# User designating layout of plates:

* Reagents will be added to >=1 Costar black 96-well plate(s).
* In a 96-well plate format, wells need to be set as either:
  + standards (dilution series of 7 concentrations)
  + unknowns
  + blanks
* All standards, unknowns, and blanks are performed in duplicate on the same plate
* [not critical] Based on the number for standards, unknowns, and blanks, the software would calculate the volumes of the necessary reagents:
  + TE buffer
  + standard DNA
  + picogreen reagent
  + Total TE needed:
    - raw\_total\_volume = TE for standards + TE for sample dilutions + TE for picogreen reagent dilution
    - volume for ALL standards:
      * 1332.5 ul
    - volume for EACH sample:
      * 99 ul
    - volume for EACH picogreen reagent dilution:
      * 110 ul
* The user would also need to designate where each unknown (nucleotide sample) is located on another 96-well plate that holds all of the unknowns.

# User-provided materials:

* reagent trough of 1x TE buffer
* empty reagent trough for preparing picogreen working stock
* micro-cfg tube with picogreen reagent
* micro-cfg tube with standard template
* 7 micro-cfg tubes for preparing the standards (per 1 plate)
* 96-well plate(s) containing unknowns (samples)
* Costar black 96-well plate(s) (will contain the final reagent mixtures)

# Making standards (per plate):

* The stock standard is diluted with TE buffer to make the dilution series of standards as specified in the table below:

|  |  |
| --- | --- |
| **TE to add (uL)** | **2ug/mL stock to add (uL)** |
| 62.5 | 187.5 |
| 125 | 125 |
| 187.5 | 62.5 |
| 225 | 25 |
| 237.5 | 12.5 |
| 245 | 5 |
| 250 | 0 |

* 100 uL of each of the prepared standards is added the the Costar black 96-well plate
  + The plate/well IDs are determined from the user designation of the final plate layout

# Add unknowns:

* For all unknown wells:
  + add 99 uL of TE Buffer
* For each unknown well:
  + add 1 uL of the corresponding unknown (user-defined correspondence)

# Add blanks:

* For all blank wells:
  + add 199 uL of TE Buffer
* For each unknown well:
  + add 1 uL of the corresponding unknown (user-defined correspondence)

# Making working stock of picogreen reagent (1x concentration):

* Calculate volume (ul) of TE buffer and 200x picogreen needed:
  + number\_unk\_std = number\_unknowns + number\_standards
  + total\_volume\_working\_stock = 110 \* number\_unk\_std
  + total\_volume\_200x\_picogreen = total\_volume\_working\_stock \* 1/200
  + total\_volume\_TE\_buffer = total\_volume\_working\_stock \* 199/200
* Transfer the necessary volume of TE buffer to the empty reagent trough
  + total\_volume\_200x\_picogreen
  + total\_volume\_TE\_buffer
* Transfer the necessary volume of 200x picogreen to the empty reagent trough
* Mix reagents in trough by pipetting up and down 5x
* Transfer 100 ul of 1x picogreen reagent into each unknown and standard well
* Mix reagents in trough by pipetting up and down 5x
* Alert the user that the method is nearly complete (5 min remaining)
* Allow plate(s) to incubate for 5 min
* Alert the user that the method is complete!