Summary

*S. aureus* Cas9:
- Is encoded by 3159 nucleotides, compared to 4104 nucleotides for *S. pyogenes* Cas9.
- Recognizes an NNGRRT PAM.
- Cleaves dsDNA with efficiency comparable to *S. pyogenes* Cas9.
- Cleaves dsDNA with gRNAs of different spacer lengths, with shorter guides generally less tolerant of sequence mismatches.

*S. aureus* Cas9 cleavage efficiency is comparable to *S. pyogenes* Cas9

*S. aureus* targets with NNGRTT and NNGRRV PAMs

Methods:
All experiments were performed via transfection into HEK293T cells using Lipofectamine 3000 in 24-well format. Transfections consisted of 750 ng/well of Cas9 (driven by CMV promoter) plasmid and 250 ng/well of gRNA (driven by U6 promoter) construct. For most of these experiments, total DNA was harvested from cells 72 hours post transfection, and NHEJ % was determined by target locus PCR followed by T7E1 cleavage assay. Alternatively, for assays involving fluorescence, GFP knockdown was measured 3.5 days post transfection.