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Editorial

An Efficient DNA Sequencing Strategy

Maria Luisa Chiusano[1]

[1]*Department of Agricultural Sciences, University of Naples Federico II, Via University, Portici (NA), Italy*

INTRODUCTION

Efficient DNA sequencing of the genomes of individual species and organisms is a critical task for the advancement of biological sciences, medicine and agriculture. Advances in modern sequencing methods are needed to meet the challenge of sequencing such mega base to gig abase quantities of DNA. Two possible strategies for DNA sequencing exist: direct methods, in which each base position in the DNA chain is determined individually (e.g., gel sequencing or pyro sequencing), and indirect methods, in which the DNA sequence is assembled based on experimental determination of oligonucleotide content of the DNA chain. One promising indirect method is sequencing by hybridization (SBH), in which sets of oligonucleotides are hybridized under conditions that allow detection of complementary sequences in the target nucleic acid. The unprecedented sequence search parallelism of the SBH method has allowed development of high-throughput, low- cost, and miniaturized sequencing processes on arrays of DNA samples or probes. Newly developed SBH methods use DNA ligation to combine relatively small sets of short probes to score potentially tens of millions of longer oligonucleotide sequences in a target DNA.

In addition to the applications of oligomer hybridization for physical mapping and complete DNA sequencing, a third application, called partial-SBH, has been proposed by one of us (R.D.) It is based on the idea that the incomplete lists of constituent oligomers will be sufficient to discern biologically relevant information in a comparative analysis of sequences. Partial-SBH should provide direct data for statistically significant inferences of the similarity of unknown DNA fragments to known sequences, to sequences constrained by some imposed rule, or to other unknown sequences. Our expectation is that partial-SBH could localize and define genes on chromosomes with 30-100 times less hybridization data.

Than required for complete sequencing. Partial-SBH could also be used profitably for fingerprinting cDNA libraries and finding and simultaneously following numerous DNA polymorphisms. The data on cDNAs can be used to count and group the expressed genes, assemble complete cDNAs by overlap, compare the popular one was introduced by BÃla˙zewicz et al. in This instance set consists of 40 real DNA target sequences of length 109, 209, 309, 409, and 509 (altogether 200 instances). Based on real hybridization experiments, the spectra were generated with probe size l = 10. All spectra contain 20% negative errors as well as 20% positive errors. For example, the spectra concerning the target sequences of length 109 buffer and blocked with 0.5ml of blocking buffer at 25o C for 5 minutes. 0.5ml of diluted alkaline phosphatase conjugate (BioRad) was added and incubated at 25o C in the device for 3.5 minutes. Unbound conjugate molecules were removed by three direct washes with 0.75ml of washing buffer (0.1M NaCl, 0.1M Tris-HCl, 3mM MgCl2, 0.5% Tween 20 v/v, pH 7.5) and color development was performed by incubating 0.5ml of NBT/BCIP substrate (BD Biosciences) at 37o C for 8 minutes and the reaction was stopped by the addition of 0.5ml of TE. Membranes were rinsed three times with 0.75ml of ambient washing buffer. Developed membranes were interpreted within 5 minutes after NBT/BCIP substrate incubation. All steps of hybridization, washing and color development were completed in the R2-M Flow-through system (DiagCor Bioscience Incorporation Limited) by simply pipetting the corresponding solutions into respective reaction chamber wells, followed by built-in pump removal. Since the reaction chamber is accurately maintained at an appropriate temperature together with solutions of controlled ingredients to provide suitable stringency, highly specific and reproducible results were achieved.

***Correspondence to:** Maria Luisa Chiusano, Department of Agricultural Sciences, University of Naples Federico II, via University, Portici (NA), Italy

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