



Supplementary information, Figure S5 Pre-annealing of chimeric RNA improved the cutting efficiency of Cas9/RNA. EGFP-A chimeric RNA was denatured and re-annealed in thermocycler, then 100 ng/ μ l EGFP-A chimeric RNA was co-injected with 400 ng/ μ l Cas9 mRNA and 20 ng/ μ l pEGFP-N1 plasmid. T7EN1 assay was performed as described in materials and methods. Arrows indicated two clearer cleavage bands (about 295 bp and 198 bp) from pre-annealed chimeric RNA (Left). Sequencing results of T-A clones of targeted fragments amplified from (Left) showed there were 3 mutant clones out of 46 sequenced. Targeted sequences were capitalized and highlighted in red. Red lower case represented mutant sequence (Right).