StabyCloning™ based Molecular Methods for the Identification of novel Microbial Strains

Delphi Genetics Inc.
Sharpened Tools for Lifescience Discoveries

Technical Note

Technical notes provide customers with innovative applications and clear protocols specifically designed for Delphi Genetics products.

INTRODUCTION

The human intestinal flora is composed of a highly diversified population encompassing the three domains of life: Eukaria, Bacteria and Archaea. It is generally believed that 80% of the later are still unidentified to date. We herein describe a molecular method for the identification of unknown microbial strains, especially archaeons. A special focus is put on methanogens, in which it is possible to amplify a specific molecular marker for this metabolic group (*mcr*, the operon encoding Methyl-coM reductase, the last enzyme of methanogenesis) using degenerated PCR primers. StabyCloning™ is a very efficient Kit for this task, as it allows for the construction of representative libraries and the efficient screening of those libraries. All colonies are independent clones and the insert is correctly oriented. StabyCloning™ is very effective for a large range of insert sizes: from 1 base pair to more than 14000bp. As a result, each unique clone corresponds to a strain, different for every RFLP profile obtained after digestion with selected restriction endonucleases.

This approach limits the number of sequencing reactions that have to be performed as it allows for the clear identification of each individual clone. Thereupon a phylogenetic analysis allows the researcher to reveal the relationships of these newly uncovered strains within the tree of life.

The proprietary StabyCloning™ technology has been extensively tested and validated in Prof. Alric's Laboratory at the Université d'Auvergne in Clemont-Ferrand (France) where StabyCloning™ has routinely outperformed PCR Cloning Kits from other leading suppliers for this specific application. The protocol developed by this laboratory is featured in the following section.

PROTOCOL

1. Biological samples (e.g. stool samples) are collected and DNA is extracted
2. Using PCR, molecular markers are specifically amplified from these biological samples.
3. The amplified sequences are directionally cloned in the plasmid pSTC1.2 using the StabyCloning™ Kit. The directionality of the cloning reaction is ensured by adding a specific 14bp tail to one of the primers.
4. The obtained library of clones is then used to assess the diversity of the sample. The insert is either checked using PCR with oligonucleotides targeting the vector at each side of the cloning site or by mini-preparation of DNA.

5. Clone specific PCR products (or plasmid DNA) are RE digested using *Alu*I and *Csp*6I, yielding a specific RFLP profile for each clone. *(see Fig. 1)*
6. Unique clones are sequenced and the sequences are fed into a phylogenetic software for analysis

TYPICAL RESULTS

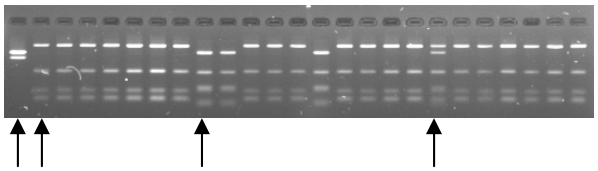


Figure 1: typical results using this methodology. Four different RFLP profiles are visible on this gel (Arrows), where each profile corresponds to a unique strain of methanogenic archaeon from the human tractus.

BENEFITS OF THE StabyCloning™ KIT FOR THE CREATION OF REPRESENTATIVE LIBRARIES AND THE SCREENING OF CLONE DIVERSISTY

- **Efficient cloning of inserts:** the positive selection process is based on gene activation rather than gene disruption. Hence, only clones bearing the desired construct are selected. It is well known that the selection of recombinants using gene disruption is not always efficient with a variety of inserts, especially small inserts. This is the case for positive selection using *a lethal gene* only (e.g. "Zero Background" vectors from Invitrogen using *ccdB or pJet vectors from Fermentas using eco47IR*) or the Blue/White selection system based on the insertional disruption of *lacZ*. Therefore the proportion of clones bearing the correct insert is increased when using the StabyCloning™ technology.

- **Orientation of the insert: A unique feature of the** StabyCloning™ system is that you can select for the correct orientation of the insert. This greatly facilitates the sequencing and the sequence comparisons when screening multiple inserts.

- **Speed of the reaction :** The whole procedure (ligation + transformation + plating) takes 1 hour. No antibiotic resistance gene expression is required, therefore the reaction can be directly plated and all the colonies on the plate are independent clones.

- **Higher number of clones** (see figure2).

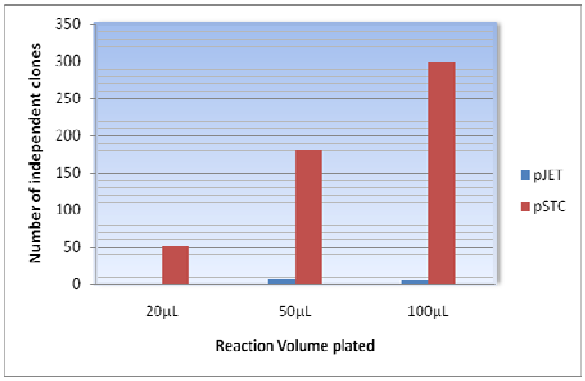
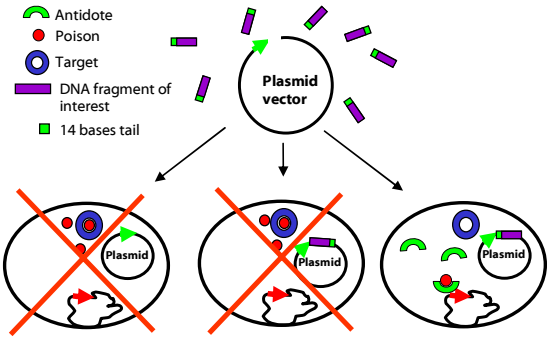


Figure 2. Number of transformants, the StabyCloning Kit is compared to Fermentas pJET Kit. 20, 50 or 100 µl transformed bacteria have been plated in each case. The insert (about 500 bp) was ligated using a vector/insert molar ratio of 1:3. 0.5µL of the ligation product (20 µL) was used for transformation (electroporation of commercial strains DG1 (Delphi Genetics) or CYS21 (Delphi Genetics)). Using StabyCloning™, in excess of 300 independent clones are obtained when using either 50 or 200 ng of vector in the ligation reaction, less than 10 independent clones are obtained using the pJET vector.

ABOUT THE StabyCloning™ SYSTEM

The StabyCloning™ Kit is designed for the rapid, precise, and efficient cloning of PCR products generated using proofreading polymerases. The system uses a proprietary positive selection strategy based on the plasmid toxin/antitoxin module *ccd*. The complete cloning procedure is performed within one hour (including plating). StabyCloning™ relies on the activation of an antitoxin gene rather than on the disruption of a toxin gene. DelphiGenetics' new selection scheme exhibits therefore unique advantages over other selection approaches. When a sequence of 14 base pairs (encoding the last 4 codons and the stop codon of the antidote gene) is added to the 5'-end of the DNA fragment to be cloned, the fusion of this sequence with the truncated antitoxin gene restores an active gene encoding a protein (CcdA) able to counteract the action of the toxin located in the chromosome of the host bacteria. The 14-bp sequence is incorporated into the target DNA fragment by using a modified PCR primer (the appropriate 14-base tail is added at the 5'-end of one of the two PCR primers).

As illustrated in the following figure, this process allows *(i)* the selection of recombinant plasmids that incorporate the fragment of interest (non-recombinant plasmids contain an inactive, truncated, *ccdA** gene, hence the host bacteria cell dies), and *(ii)* orientation of the fragment of interest (only one of the two possible orientations will restore an active, non-truncated, *ccdA* gene). Moreover, restoration of the *ccdA* gene stabilizes the plasmid into the bacterial population without the need for antibiotics (see www.delphigenetics.com for additional information on the advantages of this stabilization system).



KIT COMPONENTS

- pSTC1.3 blunt ended vector
- CYS21 bacteria
- T4 DNA ligase and reaction buffer
- Regeneration medium
- Forward and Reverse sequencing primers
- Primer Control mix

StabyCloning™ Products are available with either electro- or chemically- competent cells.

ORDER REFERENCES

- STC1-10: electro-competent cells, 10 reactions
- STC1-12: chemically-competent cells, 10 reactions
- STC1-20: electro-competent cells, 20 reactions
- STC1-22: chemically-competent cells, 20 reactions

RELATED PRODUCTS

Please visit www.delphigenetics.com for additional information on our DNA engineering and protein expression products

REFERENCES

Mihajlovski A., Alric M., Brugère J.-F. (2008). A putative new order of methanogenic Archaea inhabiting the human gut revealed by molecular analyses of the *mcrA* gene. *Res. Microbiol*. In Press.

LEGAL

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