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What a Load of Old Sequence!!!

In recent years, nucleotide sequencing has been applied to genotyping and whole genomes and is now considered a "gold standard" in its own right. As with any high-profile application, nucleotide sequencing has received both praise and criticism, and recent comments in scientific papers have suggested that certain nucleotide sequencing methods are prohibitively expensive (2, 3, 5). We wish to address this misconception, which may have been justified over a decade ago, when the development of molecular biology and recombinant DNA technology promised breakthroughs of an unprecedented level. Since these initial developments, the attitude of scientific thought has encouraged nucleotide sequencing to develop in many different areas. Unfortunately, certain perceptions have not evolved with the same vigor and accomplishment. Nucleotide sequencing systems now have many attractive features, such as high throughput, low consumable costs, and not only good intralaboratory reproducibility but also interlaboratory reproducibility (1). After initial capital start-up costs of $100,000 to $300,000, depending on throughput requirements, the nucleotide sequencing costs are low.

Recent comments have been particularly directed towards multilocus sequence typing (MLST), a method which has gained much credibility for bacterial population biology analysis since it was first described in 1998 (4). However, unjustified comments regarding its cost will reduce its uptake in laboratories. The cost for sequencing one gene, based on consumable costs alone, has been quoted as $20 (5), and other papers have used this to quote the cost of MLST as over $300 per bacterial isolate (2, 3). This cost is now grossly inaccurate; in our laboratory, e.g., MLST on seven genes costs $32 (consumable costs alone for both DNA strands). Therefore, literature which quotes any publication quoting excessive costs is misleading and may have a detrimental effect on the perception of nucleotide sequencing.

Novel techniques often require high levels of specialization and complexity and when compared to older and more familiar techniques may fuel skepticism, and therefore, initial concerns are justified. Not surprisingly, as nucleotide sequencing has become widely used and the chemistry cheaper, the actual costs involved have been reduced dramatically. Some costs have been reduced by over 60%; for example, the average cost for sequencing 600 nucleotides on both strands is as low as $4. This includes all processes involved in sample preparation, sequence setup, and data handling. In conclusion, nucleotide sequencing should now be accepted as a cost-effective method for bacterial typing which is readily available, affordable, and as easily utilized as any technique presently available.

REFERENCES

1. **Clarke, S. C., M. A. Diggle, and G. F. S. Edwards.** 2001. Semiautomation of multilocus sequence typing for the characterization of clinical isolates of *Neisseria meningitidis*. J. Clin. Microbiol. **39:**3066–3071.
2. **Goulding, J. N., J. V. Hookey, J. Stanley, W. Olver, K. R. Neal, D. A. Ala'Aldeen, and C. Arnold.** 2000. Fluorescent amplified-fragment length polymorphism genotyping of *Neisseria meningitidis* identifies clones associated with invasive disease. J. Clin. Microbiol. **38:**4580–4585.
3. **Hookey, J. V., and C. Arnold.** 2001. A comparison of multilocus sequence typing and fluorescent fragment-length polymorphism analysis genotyping of clone complex and other strains of Neisseria meningitidis. J. Med. Microbiol. **50:**991–995.
4. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
5. **Olive, D. M., and P. Bean.** 1999. Principles and applications of methods for DNA-based typing of microbial organisms. J. Clin. Microbiol. **37:** 1661–1669.

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