

### 1. Optimization of Culture Medium

- (1) Prepare the culture medium with the glucose-to-yeast extract ratios (C/N) of 10:1 (50 g/L glucose and 5 g/L yeast extract; 20 g/L glucose and 2 g/L yeast extract), 10:2 (50 g/L glucose and 10 g/L yeast extract; 20 g/L glucose and 4 g/L yeast extract), and 10:3 (50 g/L glucose and 15 g/L yeast extract; 20 g/L glucose and 6 g/L yeast extract) respectively. Sterilizing them in an autoclave at 108°C for 30°C . Inoculating the strain into the media with C/N ratios of 10:1, 10:2, and 10:3 respectively. Setting three parallels for each condition.
- (2) Activation of glycerol bacteria: Taking 100 µL of glycerol bacteria and inoculate them into the freeze-dried tubes containing 1 mL of RMG5 (containing 100 µg/mL spectinomycin) with different C/N ratios as configured above, and incubate them statically in a 30°C incubator until turbidity is achieved.
- (3) Pour the activated bacterial liquid in the cryotubes into 50 mL centrifuge tubes containing appropriate amounts of RMG5 (with corresponding antibiotics) with different C/N ratios as the fermentation seed liquid and incubate them statically in a 30°C incubator until the middle and late logarithmic phase. Inoculate them into the RMG5 (with corresponding antibiotics) media with different C/N ratios in 50 mL Erlenmeyer flasks with 80% bottling volume, controlling the initial OD600nm at 0.1, and ferment at 100 rpm and 30°C.
- (4) Take out 1 mL of samples at fixed intervals in a super-clean bench for collection. Detect the OD600nm of the bacterial liquid using a UV-1800 UV spectrophotometer and record it. Then, freeze the remaining samples at -80°C for subsequent detection.
- (5) Use Graphpad 9.0 (Insightful Science, CA, USA) software to plot the data graphs.

### 2. Analysis by High Performance Liquid Chromatography (HPLC)

Centrifuge the samples at 12,000 rpm for 4 minutes, collect the supernatant, filter it through a 0.22 µm filter, take 400 µL samples into an HPLC injection vial for the detection of the contents of glucose, ethanol, and 3-HB in the HPLC.