Product Manual

RAPD (Random Amplified Polymorphic DNA's)
Gene Mapping, Polymorphism, DNA fingerprinting
AFLP(Amplified Fragment Length Polymorphism)

Gene Detection System

GL RAPD Decamer Sets

Catalog No. 40-0001-XX

Arbitrary sequence decamer sets for rapid discerning of DNA polymorphism. There are 30 sets available, each containing 40 primers with 60-70% GC content. These primers are used in amplification reactions for determining Random Amplified Polymorphic DNA (RAPD) fragments.

GL RAPD Decamer Sets

Shipped at ambient temperature. Store at -20°C

For research use only

Not for use in diagnostic procedures for clinical purposes.

Set Contents

Arbitrary sequence decamer sets for rapid discerning of DNA polymorphism. There are 30 sets available each containing 40 primers with a 60-70% GC content. These primers are used in amplification reactions for determining Random Amplified Polymorphic DNA (RAPD) fragments.

Each tube contains 10 nmols of lyophilized primer. This is sufficient for thousands of amplification reaction. Please see Protocol section for details.

Thirty decamer sets listed below are available. Primer Data Sheet for each set is given in the Appendix and supplied with the product.

	Catalog No	Product Name
❑	40-0001-01	GL RAPD Decamer 01–AB Set
❑	40-0001-02	GL RAPD Decamer 02–CD Set
❑	40-0001-03	GL RAPD Decamer 03–EF Set
❑	40-0001-04	GL RAPD Decamer 04–GH Set
❑	40-0001-05	GL RAPD Decamer 05–IJ Set
❑	40-0001-06	GL RAPD Decamer 06–KL Set
❑	40-0001-07	GL RAPD Decamer 07–MN Set
❑	40-0001-08	GL RAPD Decamer 08–OP Set
❑	40-0001-09	GL RAPD Decamer 09–QR Set
❑	40-0001-10	GL RAPD Decamer 10–ST Set
❑	40-0001-11	GL RAPD Decamer 11–UV Set
❑	40-0001-12	GL RAPD Decamer 12–WX Set
❑	40-0001-13	GL RAPD Decamer 13–YZ Set
❑	40-0001-14	GL RAPD Decamer 14–AAAB Set
❑	40-0001-15	GL RAPD Decamer 15–ACAD Set

	Catalog No	Product Name
❑	40-0001-16	GL RAPD Decamer 16–AEAF Set
❑	40-0001-17	GL RAPD Decamer 17–AGAH Set
❑	40-0001-18	GL RAPD Decamer 18–AIAJ Set
❑	40-0001-19	GL RAPD Decamer 19–AKAL Set
❑	40-0001-20	GL RAPD Decamer 20–AMAN Set
❑	40-0001-21	GL RAPD Decamer 21–AOAP Set
❑	40-0001-22	GL RAPD Decamer 22–AQAR Set
❑	40-0001-23	GL RAPD Decamer 23–ASAT Set
❑	40-0001-24	GL RAPD Decamer 24–AUAV Set
❑	40-0001-25	GL RAPD Decamer 25–AWAX Set
❑	40-0001-26	GL RAPD Decamer 26–AYAZ Set
❑	40-0001-27	GL RAPD Decamer 27–BABB Set
❑	40-0001-28	GL RAPD Decamer 28–BCBD Set
❑	40-0001-29	GL RAPD Decamer 29–BEBF Set
❑	40-0001-30	GL RAPD Decamer 30–BGBH Set

Background

Polymorphism

The vast amount of phenotypic differences between individuals may be either due to genetic differences-meaning differences encoded at the DNA level or due to non-genetic differences particularly dependent on environmental interaction. The condition of the existence of two or more forms of DNA sequence for a particular stretch or forms of proteins that are genetically distinct and structurally different is known as polymorphism.

Genetic Polymorphism is defined as the occurrence of multiple alleles at a locus, where at least two alleles exist with frequencies greater than 1 percent. Examples of polymorphism at the protein level a) Blood group system ABO, MNSs, Rh. b) Galactosemia, galactose-1-phosphate uridyl transferase locus. c) alpha-1-antitrypsin (1).

Polymorphisms are principally of value for their use as genetic "markers" to distinguish different inherited forms of a gene in family studies.

The human haploid genome consists of ~ $3x10^9$ bases. Not all individuals have exactly the same DNA sequences. Differences in DNA sequence termed as polymorphism exists in the human population. These differences in DNA sequence may either create or abolish a site recognized by a restriction endonuclease thereby upon digestion, electrophoretic separation and southern analysis may give rise to differing hybridizing fragment lengths. DNA based variations at restriction sites detected in this manner are called **restriction fragment length polymorphism (RFLP)**. The differing fragment lengths constitute codominant alleles at a DNA locus. RFLPs may also arise from deletion or insertion of DNA, rather than from single nucleotide changes (1).

Polymerase Chain Reaction has afforded rapid progress in genetic research, Various techniques have essentially replaced the use of RFLP based analysis of polymorphism. The use of Short Tandem Repeat (STR), VNTR and microsatellite markers has generated enormous amount of polymorphic data by incorporating multi loci high throughput screening (2). The limiting factor in microsatellites is the initial generation and establishing of these markers.

A PCR based RFLP analysis is termed. Another modified approach include AFLP (Amplification fragment length polymorphism) for DNA fingerprinting (3) and genetic characterization in plants (4).

Other techniques have utilized the fact that large number of primer combination based amplification has to yield polymorphic amplified fragments. A robust random approach of using random sequence decamer primers to amplify DNA is named **RAPD (Random Amplified Polymorphic DNA**, (pronounced RAPID)) (5-7).

Polymorphisms are principally of value for their use as genetic "markers" to distinguish different inherited forms of a gene in family studies. Genetic markers are of enormous practical use in medical genetics for:

- Mapping genes to individual chromosomes by linkage analysis.
- Pre-symptomatic and prenatal diagnosis of genetic disease.
- Detection of heterozygous carriers of genetic disease.
- Evaluation of high- and low-risk persons with a predisposition to common adult disorders, such as coronary heart disease, cancer, and diabetes.
- Paternity testing and forensic application.
- Matching of donor-recipient pairs for tissue and organ transplantation.

The main context of this manual is focused on human and medical genetics. The same protocols have been used for all DNA polymorphism studies and the same implications can be extended to bacterial, plant and other organisms.

RAPD Decamer Primers

Williams et al first reported the use of arbitrary sequence short primers (5). They described a new assay for DNA polymorphism based on the amplification of random DNA segments with ***single*** primers of arbitrary sequence. These fragments amplify from one parent DNA and not from the other, signifying Mendelian inheritance. Such polymorphism can be valuable for construction of polymorphic genetic maps (5-7).

Gene Link RAPD decamer sets include arbitrary decamers of unique sequence. The individual primers are constructed to have a GC content between 60-70% with no complementary ends to minimize primer dimers. Selected sequences representing some of the combination of unique sequence without runs are spread over 30 sets, each containing 40 primers. The total number of primers spread over 30 sets is 1200.

Probability of Polymorphic Fragments

The probability of finding these short decamer primer cognate sequences in the target DNA varies and may be numerous, such that a single primer generates multiple amplification fragments. Discerning a polymorphic site is simply based on the presence in one and absence in another of the same size fragment. Repeating the amplification *should* give the same result if it is truly polymorphic. The fragment pattern generated for a particular species DNA should be constant in reproducible experimental conditions. A primer's ability to generate amplification products is dependent on its sequence and the genome size of the template. The technique is actually based on the premise of 'randomness'; the primers finding its complementary sequence randomly and possibly fortuitously polymorphic and not present in the 'other' template.

Probability calculation of the frequency of a decamer predicts between 5-10 in a genome size of 2×10^9 base pairs. Smaller genome size still generates amplification products, possibly due to mismatch miss-priming.

Nomenclature of Amplified Fragment

Gene Link RAPD decamer sets follow the same numbering pattern adopted by Operon. The similar numbering will assist customers with a universal numbering system. The primer sets are named using letters and the individual primers are named using numerals. Gene Link recommends using the convention of Paran et al. (8). Use the primer name followed by polymorphic fragment size as the suffix.

Protocol

Gene Link decamer primer sets contain 40 primers. Each tube contains 10 nmols of lyophilized primer. See protocol section below for details regarding reconstitution and use. Gene Link recommended amplification protocol given below might be different than what you may be using. It is a modified protocol with a higher annealing temperature (9). We advise you to follow the protocol already established in your laboratory. As with all initial experiments, we suggest aggressive optimization of reaction conditions and the following protocol be used only as a guideline.

Gene Link recommended protocol use a higher annealing temperature between 50 ^{o}C -55^{o}C based on a report by Atienzar et al (9). They reported that the annealing temperature avoided spurious formation of amplification products and increased the reproducibility.

Reconstitution of Primers

Gene Link recommends preparation of a stock solution of the primers to yield a concentration of 10 μM (10 pmol/μl), and a working solution of 1 μM (1 pmol/μl).

a) **Stock solution:** Add 1 ml of sterile water or TE to the supplied tubes containing the primers. This will yield a 10 μM (10 pmol/μl) solution.

b) **Working Solution**: Further 10 fold dilution to obtain a 1 μM (1 pmol/μl) solution.

PCR parameters 25 μl reaction volume

Annealing Temperature:	50 ^{o}C -55^{o}C
Mg^{++} concentration:	3-6 mM
dNTP concentration:	0.33-0.44 μM
Primer concentration:	1.0 – 2.0 μM
Taq Polymerase:	0.1 unit/μl
Template DNA:	0.2-4 ng/μl

PCR Buffer (1X): 10 mM Tris-HCl pH 8.8, 50 mM KCl, 0.08% Nonidet P40, BSA 0.1 μg/μl

Thermal Cycling:
Initial 95^{o}C for 4 min.
39 Cycles of 95^{o}C for 1 min.
Annealing at 50^{o}C for 1 min.
Extension at 74^{o}C for 1 min.
Final Cycle at 74^{o}C for 10 min.

Electrophoresis

The amplified fragments are resolved in a 1.4% agarose gel and visualized by ethidium bromide staining. Native polyacrylamide gels have been used at Gene Link for sharp resolution of short fragments between 100-400 bp. Molecular biologists find polyacrylamide gels tedious to prepare and handle but they provide a better alternate to agarose gels for resolution of short fragments.

Results and Interpretation

Obtaining good and reliable results requires meticulous and careful experimental conditions. The template quality is of paramount importance. The DNA should be predominantly of high molecular weight with very few-sheared DNA. Highly sheared DNA may loose the polymorphic site by breakage and thus not yield informative results. Thorough consistency is required to enable reproducing useful results obtained. Due to the arbitrary nature of this technique, polymorphic fragments may not be obtained even by using all the primers under stringent conditions. Under such eventuality, it is recommended that the annealing temperature be decreased gradually.

The amplified fragment gel profile may seem daunting with numerous bands. Closer visualization should clearly reveal a pattern of presence and absence of a particular band. This should be noted as possibly polymorphic and should be reproducible. Several published reports indicate the use of RAPD markers yielding polymorphic markers from template DNA from plants, bacteria and mice (10-12).

Smeared Fragment Pattern is generally a sign of mismatched priming and excess non-stringent amplification. A smear pattern is almost always converted to distinct fragments by optimizing the concentration of the primers, annealing temperature, polymerase and the template.

Use of Multiple RAPD Primers has been shown to improve discrimination over one primer used individually. This increased discrimination is limited to profiles that already show a difference. Two virtually identical patterns obtained by using one primer indicate highly related strains and cannot be more informative by using two primers (13).

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