Picogreen Assay (invitrogen, Quant-iT™ PicoGreen® dsDNA Assay Kit, cat# p7589)

1. Create standards by mixing 3 µl of Lambda gDNA with 27 µl of TE buffer. Vortex the tube and spin down the mixture. This will give you the highest concentration of DNA (1 ng/ µl). Label this tube # 1. Next, take 5 µl from the first tube and add 45 µl of TE to it. Vortex and spin down the solution. Label this tube # 2. For tube # 3, take 5 µl from tube # 2 and add 45 µl of TE. Vortex and spin this tube down as well. Finally for tube # 4, take 5 µl from tube # 3 and add 45 µl of TE to it. Vortex and spin it down too. Tubes 1-4 will be the standards with 1 being of the highest DNA concentration, and going in descending order until you reach 4 which has the lowest DNA concentration.

2. Next you will make the fluorescent dye mixture bye adding 10 µl of dye to 990 µl of TE. Vortex and spin down in the centrifuge. Keep the dye out of the light until it is ready to be used.

3. If needed (according to the readings you receive from the nanodrop machine) you may need to dilute the DNA lysate and samples 1:10. If so, take 2 µl of the sample and add 18 µl of TE. Vortex and spin down in the centrifuge. For 2nd strand cDNA, as the concentration is usually not high, we may just use 1 µl of the sample directly for measurement.

4. When placing the samples in the plate, 5 µl of the DNA samples + 20 µl of TE + 25 µl of dye goes into each well, except for the blanks which contain 25 µl of TE and no DNA. Usually wells A 1-12 contain the standard. Wells A 1-3 are from tube # 1, A 4-6 are from tube # 2, A 7-9 are from tube # 3, and A 10-12 are from tube # 4. Wells B 1-4 contain the blanks. All the other wells may be used for the samples (5 µl for diluted samples+20 µl TE buffer or 1 µl samples +24 µl TE buffer). Mix well. Be sure to centrifuge the plate and set the machine to the fluorescent setting.