TOOLBOX

Online tool boosts efficiency of popular gene editing method

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Tough choices: Researchers using CRISPR must carefully select the molecules that guide the molecular scissors on where to snip the DNA.

A new software application allows researchers to more efficiently edit genes using the popular method CRISPR. The tool, described 13 July in *Nature Methods*,ups the odds of hitting the correct target[1].

Over the past two years, CRISPR has revolutionized researchers' ability to alter specific genes.Scientists have used the approach to produce **mice and human cells** with autism-related mutations, and are working to do the same in**monkeys**.

In the method, a scissor-like protein called CAS9 cuts the genome at a specific site. Researchers instruct CAS9 on where to slice by feeding it a small sequence of 'guide' RNA (sgRNA). CAS9 carries this snippet of RNA as it searches the genome for a segment of DNA with a complementary sequence.

Once it finds the target sequence, CAS9 snips the DNA. This incision can disable genes, preventing proteins from being made. It can also allow researchers to insert sequences, producing proteins with precisely engineered mutations. (In fact, the power of the technique has sparked **serious controversy**.)

But sometimes CAS9 makes a mistake: It can cut the DNA at an undesired location or fail to hit its target. Some sequences are tucked away in tightly folded DNA and may be hard for CAS9 to reach. Others are poor targets for CAS9 for reasons that remain unclear.

One way to make CAS9 more accurate is to feed it more specific and efficient sgRNAs. The new tool, called **sgRNA Scorer**, prompts researchers to enter the sequence of a gene they wish to edit. It then spits out a list of sgRNAs designed to target various regions of that gene, ranked by how efficiently each is predicted to guide CAS9 to make cuts.

The researchers, led by geneticist **George Church**, previously developed another application, called **CasFinder**, that ranks how often specific sgRNAs target the wrong DNA sequence. Using both tools at once — which the new software supports with a single click — researchers can optimize their selection of sgRNAs.

To create sgRNA Scorer, the researchers generated sets of sgRNAs targeting 1,400 sites across the genome. They then assessed how effectively these sgRNAs direct two different versions of CAS9 to edit each site. Using an algorithm to trawl through the data, the researchers identified sequences that tend to encourage — or discourage — CRISPR cutting. Based on this information, they generated software that can forecast the activity of any given sgRNA at its target site.

To validate the program, the researchers used it to predict the activity of 10 different sgRNAs for each of the two CAS9 versions against their target sites and then checked these predictions in seven different cell types. The sgRNAs that the software flagged as being highly active frequently snip the correct sites within the cells, whereas the sgRNAs predicted to have low activity hit their targets less often.

The software should help scientists save time by using the sgRNAs best suited for their target, allowing them to more quickly generate new cell lines and model organisms.

References:

1. Chari R. *et al. Nat. Methods* Epub ahead of print(2015) **PubMed**