Acknowledgments

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Nature Biotechnology
345 Park Avenue South
New York, NY 10010-1707, USA
Fax: +1 212 696 9635
E-mail: m.francisco@natureny.com

Abundance and competition in PCR

To the editor:

It is interesting to note that while there is an awareness that the abundance distribution of mRNAs within a cell creates a problem for the sequencing of all cDNAs, there does not seem to be an awareness of the problems that abundance distribution creates for displaying lower abundance mRNAs in differential display PCR[1,2]. These problems are created by competition in PCR, whereby more abundant templates compete for reagents with less abundant ones such that the products from less abundant mRNAs are not detected[3]. This problem is particularly acute because many RNA species are rare and are present at ~0.004% of total mRNA.

Using computer simulations, López-Nieto and Nigam suggest that the use of 30 designed primers (in 370 primer combinations) allows the detection of about 75% of known human protein coding regions. However, their computer simulation does not take into account the effect of competition in PCR. The effect of competition will be to reduce the percentage of mRNAs represented by their method because a proportion of the PCR products predicted by their computer program will not be visible on the display gels. The severity of the competition can be ascertained by comparing the number of PCR products calculated in their computer simulation with the number of bands actually visible. The computer simulation shows how the use of these 30 primers gives an average of ~5 PCR products per mRNA. Therefore, using all of the 870 primer combinations on the mRNAs from a mammalian cell should produce ~56,000 bands (15,000 x 0.75 x 5) or ~65 bands per primer combination. Their figure of three typical displays (Fig. 4B) shows that the number of PCR products actually visible per primer combination is about 30 (excluding closely grouped bands that represent a single PCR product[4]). Therefore, competition in PCR seems to be responsible for the loss of about half of the PCR products predicted in their computer model. It is worth noting that this estimate of "lost PCR bands" is conservative, because the assumption of high stringency in PCR for the computer simulations, an assumption which López-Nieto and Nigam themselves recognize will not be realized in real PCR.

In summary, although the method described by López-Nieto and Nigam seems to be a large improvement on standard differential display, it is probable that they have made a considerable overestimate of the percentage of mRNAs that can be displayed using combinations of 30 primers.

Ursula H.A. Schlichter
Max-Planck-Institut für Zuchtungforschung
Köln, Germany

DavidBertioli
Area de Biotecnologia
CENARGEN/EMBRAPA
70770-Brasilia DF, Brazil

López-Nieto and Nigam reply:

Competition in PCR when multiple products are being amplified is an important issue. As pointed out in the second-to-last paragraph of our paper[1], the ability to amplify a message is a function of the message abundance and specificity of primer-template interactions. Bertioli et al.[3] have shown one of the problems of standard differential display is a limited ability to detect rare messages due to competition. They also argue that 90–95% of all messages fall into this category. From our published gel, they suggest that we are detecting slightly less than half the predicted number of bands. Thus, it follows that we must be detecting a significant number of rare messages.

Of course, these kinds of arguments have obvious limitations, and directly extrapolating from their study to ours is difficult. Our method, based on coding-region selective oligonucleotides, uses longer primers and higher annealing temperatures, so we anticipate that more specific interactions between templates and primers would improve the detection rate of rare messages. It should also be emphasized that the roughly 30 bands per lane they have counted were from experiments with human primers, the conditions for which we did not put the same effort into optimizing (for reasons described in the paper).

The number of bands obtained per primer set may turn out to be greater under more stringent PCR conditions. Under optimal priming conditions, products for abundant messages are likely reach plateau levels, while the amplification of products for rare messages may continue. In our experiments, we have seen gel bands (PCR products) not present under low stringency conditions that start to appear as the PCR annealing temperature is increased. This finding suggests that, while competition under low stringency (similar to conditions used in differential display) may limit the ability to detect rare messages, as annealing temperature increases, message abundance may be less of an issue relative to priming specificity, and, as a consequence, messages that were previously below the limit of detection can be effectively amplified. That said, the actual degree to which competition and message level limit the gene detection rate with our method remains to be established experimentally.

Carlos López-Nieto and Sanjay K. Nigam
Brigham and Women's Hospital/
Harvard Medical School, Cambridge MA

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Erratum

Collaborative Laboratories was incorrectly identified as filing a $40.5 million IPO in the August issue (*Nature Biotechnology* 14:930, 1996). The correct company is **Collaborative Clinical Research, Inc.**