

## Quantitative PCR

Aim: Determine quantity of DNA in a solution by PCR amplification and measurement of the increasing fluorescence emitted from an intercalating DNA dye

### Materials:

- Luna Universal qPCR 5X Master Mix (New England Biolabs, M3003S)
- BioRad iCycler qPCR Cycler
- 2.5  $\mu$ M Forward and Reverse Primers
- Flat cap strip tubes (Thermo Fisher, AB1191)

### Procedure

1. Pipet 1  $\mu$ l of Primers in the bottom of each well
2. Create a Master Mix, adding the following components
  - 1  $\mu$ l (c)DNA
  - 5  $\mu$ l Luna qPCR 5X Master Mix
  - 3  $\mu$ l nuclease free H<sub>2</sub>O(multiply accordingly for the wells required)
3. Add 9  $\mu$ l of the Master Mix to each well
4. Add lid, assure tight sitting
5. Spin down thoroughly using a microcentrifuge
6. Perform following program:
  - 94°C 120sec
  
  - 94°C 30 sec
  - 60°C 15 sec    X40
  - 68°C 30 sec
  - Measure Fluorescence
7. Perform a melting curve to determine presence of a singular amplification product