（1）Transformation

1. Experimental objective ：Verify the functions of sgRNA and cas9
2. Experimental steps：

BL21 competence, BL21+ Δ pand competence, dusk-cas9-puc57 plasmid and pTarget F-p15A plasmid were immersed in an ice bath for 10 minutes. Samples were added to the EP tube according to the table below and then immersed in ice bath for 30 minutes. The EP tube was placed in a 42℃ metal incubator for 90 seconds. The EP tube was immediately placed in an ice box for 1 to 2 minutes. Then the EP tube was added to a liquid LB medium preheated at 940 μl 37℃ for 1 hour in a 37℃ shaker.

Light avoiding group	Blue light group
50μl BL21 competence+5μl dusk-cas9-puc57 plasmid+5μl pTargetF-p15A plasmid	50μl BL21 competence+5μl dusk-cas9-puc57 plasmid+5μl pTargetF-p15A plasmid
50μl BL21+Δpand competence+5μl dusk-cas9-puc57 plasmid+5μl pTargetF-p15A plasmid	50μl BL21+Δpand competence+5μl dusk-cas9-puc57 plasmid+5μl pTargetF-p15A plasmid

（2）Plate culture

1. Experimental steps:

Centrifuge the EP tube 6000rmp containing 1 ml of the transformant for 5 minutes,Take 700 μl of the supernatant with a pipette near the alcohol lamp and discard it,Mix the concentrated supernatant with the precipitate,Take 50 μl of the bacterial solution in a Petri dish, pour in a proper amount of glass beads, shake it from side to side until the bacterial solution is evenly spread,pour out the glass beads,the culture dishes of the blue light group are placed in the blue light device, and the culture dishes of the light-proof group are placed in the light-proof device,they were incubated in a 37 ℃ incubator and cultured overnight.