Preassembled zinc-finger arrays for rapid construction of ZFNs

To the Editor: Since the publication of our Correspondence[1] and the reply of Joung *et al.*[2], we improved zinc-finger nuclease (ZFN) modular assembly. ZFNs are artificial restriction enzymes[3] composed of tailor-made zinc-finger DNA-binding arrays and the FokI nuclease domain, which can induce site-specific mutations[4] and large chromosomal deletions[5] in higher eukaryotic cells and organisms.

To reduce the number of ZFNs that need to be synthesized to identify a functional enzyme, we previously compared zinc fingers with equivalent DNA-binding specificity and chose ones that are often found in functional ZFNs[4]. Based on that analysis, we recommended 37 zinc fingers for use in genome editing[4]. Here we tested 33 of these fingers, which collectively recognize 39 of 64 three–base-pair subsites (15 GNN subsites and 24 non-GNN subsites, where G is guanine and N is any base; **Supplementary Table 1**). We prepared a combinatorial library of two-finger modules (**Supplementary Methods**) consisting of 1,089 (33 × 33 zinc fingers) two-finger arrays, each linked to the FokI nuclease domain. This library allowed us to construct three-finger or four-finger ZFNs in a single subcloning step (**Supplementary Fig. 1**). Previously, assembling hundreds of ZFNs to target a single gene had taken at least several weeks because up to four repetitive subcloning steps were required to make four-finger ZFNs. With our preassembled two-finger library, preparing several ZFNs to target a gene of interest took only a few days.

We assembled and tested ZFNs to target a human gene and a mouse gene (**Fig. 1**, **Supplementary Fig. 2** and **Supplementary Table 2**). Of six ZFNs, each targeting different sites at the *CDKN2A* locus, two ZFNs induced site-specific mutations in human cells. Of seven ZFNs targeting the *Gt(ROSA)26Sor* (*Rosa26*) gene, one had genome-editing activity in mouse cells. Thus, the success rate of our improved modular-assembly method was 23% (3/13 ZFNs), in good agreement with the success rate of 24% previously measured by the number of target sites at the human *CCR5* locus[4]. In this study, we tested only a single ZFN pair at each target site. Our semi-assembled zinc-finger arrays allow rapid construction of ZFNs in a few days using conventional recombinant DNA technology, and fewer ZFNs need to be synthesized to obtain a functional enzyme.

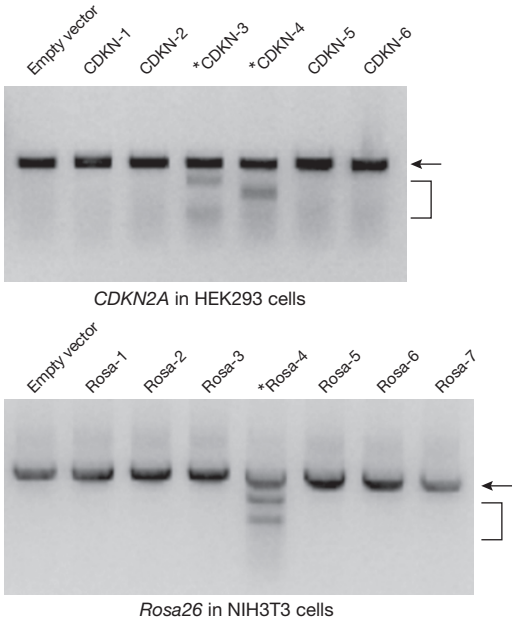

Figure 1 | Targeted genome modifications in human and mouse cells using modularly assembled ZFNs. Insertion-deletions induced by ZFNs designed for *CDKN2A* (CDKN-1 to CDKN-6) and for *Rosa26* (Rosa-1 to Rosa-7) were detected by digesting PCR amplicons of the respective genes with T7 endonuclease I, which cleaves heteroduplexes but not homoduplexes. Arrow indicates uncut bands, and bracket marks cut bands. Asterisks denote functional ZFNs.

Note: Supplementary information is available on the Nature Methods website.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemethods/.

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Live-cell dSTORM with SNAP-tag fusion proteins

To the Editor: In the September 2010 issue of *Nature Methods* we demonstrated live-cell direct stochastic optical reconstruction microscopy (dSTORM) of histone H2B proteins using a trimethoprim chemical tag (TMP tag) for genetic encoding with photostable standard fluorophores[1]. The method takes advantage of the fact that cells contain the reducing thiol glutathione—a cysteine-containing tripeptide—at millimolar concentrations, which enables reversible photoswitching of synthetic organic fluorophores[2,3]. The generality of the method can be easily understood considering that most Alexa