

In Situ Screening of Bacterial Colonies - Protocols

Patrick Eldin, Faculté de Pharmacie, Montpellier, France

Key Words

Bacterial clones, Dot Blot, Bacterial Lysis, Replication, Thermo Scientific™ Nunc™ MicroWell™ Plate, Pall Biodyne™ B Nylon Membrane, Thermo Scientific™ Nunc™ OmniTray™, Thermo Scientific™ Nunc™ TSP 96 Pin Replicator, E.coli bacteria.

Goal

The goal of this application note is to describe the use of the Thermo Scientific™ Nunc™ TSP 96 Pin Replicator when working with E.coli bacteria clones in Dot Blot assays. This system will allow selection of positive clones containing modified plasmids.

Positive clones are selected after plasmid cloning. This is often done by DNA probe hybridization in situ to plasmid DNA containing E.coli bacteria. The following protocol can be used for most plasmid cloning events in E.coli bacteria.

I - Protocol for DNA screening of bacterial colonies

Transformation is performed as usual.

A. Dot Blot of bacterial clones

1. Colonies are plated on LB Agar medium (petri dishes) with the correct antibiotic.
2. Following an overnight incubation at 37°C individual colonies are picked (with a tooth pick) and inoculated into single wells of a Nunc MicroWell Plate 96F pre-filled with LB-medium containing the appropriate antibiotic.
3. Following an overnight incubation at 37°C, a disposable TSP 96 Pin Replicator is used to replicate all individual colonies from one plate to a nylon hybridization filter (e.g. Nunc Pall Biodyne B Nylon Membrane) (Fig. 1). Allow filter to air-dry.
At this stage, add one tenth volume of glycerol to each well of the 96 Nunc MicroWell plate overnight culture to allow the storage of the colonies at -80°C.
4. The air-dried nylon filter is then placed (colonies facing up) on a Nunc OmniTray previously filled with LB Agar containing appropriate antibiotic. Incubate overnight 37°C to allow the cells to grow on the filter.



B. Bacterial Lysis on filter

1. The alkaline lysis is performed as follows:
 - 1.0 mL of the lysis solution (0.5 N NaOH) is placed in a Nunc OmniTray. The filter is placed on top of the lysis solution with the colony side up. Incubate at room temperature for 10 minutes. The solution diffuses through the filter and lyses the cells during the incubation.
 - The same incubation is repeated with a fresh lysis solution.
2. At the end of the second 10 minutes incubation the filter is neutralized using 1 mL of Tris 1M pH 5.4. Repeat this treatment twice.
3. The filter is air-dried and the DNA fixed or cross-linked on the filter, depending on the type of filter used.

The filter is now ready to be processed for hybridization with a labeled DNA probe (cDNA or oligonucleotide, radioactive or non-radioactive). When radioactive labeling with ^{32}P is used the auto-radiogram is developed (e.g. Kodak XAR film) after appropriate exposure with intensifying screen at -80°C . It is easy to identify a positive clone and to carry out further analysis starting with the corresponding frozen clones as the individual colonies are well aligned and discrete.

Additional applications

The protocol for DNA screening is used for selection of modified plasmids, but other systems can be applied as well.

At the end of the coding sequence of human β -myosin heavy chain (β -mhc) segment, a specific immuno TAG (NH₂-YEEEEYYYY COOH) was introduced against which there is a monoclonal antibody. In this way, a specific detection of the corresponding protein product after transfection in muscle cells is accomplished. After insertion of a 34 bp segment, encoding the TAG at the 3'-end of the β -mhc coding sequence in an expression plasmid, colonies were screened for correct insertion of the radio-labeled TAG oligonucleotides.

Results for one Nunc MicroWell plate 96F with two positive controls in the bottom (Fig. 2).

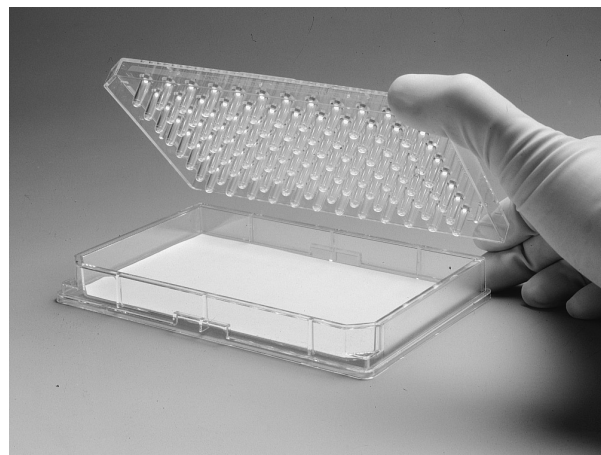


Fig. 1 - By using the disposable TSP 96 Pin Replicator all individual colonies of a plate are replicated on a nylon hybridization filter (e.g. Nunc Pall Biodyne B Nylon Membrane).

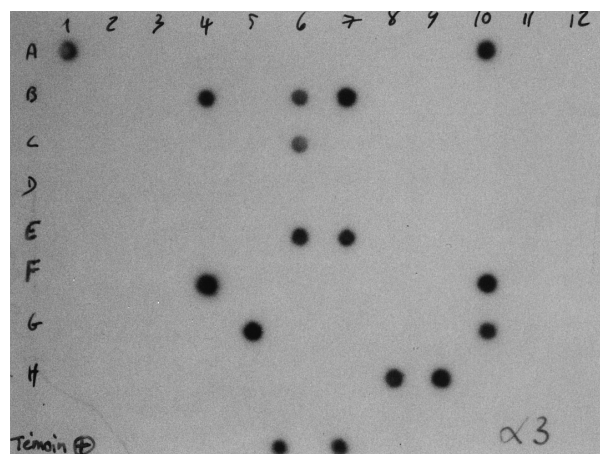


Fig. 2 - DNA screening for selection of modified plasmids. Positive clones are A1,10; B4, 6, 7; C6; E6, 7; F4, 10; G5, 10; H8, 9. At the bottom two positive controls are shown.

II - Protocol for in situ immunoscreening of bacterial colonies

When an inducible expression vector is used, the same colonies can be screened with monoclonal or polyclonal antibodies with slight modifications of the bacterial lysis.

A. Dot Blot of bacterial clones

Procedure is the same as for in situ DNA screening previously presented, except in this case an inducible expression vector is used (e.g. a temperature inducible expression vector such as the pEX vectors).

B. Induction of recombinant protein expression on filters

Induction of expression must be made on the filter (after step I.A.4 above) by adding the specific inducer (IPTG for example) or by switching temperature from 30°C to 42°C if you use a pEX vector, dependent on the promoter used for expression.

C. Bacterial Lysis on filter

1. The lysis is performed by placing the filter containing bacterial colonies face up (Nitrocellulose Millipore HATF) on three sheets of Whatman 3 mm paper previously soaked in 5% SDS followed by heating in a microwave oven for one minute at full power (approximately 650 W).
2. SDS is then removed by an electro-transfer in Tris 20 mm, Glycine 160 mm, in a 2x2 Whatman sheet sandwich, at 50 volts for 30 min. Place the colony side facing the negative electrode.
3. Blockage of non-specific binding is performed by overnight incubation at 4°C in TBS-Tween containing 1% BSA and DNase I grade II at 10 mg/mL. The filter can then be treated in an ordinary Western blot procedure (e.g. primary antibody incubation, washings, secondary antibody coupled to alkaline phosphatase or peroxidase incubation and visualization with the adapted substrates for color development).

Buffers

TBS-Tween: 10 mm Tris-HCl, pH 8.0, 150 mm NaCl, 0.05% Tween 20.

AP: 100 mm Tris-HCl, pH 9.5, 100 mm NaCl, 5 mm MgCl₂.

NBT/BCIP substrate: for 10 mL solution add 66 µL of NBT solution (Nitro Blue Tetrazolium at 75 mg/mL in 70% dimethyl formamide) and 33 µL of BCIP solution (5-bromo-4-chloro-3-indolyl phosphate, at 50 mg/mL in 100% dimethyl formamide) to 10 mL of AP buffer.

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