Gel electrophoresis for SSR genotyping

The main advantage of this protocol is that it uses ethidium bromide staining, instead of the more time-consuming silver nitrate procedure. A special apparatus with one plate transparent to UV allows the gel to be photographed without removing it from the sandwich assembly. Additionally, the gel can be re-used up to four times.

Equipment: Mega-Gel Dual High-Throughput Vertical Electrophoresis Unit from CBS Scientific. (http://www.cbssci.com/). Catalog #: C-DASG-400-50 (http://www.cbssci.com/catalog/71.html).

Gel mix (for 6% non denaturing PAGE)**:**

- 22.5ml 40% Acrylamide-Bis (29:1)
- 7.5ml 10X TBE
- Adjust volume with deionized water to 150 ml
- Just before use add:
  1 ml 10% APS solution
  110 µL TEMED

Procedure:

1. **Casting the gel:** pour the gel directly into the plates in vertical position. Avoid the formation of air bubbles. When the volume is almost full, place the comb and clamp it to both plates (do not push the comb). Finish pouring the rest of the gel using a large syringe. Let it solidify for 60 min.
2. **Pre-running:** Pre run for 45-60 min at 350V with running buffer (0.5X TBE). Add approx. 20 µL of 10mg/ml ethidium bromide to the buffer in the lower tank.
3. **Loading samples:** Add 4 µL of 6X loading buffer (1) for 25 µL PCR reaction. Load 15 µL of sample per well. Add one molecular size marker every 24 lanes, this will still leave enough room to load one full 96-PCR plate.
4. **Run** for 100-120 min at 300V. As an alternative, gels can be run overnight (12-14 hours) at 60 V.

The gel can be reloaded, four times on average. The buffer should be replaced every two runs.

[1] 6X loading buffer:

10mM	Tris-HCl, pH7.5
50mM	EDTA
10%	Ficoll 400
0.25%	Bromophenol blue
0.25%	Xylene cyanol FF

Experimental conditions presented here should be consider only as a starting point. It is possible that the procedures need adjustments to work adequately in a given lab.

Mention of trademark or proprietary products does not constitute a guarantee or warranty by the authors and does not imply its approval over other suitable products.