To test the efficiency of pCRISRPi-*ccdB* for sgRNA cloning and counter selection, sgRNA targeting different genes were cloned by BsaI mediated Golden Gate Assembly. Two 24-nt oligonucleotides with 5'-ends of TATA and AAAC, respectively, were synthesized for each guide sequence and annealed in TEN buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 8.0) in a thermocycler. The annealing process involved incubation at 95°C for 5 minutes and then slowly cooling them to room temperature (approx. 30 min) to allow for proper pairing. The annealed oligonucleotides and pCRISPRi with *ccdB* vector were assembled based on the One-step Golden Gate protocol , using DNA restriction enzyme BsaI (Lablead, F5518S, China) and ligase T4 (Vazyme, C301-01, China). The assembly mixtures were transformed into *E. coli* WM3064 and plated on LB agar plates containing 30 μg/mL apramycin and 300 μM DAP for selection of successful assembly clones. As a control to test the efficiency of CcdB counter selection, the intact pCRISPRi-ccdB plasmid without sgRNA cloning was transformed into *E. coli* WM3064 in parallel.