CRISPR-Cas systems enable bacteria and other microbes to acquire immunity against viruses by capturing snippets of their DNA. Marraffini investigates the molecular mechanisms that make CRISPR immunity possible, as well as its evolutionary implications. His lab also explores genome editing and other potential applications for CRISPR-Cas systems.

Sequence-directed genetic interference pathways control gene expression and preserve genome integrity in all kingdoms of life. In many bacteria and most archaea, CRISPRs—clustered, regularly interspaced, short palindromic repeats—specify a recently discovered genetic interference pathway that protects cells from phages and conjugative plasmids. Within CRISPR sites, the repeats are separated by short spacer sequences that match phage or plasmid genomes and specify the targets of interference.

Spacer sequences are transcribed into CRISPR RNAs (crRNAs)—small RNAs that, through base-pairing interactions with the target sequence, guide Cas nucleases to the invasive nucleic acid. Upon infection, CRISPR arrays can acquire new spacer units that match the sequence of the infecting phage or plasmid. In this way, CRISPR-Cas systems provide adaptive and inheritable immunity to the bacterial cell. The spacer content of CRISPR arrays reflects the many different invaders encountered by the host and can be expanded rapidly in response to new ones. Accordingly, CRISPR loci constitute a form of genetic memory that ensures the rejection of new, returning, and ever-present invading DNA molecules.

Marraffini uses Staphylococcus epidermidis and Streptococcus pyogenes as model systems for studying CRISPR immunity. The clinical isolate S. epidermidis RP62a harbors a CRISPR spacer that matches the nickase gene (nes) that is present in nearly all staphylococcal conjugative plasmids and prevents their spread. Using this system, Marraffini revealed that the CRISPR-Cas machinery targets DNA, rather than RNA, directly. Work in the Marraffini lab also demonstrated that the S. pyogenes crRNA-guided Cas9 DNA nuclease constitute a formidable tool for genetic engineering.

Marraffini’s current research employs molecular genetic and biochemical approaches to analyze the genesis and function of CRISPR-Cas systems. He ultimately hopes to answer fundamental questions about how CRISPR-Cas systems destroy their targets, how the genetic memory is generated, and how CRISPR-Cas immunity affects the evolution of bacteria and archaea.