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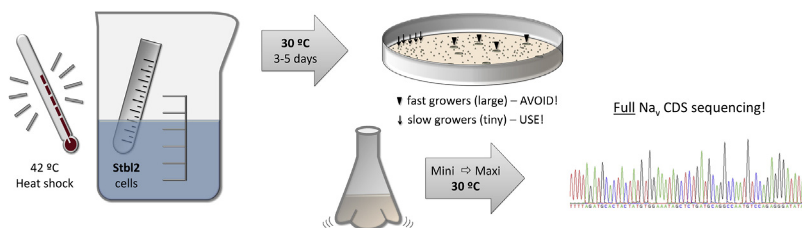
# The $Na_v$ channel bench series: Plasmid preparation☆

Daniel H. Feldman, Christoph Lossin *

*Department of Neurology, University of California - Davis, United States*

GRAPHICAL ABSTRACT




ABSTRACT

Research involving recombinant voltage-gated sodium ($Na_v$) channels has unique challenges. Multiple factors contribute, but undoubtedly at the top of the list is these channels' DNA instability. Once introduced into bacterial hosts, $Na_v$ channel plasmid DNA will almost invariably emerge mutagenized and unusable, unless special conditions are adopted. This is particularly true for $Na_v1.1$ (gene name *SCN1A*), $Na_v1.2$ (*SCN2A*), and $Na_v1.6$ (*SCN8A*), but less so for $Na_v1.4$ (*SCN4A*) and $Na_v1.5$ (*SCN5A*) while other $Na_v$ channel isoforms such as $Na_v1.7$ (*SCN9A*) lie in between. The following recommendations for $Na_v$ plasmid DNA amplification and preparation address this problem. Three points are essential:

- Bacterial propagation using Stbl2 cells at or below 30 °C.
- Bias toward slow-growing, small bacterial colonies.
- Comprehensive sequencing of the entire $Na_v$ channel coding region.

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* Corresponding author at: 4635 Second Avenue, Room 1004A, Sacramento, CA 95817, United States. Tel.: +1 916 703 5511; fax: +1 916 703 5512.

*E-mail address:* lossinc@gmail.com (C. Lossin).

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## Method details

*Step 1: transformation*

*Materials*

- high quality starter $Na_v$ plasmid DNA
- Max Efficiency® Stbl2 competent cells (Life Technologies)
- LB plates (with antibiotic at half-standard concentration)
- SOC or comparable outgrowth medium [8]
- dedicated 30 °C incubator and shaker

  *Note*: This list includes only non-standard items. Generic components such as water baths and baffled flasks are assumed to be available.

Analyses of $Na_v$ channel function begin with the construction or purchase of an expression vector (e.g., pCMV-Scrip, pCI-neo) containing a $Na_v$ channel's coding sequence. Alternative splicing is common among $Na_v$ channels, creating different proteins from the same gene with distinct spatial and temporal expression patterns [9–12]. Careful planning is therefore necessary to determine exactly which isoform and coding sequence are needed to address the questions of interest. With approximately 6 kb in length, $Na_v$ channel coding sequences are long, making final insert-vector plasmid construct sizes in excess of 11 kb common. Regardless of its origin, be it a gift from a colleague or purchase from a commercial supplier, comprehensive sequence information ought to be part of the plasmid construct transaction. This will allow the end user to be fully informed when it comes to restriction analysis of the plasmid, which should always be the first step upon the DNA's arrival. Simple electrophoresis of small aliquot of plasmid DNA digested with a reliable enzyme that produces a known banding pattern will show whether the plasmid is correct or not. This may seem trivial, but cases where providers less experienced with $Na_v$ channel handling send corrupted DNA are not uncommon. If above mentioned restriction digest does not deliver the expected results (be selective here; minimal changes can be telltale signs of problems), digest with an alternative enzyme to confirm.

Once the restriction enzyme digestions delivered the appropriate restriction fingerprint, bacterial transformation can begin. We have had good success with chemical transformation as described below, but electroporation of suitable bacterial strains may be a viable alternative. Contrary to standard transformations, competent cell choice is critical when comes to $Na_v$ channels. Among the cell lines known to be susceptible to produce problems are JM101, DH5α, One Shot Top10®, to name but a few. Transforming these cells according to the manufacturer's instructions has a high chance of producing a negative outcome, although in select cases success appears to be possible (JM109, unpublished data). In our hands, by far the most reliable and successful is the Stbl2 bacterial strain, whose genotype favors cloning of problematic plasmid DNA. Although somewhat costly, it is possible to refreeze them at −80 °C in 25-μl ready-to-use aliquots using immediate immersion in ethanol/dry ice without losing too much efficiency, should a more economical solution be necessary.

Once the transformation is completed, the bacteria are propagated on LB plates with an appropriate antibiotic, at approximately half-standard concentration (e.g., 50 vs. 100 μg/ml ampicillin), at 27–30 °C. Because this temperature is lower than the 37 °C incubation used in standard procedures, using a lower concentration of antibiotic will allow faster bacterial growth without compromising the selection of resistant clones. It is very important at this point to emphasize the use of a dedicated and appropriately labeled 30 °C incubator. Any temperature elevation of Stbl2 cells above 30 °C will inevitably corrupt $Na_v$ channel DNA through host-mediated mutation (personal observation). At this temperature, the growth of bacteria harboring $Na_v$ channel DNA is very slow, and incubations of 3–5 days are often necessary. At no time during this period can the temperature exceed 30 °C. Appropriate signage ought to be attached to the incubators to ward off secondary users who may briefly change the incubator temperature to the standard 37 °C unbeknownst to the $Na_v$ channel researcher.

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