Amplified fragment length polymorphism (AFLP) markers are used for a variety of genetic applications including population genetic studies (1,2), mapping (3), and gene discovery (4). Genomic DNA or cDNA is digested with restriction enzymes in the presence of synthetic adaptors in a “restriction/ligation” reaction that produces a population of anonymous DNA fragments with known ends. Selective primers complementary to the adaptors with additional 3’ nucleotides are then used to amplify specific subsets of the modified fragments under stringent PCR conditions. Typically, the resulting AFLP profiles are separated using polyacrylamide, and the markers are scored for presence or absence (5).

One of the advantages of AFLP is that a large number of markers can be generated based on differential combinations of selective primers (5). The use of fluorescently labeled selective primers in conjunction with a capillary sequencing device provides a robust platform for resolving fragment profiles, but a limiting factor for this type of resolution is the cost of fluorescently labeled selective primers. Cost includes the relatively short lifespan of fluorescently labeled primers. We report here a strategy for labeling selective AFLP products using a single fluorescently labeled AFLP primer. This strategy requires no new technical skills on the part of the investigator and provides greater consistency as batch-to-batch variation in primer synthesis is lessened.

Our AFLP protocol is very similar to the original description of AFLP by Vos et al. through the selective amplification step with the addition of a labeling step (6). Specifically, unlabeled selective reactions are diluted 500x and used as template for an additional nonselective reaction using a fluorescently labeled preselective primer. We have tested this strategy on a variety of organisms including Phytophthora capsici, horseweed (Conyza Canadensis), dogwood (Cornus florida), and soybean cyst nematodes (SCN) (Heterodera glycines) comparing AFLP profiles generated using fluorescently labeled selective primers versus AFLP profiles labeled using nonselective primers. In each case, the profiles generated were identical (Figure 1).

In brief, high molecular weight DNA was extracted from approximately 10 mg of freeze-dried material using DNeasy® mini-kits (Qiagen, Valencia, CA, USA). Freeze-dried starting material consisted of Phytophthora mycelium grown in antibiotic-amended V8 broth, SCN eggs, and leaf discs of horseweed and dogwood leaves. DNA was quantified on an agarose gel with known standards. EcoRI was obtained from Invitrogen (Carlsbad, CA, USA), while MseI and T4 DNA ligase were obtained from Takara (Madison, WI, USA). EcoRI and MseI adapters and primers for ligation and amplification reactions were obtained from Integrated DNA Technologies (Coralville, IA, USA). For sequences for the adapters and primers, see Vos et al. (6). The fluorescently labeled primers were obtained from Proligo (Boulder, CO, USA) and are comprised of the WellRED D4-PA label sequence with and without any selective nucleotides and either the WellRED D4-PA label (Proligo) or the WellRED D3-PA label at the 5’ end.

Restriction and ligation reactions were carried out simultaneously in 11-μL volumes containing 150 ng of genomic DNA in 1x T4 ligase buffer, 0.5 M NaCl, 0.045 M bovine serum albumin (BSA), 0.5 μM EcoRI adapter, 5.0 μM MseI adapter, 5 U EcoRI, 1 U MseI, and 1 U T4 DNA Ligase. Reactions were placed on a Dyad® PTC-220 thermal cycler (MJ Research,


BENCHMARKS

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