Plasmid generation

1. Amplify the *wsp* and *rps6* gene fragments using the extracted Aa23 cell DNA as a template, and perform the PCR reaction.
2. Purify the PCR product using an extraction kit (see the **Table of Materials**), ligate it into pMD18-T, and use the ligated vector to transform *Escherichia coli* DH5α.
3. Spread the transformed bacteria on 100 μg/mL Amp/X-gal/IPTG LB plate medium, pick single clones after culture, identify the fragment size by PCR, and send it for sequencing.
4. Use the single clone with correct sequence as the strain, and inoculate 1:100 (v/v) into LB (containing 100 μg/L of ampicillin) to obtain the standard plasmid.

Anti-wsp antibody preparation

1. Collection of mouse serum as a negative control

Collect 0.5–1 mL of blood from the eyeballs of unimmunized mice, inactivate the blood at 37 °C for 30min, and coagulate it overnight at 4 °C to release the serum. Centrifuge it at 1,000 × *g* for 10 min at 4 °C; collect the supernatant and store it at -80 °C.

2. Mouse immunity

2.1. For the first immunization, mix 1 mL of Freund's complete adjuvant with the prepared 1 mL of antigen (containing 40 μg of immune protein), and inject mice subcutaneously at multiple points.

2.2. For the second immunization two weeks later, mix 40 μg of antigen and equal volume of incomplete adjuvant, and inject the mice subcutaneously at multiple points.

2.3. Perform the third and fourth immunizations similar to the second.

2.4. Extract a little venous blood 7–14 days after each immunization, and separate the serum for testing the immune effect. Test blood samples 5–7 days after the fourth immunization.

3. Detection of immune titer

3.1. Collect 50 μL of blood from the tail vein of mice, and separate serum for antibody titer detection.

3.2. When the antibody titer is greater than 1:5,000 (diluted antigen) by the ring precipitation method, collect blood from mouse eyeballs, and separate the serum for antibody purification and detection.

4. Antibody storage

4.1. After inactivating the immune serum by heating at 56 °C for 30 min, add the appropriate preservatives, label the serum samples, and store them at a low temperature below −20 °C.