Failure to detect DNA-guided genome editing using *Natronobacterium gregoryi* Argonaute

To the Editor:

DNA-guided DNA cleavage using Argonaute-family proteins was previously reported but of limited practical utility due to the requirement for supraphysiological conditions, such as high temperature[1,2]. Recently, Gao *et al.*[3] reported an Argonaute protein isolated from *Natronobacterium gregoryi* (NgAgo) as a genome engineering tool for editing the human genome. After transfecting human cell lines with a plasmid DNA encoding NgAgo and a 24-nucleotide, 5′-phosphorylated single-strand guide DNA (gDNA), they showed gene editing at endogenous targets. Here, we report the results of three different groups (Cathomen, **Supplementary Fig. 1** and **Supplementary Methods 1;** Ekker, **Supplementary Fig. 2** and **Supplementary Methods 2**; and Kim, **Supplementary Fig. 3** and **Supplementary Methods 3**), independently attempting to reproduce the original findings of Gao *et al.*[3], specifically focusing on the evidence for DNA edits in cultured human cell lines. All three groups synthesized the same 5′-phosphorylated gDNA sequences and used the NgAgo vector provided by Gao *et al.*[3] via Addgene (Cambridge, MA, USA) to transfect the same cell lines, and analyzed the genomic DNA for signs of gene editing. Controls confirmed efficient delivery of both plasmid DNA and gDNAs as well as expression of the NgAgo protein. Despite various attempts to optimize NgAgo-mediated genome editing in three of the reported cell lines, no evidence of successful editing of endogenous target sequences was detected.

Gao *et al.*[3] used an episomal enhanced green fluorescent protein (EGFP) marker gene as a target to optimize the NgAgo platform. The Cathomen group used the same experimental setup to disrupt an episomal EGFP expression plasmid in HeLa and HEK293T cells, but failed to detect any statistically significant decrease in EGFP expression (**Supplementary Fig. 1a**). Moreover, they were not able to detect any significant disruption of an integrated EGFP marker gene in human U2OS cells using either the NgAgo-encoding plasmid obtained from Addgene or another plasmid, generated by subcloning the NgAgo sequence in a mammalian expression vector (**Supplementary Fig. 1b**,**c**). In contrast, cells transfected with plasmids encoding an RNA-guided nuclease based on *Streptococcus pyogenes* Cas9 (SpCas9) and a specific gRNA decreased EGFP expression 14-fold in the episomal assay in HeLa and HEK293T (not shown) cells and knocked out EGFP in ~80% of transfected U2OS cells, demonstrating efficient delivery of, and endonuclease expression from, plasmid DNA in these cell lines.

Gao *et al.*[3] demonstrated the ability of NgAgo to robustly edit the human genome under physiological conditions by testing several targets on the gene *DYRK1A* in four human cell lines[3]. Of these four, the Ekker group used HEK293, HeLa and K562 cells and transfected them with NgAgo expression plasmid and five gDNAs corresponding to G5, G6, G10, G12, and G13 in the original report. To test for NgAgo-induced gene editing, genomic DNA was extracted from transfected cells, PCR amplified and subjected to TIDE (tracking of indels by decomposition) analysis[4] (**Supplementary Dataset 2)**. In all samples ($N = 3$ per gDNA, for a total of 45 samples), analysis of the DNA chromatogram failed to show any evidence of robust DNA sequence alterations above the detection threshold (2%; **Supplementary Fig. 2a**). Given that a gene editing efficiency of >20% was reported with NgAgo for these guides in the original report[3], this detection threshold would be sufficient to identify comparable gene editing activity. Assuming a normal distribution of the efficiency of generating insertion/deletion (indel) mutations in these samples, the probability that none of these would show any evidence for *de novo* indels is $P < 0.0001$. Assuming the true prevalence of indels induced by NgAgo is 0.20 with a normal distribution, our null hypothesis is "NgAgo does not induce indels in human cells at or greater than the rate of 0.20." The probability of us not observing any evidence of indels after testing 45 independent samples is $p = 0.8^{45} = 4.4 \times 10^{(-5)}$. In addition, parallel sequence analysis of 45 subcloned colonies from HeLa cell samples also failed to show any evidence of indels (data not shown). These independent replicates failed to detect any NgAgo-induced editing despite clear evidence of cellular delivery of each of the listed components for this system.

Gao *et al.*[3] reported successful gene editing on other human loci, including *EMX1*, *GATA4*, and *GRIN2B* (**Supplementary Fig. 3a**). The Kim laboratory delivered gDNA and NgAgo expression plasmids to HEK293 cells or HeLa cells either by lipofection (**Supplementary Fig. 3b**–**e**) or electroporation (**Supplementary Fig. 3f**–**j**). They also added Mg^{2+} to the culture medium because NgAgo requires this cation for catalytic activity (**Supplementary Fig. 3k**). T7E1 (T7 endonuclease I) assay[5] and targeted deep sequencing (NCBI SRA: SRX2161446) was applied to detect NgAgo-induced mutations. NgAgo failed to generate indels at any of the four analyzed loci (*DYRK1A*, *EMX1*, *GATA4*, and *GRIN2B*), with frequencies above sequencing error rates (~0.1%). Moreover, the Cathomen group was not able to detect signs of gene editing at the *CCR5* locus either by the T7E1 assay[5] or by TIDE analysis[4] (**Supplementary Fig. 1c**,**d, Supplementary Dataset 1**). In contrast, SpCas9 nucleases, which were used as controls in these experiments, induced indels at frequencies that ranged from 3% to 73% (**Supplementary Figs. 1d** and **3b**–**k**).

To confirm that the gDNA molecules used were chemically phosphorylated at the 5′ end, the Kim lab performed mass spectrometry (**Supplementary Fig. 3m**). To confirm efficient co-delivery of plasmid DNA and the putative guide DNA, they co-transfected 3′-Alexa Fluor 594-tagged gDNA bearing the same targeting sequence as G5 along with

the EGFP expression plasmid (pEGFP-N1) driven by a cytomegalovirus (CMV) promoter. As the expression of NgAgo modified at its N terminus with a nuclear localization signal (NLS; NLS-NgAgo) is also driven by a CMV promoter, the Ekker lab used EGFP expression as a surrogate marker of NgAgo in these cells. At 4, 12, and 24 h after lipofection, HeLa cells and HEK293 cells were fixed with paraformaldehyde and the coverslips mounted on slides. Strong red fluorescence corresponding to Alexa Fluor 594 was observed inside the cells by 4 h and persisted for at least 24 h (**Supplementary Fig. 2b**). The distribution of red fluorescence was diffuse within cells at 4 h, but later became more punctated. As expected, some EGFP fluorescence was observed by 4 h, with a much stronger signal detected at 12 and 24 h after transfection. Delivery of NgAgo plasmid was also confirmed by PCR assays (**Supplementary Fig. 2c**). To confirm expression of NgAgo in transfected cells, the extent of RNA or protein expression was determined either by qualitative reverse transcription PCR (RT-PCR; **Supplementary Fig. 1e**), immunoblot analysis of hemagglutinin (HA)-tagged versions of NgAgo (**Supplementary Figs. 1f** and **3n**), or flow cytometry of a DsRed-tagged version of NgAgo (**Supplementary Fig. 1f**). Taken together, these data suggest that plasmid DNA and gDNA are efficiently delivered to the human cell lines used here and that all the tested cells are capable of expressing NgAgo.

On the basis of the above data, we conclude that in conditions designed to replicate those in Gao *et al.*[3], co-delivery of plasmid DNA encoding NgAgo and a 5′-phosphorylated single-strand gDNA alone is insufficient to induce gene editing at the indel frequencies in cultured human cells reported in the original study.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nbt.3753).

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper (doi:10.1038/nbt.3753).

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Should you profit from your genome?

To the Editor:

As increasingly detailed information about personal health and wellness becomes available, the commercial sector is seeking to develop products based on personal health profiles that exploit growing markets for medical data. Genetic and genomic information is precisely the kind of valuable medical data that companies are eager to buy and sell. However, individuals express distrust of for-profit entities and have reservations regarding sharing their data as a result[1,2]. To encourage participation, some genetic and genomic testing companies are considering paying people to share their data. These companies hope that offering economic incentives will lead more individuals to make their genetic and genomic data available on this new market.

But should a person profit from her genome? And if companies compensate people for sharing data, how can they structure that compensation to promote fairness and transparency? Here, we outline the benefits and challenges that this cutting-edge business model may raise.

In the past, genetic and genomic testing industries have primarily made money from selling tests. However, some have realized that they can generate a much greater profit by selling their customers' genetic and genomic information. Genetic testing companies then have two sets of potential clients: the people who buy tests and the entities that purchase data. Of course, selling access to an extensive database of customer information is nothing new. Direct-to-consumer (DTC) genetic testing company 23andMe (Mountain View, CA, USA) raised eyebrows by doing just that last year. What makes the latest business strategies novel is that the companies hope to gain a competitive advantage in the data-selling industry by providing financial incentives to their consumers.

One new consumer genomics firm, Genos (San Francisco), will compensate individuals for licensing their genomic data to biotech and pharmaceutical companies. Although Genos will sequence its customers' exomes for $399, it will not interpret those data. When consumers get their data, they can then share them for compensation. This new business model centers not on selling the $399 tests—in fact, people who have already been sequenced can upload their data for free—but on brokering access to the genetic information. According to executive chairman Cliff Reid, "Our business is not to make money sequencing people, our business is to make money enabling researchers and individuals to connect and transact with each other"[3].

San Francisco–based Invitae is also considering this new approach. Invitae is offering to sell "genome management" services, including the lifetime storage and analysis of its customers' genetic information. Once the company accrues a sizable consumer database, Invitae plans to sell those data to third parties and to share the profits with individuals who are willing to contribute their information.