

## **Writing the result section** (shown for a single figure)

Adapted from MIT Biological Engineering Communication Lab: <https://bit.ly/2PJiusp>.

### **Subheading states conclusions.**

Cpf1-Containing CRISPR Loci Are Active Bacterial Immune Systems.

### **Section content corresponds to Fig. 1.**

Cpf1 was first annotated as a CRISPR-associated gene in TIGRFAM and has been hypothesized to be the effector of a CRISPR locus that is distinct from the Cas9-containing type II CRISPR-Cas loci that are also present in the genomes of some of the same bacteria, such as multiple strains of *Francisella* and *Prevotella* (Schunder et al., 2013, Vestergaard et al., 2014, Makarova et al., 2015) (Figure 1A). The Cpf1 protein contains a predicted RuvC-like endonuclease domain that is distantly related to the respective nuclease domain of Cas9. However, Cpf1 differs from Cas9 in that it lacks a second, HNH endonuclease domain, which is inserted within the [...].

### **Experimental rationale + methods description without too much detail.**

To simplify experimentation, we cloned the *Francisella novicida* U112 Cpf1 (FnCpf1) locus (Figure 1A) into low-copy plasmids (pFnCpf1) to allow heterologous reconstitution in *Escherichia coli*. [...] Given the completely uncharacterized functionality of the FnCpf1 CRISPR locus, we adapted a previously described plasmid depletion assay (Jiang et al., 2013) to ascertain the activity of Cpf1 and identify the requirement for a PAM sequence and its respective location relative to the protospacer (5' or 3'). [...]

### **Quick description of how assay was interpreted.**

Using this assay, we determined the PAM sequence and location by identifying nucleotide motifs that are preferentially depleted in cells heterologously expressing the FnCpf1 locus.

### **Statement of findings.**

We found that the PAM for FnCpf1 is located upstream of the 5' end of the displaced strand of the protospacer and has the sequence 5'-TTN. [...]

### **Conclusion (I).**

Beyond the identification of the PAM, the results of the depletion assay clearly indicate that heterologously expressed Cpf1 loci are capable of efficient interference with plasmid DNA.

### **Transition + rationale + methods.**

To further characterize the PAM requirements, we analyzed plasmid interference activity by transforming cpf1-locus-expressing cells with plasmids carrying protospacer 1 flanked by 5'-TTN PAMs.

### **Findings.**

We found that all 5'-TTN PAMs were efficiently targeted (Figure 1E). In addition, 5'-CTA, but not 5'-TCA, was also efficiently targeted (Figure 1E),

### **Conclusion (II).**

... suggesting that the middle T is more critical for PAM recognition than the first T and that, in agreement with the sequence motifs depleted in the PAM discovery assay (Figure S1D), the PAM might be more relaxed than 5'-TTN.

Zetsche et al., "Cpf1 is a single RNA-guided endonuclease...", *Cell* 2015

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