

### ***Dictyostelium discoideum* (orf+ cells) genomic DNA isolation**

Overnight culture of 100ml orf+ cells were harvested and washed in sterile water. Cell pellets were resuspended in 300 µl DNA Extraction buffer (2% Triton<sup>®</sup> X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). Glass beads (acid washed, 425-600 µm) was added to the cell suspension. After 300 µl phenol:chloroform:isoamyl alcohol 25:24:1 (PCI) was added to the suspension (pH was check to be  $7 \leq$ ), it was wortexed for 30 s and shacked for 30 min at 4°C. Lysed cells were centrifuged at 12,000 rpm for 5 min and the supernatant was transferred into a new tube. 5 µl RNase A (10 mg/ml) was added and was incubated for 30 min at 37°C. The PCI extraction was repeated and the supernatant was transferred into a new tube. 500 µl isopropanol was added and DNA was precipitated for 10 min at -20°C. After centrifugation at 12,000 rpm 10 min isopropanol was removed and pellet was resuspended in 300 µl sterile water at 55°C. PCI extraction was repeated and centrifuged at 12,000 rpm 8 min, and supernatant was added to a new tube. DNA was precipitated with 500 µl isopropanol for 10 min and centrifuged 12,000 rpm 10 min. Pellet was washed with 500 µl 70% EtOH and centrifuged at 12,000 rpm 8 min. Pellet was dried and resuspended in 250 µl sterile water and stored at 4°C. The  $A_{260}/A_{280}$  ratio was 1.845 and the genomic DNA concentration was  $\sim 3$  µg/µl. 1 µl from the isolated DNA was used to a 100 µl PCR reaction.