BioFeedback

The Biofeedback section accommodates letters to the editor, addenda, errata and other comments on previously published articles.

Cost Savings Using Automated DNA Sequencing

BioTechniques 29:544 (September 2000)

Confirmation of mutations mostly involves DNA sequencing. The conventional manual radioactive labeling sequencing protocol may be conceived as the method of choice because of the perception that the capital and operational costs of automated sequencers are high. Velickovic et al. (3) reduced the cost of DNA sequencing by running 48 or 64 samples on a 36-lane model 377-01 ABI PRISM™ automated sequencer (PE Biosystems, Foster City, CA, USA). We found that a cheaper model, the 18-lane model 377-18 ABI PRISM, can run 37 samples on one gel. We also found that the volume of the

most costly reagent, dye terminator reaction mixture, can be reduced to cut operating costs by 69%. As a result, automated sequencing is more feasible for use in research or clinical service.

At least 37 samples can be loaded on a 377-18 sequencer by using lanes 15–51 of an aligned 64-well shark's-tooth comb. Since the wells are narrower than for an 18-well comb, the amount of sample required to get the same intensity of signal is reduced. Purified PCR products were cycle sequenced using the dRhodamine Dye Terminator FS Cycle Sequencing Ready Reaction Kit (PE Biosystems) in a total volume of 5 µL instead of the suggested 20 µL. We detected a heterozygous mutation in either a 1.5-µL aliquot from a 20-µL reaction loaded on an 18-well comb or a 0.7-µL aliquot from a 5-µL reaction loaded on a 64-well comb (data not shown). The signal quality remained the same. By our protocol, we screened over 2000 PCR samples for novel mutations in the *TIGR* (2) and *PAX6* (1) genes.

Both capital and reagent costs are reduced. A 36-lane 377-01 sequencer costs about \$184 000 US, while a 377-18 costs about \$100 000 US, a capital cost reduction of 46%. The PE Biosystems protocol costs \$6.96 US per sample (\$6.36 for dRhodamine dye terminator, \$0.40 for *ExoI* and SAP and \$0.20 for acrylamide and other consumables), while our protocol costs

\$2.19 US (\$1.59 for dye terminator and \$0.60 for the rest), a reagent cost savings of 69%.

Automated sequencing has advantages over manual methods, in addition to those mentioned by Velickovic et al. (3). It avoids the higher cost and environmental hazards of radioactive labeling. It can read longer sequences and requires fewer reactions to cover the same length. Our modifications increase the capacity of the machine and reduce operating costs, allowing laboratories with limited budgets to more readily achieve their research targets.

REFERENCES

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2. Lam, D.S.C., Y.F. Leung, J.K.H. Chua, L. Baum, D.S.P. Fan, K.W. Choy and C.P. Pang. 2000. Truncations in the *TIGR* gene in individuals with and without primary open angle glaucoma. *Invest. Ophthalmol. Vis. Sci.* 41:1386-1391.
3. Velickovic, Z.M., M.B. Velickovic and S.K.G. Grebe. 1999. Increasing sample throughput on a standard 36-lane model 377 DNA sequencer to 48 or 64 samples per run. *BioTechniques* 27:652-654.

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Corrigendum

It has been brought to the editor's attention that relevant previously published work was not cited in a recent *BioTechniques* article (Qi, J., C.J. Link and S. Wang. Direct observation of GFP gene expression transduced with HSV-1/EBV amplicon vector in unfixed tumor tissue. *BioTechniques* 28:206-208, 2000). Chishima and colleagues had already reported cellular expression of GFP in live tumor tissue (Chishima, T., Y. Miyagi, X. Wang, H. Yamaoka, H. Shimada, A.R. Moossa and R.M. Hoffman. Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression. *Cancer Res.* 57:2042-2047, 1997). The same group followed this report with a series of 15 articles that described the use of GFP-expressing tumor cells to visualize cancer progression and metastasis. Qi et al. focused on in vivo gene transduction (direct injection of tumors with the GFP-containing vector). The work of Chishima and co-workers involved delivery of the GFP-containing vector into cancer cell lines in vitro, followed by subcutaneous inoculation into mice and subsequent orthotopic implantation of the growing tumor.

Corrigendum

In an article published in March (*BioTechniques* 28:426-432, 2000), reference number two was incorrect. Dr. Kurien's initials should be B.T. rather than B.J.

Corrigendum

The authors of an article that appeared in June (*BioTechniques* 28: 1220-1225, 2000) have requested that certain editorial errors be corrected and clarified. Endogen, Inc. no longer markets Xplore® assays. Inquiries about Cleavase® enzymes and Invader™ probe technology should be directed to Third Wave Technologies, Inc. (Madison, WI, USA). ELISA kits for the determination of IL-1β and TNF-α protein were manufactured by Endogen, Inc. (Woburn, MA, USA).

Corrigendum

An equation published in an article in the May issue (*BioTechniques* 28:921-926, 2000) was incorrect. Equation 2 on page 922 should read

$$y(x) = \frac{100\%}{1 + \exp[(x_C - x)/b]}$$

and not

$$y(x) = y_{\text{MIN}} + \frac{100\%}{1 + \exp[(x_C - x)/b]}$$

Addendum

The corresponding author of the following article, which appeared in the April issue, has asked that both of the first two authors be considered equally as first authors because they contributed equally to the work reported.

Täpp, I., L. Malmberg, E. Rennel, M. Wik and A.-C. Syvänen. 2000. Homogeneous scoring of single-nucleotide polymorphisms: comparison of the 5'-nuclease TaqMan® assay and Molecular Beacon probes. *BioTechniques* 28:732-738.