Citations

TALEN scout

Genome engineering via programmable nucleases has taken the life science community by storm. Transcription activator-like effector nucleases (TALENs) are designer nucleases that work in pairs, with each partner recognizing a DNA sequence 16-20 bp long and cleaving one of the DNA strands in the spacer region between the recognition sites. The resulting double-strand breaks often lead to small insertions or deletions, disrupting the genes in which they occur. Now, a team from Seoul has created a plasmid library for producing TALENs with a high probability of efficacy for every gene in the human genome. This work, published by Kim et al. in *Nature Biotechnology*, began by optimizing TALEN architecture to more precisely set the acceptable spacer length within the target site, which should increase specificity. The authors then developed an algorithm to search for target sites in every human gene. The search criteria were rigorous, requiring 40 bp target sequences with 12 or 13 bp spacers, at least 7 single-base mismatches with any off-target site, localization within the first 70% of the coding sequence and, for alternatively spliced genes, a target site within a common exon. With these restrictions, at least 1 target site could be found for 91% of human genes. Using modestly relaxed criteria, target sites for 18,740 genes were identified. After preparing a set of 424 plasmids containing different TAL effector repeat domains, Kim and colleagues used PCR-free Golden Gate cloning to combine the appropriate sequence recognition modules with a nuclease domain to assemble 37,480 TALEN plasmids, representing TALEN pairs for all 18,740 genes. To test TALEN activity, the researchers transfected 104 TALEN pairs into HEK293 cells, finding 98.1% functional with an

Schematic of modular, Golden Gate-based cloning of TALEN plasmid library spanning the human genome. Source: *Nature Biotechnology*

average mutation frequency of 16%. No off-target effects were detected. To demonstrate the potential of this resource, the authors created knockouts of genes associated with NK- κ B signaling in cultured cells. In contrast to siRNA-treated cells, the TALEN-mutated cells showed complete suppression of NF- κ B signal transduction. Researchers eager to create their own knockout clones should visit www.talenlibrary.net. There, users need simply to search for a gene to pull up order information for the associated TALEN plasmid. Material transfer agreements and a fee to cover costs for the nonprofit entity providing the TALEN library resource apply.

Y. Kim et al. 2013. A library of TAL effector nucleases spanning the human genome. *Nat Biotechnol.* 31:251-8.

Disruptive technology

Could switching the reagent used to disrupt cell membranes reset the standard protocol for proteomic analysis? That's the contention explored by a group from the University of Ottawa in an article appearing in the *Journal of Proteome Research*. Traditionally, cells are lysed using a detergent such as SDS, which breaks open the cells, denatures the proteins, and solubilizes those that are hydrophobic. Because detergents tend to interfere with proteolytic enzymes, the surfactant is generally replaced by a chaotrope such as urea before trypsinization, keeping the proteins in solution without blocking proteolysis. However, since mass spectrometry is disrupted by most chaotropes, these agents must also be removed. Prior efforts to simplify this workflow have not caught on since the proposed detergent/chaotrope replacements have their own limitations, such as incomplete solubilization and safety concerns. Now, Ning et al. propose using Amphiphols (APols), which are commer-

cially available polymers with some subunits functionalized with hydrophobic and hydrophilic groups: the former interact strongly with hydrophobic regions of proteins, the latter keep the complex soluble. To date, APols have been used in structural analysis of proteins, but Ning and colleagues hypothesized that the polymers, with a little help from sonication, might be capable of lysing cells and solubilizing proteins for trypsinization. Indeed, APols proved equivalent to RIPA buffer for lysing cultured cells, while only minimally affecting trypsin activity. Next, the authors compared sample preparation with traditional methods versus the APol-based method. The former comprised SDS extraction, acetone precipitation to remove the detergent, reconstitution in urea for trypsin digestion, desalting, and vacuum drying prior to mass spectrometry. By contrast, in the APol method, trypsin was added directly to the detergent-containing solution; after digestion, APols

were removed by adding formic acid (at pH 3-4, APols aggregate and can be pelleted by centrifugation). Any concern that the accelerated protocol would compromise mass spectrometry was dismissed by data showing 30%-40% higher signal intensity and 40% more unique peptides in proteomic samples prepared using APols. The authors attribute this difference to sample loss during the protein precipitation and desalting steps in the traditional protocol.

Z. Ning et al. 2013. From cells to peptides: "one-stop" integrated proteomic processing using amphiphols. *J Proteome Res.* 12:1512-9.

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