MethodsX 5 (2018) 669–675

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

A novel method for rapid and sensitive metagenomic activity screening

Meiling Shang[a,1], Victor J. Chan[a], Dominic W.S. Wong[a,*], Hans Liao[b]

[a] Western Regional Research Center, USDA-ARS, Albany, CA, USA
[b] Cargill Biotechnology Development Center, Minneapolis, MN, USA

A B S T R A C T

Direct cloning of metagenomes has proven to be a powerful tool for the exploration of the diverse sequence space of a microbial community leading to gene discovery and biocatalyst development. The key to such approach is the development of rapid, sensitive, and reliable functional screening of libraries. The majority of library screen have relied on the use of agar plates in petri dishes incorporating the target enzyme substrate for activity detection of positive clones (Iqbal et al. [1], Knietsch et al. [2], Popovic et al. [3]). In this article, a novel method is described consisting of: (1) formulation and application of substrate gel microtiter assay plates, (2) screening of libraries of clones in split pools in the wells of the assay plate, and (3) progressive enrichment and isolation of individual positive clones. The method has been successfully used in the rapid discovery of novel genes and enzymes from rumen microbial metagenome with high efficacy.

- Novel substrate gel assay plates for activity screening with localized and intensified signals.
- Rapid and complete screening of library clones in split pools.
- Progressive enrichment scheme as a refining step for isolating target gene.

A R T I C L E I N F O

Keywords: Metagenomic library, Functional screening, Activity screening, Split-pool sub-library screening, Substrate gel microtiter assay plate

Article history: Received 13 April 2018; Accepted 10 June 2018; Available online 20 June 2018

* Corresponding author.
E-mail address: Dominic.Wong@ars.usda.gov (D.W.S. Wong).
[1] Present address: Abbot Laboratories (Hematology Business Unit, Diagnostics Division), 4551 Great American Pkwy, Santa Clara 95054, USA.

https://doi.org/10.1016/j.mex.2018.06.011

Method details

Background

Microbial diversity provides a vast genetic resource for the discovery of new genes, enzymes, natural products, and bioactives with impacts on industrial and biotechnological applications. However, only less than 1% of the microorganisms in the natural environment can be cultured in traditional laboratory conditions. For the last two decades, metagenomic research has demonstrated that direct cloning of collective genomic DNA provides a powerful tool for exploring the diverse sequence space of uncultured microbes [4]. The general methodology involves sampling of environmental microbes, direct extraction of the metagenome, restriction of the total DNA, cloning of the DNA fragments into a suitable vector, and transformation of the recombinant DNA into a host for construction of metagenomic libraries [5]. This is followed by a key step of sequence-based or function-based screening of the library [5–7]. The development of next-generation sequencing technology and advanced bioinformatics has significantly improved the efficiency of sequence-based screening. However, sequence-based search is limited to the screening for homologs of already known sequence motifs, and quite often obtaining incomplete gene sequences. In contrast, function-based screening involves cloning and functional expression of gene-containing fragments in a heterologous host, and allows the detection of active clones, which is particularly desirable for identifying novel biocatalysts [8]. The enzyme activity and biochemical parameters can often be revealed during the screening. One challenge of functional metagenomic is the development of sensitive, reliable and rapid assays for screening libraries. This report describes a novel method for rapid and sensitive screening and isolation of genes/enzymes of interest from complex metagenomes, with the following unique features: (1) the formulation and application of substrate gel microtiter assay plates for rapid screening of library of clones in split pools, and (2) a progressive enrichment and isolation of individual positive clone and the target gene.

Preparation of substrate gel microtiter assay plate

In the general scheme, a metagenomic library was split into small pools (sub-libraries) for culturing in 96-well microplates (culture plates). The sub-libraries grown overnight in the culture plate were then transferred to premade substrate gel microtiter assay plates. The substrate gel was formulated to enable the localization and confinement of the reaction products, resulting in intensified signal for detection [9].

The following is a typical protocol for preparing substrate gel microtiter assay plates. In one test tube, low-melt agarose of 1.5% (w/v) was melted into Luria Bertani (LB) or other suitable growth medium and equilibrated to 50 °C. In a separate tube, buffer, detergent, antibiotic, inducer, and substrate were mixed in the growth medium and equilibrated to 50 °C A typical example would include 50 mM buffer, pH 6.5, 1.67% (v/v) detergent (Triton X-100), 50 μg/ml antibiotic, 2 mM expression inducer, and 2 mM chromogenic substrate added to LB medium to a final volume of 25 ml and equilibrated to 50 °C. The two tubes were combined, mixed and poured in 10 ml aliquots into a reservoir (for multichannel pipettors). Using an 8-channel pipettor fitted with 1.2 ml tips, the combined mixture was quickly dispensed at 50 μl per well to 96-well microtiter plates (half area, flat bottom, polystyrene). A volume of 50 ml would be sufficient for preparing 9 substrate gel microtiter assay plates. After the gel was set, the assay plates were used immediately, or could be stored at 4 °C for several weeks. The substrate gel mix should contain three basic components: (1) a buffer at a suitable pH (dependent on the pH optimum of the enzyme activity to be detected), (2) a detergent Triton X-100, (3) a suitable substrate for detecting expression of the target gene. The type of substrate could be chromogenic, flourogenic, or soluble/insoluble dye-crosslinked, enabling the enzyme reaction product to be quickly visualized and/or measured by a plate reader. The inclusion of antibiotics and inducers would depend on the type of libraries, which can be constructed based on *Escherichia coli*, bacteriophage, phagemid, and so on.