wet lab QC

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Wetlab QC

- sample concentration results summary
  - bacterial quant (http://www.zymoresearch.com/dna/dna-analysis/femto-bacterial-dna-quantification-kit)
- Mixture QC
  - ERCC qPCR

- 16S PCR QC
- Gel
  - Need gel info - gel concentration, run info - V and time, ladder info - manufacturer and band sizes.

The initial 16S PCR product was first verified for amplification and amplicon size using gel electrophoresis @ref(fig:gel_16S).

The concentration of the PCR product was also assessed using pico green, samples with negative measured concentration values (excluding no template controls (NTC)) were also check using Qubit. Only one of the 180 samples did not successfully amplify E01JH0016 dilution 5 (postion F9, plate 1). All but one of the no template control concentration measurements was less than 1 $ng/\mu l$, when the one sample was checked with Qubit the concentration was too low to measure.

```
pcr_16S_pico %<>% filter(pos != "Well", !is.na(pos))
pcr_16S_qc <- sample_sheet %>% filter(barcode_lab == "JHU", seq_lab == "JHU") %>%
    rename(pcr_16S_plate = "plate") %>%
    select(sampleID, dilution, plate, pos) %>%
    mutate(pos = str_replace(pos, "_","")) %>% right_join(pcr_16S_pico)
```

```
## Joining, by = c("plate", "pos")
```

```
pcr_16S_qc %>% ggplot() +
    geom_point(aes(x = factor(dilution), y = conc_ngul)) + theme_bw() +
    facet_wrap(~sampleID, scales = "free_x", nrow = 1) +
    geom_text(aes(x = factor(dilution), y = conc_ngul, label = qubit),nudge_y = -0.25, nudge_x = 1)
```

```
## Warning: Removed 186 rows containing missing values (geom_text).
```

Index PCR

- Post clean-up DNA concentrations

Normalization

- Picogreen concentration
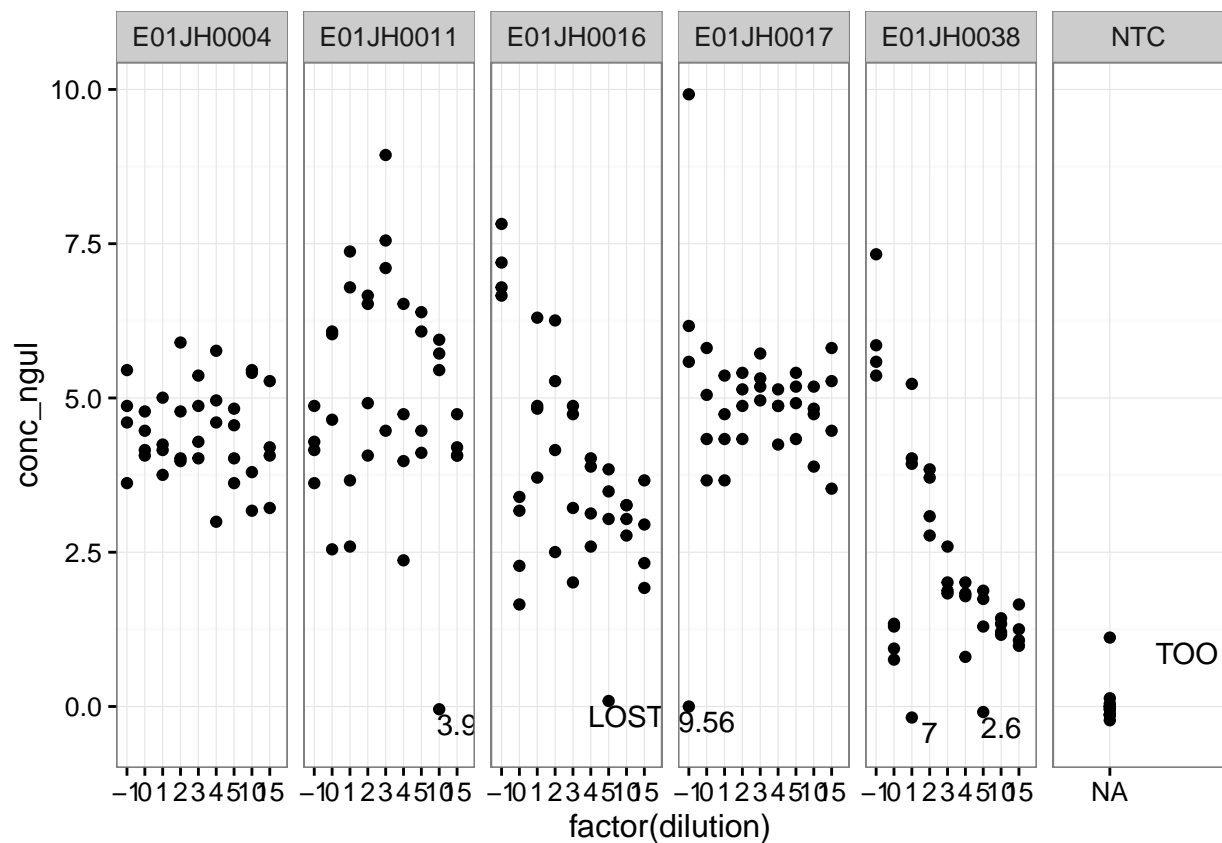
Library Pooling

- Assay methods

- QC

Figure 1: Concentration measured using picrogreen of 16S PCR products post-cleanup. Low concentration samples were remeasured using QUBIT, and concentration values (ng/ul) are indicated as text near pico green data point.

- methods

- results

```
# Concentraiton results after PCR clean-up
pcr_16S_pico %>% DT::datatable()
```

NOTES Modify names for consistency and merging with sample sheet summary plot showing outliers plot of standard curve

END of NOTES