Quantification of DNA using PicoGreen

Reagent Preparation

Prepare 1x TE using 20x TE supplied with PicoGreen kit

Preparation of Samples for Standard Curve

To standardize the DNA concentrations we must prepare a dilution of known concentrations. This is done using the λ-DNA supplied with the PicoGreen reagent.

In one 8 tube strip:

- **Prepare λ-DNA following the table below**

Well number	*DNA conc. (ng/ul)*	*Amount of **λ-DNA** to H_2O (total 100ul)*
1	100	100:0
2	80	80:20
3	60	60:40
4	40	40:60
5	30	30:70
6	20	20:80
7	10	10:90
8	0	0

In a new Costar White, flat-bottom plate:

- **Add 100ul 1x TE to 8 wells on a plate**
- **Transfer 2ul λ-DNA from the 8 tube strip**

Set standard samples aside. PicoGreen will be added later.

Preparation of DNA samples

DNA samples in plate format. Prepare DNA plate by thawing and spinning down.

In a new Costar White, flat-bottom plate:

- **Add 100ul 1x TE to each well**
- **Transfer 1ul from stock DNA**
- **Set aside**

Preparation of PicoGreen

Prepare PicoGreen in a foil cover bottle. PicoGreen should be protected from light.

For each DNA plate to be quantified add:

- **10 ml 1x TE**
- **50ul PicoGreen**

Mix by swirling bottle.

(An additional 1 ml TE with 5ul PicoGreen may need to be prepared for the standard DNA samples)

Add PicoGreen to DNA samples

- **Add 100ul TE w/ PicoGreen to each well of the DNA plate** and the standard samples without contaminating tips.

- **Mix PicoGreen and DNA by pippetting up and down 5-10 times.** (The same tips can be used across the plate provided that the samples are fully expelled between mixings to minimize transfer.)

Cover the plate to protect from light.

Read florescence of quantification plates

1. **Open Cary Scan**
   a. **> Setup**
   b. **> Accessories**

2. **Accessories**:
   a. **Check well plate**
   b. **Format: Whole plate**
   c. **Wavelength:**
      i. EX: 480 (excision)
      ii. EM: 520 (emission)
      iii. Stop: 520
   d. **Use low voltage**

3. Click **Start** to run

4. **Save files**
   a. **Choose "Save as" and choose spreadsheet.csv as the file format**.
   b. **Save data as "q" + plate_id + "_Plate Name" (e.g. qDNA100811P01_U6200.csv)**

5. To read another plate, close program and repeat.

Use DNAquantifier.jar