Supporting Information

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SI Methods

Real-Time RT-PCR. About 3 million CHO cells were harvested for preparation of total RNA, which was obtained using TRIzol reagent (Invitrogen), according to the manufacturer's protocols. RNA was extracted from cells 48 h after transfection with plasmids that express zfGCA and zfGCT. Following DNA degradation with amplification grade DNase I (Invitrogen), cDNA was synthesized from 1 μg of RNA per 20-μL reverse transcriptase reaction, using SuperScript III (Invitrogen) and oligo(dT) primers as described in the manufacturer's protocols. For quantitative PCR, ≈25 ng or less of cDNA was used per 50 μL of reaction. Final qPCR reaction conditions were as follows for a 50-μL reaction: 1.5 × standard PCR buffer, 15 pmol per primer, 10 nmol of each dNTP (Invitrogen), 0.2 X SYBR green (Invitrogen), 7.5 units of Taq Polymerase (New England Biolabs), and the cDNA template. The qPCR reaction was analyzed on an Opticon 2 DNA Engine (continuous fluorescence detector) (MJ Research). Real-time RT-PCR primers were 5′-GAA CTT TGC AGT TTC TGC TT and 5′-CAC TTA TCA CCA GCC TCA TT for CHO β-actin and 5′-CGG ATC TCC TAT TGA TTA CG and 5′-CAC CAT TCA TTA GGG TTG AT for the FokI domains of the ZFNs, which are identical in zfGCT and zfGCA. Conditions for quantitative RT-PCR were 96 °C for 1 min, followed by 40 cycles of 96 °C for 15 sec, 53 °C for 20 sec, and 72 °C for 30 sec. The efficiency of amplification per cycle (E) was measured for actin and the ZFNs: E_{actin} was 1.86 ± 0.05 and E_{zf} was 2.34 ± 0.10. The relative levels of mRNA were calculated by comparing number of cycles at which the PCR products became detectable above the basal threshold, which is defined as the crossover point (CP) (1). CP_{zfGCT} was 20.34 ± 0.22, CP_{actinT} was 28.72 ± 0.34, CP_{zfGCA} was 20.57 ± 0.09, and CP_{actinA} was 28.78 ± 0.45. The ratio of zfGCA to zfGCT was calculated according to Equations 1 and 4 in reference (1). Eq. **4** is ratio = $2\exp[(CP_{zfGCT} - CP_{zfGCA}) - (CG_{actinT} - CP_{actinA})]$. It assumes that the efficiency of amplification is the theoretical value of 2. Substituting measured values into this equation give a ratio of 0.89; that is, that zfGCA is expressed at 89% the level of zfGCT. Eq. **1** is ratio = $\{[(E_{zf})\exp(CP_{zfGCT} - CP_{zfGCA})]/[(E_{actin})\exp(CP_{actinT} - CP_{actinA})]\}$. This equation uses the actual values for amplification efficiency. Substituting values into this equation gives a ratio of 0.85, indicating that zfGCA is expressed at 85% of the level of zfGCT. Thus, expression of zfGCA is about 85–90% the level of expression of zfGCT.

1. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45.