Gen GT system, PowerPac 3000 Power Supply, PowerPac temperature probe, and PowerPac instruction manual.

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