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Eurofinsgenomics RAPD 10-mer Kits and Primers

Product Description

Eurofinsgenomics RAPD 10-mer Kits contain 10-base oligonucleotide primers for use in genetic mapping (Williams et al.[1]) and DNA fingerprinting (Welsh and McClelland[2]). Eurofinsgenomics presently has 1,200 different 10-base primers in stock. These primers are sold in kits of 20 sequences each and are designated "Kit A" through "Kit Z," "Kit AA" through "Kit AZ," and "Kit BA" through "Kit BH." The oligos were selected randomly from a group of sequences with a 60% to 70% (G+C) content and no self-complementary ends.

Principle of Technique

A single 10-base oligonucleotide primer is used to amplify genomic DNA. A DNA amplification product is generated for each genomic region that happens to be flanked by a pair of 10-base priming sites (in the appropriate orientation), which are within 5,000 base pairs of each other. Amplification products are analyzed by electrophoresis. Genomic DNA from two different individuals often produce different amplification fragment patterns. A particular DNA fragment which is generated for one individual but not for another represents a DNA polymorphism and can be used as a genetic marker. These markers are inherited in a Mendellian fashion(1). In mapping studies, the segregation of these markers among the progeny of a sexual cross can be used to construct a genetic map. In fingerprinting studies, the banding patterns are compared directly to allow strain determination, usually without the need to correlate band differences with particular properties.

Methods

Each Eurofinsgenomics 10-mer sample tube contains enough primer for approximately 1000 amplification reactions. Each 10-mer sample tube should be resuspended in sterile water or TE buffer at pH=7. For long term stability, we recommend that you subdivide each 10-mer sample into several aliquots, dry each aliquot, and store at -20°C. Use clean disposable plasticware for all transfers.

For DNA amplification, the following conditions are those originally recommended by Williams et al.(1)

Amplification reactions are performed in a volume of 25 µl containing 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 2 mM MgCl2, 0.001% gelatin, 100 µM each of dATP, dCTP, dGTP, and dTTP, 5 picomoles [add

1 mL, take out 1 µL and add 9 µL of water. Of this solution take 1 µL for the reaction] of a single

10-base primer, 25 ng of genomic DNA, and 0.5 units of Taq DNA polymerase (Perkin Elmer Cetus). Amplification should be performed on a top quality thermal cycler programmed for 45 cycles of 1 minute at 94°, 1 minute at 36°, and 2 minutes at 72°. Amplification products are analyzed by electrophoresis in 1.4% agarose gels and detected by staining with ethidium bromide.

More recently published methods recommend an annealing temperature between 33° and 35°.

Nadeau et al.[9], have recommended slightly different conditions than those of the original procedure. Nadeau recommends the use of 25 picomoles [add 200µL, take out 1 µL and add 9 µL of water. Of this solution take 1 µL for the reaction] of primer and 100 ng template (a fivefold and fourfold increase over Williams' recommendations, respectively.) These conditions may improve the reproducibility of the results of this method.

Number of Amplification Products

The number of different amplification products for each reaction depends upon the primer sequence, the genomic sequence, and the genome size. Assuming that the priming sites are randomly distributed throughout a genome, probability theory predicts that the number of amplification products will be approximately $2.5 \times 10^{-9} \times G$, where G is the size of the haploid genome in base pairs. For example, lettuce has a haploid genome size of approximately 2×10^{9} base pairs, so the above formula predicts that a typical reaction should yield between 5 and 10 bands, depending on the extent of genetic heterozygosity. This prediction is in close agreement with actual results in lettuce obtained by Michelmore et al. (5). However, for much smaller genome sizes, such as that of *E. coli* ($G = 4 \times 10^{6}$ base pairs), the above formula predicts that most primers should generate no bands at all. Nevertheless, several laboratories have reported multiple amplification products from prokaryotic DNA. Such results can only be explained on the basis of mismatch between the primer and the DNA template[1].

Naming of Primers and Markers

In order to prevent ambiguity in the naming of primers from different sources, Eurofinsgenomics attaches the prefix "OP" to the names of all of its primers. For example, the fourth sequence in Kit H is labeled "OPH-04." To refer to a specific polymorphic amplification product, we recommend the convention used by Paran et al.[3] which is to add a subscript denoting its size to the primer name. For example, an 800 bp amplification product produced by primer OPAC-01 would be called

"OPAC-01800."

Troubleshooting

Not all amplifications products arise from perfect pairing between primer and DNA template.

Amplification products arising from mis-pairing may still be reproducible and may be useful genetic markers. However, we suspect that these mismatched markers are more sensitive to slight changes in the temperature cycle, so we strongly suggest using identical amplification conditions when comparing results.

The DNA amplification method described above is unusual in that it uses very short (10-base) primers, which have less specificity than longer primers. As a result, this method is quite sensitive to small variations in the temperature cycle, particularly the annealing temperature. Since the actual temperatures delivered to the tubes by different thermal cyclers may differ significantly, it is often necessary to refine the recommended temperature program in order to optimize this method to your particular thermal cycler. If no amplification products are seen, it may be necessary to adjust temperatures downward. If too many products are seen, it may be necessary to adjust temperatures upward.

Occasionally, a "smear" of amplification products is observed, and this may be converted to discretely sized bands by adjusting the concentration of the polymerase, the primer, or the genomic DNA.

It is important that your genomic DNA is relatively free of single strand breaks since such breaks will prevent amplification. Treat your genomic DNA very gently to prevent shearing. In particular, avoid repeated boiling or freeze-thawing of your genomic DNA samples.

Although we have chosen oligonucleotide sequences which do not have self-complementary ends, some of our 10-mers are still capable of forming complicated secondary structures which can lead to the production of amplification artifacts. These artifacts tend to appear only if one intentionally omits the genomic DNA to test a "reagent blank." These artifacts have now been directly observed[(10)]. Fortunately, such artifacts are not produced when genomic DNA is included.

Uses in Genetic Mapping

Of those kits already tested, our customers report getting useful genetic markers from about 50% to 98% of our 10-mer sequences, depending on the species.

A variety of mapping and fingerprinting strategies which employ this technique have appeared in the scientific literature and are listed below (1-10). This technology has been reviewed by Rafalski et al.[(6)].

References

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