Nucleic acid stain-dependent single strand conformation polymorphisms

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Single strand conformational polymorphism (SSCP) is a mobility shift analysis of single-stranded DNA (ssDNA) by nondenaturing polyacrylamide gel electrophoresis to detect DNA sequence polymorphisms (1). Typical SSCP protocols detect single strands by autoradiography (2–4) or by treatment with a DNA stain after electrophoresis (5–12). We report here that treatment with a nucleic acid stain before electrophoresis reveals polymorphisms undetected by staining after electrophoresis.

Our SSCP protocol includes four phases. In phase I, double-stranded DNA (dsDNA) is denatured into ssDNA by heating for 5 min at 95°C in loading buffer (95% formamide, 20 mM NaOH, 0.05% xylene cyanol, 0.05% bromophenol blue, 10 mM EDTA). Phase II stabilizes ssDNA by rapid cooling on ice. In phase III, ssDNA fragments are separated by electrophoresis in a nondenaturing gel. Phase IV is staining (if necessary) and imaging.

SYBR® Gold is a proprietary, cyanine-based nucleic acid stain from Molecular Probes (Eugene, OR, USA) and is characterized by Tuma et al. (13). We recently adopted it for detecting DNA in gels. It is more sensitive than ethidium bromide and more time efficient than silver staining. To achieve additional efficiency, we include SYBR Gold in the phase I buffer at the manufacturer's suggested stain bath concentration of $1\times = 1/10,000$ dilution of stock stain. This pre-electrophoretic (pre-staining) eliminates a phase IV staining step (post-staining), decreasing by 1–4 orders of magnitude the amount of SYBR Gold required

to detect DNA by submerging an entire gel in staining buffer.

Testing of identical samples with pre- and post-staining protocols led to the serendipitous discovery that pre-staining with SYBR Gold reveals SSCPs (Figure 1, compare lanes 2 and 3 to lanes 4 and 5) undetected by post-staining with SYBR Gold ($1\times$ stain bath; Figure 1, lanes 12–15) or ethidium bromide ($0.5\ \mu\text{g/mL}$ stain bath; Figure 1, lanes 17–20). Pre-staining with ethidium bromide ($0.5\ \mu\text{g/mL}$ phase I buffer) failed to detect these same polymorphisms (Figure 1, lanes 7–10).

We screened PCR products of nine sequence-tagged sites (STS) amplified from two distantly related strains (*mas p au*, *Tiw-1*) of the red flour beetle, *Tribolium castaneum*. The post-staining protocol using ethidium bromide detected three polymorphisms (Figure 2B, Post). The pre-staining protocol using SYBR

Table 1. STS Polymorphisms

STS	Size (bp)	SNP (No.)	Indel (bp)
H09g	401	3	
H115	138	1	
M2s	317–320	2	3
A011	251	2	
B16s	157–169	3	10, 2
F2s	309–310	3	1
Tsh	236	0	
J1S	142	0	
CHSA	467	0	

Shown are the size, number of single nucleotide polymorphisms (SNPs), and size of insertions/deletions (Indel) underlying SSCPs detected between the *mas p au* and *Tiw-1* strains of *Tribolium castaneum*. STS H115 was designed to isolate a single SNP of STS H09g. STS, sequence-tagged site; SSCPs, single strand conformational polymorphism.

Gold detected the same three polymorphisms (Figure 2B, Pre) plus three additional polymorphisms (Figure 2A, Pre). In no case did post-staining reveal a polymorphism that was not revealed by pre-staining. In some cases, neither protocol detected a polymorphism (Figure 2C). Sequence analysis corroborated the absence of sequence polymorphism in these latter cases and the presence of sequence polymorphism in all other cases (Table 1). One case found resolution

Figure 1. Four single strand conformational polymorphism (SSCP) staining methods in a single polyacrylamide gel. SSCP of H09g marker for two *Tribolium castaneum* strains (M2, *mas p au*; T2, *Tiw-1*) show duplicate applications in each of four treatments (EB, ethidium bromide; SYB, SYBR Gold). λ DNA (50 ng) loaded into lanes marked "L" served as fluorescent controls. This 10% polyacrylamide gel [99:1 methylene-bis-acrylamide in $1\times$ Tris-borate EDTA (TBE)] was run in a vertical position at 10°C (5°C water jacket) with $0.5\times$ TBE running buffer for 60 min at 180 constant volts. Pre- and post-stained samples were run on the same gel and physically separated for postelectrophoretic treatment.

Figure 2. Pre-stain versus post-stain single strand conformational polymorphism (SSCP). Negative fluorescent images are shown for two strains of *Tribolium castaneum* (M, *mas p au*; T, Tiw-1), two staining methods (SYBR Gold pre-stain, ethidium bromide post-stain), and nine molecular markers. Markers are grouped as: (A) stain-dependent polymorphisms (H09g, H115, M2s); (B) stain-independent polymorphisms (A011, B16s, F2s); and (C) nonpolymorphic (Tsh, J1s, and CHSA). Polyacrylamide gel electrophoresis conditions were same as Figure 1 and run for 90 min. Pre- and post-stained samples were run on the same gel and physically separated for postelectrophoretic treatment. STS, sequence-tagged site.

of the polymorphism to be sensitive to $MgCl_2$ concentration in the phase I buffer. Pre-stain loading buffers effectively stained DNA up to 14 days if held at 5°–6°C. In addition we have successfully reproduced SSCP patterns from PCR-amplified DNA stored for 18 months at 5°–6°C in the phase I buffer containing SYBR Gold (data not shown).

We surmise that SYBR Gold interacts with ssDNA in a sequence-specific way to reveal SSCPs. Cyanine-based nucleic acid stains have been shown to alter the molecular character of DNA both electrostatically and structurally and as a result modify electrophoretic properties (14). Our results further support this premise with the influence upon mobility shifts and show sensitivity to a single nucleotide polymorphism within an ssDNA fragment of 138 nucleotides, and to larger fragments (401 nucleotides) with multiple polymorphisms. Pre-stained PCR products show SSCP pattern reproducibility within gels and across gels over time. Investigation is under way to determine sequence characteristics of the pre-stain dependency.

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Microsoft® Excel® spreadsheet-based animal colony management for genetically altered animals

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Animal research has significantly advanced our understanding of mammalian systems and disease. The need for genetically altered animal models, particularly mice, is expected to increase due to the Human and Murine Genome Projects that plan to identify all genes, and explore their role in relation to other genes. It has been these authors's experience, that despite the anticipated value of gene targeted and transgenic animal studies toward this goal, many institutions do not maintain a transgenic mouse colony, in part due to the high costs of genotyping (and associated housing of animals during the genotyping delay) and extensive record keeping required with these colonies. Federally mandated regulatory oversight of animal colonies also dictates a high quality of animal care, including health monitoring and record keeping. Thus, these facilities are a major fiscal burden to every institution that maintains research animals.

In addition to routine animal care expenses, genetically altered animal colonies incur additional costs associated with the need for rapid and accurate assessment of the genotype for every animal produced. A tissue sample (tail snip or blood sample) is obtained from the progeny, the chromosomal DNA is isolated, and genotyping data is generated to identify progeny harboring the perti-

nent genetic alterations. The genotyping information is essential to identify mice that will be designated as experimental or breeding stock. Breeding strategies can be very complex, depending on the genotype of the breeding stock and the desired genetic make up of the progeny. Genotype considerations are not limited to the induced chromosomal changes, but can also include the genetic background strain of the line that harbors the genetic alterations. Thus, essential to the efficient management (and financial survival) of a transgenic colony is the ability to rapidly generate and communicate genotyping data, breeding instructions, and breeding productivity between the end user(s), the animal care staff, and the genotyping staff.

Animal colony management has been an issue of concern for many years (1-8). There are a number of animal colony management software programs available, each with their own advantages and special adaptations. Colony management software costs can range from gratis [CoPE (a collaborative pedigree drawing environment) Software. Retrieved December 8, 2003 from <http://www.nslj-genetics.org/soft/cope.pdf>] to greater than \$1 million for one license (Progeny Software, LLC. <https://www.progeny2000.com/products.html>; Southwest Foundation for Biomedical Research.

Genetic Analysis and Pedigree Management Software. <http://www.sfb.org/sfbr/public/software/software.html>; colony management databases. <http://maillists.uci.edu/mailman/public/uci-mouseusers/2001-December/000007.html>). In each case, employees must be trained in the software operation, and if a problem arises, the facility is solely dependent on the technical support of the originator. A nightmare scenario is that the researcher or colony manager is unable to access the database after they are dependent on it. Cost-effectiveness and ease of operation are also important factors when deciding on animal colony management software, especially in view of potentially high turn over of animal care staff and limited training resources. The process described here is a very basic system that is inexpensive, well documented, and readily modified to meet the special needs of any colony/end user. The only software requirement is Microsoft® Excel®, and the only hardware requirement (in addition to a personal computer) is a mechanism to allow the same Excel file to be accessed by multiple users (animal care staff, genotyping facility, and end user staff). In the Excel workbook, related information is stored in one database (also called a file) that can contain many spreadsheets, which organize, list, and analyze related information. Group access to the database is easily accomplished on any local area network.

Figure 1 describes the overall process, illustrating the interactions and the information flow between all staff and the two databases (breeding and genotyping) that are maintained as two separate Excel workbooks. Entries into the genotyping database are initiated by the animal care staff when a litter is weaned, ear-tagged, and a tissue sample is sent to the genotyping facility (which might also be the end users laboratory). The animal care staff enters the weanling data [date of birth (DOB), unique identifier or tag number, sex, parents] for all progeny (this data set can be modified to meet the needs of each facility and can include other information deemed essential, such as cage #, room #, rack #, name of animal care staff entering data, etc.). See Figure 2A for sample genotyping database entries. Results of genotyping assays (PCR or Southern blot analysis) are entered by the genotyping facility and should