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Reply to “Successful genome editing with modularly assembled zinc finger nucleases”

J. Keith Joung^{1,2}, Daniel F. Voytas³, and Toni Cathomen⁴

¹Molecular Pathology Unit, Center for Cancer Research, and Center for Computational and Integrative Biology, Massachusetts General Hospital, Charlestown, MA USA

²Department of Pathology, Harvard Medical School, Boston, MA USA

³Department of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, Minneapolis, MN USA

⁴Department of Experimental Hematology, Hannover Medical School, Hannover, Germany

To the Editor:

The publications cited by Kim et al. describing successful construction of ZFNs by modular assembly only further support our original conclusion that this method has a high failure rate for engineering functional zinc finger arrays.¹ Two of the three reports cited provide data that enable calculation of failure rates for modular assembly.^{2, 3} Although it is true that modular assembly yielded ZFNs for ~25% of the DNA sites targeted, failure rates measured instead by the number of zinc finger proteins tested are remarkably consistent with those reported in our original Correspondence.¹ For example, at the human *CCR5* gene, Kim et al. screened 315 pairs of ZFNs for activity;³ this large-scale effort yielded only a small number of functional ZFN pairs (93.3% failure rate for ZFN pairs tested). Similarly, for the tobacco *SuRB* gene,² we tested 32 zinc finger arrays *in vitro* but identified only three with functional activity (91.6% failure rate for zinc finger arrays tested). These data are consistent with our original predicted failure rates of ~94% and ~76% for modularly assembled ZFN pairs and zinc finger arrays, respectively.¹ We believe that failure rates measured by numbers of zinc finger arrays or ZFN pairs tested rather than by numbers of DNA sites targeted are more relevant statistics for potential ZFN users because these influence how many proteins must be modularly assembled and tested for each potential site.

We also respectfully disagree with various statements Kim et al. make regarding Oligomerized Pool ENgineering (OPEN), a selection-based method for engineering zinc finger arrays.⁴ Kim et al. contend that our recent report² shows that “*modularly assembled ZFNs...outperformed OPEN ZFNs in terms of mutation frequencies*” and that “*each method [modular assembly and OPEN] gave rise to successful genome modification at one out of four target sites.*” However, the modularly assembled and OPEN ZFNs in our study were designed to recognize different DNA target sites and therefore Kim et al.’s conclusion is based on an indirect comparison. For the single site where direct comparison was possible, only the OPEN approach yielded functional ZFNs. In addition, we found in a different direct comparison of OPEN and modular assembly at five different target sites in the *EGFP* gene that OPEN ZFNs were active at four sites whereas modularly assembled ZFNs showed activity at only one site.⁴ Furthermore, the OPEN ZFNs outperformed the modularly assembled ZFNs at this one site. We believe that the

higher activity and success rate of OPEN ZFNs is most likely due to the method's explicit consideration of the well-established context-dependent behavior of zinc fingers,^{1, 4} a parameter that is largely ignored by modular assembly.

Kim et al assert that “*OPEN ZFNs thus far are largely limited to target GNN repeat sequences (i.e. 5'-GNN GNN GNN-3')*”. However, ~40% (11 out of 28) of the half-sites successfully targeted in endogenous genes by OPEN ZFNs to date actually contain one or more non-GNN subsites (Supplementary Table 1). Furthermore, potential users of the ZFN technology should be aware that with both modular assembly and OPEN it is easiest to target sequences composed entirely of GNN subsites.

Kim et al. further suggest “*that careful choice and use of reliable modules could improve success rates [of modular assembly]*.” However, given that our original Correspondence showed that modular assembly has considerably higher failure rates for target sites harboring one or more non-GNN subsites than for those composed solely of GNN subsites,¹ taking this approach could also substantially restrict the targeting range of the method.

In summary, we advise potential users to carefully weigh the effort required both to engineer and to validate ZFNs. Although modular assembly is simpler to perform than the selection-based OPEN method, our direct comparisons suggest that OPEN is more efficient than modular assembly for engineering functional ZFNs. As researchers who have practiced both methods, 2·4^{–6} we have concluded that modular assembly requires as much (if not more) time and effort to use than OPEN when one considers the requirement to screen hundreds of largely non-functional modularly assembled ZFNs for cellular activity. Nonetheless, the Zinc Finger Consortium continues to make reagents and software for both modular assembly and OPEN available to academic scientists (<http://www.addgene.org/zfc>; www.zincfingers.org/software-tools.htm). We believe that rather than attempting to improve the success rate of modular assembly, future efforts should instead focus on further simplification of selection-based techniques or development of more effective design-based methods that account for the context-dependent behavior of zinc finger domains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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