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They should be addressed to:
Correspondence
Nature Biotechnology
345 Park Avenue South
New York, NY 10010-1707, USA
or sent by e-mail to biotech@natureny.com
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Chimeraplasty validation

To the editor:

Initial studies of gene correction using chimeric RNA/DNA oligonucleotides (RDOs), also termed chimeraplasts, reported exciting rates of correction of point mutations (up to 40% in one *in vivo* model[1]). However, the technique has not yet fulfilled its promise in the fields of transgenics and gene therapy, and some groups have reported persistent failures[2,3]. In this context, high hopes for the outstanding potential of the technique were raised again when a group of independent researchers recently reported success[4,5]. We believe, however, it is wise not only to share negative results, as Van der Steege *et al.*[3] highlighted in the April issue, but also to propose criteria for further experiments intending to validate RDO technology.

We have determined four criteria that are necessary to address the criticisms[6,7] and cautionary remarks[8] expressed in early reports dealing with RDOs. Briefly these criteria are as follows:

- First, the mutational activity of an RDO must be established for gene conversion from a wild type to a rare mutant genotype in order to obviate spontaneous reversion events.
- Second, the specific mutation to be introduced should be absent in the cell line used for experiments. Ideally, cell lines harboring the mutated genotype should not be present in the laboratory, to avoid cross-contamination artifacts.
- Third, mutated clones must be grown and studied individually, as pooled-cell extracts containing large molar amounts of reagent oligonucleotides are poorly suited to estimate rates of chromosomal gene conversion, as well as to confirm gene conversion by sequencing and to avoid other causes of PCR-based artifacts.
- Fourth, gene conversion rates should be confirmed at the protein level in pools of cells at different time points after the RDO treatment, in order to check for the artifacts in selection of very rare mutation events through multiple cycles of cell culture.

Designing our experiments with these criteria in mind, we have targeted the hypoxanthine phosphoribosyltransferase *HPRT* gene. $HPRT^+$ and $HPRT^-$ cells can be readily selected in HAT (hypoxanthine + aminopterine + thymidine) medium and in 6-thioguanine (6-TG) medium, respectively. The gene is expressed ubiquitously and is located on the X chromosome so that only one allele is to be mutated in male (XY) cells. We aimed at introducing a mutation previously described in a human patient with HPRT deficiency, G310→C at the level of the HPRT complementary DNA ($HPRT_{Yale}$; ref. 9). This mutation leaves virtually no residual enzymatic activity in functional assays; it produces a messenger RNA (mRNA) in normal amounts yielding a protein recognized by a monoclonal and polyclonal antibodies[10], and it generates a restriction site for enzyme *Hha*I, which eases detection at the genomic level.

A chimeric RDO was designed according to published data[11] to produce the $HPRT_{Yale}$ genotype. It was introduced in human male, diploid cell lines HCT-15 and HBE4-E6/E7 (ATCC numbers CCL-225 and CRL-2078, respectively) either by transfection with Fugene 6 (Roche, Brussels) or polyethylenimine (Fluka, Brussels), and by direct microinjection. $HPRT^-$ mutants were selected in 6-TG medium and resistant clones were isolated. Experimental procedures were as reported[12,13]. Controls included cells treated with various molar concentrations of a wild-type RDO, and cells exposed to the transfectant reagents alone or microinjected with buffer only.

In all experiments, we failed to observe any significant difference in the number of 6-TG-resistant clones between controls and RDO-treated cells. Moreover, resistant clones did not result from specific mutagenesis but merely from the background noise of the selection process, as the G310→C mutation was not observed either by PCR-RFLP analysis or by direct sequencing of genomic DNA from these clones. Thus, in our hands RDOs were not able to produce a *HPRT* gene conversion rate above background level.

Experiments meeting the above requirements are mandatory to validate the outstanding rates of gene conversion reported with RDOs. In reviewing the 20 original studies published on chromosomal gene conversion using RDOs, we found none fulfilling all four of our criteria. In view of potential artifacts and the lack of reproducibility of published reports[3], we consider that conversion mediated by chimeric RDOs still awaits validation. We hope that confrontation of negative as well as positive results will help solving the problem of reproducibility of this potentially promising methodology.

João Albuquerque-Silva[1,2], Gilbert Vassart[1], João Lavinha[2], and Marc J. Abramowicz[1]
[1]Laboratoire de Génétique Médicale–IRIBHN and Hôpital Erasme, Université Libre de Bruxelles (ULB), 808 Lennik St., B-1070 Brussels, Belgium (marcabra@ulb.ac.be)
[2]Instituto Nacional de Saúde Dr. Ricardo Jorge, Av. Padre Cruz 1649-016 Lisbon, Portugal

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Accuracy in amplification

To the editor:

Sensitive quantitative PCR techniques based on coamplification of multiple target templates[1–3] as well as kinetic PCR[4–6] have been used to quantify tiny amounts of viral or ectopic DNA or mRNA expression, even in single isolated cells. As kinetic PCR systems measure the absolute amounts of a single nucleic acid in a sample, many applications, such as accurate mRNA expression analysis, often require the use of a reference template

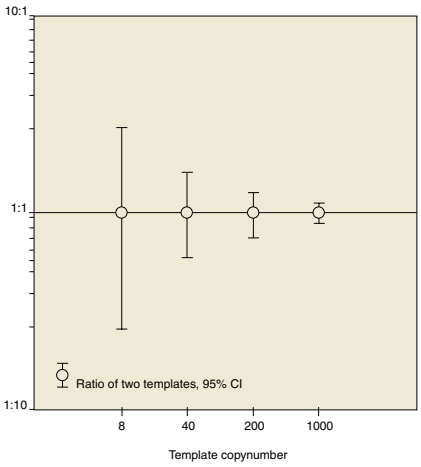

Figure 1. Poisson-distribution-based estimates of the ratio of two templates in an aliquot pipetted from a highly diluted solution containing the templates in a 1:1 ratio.