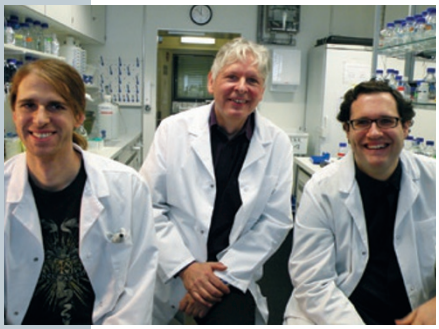
THE AUTHOR FILE

Rolf Zeller and Javier Lopez-Rios

Gene cutting and pasting just got a whole lot faster.

It is one of the arbitrary facts of nature that some genes are more amenable to tinkering than others. Unfortunately the gene that Marco Osterwalder, a graduate student at University of Basel Medical Faculty, was most interested in studying was in the latter category. *Hand2* is essential for heart and limb development, and replacing the endogenous mouse gene with an engineered construct, he thought, would help identify a wealth of interaction partners.



Aimee Zuniga, University of Basel

First author Marco Osterwalder (left) is pictured with corresponding authors Rolf Zeller (middle) and Javier Lopez-Rios (right).

But replacing endogenous genes is a tedious, exacting process, explains Javier Lopez-Rios, a postdoc who works with Osterwalder. Constructing a vector containing the replacement construct can take a month or two, and using it to transfect mouse embryonic stem cells takes another couple of weeks. Then screening is necessary to identify the very few cells modified correctly. "You have to pick hundreds of clones," says Lopez-Rios. Each clone is checked for appropriate modification using PCR or Southern blotting, and if no appropriate clones are found, the process starts over again. Creating a mouse with a new version of the gene by conventional homologous recombination can take up to 18 months.

Generation of the *Hand2* 'conditional knockout' by homologous recombination involved screening ~1,500 clones, which took three scientists several months. "It was kind of the story in the lab; this is really the locus from hell," Lopez-Rios recalls. Therefore, nobody in the group except Osterwalder was keen on modifying this locus any further.

And then Lopez-Rios had an idea. The *Hand2* 'conditional knockout' encodes sites that allow two DNA recombinases to excise the protein-coding region on cue, but some sites remain even after the gene is deleted. Maybe, Lopez-Rios thought, these left-over sites could serve as an anchor to pull a new gene construct in. Even better, the International Knockout Mouse Consortium has engineered 'conditional knockouts' for thousands of genes using the same configuration of recombinase sites.

Lopez-Rios and Osterwalder sketched out a possible approach and showed it to Rolf Zeller. "I said it looks like it would work on the drawing board," Zeller recalls, "but if it would be so easy, why hasn't anyone done it?" The scientists were not keen to spend another year and a half doing homologous recombination, so they decided to give it a try. "It worked just like we drew it on the blackboard of the seminar room," Zeller recalls. "Per construct, you pick many fewer colonies. It's a gain of time, it's less stress, and you get your result much quicker. It's one of those cases where laziness makes for good science," he laughs.

Osterwalder is now back studying limb development and can create more tools than Zeller originally considered possible. The goal of his project is to modify the Hand2 protein with a tag that will capture its interaction partners, and now Osterwalder is screening several tags to find the most effective one. "It's something we never would have thought of doing if we had to use homologous recombination," says Zeller. He believes there is even potential for the technique to be used in high-throughput screening to test the effects of various mutations on protein function.

"It's one of those cases where laziness makes for good science."
—Rolf Zeller

What's more, the technique is easy to learn. A graduate student who has never worked with embryonic stem cells is already inserting different tags into an endogenous gene, says Zeller. In contrast, the use of homologous recombination would be tedious and require copious training, he explains. He has previously been reluctant to push such projects for fear that part of a young scientist's career might be wasted with a long-term project that didn't work.

Plasmids with the insertion vectors have already been deposited with AddGene for public distribution, says Zeller. And, given that the International Knockout Mouse Consortium has created conditional knockouts for about half of the mouse genome, the technique could be quickly applied to thousands of genes, he says. "What we really hope is that this technology will be useful for a lot of people."

Monya Baker

Osterwalder, M. *et al.* Dual RMCE for efficient re-engineering of mouse mutant alleles. *Nat. Methods* **7**, 893–895 (2010).