Optimal CRISPR guide RNA design for gene editing in corals

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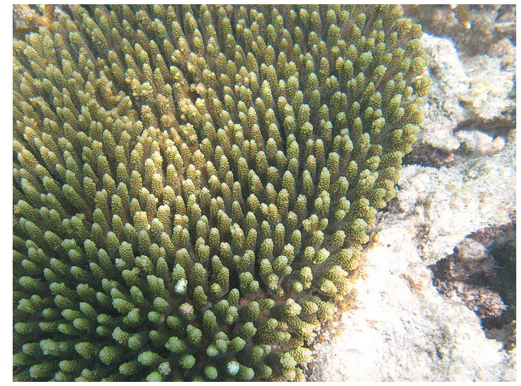

A Genetic Tool for Coral Research

Coral reefs are biodiversity hot spots of great ecological, economic, and aesthetic importance. Their global decline due to climate change and other stressors has increased the urgency of understanding the molecular bases of corals' responses to stress.

Analyses of coral genomes and gene-expression patterns have identified many genes that may be important in stress resistance, prompting a major focus of research to understand both (i) the mechanisms that lead to heat-induced bleaching and death and (ii) those that may protect against it.

However, without tools to enable accurate genetic modifications, it is difficult to validate hypotheses. CRISPR has the potential to address this gap, provided that we have a method to generate high-quality guide RNAs for gene editing in corals. We want to: (i) limit the off-target risk (to really test the hypotheses of interest rather than the impact of random modifications), and (ii) maximise the efficiency of on-target modifications (as the annual spawning rhythm is a major bottleneck for experiments).

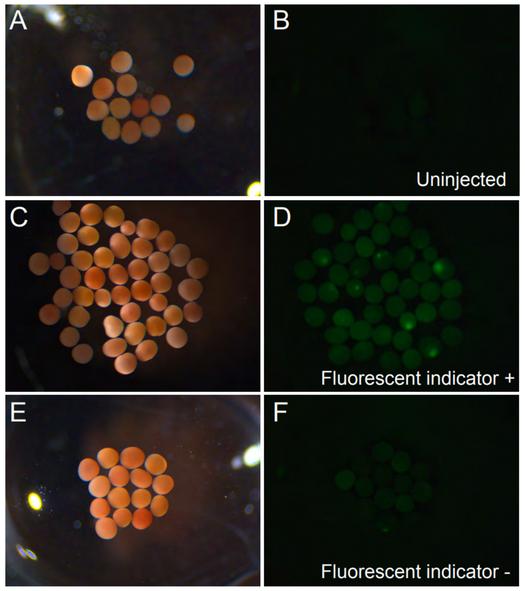

Figure 1: Highly Effective Disruption of a Coral HSF1 Gene Using Crackling-designed sgRNAs

Identification of successfully injected A. millepora larvae using a fluorescent indicator. Bright-field and fluorescence images of representative uninjected (A-B) and injected (C-F) larvae. The injected larvae were sorted at ~12 h after fertilization into groups with (C and D) or without (E and F) visible fluorescence from the Alexa Fluor 488-dextran injection marker. Larvae were imaged immediately after sorting. The faint fluorescence seen in F was not visible during sorting, so these larvae were treated as not successfully injected. See Figure S2 in [1].

Intelligent CRISPR sgRNA design using the *Crackling* pipeline

Here, we present Crackling, a sgRNA design pipeline that fulfills the need for a tool that can design sgRNAs with precision and speed, whilst remaining practical to use on large genomes.

Crackling utilises Inverted Signature Slice Lists (ISSL) to identify possible off-target sites. ISSL performs constant-time approximate nearest neighbour searches in an index of bit-encoded, locality-sensitive signatures.

Each site is encoded as a signature and portioned into $n + 1$ slices, where n is the maximum number of mismatches (here, $n = 4$). The position of the slice is retained in the index, thus yielding each slice as the locality-sensitive signature. Given that n mismatches are allowed and there exist $n + 1$ slices, one slice will be shared between a candidate guide and a potential off-target site. This strategy identifies a neighbourhood of sites, from which we can extract that are at most n mismatches away. They are then evaluated using the Zhang score.

To identify efficient sgRNAs, Crackling only recommends candidate guides that have been accepted by at least two of three scoring approaches, which is more precise than using a single method.

Crackling is available at: github.com/bmds-lab/Crackling

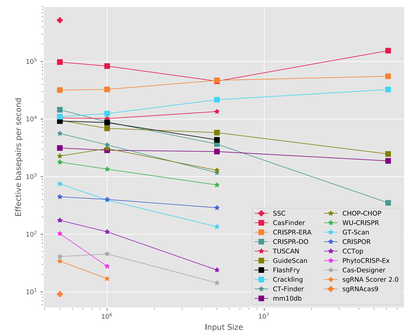

Figure 2: Crackling is amongst the fastest available guide design pipelines

Previously, we benchmarked leading CRISPR guide design methods using custom datasets derived from the mouse genome. We reported a measure, *effective base-pairs per second* (EFPS), which uses the length of the coding regions used for candidate extraction. Crackling places amongst those top performing pipelines, in terms of overall speed.

Figures 2 and 3 are derived from [3].

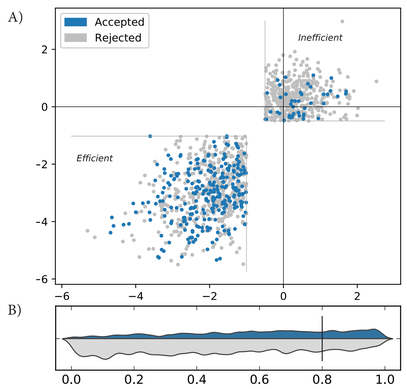

Figure 3: Optimal consensus approach provides precision of up to 86%

Panel A: blue points indicate positive predictions. Here, some experimentally inefficient guides have been accepted by the consensus approach.

Panel B: the blue distrubtion shows the number of guides that have been accepted by the consensus approach. The grey distribution shows the number of guides rejected. Guides ranked above 0.8 were deemed experimentally efficient.

Decreased Heat Tolerance of Coral Larvae Carrying Mutations in HSF1

Crackling is fast enough to allow the systematic identification of all suitable sgRNAs in any genome of interest. This is important in a context where there is not a single reference genome.

As a demonstration of the value of Crackling, we report here on a knock-out experiment in A. millepora, where we targeted the HSF1 gene [1].

Overall, it appeared that up to ~99% of the sgRNA/Cas9-injected animals yielded mutant HSF1 copies (at one or both sites). In contrast, no mutant sequences were detected in any of the control animals.

Wild-type larvae survived at 27°C and 34°C. KO larvae similarly survived at 27°C, but rapidly died at 34°C.

This highlights the role of HSF1 in heat tolerance in corals. It also demonstrates the value of CRISPR-based gene editing in coral research, and the benefit of having an efficient method for sgRNA design.

We are now in the process of systematically analysing a number of coral genomes.

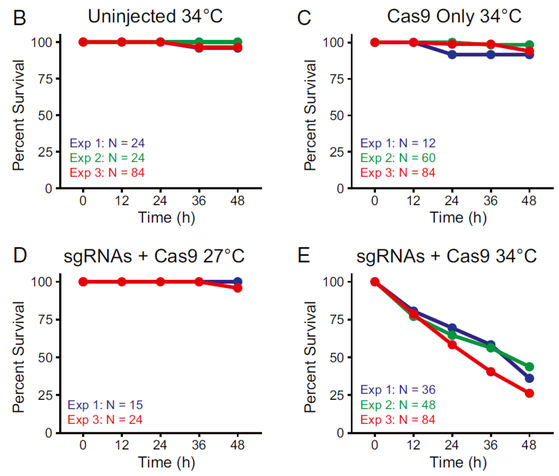

Figure 4: Decreased Heat Tolerance of Coral Lravae Carrying Mutations in HSF1

The numbers of individual larvae monitored in each experiment are indicated.

(B) and (C): Survival curves (percentages of surviving animals) for uninjected (B) and Cas9-injected (C) larvae during incubation at 34 °C.

(D) and (E): Survival curves for sgRNA/Cas9-injected larvae during incubation at 27 °C (D) or 34 °C (E).
See full Figure (as Figure 2) in [1].

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