(1) Inoculated culture

1. The purpose of the experiment： prepare for the extraction of plasmids

(2) Extraction plasmid

1. The purpose of the experiment:prepare for PCR

2. Experimental steps:

5ml overnight cultured liquid was taken and centrifuged at 8000 × g for 2 minutes to collect the bacteria and discard the culture medium. Adding 250μl Buffer P1 to the precipitate, suspending the bacteria thoroughly. Add 250μl Buffer P2, immediately gently reverse EP tube 5-10 times, leave at room temperature for 2-4 minutes. After adding 350μl Buffer P3, the EP tube was reversed for 5-10 times and centrifuged at 12000 × g for 5-10 minutes. Transferred the supernatant fluid to the adsorbent column and centrifuged at 8000 × g for 30 seconds, and the liquid in the collecting tube was emptied. Add 500μl Buffer DW1, centrifuged at 9000 × g for 30 seconds and the liquid in the collecting tube was emptied. Add 500μl Wash Solution, centrifuged at 9000 × g for 30 seconds and the liquid in the collecting tube was emptied. Add 500μl Wash Solution, centrifuged at 9000 × g for 30 seconds and the liquid in the collecting tube was emptied. The empty adsorption column was centrifuged for 1 minute at 9000 × g. The adsorption column was placed in a clean 1.5ml EP tube and 30μl ddH_2O was added in the center of the adsorption membrane. Centrifuged at 9000 × g for 1 minute. Then the liquid in EP tube was sucked out by liquid transfer gun, and added to the center of adsorption membrane and added 30μl ddH_2O. After leaving at room temperature for 1 minute, centrifuged at 9000 × g for 1 minute, and the plasmid DNA solution in the tube was preserved.

PS: 1. ddH_2O preheated in the oven at 55 ℃.

(3) measurement of plasmid sample concentration

1.The purpose of the experiment: to provide the basis for determining the formula of PCR system.

2. experimental steps:

1μl ddH_2O was added to the measuring platform with a liquid transfer gun, and the DNA concentration was measured (when the concentration was > 0.1 ng/μl, the measurement platform was not cleaned and ddH_2O was re-measured until the concentration was lower than 0.1 ng/μl). The DNA concentration of 1μl solution was measured and the concentration was recorded.

3.experimental result

	dusk-Cas9-pUC57①
DNA concentration（ng/μl）	58.2692