A CRISPR immune systems target DNA or RNA in microbes Bacteriophage Microbe (virus) Protospacer (viral gene) Viral DNA PAM Protospacer Adjacent Motif Acquisition of Microbial DNA CRISPR spacer New spacer Pre-crRNA transcripts Transcription and formation of Cas-RNA complexes -Guide RNA Surveillance complexes Cas protein-Same viral gene Custom-designed Activation of CRISPR-Cas9 system **DNA** cutting mechanism Eukaryotic cell Target RNA Guide RNA Cas9 -Reinfection B CRISPR-Cas9 is the Cas9 seaches the DNA for PAM canonical genome editing binding sites. DNA tool for RNA-guided 1 If PAM is found, genetic manipulation Cas9 binds and forms an R-loop with the DNA. Cas9 generates a double-stranded DNA break. The DNA is left for repair by other enzymes.