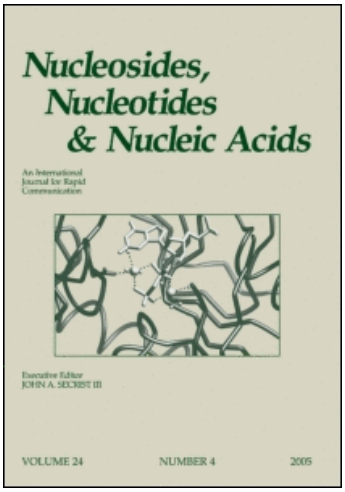

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ROLLING CIRCLE AMPLIFICATION FOR SCORING SINGLE NUCLEOTIDE POLYMORPHISMS

A. Rösler,[1,*] L. Bailey,[1] S. Jones,[1] J. Briggs,[1] S. Cuss,[1] I. Horsey,[1] M. Kenrick,[1] S. Kingsmore,[2] L. Kent,[1] J. Pickering,[1] T. Knott,[1] E. Shipstone,[1] and G. Scozzafava[1]

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ABSTRACT

The analysis of the genetic basis of phenotypic traits is moving towards the complex diseases prevalent in wealthy populations. There is an increasing requirement for the detection of different types of sequence variation, particularly single-nucleotide polymorphisms (SNPs). SNPs occur about once every 100 to 300 bases. High-density SNP maps will help to identify the multiple genes associated with complex diseases such as cancer, diabetes, vascular disease, and some forms of mental illness.

There are a number of key criteria that must be addressed when developing a SNP typing technology. These include accuracy, flexibility, cost, throughput, sensitivity, and the capacity to use genomic DNA rather than Polymerase Chain Reaction (PCR) products. SNiPer^{TM} has therefore been developed with a ligation based allele discrimination step on genomic DNA. Detection of ligated products is by rolling circle amplification (RCA) (1,2) and fluorescence based end-point detection, without the need for any purification steps.

The SNiPer RCA assay uses allele-specific circular probes to provide highly accurate allele discrimination. A positive match depends on two hybridization

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events combined with a ligation event, a process that confers a high degree of specificity to the assay. Generic amplification primers are used that do not require redesigning for each SNP, thereby reducing the cost per assay. The primers incorporate fluorescence resonance energy transfer (FRET) dyes, which eliminate the need for purification steps. RCA delivers an amplification level of 10^9–10^{12} fold. This level of sensitivity enables the assay to be conducted directly on genomic DNA samples. All reactions are isothermal, making the assay amenable to automation and miniaturization within a microtitre plate format.

SNiPer System

SNiPer combines the RCA assay described above with robotic liquid handling and automated plate manipulation into a fully integrated SNP scoring system. This is a scaleable system capable of handling 10,000 to 500,000 genotypes per day in 384 well microplate formats. SNiPer software enables automated design of long open-circle oligonucleotide probes and includes a direct link to oligonucleotide ordering services. Assay set up is automated and flexible allowing many samples to be typed for one SNP or one sample to be typed for many SNPs. The laboratory workflow system (LWS) registers tracks and manages samples and reagents. LWS also translates data into results for subsequent analysis and quality checks using SNP calling software.

CONCLUSION

SNiPer is a sensitive, fully automated, high-throughput SNP scoring system designed for the fluorescent detection of SNPs directly from genomic DNA. The incorporation of RCA technology enables isothermal, geometric amplification of circular oligonucleotide probe and provides a homogenous fluorescent end-point that eliminates the need for purification steps. Two alleles are scored per well maximizing accuracy while a microtitre plate format enables scalable throughput. The use of generic probes in a highly sensitive, non-PCR-based system provides a cost-effective innovative technology that requires minimum consumption of precious samples.

REFERENCES

1. Lizardi, P.; Huang, X.; Zhu, Z.; Bray-Ward, P.; Thomas, D. and Ward, D. Mutation detection and single-molecule counting using isothermal rolling-circle amplification. *Nature Genetics* 1999, 19, 225–232
2. Baner, J.; Nilsson, M.; Mendel-Hartvig, M.; and Landegren; U. Signal amplification of padlock probes by rolling circle replication. *Nucleic Acids Research* 1998, 26, 5073–5078.

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