RESEARCH NEWS

Making the perfect mismatch

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Scanning for point mutations and small base pair deletions or insertions in disease genes is a notoriously difficult undertaking. Mutation detection for these alterations falls into two general categories. The first is detection of either mutations that are conserved among ethnic groups (e.g., the $\Delta F508$ cystic fibrosis mutation in individuals of Northern European ancestry¹) or common mutations occurring in a large collection of patients without ethnic predisposition (e.g., hemophilia¹). For these cases, current methodologies are both useful and applicable for most scenarios (for review, see refs 1,2).

The second category of mutation detection is, however, more complex. This involves the detection of multiple small alterations in a rather large gene with no ethnic predispositions. One of the best examples of this is growth hormone insensitivity (or Laron's) syndrome, in which numerous "private" mutations (i.e., those occurring within individual families) in the growth hormone receptor locus have been described³. Mutation detection in such syndromes becomes a laborious and tedious process. What, then, is the best approach for inherited disorders that fall into both of these categories? One answer may be the new technique described by Guo et al. in this issue⁴.

The past decade has witnessed the development of a variety of mutation scanning techniques (for review, see refs 1,2,5). Those commonly used include single-strand conformational polymorphism (SSCP), heteroduplex analyses and denaturing gradient gel electrophoresis (DDGE). Both SSCP and DDGE operate on the principle that, under certain conditions, single-stranded DNA (ssDNA) has a defined secondary structure, which can be altered by base pair substitution. Specific electrophoretic conditions are employed to detect thermolabile differences in ssDNA molecules, with SSCP being the simpler and more adaptable technique. Heteroduplex analysis, like SSCP, is based on electrophoretic separation of mutant alleles, but uses double-stranded DNA rather than ssDNA². Although SSCP and heteroduplex

analysis are in general inexpensive and straightforward techniques, their major drawback is the mutation detection rate, which is only ~80%.

More advanced techniques have been developed to increase the mutation detection rate to 90% and above². Several of these focus on allele-specific PCR amplification, with variations on the central methodology of placing one PCR primer of a primer pair at the site of a known mutation. The primer is either designed as the wild-type or mutant sequence and thus amplifies the corresponding gene sequence. Many of these techniques have now become automated², allowing multiple mutation screening in large cohorts of patients. Again, the mutation being screened for must be known.

The method described by Guo et al. is a twist in mutation detection. It is most closely related to the ARMS (amplification refractory mutation system) technique, in which artificial mismatches are introduced at the terminal 3' nucleotide of a PCR primer. In the ARMS technique, the primer is designed in two forms: The wild-type (which is refractory to PCR of the mutant allele), and the mutant (which is refractory to PCR of the wild-type allele). Through use of specific combinations of primers, one can detect mutant and wild-type alleles⁶.

Guo et al. modify this approach of artificial mismatch by introducing a "universal" nucleoside substitute, 3-nitropyrrole⁷, which forms weak hydrogen bonds with all four bases in DNA without steric disruption of the DNA duplex. Using this approach, the authors elegantly demonstrate that the introduction of artificial mismatches (see p. 331, Fig. 1B and C) into oligonucleotide probes significantly enhances the detection of single nucleotide changes over conventional allele-specific mismatch ("true mismatch," p. 331, Fig. 1A) oligonucleotide probes.

Although the majority of the experiments discussed in their report focused on the use of oligonucleotide hybridization for mutation detection [i.e., allele-specific oligonucleotide (ASO) assays]. The authors clearly demonstrate that the introduction of one or more artificial mismatches at least three bases from a "true" mismatch enhanced the discrimination between wild-type or mutant alleles, altering the T_m as much as 8°C when the spacing between the

two mismatches was optimal. Furthermore, when two artificial mismatches were introduced (Fig. 1C), and spacing between the two 3-nitropyrrole groups was 8–12 bases, a 50% increase in T_m was observed, indicating that the ability to extend the range of artificial mismatch hybridization to longer oligonucleotide sequences may be quite important for detecting specific DNA alterations in complex genetic backgrounds. Clearly, the technique is useful for improvement of current oligonucleotide hybridization techniques.

The use of artificial mismatch in allele-specific PCR amplification was also addressed (see Fig. 7 in their paper). In these experiments, the authors used one primer that perfectly matched the target DNA, whereas the second primer contained either one or two 3-nitropyrrole mismatches in combination with either a true mismatch or a wild-type sequence at a specific polymorphic base. Again, the authors claim that this technology improves the specificity of conventional allele-specific PCR amplification.

In summary, Guo and colleagues present an eloquent discussion of a new technology that should prove useful in allele-specific mutation detection. The general applicability of the technique remains to be determined, especially in relation to the cost of synthesizing sequence-specific primers with various 3-nitropyrrole substitutions. One should emphasize that, at this time, this technique, and many other related technologies, can be applied only to the detection of known mutation alleles. Improved methodologies for the detection of varying and private disease-causing mutations await further development.

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