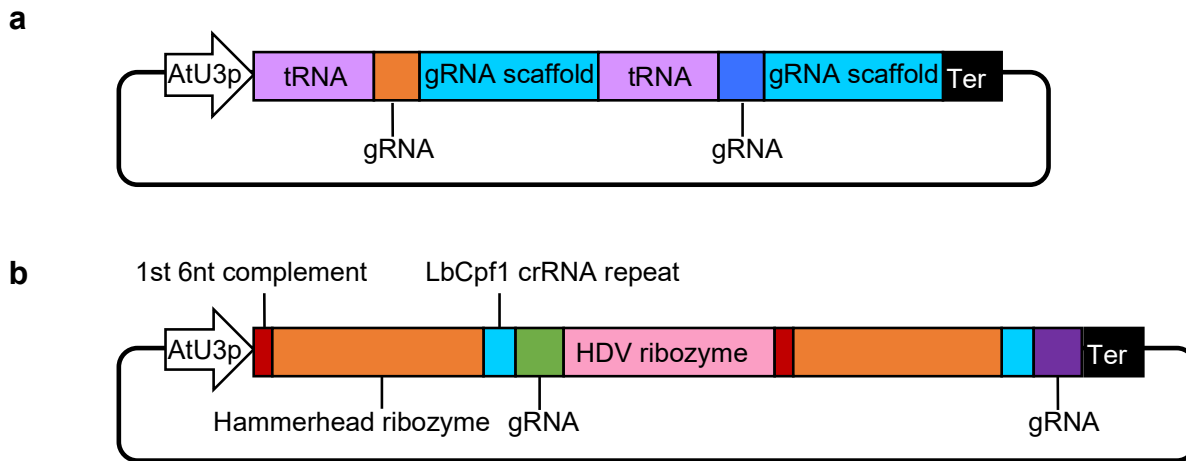


## Supplemental Materials

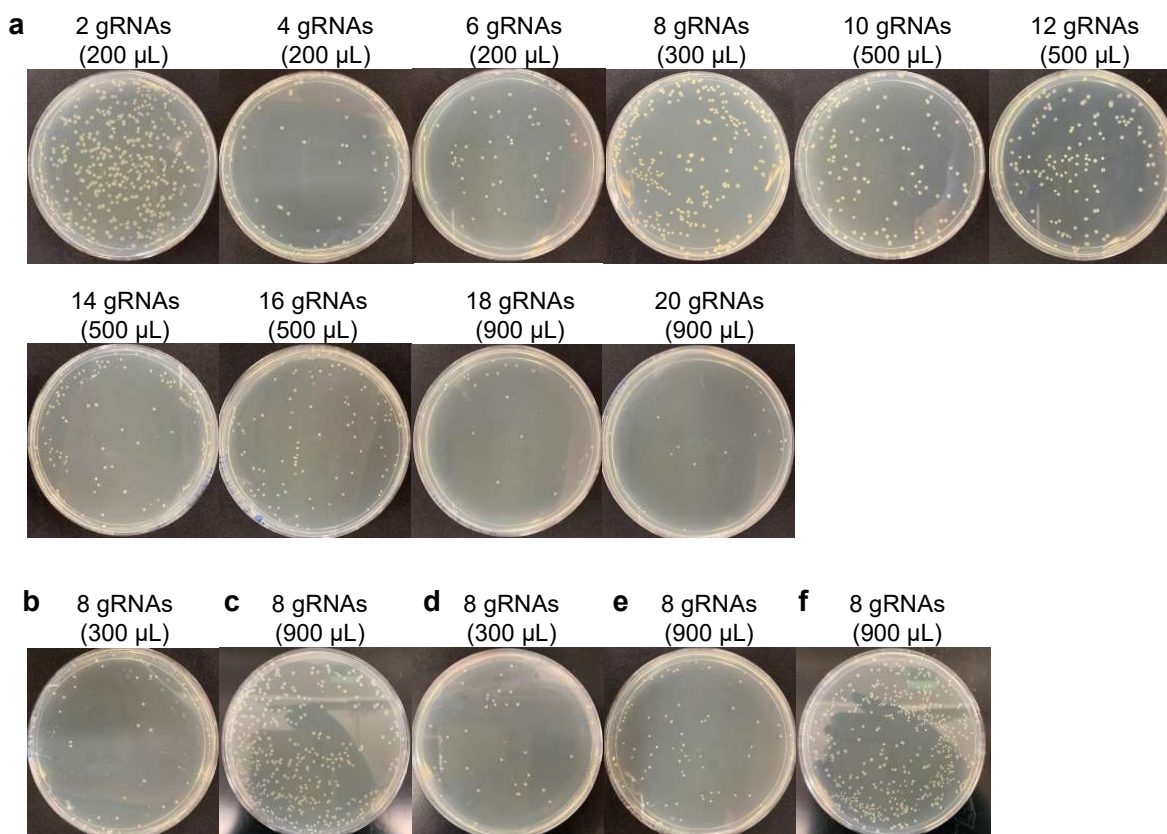
### Supplemental Figures



**Figure S1. Structure of template vectors for PCR amplification of component DNA fragments. (a) Template vector type I. (b) Template vector type II.**



**Figure S2. PCR products for the assembly of 8 gRNAs in a plant tRNA system.**  $[n+1]$  fragments are required for assembly of  $[n]$  gRNAs. Two PCR bands were generated for first fragment F[1] and last fragment F $[n+1]$ , and red circled bands are the expected fragments.

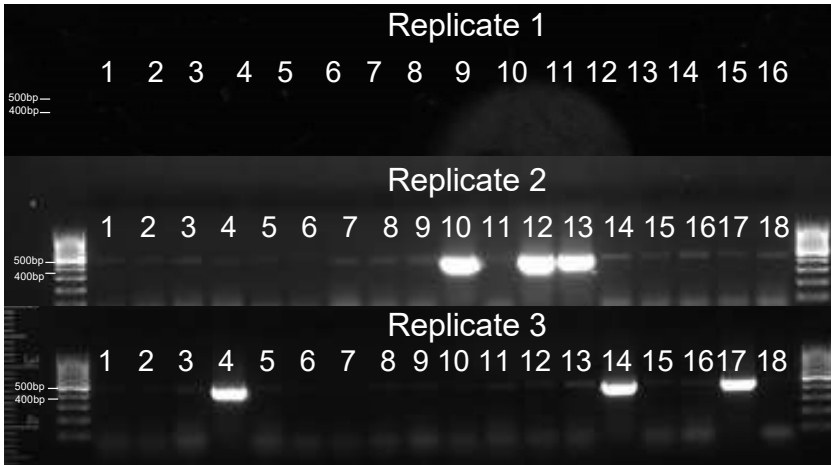


**Figure S3. Colony plates for the assembly of different numbers of gRNAs in different expression systems.**

**(a)** Colony plates for the assembly of different numbers of gRNAs in the plant tRNA expression system using PCR products. Loading volume varies in different plates. **(b)** Colony plates for the 8-gRNA assembly in the RB expression system using PCR products. Loading volume = 300 µL. **(c)** Colony plates for the 8-gRNA assembly in the Csy4 expression system using PCR products. Loading volume = 900 µL. **(d)** Colony plates for the 8-gRNA assembly in the HDV-HH-RB expression system using PCR products. Loading volume = 300 µL. **(e)** Colony plates for the 8-gRNA assembly in the HDV-HH-RB expression system using gBlocks. Loading volume = 900 µL. **(f)** Colony plates for the 8-gRNA assembly in the plant tRNA expression system using PARAwab-based PCR products. Loading volume = 900 µL. The total volume of the outgrowth medium is usually 900 µL.

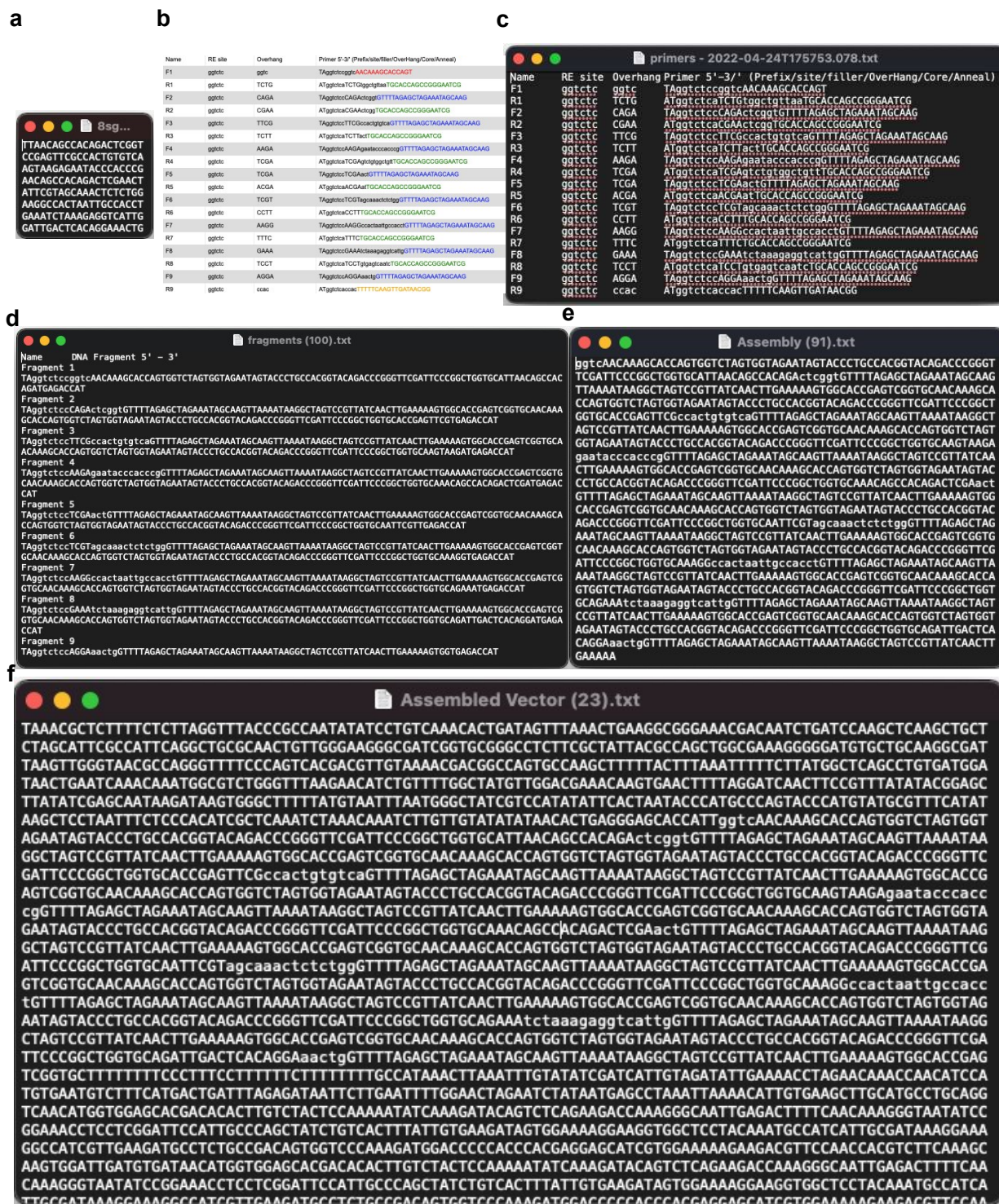


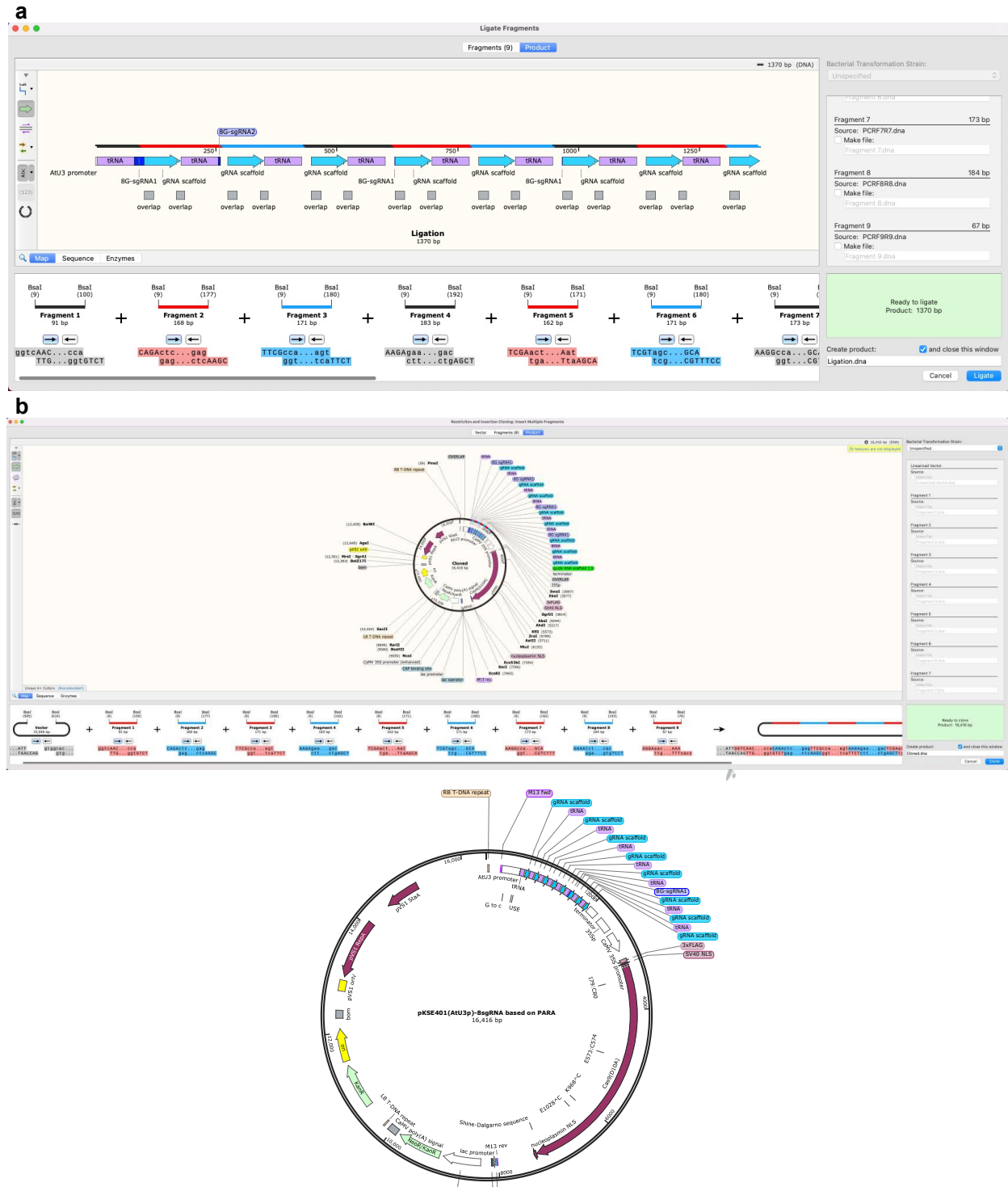
**Figure S4. Colony PCR for the screening of transformants for the plant tRNA expression system. (a)** Colony PCR of the 2-gRNA assembly. **(b)** Colony PCR of the 4-gRNA assembly. **(c)** Colony PCR of the 6-gRNA assembly. **(d)** Colony PCR of the 8-gRNA assembly. **(e)** Colony PCR of the 10-gRNA assembly. **(f)** Colony PCR of the 12-gRNA assembly. **(g)** Colony PCR of the 14-gRNA assembly. **(h)** Colony PCR of the 16-gRNA assembly. **(i)** Colony PCR of the 18-gRNA assembly.

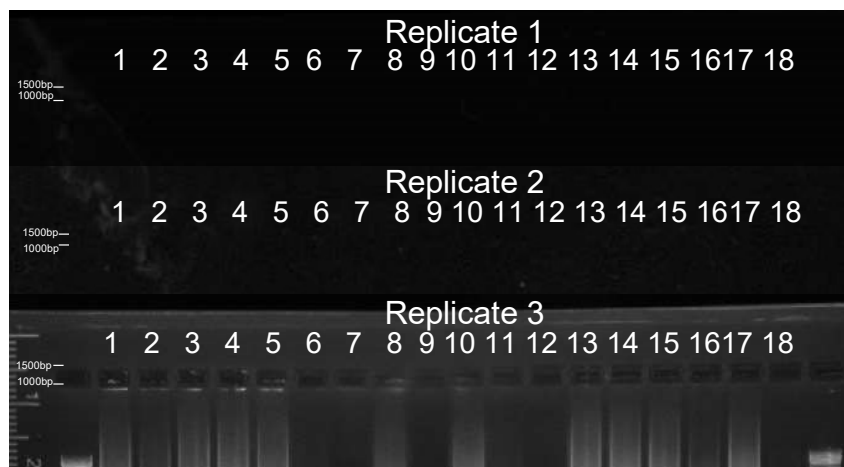


**Figure S5. Colony PCR for the screening of transformants for the plant HH-HDV-RB expression system.** Colony PCR of the 8-gRNA assembly using synthesized DNA fragments.









**Figure S8. Colony PCR for the screening of transformants for the plant tRNA expression system.**

Colony PCR of the 8-gRNA assembly using PARA-designed PCR products.