**Polyacrylamide electrophoresis for small DNA fragments.**

DNA fragments smaller than 200 bp can be separated using PAGE analysis.  The following protocol is performed using BIORAD protean3 system.

Gel concentration

|  |  |
| --- | --- |
| Band of interest | Acrylamide concentration |
| 100-200 bp | 10 % |
| <100bp | 12 % |

Reagent
30% Acrylamide: Bis (29:1) BIORAD
5x Tris-Borate-EDTA

|  |  |
| --- | --- |
| Tris base | 5.4g |
| Boric acid | 2.75 |
| 0.5 M EDTA | 2ml |
| DW | total 100 ml |

10% ammonium persulfate (APS)

Gel preparation

1. Assemble gel casette.
2. Prepare the gel solution (6.5 ml per gel) and mix well, do not add TEMED till step 3.

|  |  |  |
| --- | --- | --- |
| **Percentage** | **10 %** | **12 %** |
| 30% acrylamide:Bis | 2.17 ml | 2.6 ml |
| 5x TBE | 1.3 ml |
| 10% APS | 45 µl |
| DW | 2.78 ml | 2.35 ml |
| TEMED | 5 µl |

1. Add TEMED, mix well and pour to the top of gel casette.  Carefully insert comb to avoid bubbles.
2. Wait about 20 min for gel to solidify.  If gel solidify poorly, Increase the amount of APS 2 times.

Electrophoresis