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Research Article

Fast Plasmid Slot Lysis and Gram-Negative Bacteria Ghost Preparation Protocol

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Abstract

Plasmid isolation is an important tool for the success of most of the genetic engineering based research. Alkaline lysis is a popular protocol for plasmid isolation. However, in some cases there are need for screening many colonies for the existence of the right clone. In cases where there is no selective marker for the cloned gene slot lysis is the most accepted solution. Some cloning strategies do not contain antibiotic markers or white/blue selection. In such cases one should screen nearly all the colonies he get till find the correct clone. Preparing plasmid from each clone will need large efforts. This slot lysis protocol for screening large library. The electrical current is used for evacuating the cells from their cytoplasmic content. Dialysis pages were used for large preparation. Such protocol succeeded to screen gene in plasmid which did not contain any selective marker.

Keywords: Fast slot lysis; Bacterial ghosts; pT7-7

Introduction

Recently Amara et al. (2013) introduced a simple protocol for evacuating microbial cells based on using the critical chemical concentration of effective chemical compounds as well as enzymes [1-3]. Such protocol succeeded to evacuate the microbes from their cytoplasmic content. Amara introduced the idea of preparing the DNA and the protein from microbes using the same concept by using the minimum inhibition concentration of the used chemical compounds in the Sponge-like protocol [1-11].

Slot lysis is another protocol applied for screening a large number of clones. In this study, a simple new protocol is introduced. The protocol guarantees fast and simple plasmid isolation from *E. coli*. Those who are interested in isolating plasmids from gram positive strains and full maintain the evacuated cells they can determine the MIC of SDS and NaOH in the lysis buffer.

The protocol is simple fast and use only the GET-solution and the SDS-NaOH solution of the alkaline lysis protocol.

Material and Methods

Used plasmid and gene

pT7-7 plasmid which did not contain white/blue selection was used to clone gene x (data not shown).

Lysis and stop-mix solution

GET-solution: (25 mM Tris/HCl pH 8), 10 mM EDTA and 50 mM Glucose.

SDS-NaOH solution: 200 mM NaOH and 1% (w/v) SDS (in water).

Stop-Mix: 4M Urea, 50 mM EDTA, 50% (w/v) sucrose and 0.1% (w/v) Bromophenol blue.

The slot lysis protocol

After transformation and overnight incubation prepare LB plates (or other suitable medium) and sterile toothpick (wood or plastic and plastic is preferable).

In 1 ml appendorf tube put 5 µl of the GET-solution and 10 µl of SDS-NaOH solution. During preparing the SDS-NaOH solution it is recommended to prepare it fresh and not put the SDS on NaOH directly but put them in sequence in the water. By the toothpick, touch a colony from the original plate till the colony stack well. Touch genteelly (did not allow full loss of the colony) the new LB medium plate and number the site of the touch with number. Transfer the toothpick which still contains the rest of the touched colony in an appendorf tube which contain both of GET-solution and the SDS-NaOH solution. Incubate for 10 minutes. One could keep the toothpick or allow slight vortex and remove it before the incubation period. Do that for as much as you could for the colonies in the original plates.

Cultivation of the tested colonies

The plates which contain the used colonies are incubated overnight at 37˚C so the colonies which proved to have the right clone could be retrieved again.

Searching for the right clone(s)

For searching right clone standard agarose gel electrophoresis method is used. 10 µl of the stop-mix is added to 10 µl of each colony lysis mixture and then loaded to the agarose gel. After the end of the experiment, the gel is stained using standard criteria by ethidium bromide solution and the result visualized using the UV-transilluminator.

Results and Discussion

There is always a need for modifying and simplifying the molecular biology tools and techniques. Not all of us have in their labs all the needed chemicals and facilities. Even in well instrumented labs one could need to use simple protocols. This study introduces a case where pT7-7 was used to clone a gene. pT7-7 do not enable white/blue

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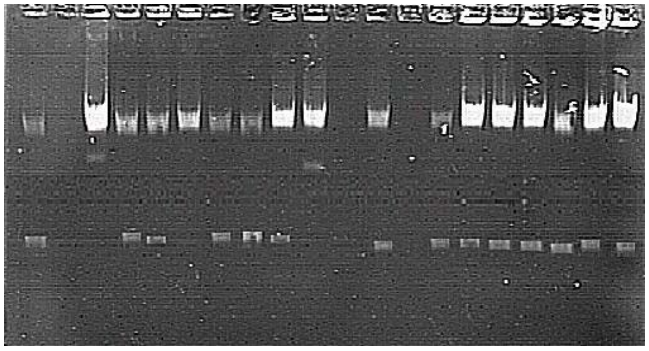

Figure 1: The result of the Fast slot lysis protocol. In the image two colonies show right clone.

selection. Some other plasmids even did not allow antibiotic selection. I used to use such simple protocol not only for screening library for the right clone but also for screening wild type gram negative bacteria for wild plasmids. The idea come from my awareness with the alkaline lysis protocol. If one use only the GET-solution and the SDS-NaOH that will guarantee cell lysis. One can dilute the lysis buffer to obtain non-deformed cells as well. Or using the concept of the Sponge-Like protocol to determine the concentration of both of the MIC of the NaOH and SDS.

The agarose gel electrophoresis will guarantee the migration of the cytoplasm from the injured cells due to the effect of the electrical current. The cells will be evacuated and turn to ghost cells while the cytoplasm which contains the plasmid will migrate through the gel. In (Figure 1) the colony number 3 and 10 contain the right clones. The colony number 2, 11 and 13 did not appear due to strong touch to the LB plates which lead to removeal of the cells. By making a viable reference of the used colonies the colonies, which contain the right clones could be retrieved again. The protocol is used for microbial ghosts' preparation using the electrophoresis current to force the cells to evacuate the cells. The electrical current will substitute the centrifuge in the original protocol. Suitable containers such as the dialysis bags are used for large preparation (data not shown).

This study introduce a simple and fast method for slot lysis and microbial ghost preparation.

Conclusion

This study introduced for the first time a one-step technique for plasmid slot lysis preparation. The protocol could be modified to prepare microbial ghosts by minimizing the effect of the lysis buffer and using the agarose electrophoresis's electrical current and suitable container like the dialysis pages.

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