Methods and reagents

Preparing ultra-competent *Escherichia coli*

Methods and Reagents is a unique monthly column that highlights current discussions in the newsgroup **bionet.molbio.methds-reagnts**, available on the Internet. This month's column discusses how to get the most out of your transformation procedure and how to prepare your own ultra-competent *Escherichia coli* cells. For details on how to partake in the newsgroup, see the accompanying box.

Do you lack consistency with your transformation protocol or find your preparation time is far too long? Then, maybe it's about time you switched protocols. Procedures based on a simple $CaCl_2$-wash method provide a fair number of transformants when you only need to change bacterial host strains or if you receive plasmid DNA from someone else and need to propagate it.

The hare

The simplest methods[1-3] are quick and dirty, but rarely yield more than 1×10^4 to 1×10^6 transformants per μg of purified plasmid DNA, which could amount to a substantial loss of recombinants when efficiency is of the utmost importance, for example when constructing a random library.

The most widely known procedure used for commercial preparations of ultra-competent *E. coli* uses techniques based on the work of Douglas Hanahan[4,5], in which he optimized many different parameters. By washing the cells in many different divalent cations and by adding reducing agents, chemical transformation can be quite efficient, and because the Hanahan procedure usually yields from 1×10^5 to 1×10^8 transformants per μg of DNA, some people are convinced that this is still the best protocol available.

However, not only have others tried to reproduce much of Hanahan's work with mixed results[6], but also they have addressed some other factors that have not been previously tested and that have since been shown to increase competency. Methods other than those above can be faster and simpler, with minimal loss of competency. For example, the use of a single solution method can provide competent cells in less than 20 min.

Chung *et al.*[7] showed that JM109 cells prepared in a single transformation and storage solution composed of LB media with 10% PEG, 5% DMSO and 50 mM Mg^{2+}, pH6.5 (TSS) were able to produce 5.12×10^7 transformants per μg of pUC19 DNA when made fresh, and after being frozen at −70°C for 1–18 weeks, gave 6.17×10^7 transformants per μg of DNA. Another study[8] extended this, showing that 36% glycerin and 12% PEG added to the media allowed 1.07×10^8 JM103, 1.17×10^8 DH1, and 1.29×10^8 HB101 transformants per μg of pBR322 DNA.

Epicentre Technologies now prefers the use of this method for preparing their frozen competent cells and claim the standard TSS gives an efficiency of 2.4×10^6, while their own modified TSS protocol gives 7.5×10^6 HB101 transformants per μg of pGEX-2T DNA using 5 ng. In addition, one netter said that he found an increase of about 50- to 100-fold in efficiency when he switched from the standard $CaCl_2$ method to the single-step TSS protocol.

The tortoise

By far, the most preferred method of netters for producing ultra-competent *E. coli* cells by a chemical method is that of Inoue *et al.*[9] Interestingly, a very important step included in this protocol seems to be the growth of cells at 18°C. It was reported that 1.44×10^9 JM109, 1.84×10^9 DH5α, and 1.00×10^9 HB101 transformants per μg of pBR322 DNA were obtained with this method. In practice, however, one netter said that an efficiency of between 2×10^8 and 3×10^8 was more typical and that only occasionally might one expect to see upwards of 2×10^9 XL1-blue cells transformed per μg of pBluescript DNA. On the other hand, some netters have reported that 1×10^8 to 1×10^9 for DH5α and XL1-blue strains is common, and some have reported obtaining efficiencies as high as 1×10^{10} on occasion.

Although the efficiencies using this method are among the highest, the down side is the requirement for a cooled incubator, and owing to the lower temperature of incubation, the growth of the bacterial culture takes much longer to arrive at the desired cell density, which makes this a much more lengthy protocol. Also, several washing steps must be performed adding to the overall prep time.

Netters feel that the tradeoff in time is worth it and that preparing a large batch of cells and freezing aliquots of them at −70°C can limit the number of preparations done in a year. Also, the long wait can be avoided by determining the doubling time (usually 3 to 4 hours for DH5α and XL1-blue) and by inoculating late in the evening, then harvesting the cells early the next day. Netters also agree that the culture can be grown at room temperature (20–22°C) without significant loss of efficiency, thereby avoiding the use of the cooling equipment. In addition, the longer doubling time might help alleviate the difficulty in catching the cells at the more optimal cell density of an OD_{600} of 0.94 (Ref. 10) as discussed in a previous *Methods and reagents* column (*TIBS* 19, 426–427), although this has not yet been thoroughly investigated.

As nobody knows why this lower temperature growth works so well, there are some gaps in the knowledge of exactly how the cells become competent. One idea put forth by Mikhail Alexeyev (**malexeyev@biost1.thi.tmc.edu**) is that *E. coli* might be most susceptible to chemical treatment that induces competency only at a certain stage of its cell cycle. The slower growth of the cells at lower temperatures could allow more cells to be captured at this stage and maybe this temperature somehow synchronizes them and they can be caught at the correct stage more easily. This idea could be tested quite easily

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by synchronizing cells at different stages and then testing their competency.

Although some might think that the development of bacterial transformation protocols has been through its heyday long ago, the ability to consistently produce highly competent *E. coli* cells still remains somewhat elusive. Variability in transformation efficiencies seen by netters suggests that many parameters still need to be explored.

Overall, a single method can be chosen from the many methods available, but a combination of various different methods is most likely the best bet for increasing efficiency beyond 1×10^9 transformants per μg on a regular basis. For example, one of the parameters not addressed in the work of Inoue *et al.*[9] is the inclusion of reducing agents in the transformation reactions, as noted in earlier work.

Zophonias O. Jonsson (**zjons@vetbio.unizh.ch**) wrote that he did a comparison of BL21(DE3)plysS transformed with a pET derivative using cells grown at 23°C that were prepared by the Inoue method. When treated with reducing agents as per the Hanahan procedure, β-mercaptoethanol increased efficiency five-fold while dithiothreitol did so by 20-fold.

Increasingly, commercial suppliers are offering frozen ultra-competent cells that are claimed to provide the best efficiency for transformation. It appears that the companies have tweaked the process for increased transformants and, having created their own slight modifications, refuse to reveal exactly how they have pushed the limits of competent cells in order to remain competitive. Netters are not only distraught by this type of action, but think that it is time a new protocol be made publicly available that can push the competency level one step closer to that obtained by electroporation. Most feel that many different parameters still need to be explored, and that it is probably worth some effort to develop a new and better protocol. They are convinced that the high transformation efficiencies of using electroporation might some day be replaced with an ultra-competent-producing chemical method pushed through the 1×10^{10} efficiency envelope (for a discussion on electroporation, see *TIBS* 20, 248–249).

Breaking the cell competency barrier could all but eliminate the need for expensive equipment and cut the high cost of frozen electro-competent cells and special cuvettes now sold by the suppliers. Netters feel that one day soon ultra-competent cells will be able to be prepared in-house by chemical means and that this capability might be just around the corner.

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PAUL N. HENGEN

National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, USA.
Email: pnh@ncifcrf.gov

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