

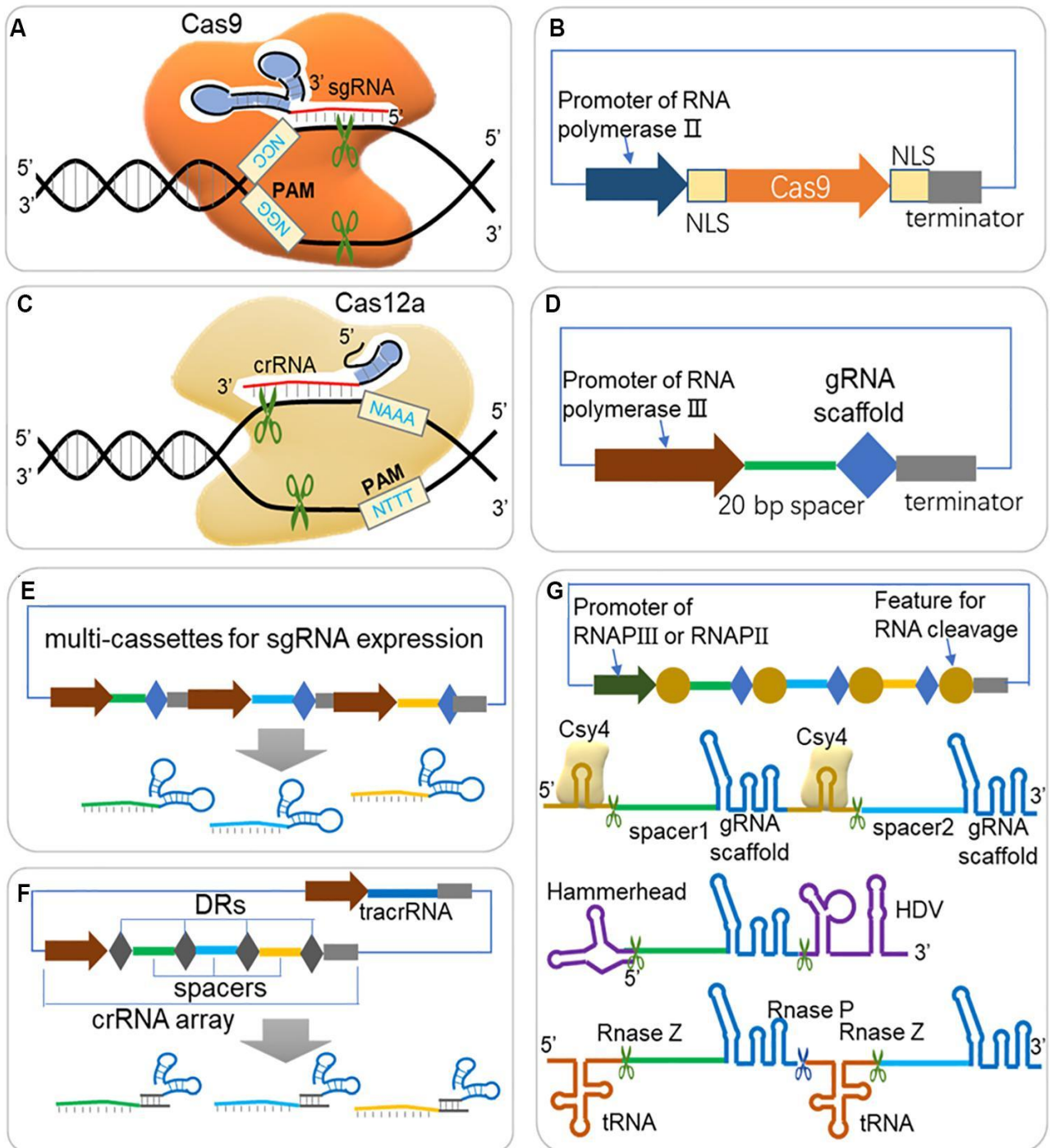


Figure 3 Guidelines for expression of Cas protein and sgRNA in CRISPR/Cas system (Adopted from Ding et al., 2020)

Image caption: (A) Scheme of CRISPR/Cas9 system. The Cas9-sgRNA (or Cas9-crRNA-tracrRNA) complex binds to DNA target arising from Watson-Crick base pairing of spacer sequence, and triggers double strand break (DSB) when next to a short protospacer adjacent motif (PAM, 'NGG' for Cas9 from *S. pyogenes*). (B) Expression cassette for Cas9. For efficient targeting to nucleus in eukaryotes, the Cas9 should be fused to NLS (nuclear localization sequence) at one end or both ends. (C) Scheme of CRISPR/Cas12a (Cpf1) system. Cas12a triggers DSB through a similar scheme of Cas9, but depends on different PAM ('NTTT') and less folded crRNA, and creates a sticky end at 18–23 bases away from the PAM. (D) Expression cassette for sgRNA. A promoter of RNA polymerase III (RNAP III) is usually required for directing sgRNA in nucleus and with less modification. (E) Multi-sgRNA expression through multi-cassettes. Repeated elements, such as promoters, gRNA scaffold and terminators are repeated for different spacer sequences. (F) Multi-sgRNA expression through crRNA array and tracrRNA (HI-CRISPR system). (G) gRNA multiplexing strategies. Both RNAP II and RNAP III promoter can be used for expression the sgRNA array, where sgRNAs are separated by features for RNA cleavage (Adopted from Ding et al., 2020)