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Approaches to Library Screening

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Abstract

Fuelled by the drive to complete the Human Genome Project, many laboratories have developed new methods of screening clone libraries. From PCR-based strategies to pooling schemes and increased automation, the tedious task of library screening has become less labour-intensive and more cost-efficient. Currently, two main screening methods dominate: hybridization and polymerase chain reaction (PCR). In the following article, we present a brief overview of hybridization and PCR-based screening of yeast and bacterial libraries. Multi-faceted approaches combining different techniques, as well as less frequently employed methods such as fingerprinting are also described.

Introduction

Prior to the completion of a working draft of the human genome on June 26, 2000 (Meldrum 2000), there was a strong drive to advance genetic and molecular biology genomic analysis methods. Other current large-scale sequencing projects are continuing to provide the impetus to further improve such methods. One of the most common rate-limiting steps in genomic analysis is the screening of library clones. Traditional methods relied upon the Benton-Davis technique (1977) for phage plaques and the Grunstein and Hogness method (1975) for *Escherichia coli* colonies. These protocols involve immobilization of recombinant DNA molecules on solid supports, followed by DNA/DNA or RNA/DNA hybridization with specific probes to detect clones containing sequences of interest. Although effective, these protocols are laborious and prone to artefacts such as false positives. Over the past decade, a number of new strategies have been devised. Such strategies include PCR-based methods, pooling schemes, combination approaches, and increased automation. Additionally, initial hybridization methods have been modified. These new approaches have greatly decreased time and cost requirements of library screening. The following article presents a brief overview of current commonly used strategies to screen yeast and bacterial libraries.

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Hybridization

Screening large numbers of clones to identify those containing desired DNA sequences is a key step in many applications of recombinant DNA technology. One of the first large-scale screening methods introduced, hybridization, quickly became a standard technique that is still in widespread use today (Figure 1a; Grunstein and Hogness 1975, Benton and Davis 1977, Coulson *et al.* 1988, Evans and Lewis 1989, Traver *et al.* 1989, Shizuya *et al.* 1992, Ioannou *et al.* 1994, Nilsen *et al.* 1997, Asakawa *et al.* 1998, Han *et al.* 2000). Hybridization-based screening can be performed against high-density gridded macroarrays of a whole library using either a single type or mixtures of different types of radioactively labelled probes. The most frequently used probes include sub-cloned DNA fragments, PCR amplimer products, or DNA oligonucleotides (Han *et al.* 2000). Several variations upon the common theme of hybridization have been developed including “hybridization fingerprinting” (Craig *et al.* 1990), pulsed-field gel southern blot analysis of pooled clones (Mendez *et al*. 1991), and two-dimensional overgo hybridization (Han *et al.* 2000). The most rapidly-evolving aspect of hybridization-based screening, however, has been increased automation. From robotic colony and plaque pickers to full-fledged automated workstations, there is an increasing emphasis being placed upon mechanical labour-saving devices (Nizetic *et al.* 1991, Uber *et al.* 1991, Olsen *et al.* 1993, Panussis *et al.* 1996, Meldrum 2000). As new technologies continue to force prices lower, such computer-controlled systems will not be limited to large genome centers, but will also be available to smaller laboratories, permitting rapid hybridization-based screening and increased productivity.

Polymerase Chain Reaction

PCR is one of the most widely used techniques in molecular biology. It has been applied to mutation analysis, nucleotide quantification, forensic DNA fingerprinting, and genomic analysis. PCR-based strategies now rival hybridization-based strategies as the method of choice when screening yeast and bacterial libraries. A single PCR can be used to determine insert presence, size, and orientation without the need for purification, restriction digestion, or hybridization. Some of the more frequently used PCR-based screening approaches include sequence-tagged site (STS)-PCR (Green and Olson 1990a), interspersed repetitive sequences (IRS)-PCR (Liu *et al.* 1995), amplified fragment length polymorphism (AFLP; Vos *et al.* 1995, Klein *et al.* 2000), and island rescue PCR (IRP; Valdes *et al.* 1994). Numerous PCR-based screening protocols have demonstrated that crude cell lysate from whole cells or phage is sufficient for analysis (Gussow and Clackson 1989, Zon *et al.* 1989, Bloem and Yu 1990, Isola *et al.* 1991, Israel 1993, McAlinden and Krawetz 1994, Ling *et al.* 1995, Campbell and Choy 2001, Sambrook and Russell

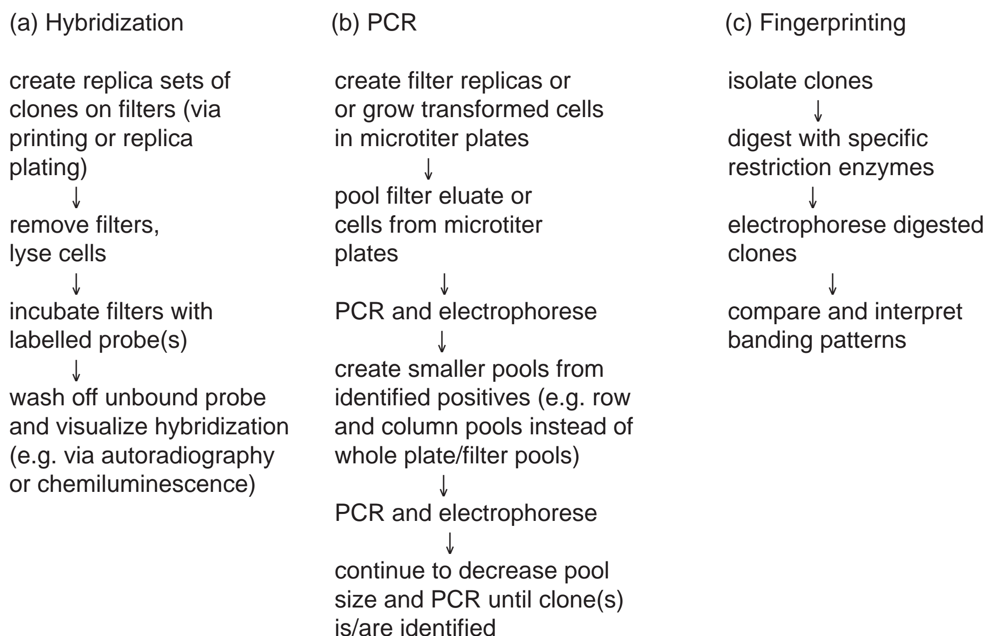

Figure 1. General overview of common library screening methods.

2001). However, many strategies still include a template purification step. Most PCR- based screening methods share a common theme. This involves initial large-scale screening by PCR-amplifying pooled sample from rinsed library filters or microtiter plate cultures, followed by repeated rounds of screening of incrementally smaller pools (i.e. smaller numbers/sections of filters or greater serial dilutions) from which a positive signal has been observed (Figure 1b; Yu and Bloem 1996). The majority of pooling designs involve plate/row/column pools (Kwiatkowski *et al.* 1990, Chumakov *et al.* 1992, Libert *et al.* 1993, Bruno *et al.* 1995, Alphey *et al.* 1997), though variations such as two-step PCR screening (Asakawa *et al.* 1997) and sixfold pooling (Klein *et al.* 2000) have been described. As with hybridization-based library screening strategies, PCR-based techniques have benefited from increased automation. Recent advances in thermal cyclers, for example, have focused on scaling sample volumes down, increasing the number of samples run simultaneously, and decreasing overall required cycling time (Meldrum 2000).

Combination and Other Methods

Though both hybridization and PCR are sufficient screening methods when used alone, it is often desirable to use more than one approach to obtain reliable and robust results. While hybridization offers the advantage of screening large numbers of clones in parallel, it often yields multiple false positives/negatives and requires working with large numbers of filters. PCR, on the other hand, greatly decreases the number of false positive/negatives and the amount of filter preparation, but requires sequence information and primer generation for each probe (Mendez *et al.* 1991). Therefore, it not surprising that the majority of genomic studies use both hybridization and PCR-based methods (Edwards *et al.* 1992, McAlinden and Krawetz 1994, McCormick *et al.* 1993, Brodyanskii *et al.* 1995, Gingrich *et al.* 1996, Kim *et al.* 1996, Umehara *et al.* 1996, Hunt *et al.* 1999, Crooijmans *et al.* 2000). Some reported screening protocols combine both hybridization and PCR into one continuous method, instead of performing each separately. One combination technique, first presented by Green and Olson (1990b), involves rounds of pooled PCR screening with a final hybridization step. This method has been utilized in numerous studies since then (Anand *et al.* 1990, Riley *et al.* 1990, Strauss *et al.* 1992, Bonemma *et al.* 1996, Michalek *et al.* 1997, Gosele *et al.* 2000, Sambrook and Russell 2001). Another combination technique, termed *Alu*-PCR, utilizes primers to conserved *Alu* repeats and selectively amplifies sequences between properly oriented *Alu* segments. These amplified sequences are then identified solely by hybridization (Nelson *et al.* 1989, Nelson *et al.* 1991, Amemiya *et al.* 1992).

Aside from PCR and hybridization, other screening methods have been employed. One such method is "fingerprinting" (sometimes alternatively referred to as "restriction mapping"). In general, this involves clone isolation, restriction digestion, electrophoresis, and banding pattern visualization/comparison (Figure 1c; Silverman *et al.* 1989, Marra *et al.* 1997, Cao *et al.* 1999, Vollrath and Jaramillo-Babb 1999, Zhu *et al.* 1999). Fingerprinting techniques have two main advantages: a small increase in the mapping effort per clone with the size of the project (proportional to the logarithm of the number of clones) and insensitivity to interspersed repetitive sequences (Craig *et*

al. 1990). As with PCR and hybridization, however, fingerprinting is rarely used as the lone screening method, but is instead usually part of a multi-faceted screening approach to ensure data validity and reliability.

Conclusions and Future Perspectives

Over the past decade, there has been an exponential increase in the development of new library screening methods. The efficiency of PCR-based techniques has been increased with the introduction of new primer design tactics, pooling schemes, and improved thermal cyclers. Hybridization-based approaches have been stream-lined due to the creation and modification of labour-saving devices such as robotic plaque/colony pickers and gridders. Though a working draft of the human genome is now complete, other large-scale sequencing projects are continuing to provide the drive to further advance screening techniques. It is likely that increased automation will play a key role in developing new methods and revising past protocols.

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