Analysis method

To determine certain properties of proteins or contructed DNA fragments such as BioBricks, we have used different analytical methods. All used methods are listed below.

1. Agarose Gel Electrophoresis

The Agarose Gel Electrophoresis is used for separation of DNA fragments (e.g. after a PCR).

1. Take 5 µl of the PCR product.
2. Mix with 1 µl 6xDNA loading buffer(Tiangen).
3. Apply onto agarose gel together with a marker.
4. Run at 110 V for 30 minutes for a full gel.

2. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE is used to determine certain features of the cells' proteome such as the strength of expression of a desired protein.

Cell Preparation

1. Splitting cell by ultrasonic disruption.
2. Centrifuge for 45 min at 13.000 rpm.
3. Mix the supernatant with 5x lammli buffer with β-mercaptoethanol.
4. Denatured for 5 min at 95°C.
5. Sample to the gel.

12%SDS gels were made as described below:

COMPONENT	12% Separation gel	4%Upper gel
ddH_2O	3.34mL	3.625mL
30%Acr/Bis	4.0mL	0.67mL
Buffer	2.5mL(pH=8.8)	0.625mL(pH=6.8)
10%SDS	100	50
10%APS	50	20
TEMED	15	10

6. Apply the prepared samples together with a protein marker on the gel.
7. Run the gel for 30 min at 80 V and after that for 100 min at 120 V.

3. Verification Digest

1. In a tube, add (per reaction):

COMPONENT	Volume
Plasmid purified DNA	2µL（up to 1µg）
10xgreen buffer	2µL
enzyme 1	1µL
enzyme 2	1µL
ddH2O (deionized water)	14µL

2. Incubate at 37°C for 30min
3. Run 20µL in a 1% agarose gel at 110V

4. ABTS method

1. In a test tube, component as follow (per reaction):

Component	Volume (3mL)
0.1M acetate buffer(pH 4.5)	3mL

10 mM ABTS	20μL
Laccase extracts	8μL

2. Add 20μL ABTS into 3mL 0.1M acetate buffer, 60 °C incubated for 10min.
3. Add 8μL laccase (crude extract) to the solution, stirring 10s.
4. Use the UV spectrophotometer detect the absorbance at 420nm (measured by time).

5. EBFC's establishment and detection

1. Component of the EBFC as follow：（100ml）

Component	Anode	Cathode
Buffer	0.2M PBS	0.1M CH_3COONa
Substrate	15mM glucose	200ul 1mM ABTS
Enzyme	7 mg glucose oxidase	200ul Laccase extracts

2. Add buffer and substrate into the two cells respectively.
3. Connect the multimeter and oscilloscope with the electrode correctly.
4. Add enzymes into the two cells and make it mixing.
5. Record the voltage.

6. Transmission electron microscope (TEM)

Free cell collection and immobilization:

1. Take 2-4mL cultured *E.coli* into microcentrifuge tubes.
2. Centrifuge for 10-15 min at 2000 rpm and discard the supernatant liquid.
3. Add 0.5% glutaraldehyde stationary liquid into the tube gently, stewing at 4°C for 10min.
4. Centrifuge for 10-15 min at 12000 rpm and discard the supernatant liquid.
5. Add 3% glutaraldehyde stationary liquid into the tube gently.
6. Storage the sample at 4°C or send the sample to embed and section.