Gel electrophoresis (Southern)

Usage amount per slot:

Material	Concentration	Final concentration	Location
PCR product	--	--	4°C
TAE buffer ※	50X	0.5X	II
Agarose gel †	--	3%	II
SYBR® stock solution *	1000X	1X	II
Loading Dye	6X	1X	4°C (c)
RTU-100 (Ladder marker)	--	--	4°C (c)

※ To prepare the 0.5X TAE buffer, make at least 1 L, as each run requires at least 500 mL 0.5X TAE buffer.

† The big gel slot requires around 40 mL gel solution; the small gel slot requires 20 mL gel solution. For example, to make a 3% big gel, add 1.2 g agarose powder (ultra pure grade) to 40 mL 0.5X TAE buffer.

* To prepare 1000X SYBR stock solution, dilutes 100 µL SYBR® raw solution with 900 µL DMSO. This chemical is used to stain the DNA and detected by UV radiation.

Procedure

Step 1: Make gel

1. Prepare the gel. After adding agarose powder into the 0.5X TAE buffer, melt them with microwave. Gently shake the vial during each time points.

> - use mid-low firepower, 1 mins → 30 secs → add ddH_2O → 20 secs → 10 secs

2. Add 0.001 volume of SYBR® stock solution into the gel solution immediately (1000X → 1X), gently shake to mix them well;

> - For example, 40 mL gel requires 40 μL 1000X SYBR stock solution.

3. Pour the gel into the gel container and wait for solidification (around 15 mins);

Step 2: Set up the electrode machine

1. Prepare the electropheresis machine, the electrode direction should be from negative to positive;

2. After the gel was solidified, put the gel container together with the gel into the elctrophoresis machine;

3. Add some TAE buffer (same strength with the gel; 0.5X in this case) into the electropheresis machine and make sure the gel was fully submerged in the buffer;

Step 3: Load sample

1. Add loading dye to the PCR product. The loading dye final concentration should be 1X. For example, 2 µL 6X loading dye + 10 µL PCR product;
2. Gently inject 2 µL ladder marker into the first and the last wells of the gel;
3. Gently inject the blank and the PCR products (around 6 µL) into the wells;

Step 4: Start running

1. Set the voltage as **135 V** and the runtime as **40 mins**;

> - 5 ~ 10 V / cm for DNA size < 1 kb;
> - 4 ~ 10 V / cm for DNA size between 1 ~ 12 kb;
> - 1 ~ 3 V / cm for DNA size greater than 12 kb.
>
> *For our machine, the distance between the electrodes is around 13.5 cm.*

Step 5: Gel-imaging

1. Turn on the machine (Bio-Rad Gel Doc XR+).
2. Put in the gel.
3. Start up the `Image Lab` software.
4. `New protocol` → `Gel imaging`.
5. `Application` → `Select` → `Nucleic acid` → `SYBR® Safe`.
6. `Position Gel` → `Filter 1` → adjust gel position.
7. `Run protocol`.
8. `Export for publication` (Export for analysis is 16-bit images).

Done !!!
