Cell cycle analysis

DNA staining with Propidium Iodide (PI)

1. Fix cells by slowly adding ice-cold 70% ethanol and keep at at 4°C (or -20°C) for >4h. At this point cells can be stored for months.
2. Spin down the cells (500xg, 5 min) and wash the pellet once with PBS + 2%FCS. Cells fixed with ethanol usually requires a longer centrifugation time. Be careful when removing the supernatant, the pellet is easily disintegrated.
3. Treat cells with RNase A. Add 100µl RNase A (for $1x10^6$ cells) at a concentration of 1 mg/ml to the cells and incubate at 37°C for 30 min.
4. Stain the cells with 5-20µg PI in 0,5-1 ml depending on your cell amount. incubate for ≥30 min at RT protected from light.
5. Analyze your sample on the Accuri, use the flow rate slow and make sure the flow stays under 500 events/s. Gate your cell population in the scatter plot and view the cell cycle as FL3-H using a linear scale.

NOTE

PI also stains RNA, the RNase treatment is crucial for a nice cell cycle analysis.
PI can also be used as viability dye since PI does not cross the intact cell membrane but enters dead cells that have damage plasma membranes and stains the RNA and DNA in these cells.
PI is a carcinogen and should be handled with care!