wet lab QC

Nate Olson

2017-01-19

Wetlab QC

16S PCR Concentration Post-Cleanup

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 picogreen, samples with negative measured concentation values (excluding no template controls (NTC)) were also check using Qubit. The DNA concentration of the 16S PCR product after clean up samples after ranged from below the limit of detection to 49.6 ng/ml @ref(fig:16S_pcr_quant). The DNA concentration of samples with low DNA concentration measured using picrogreen ($< 1\ ng/\mu L$.) was also measured using Qubit (Table **QUBIT**). Only one sample had an undetectable DNA concentration using the Qubit. Gel electrophoresis of the 16S PCR products indicated that the sample failed to amplify during the initial PCR @ref(fig:16S_pcr_gel). **TODO** Need gel info - gel concentration, run info - V and time, ladder info - manufacturer and band sizes. Despite failure to amplify the sample was processed through the sequence pipeline with the rest of the samples.

```
tstWetLabQC %>% left_join(tstPcrSampleMetaData) %>%
      mutate(titration = as.factor(titration)) %>%
      ggplot(aes(x = biosample_id, y = conc_ngml, color = titration)) +
      geom_point() +
      theme_bw() +
      labs(x = "Biological Replicate",
           y = "DNA Concentration (ng/mL)",
           color = "Titration Factor")
```

```
## Joining, by = c("pcr_16S_plate", "pos")
```

```
tstWetLabQC %>% left_join(tstPcrSampleMetaData) %>%
      filter(!is.na(qubit), biosample_id != "NTC") %>%
      select(biosample_id, titration, pcr_16S_plate, pos, conc_ngml, qubit) %>%
      kable()
```

```
## Joining, by = c("pcr_16S_plate", "pos")
```

biosample_id	titration	pcr_16S_plate	pos	conc_ngml	qubit
E01JH0038	1	1	B11	-0.8939313	7
E01JH0016	5	1	F9	0.4467310	LOST
E01JH0011	10	1	G8	-0.2236001	3.9
E01JH0017	20	2	E12	-0.0001564	9.56
E01JH0038	5	2	F11	-0.4470438	2.6

Index PCR

- Post clean-up DNA concentrations **TODO** Script to extract concentration information

Normalization

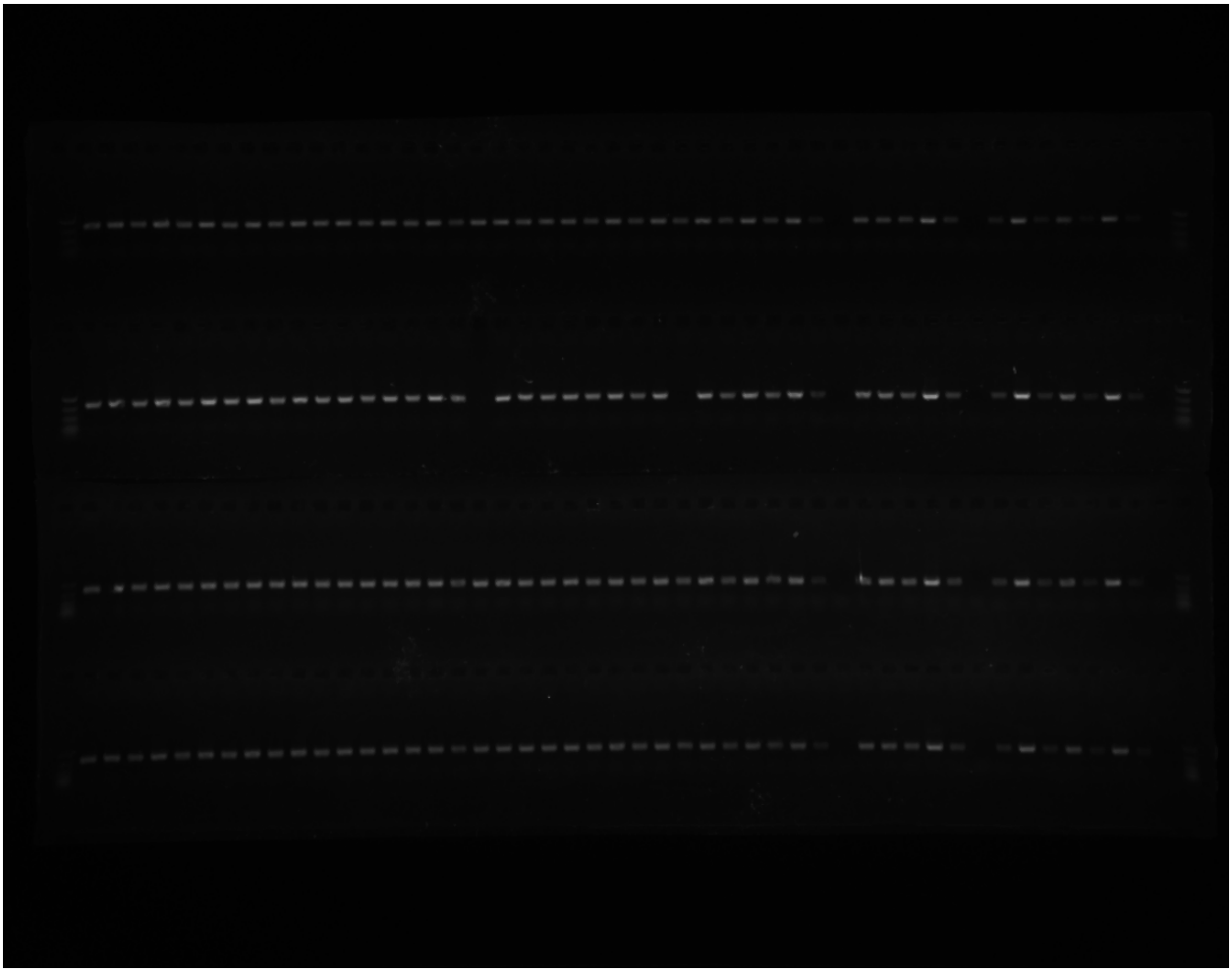

Figure 1: Agarose gel electrophoresis of 16S PCR products.

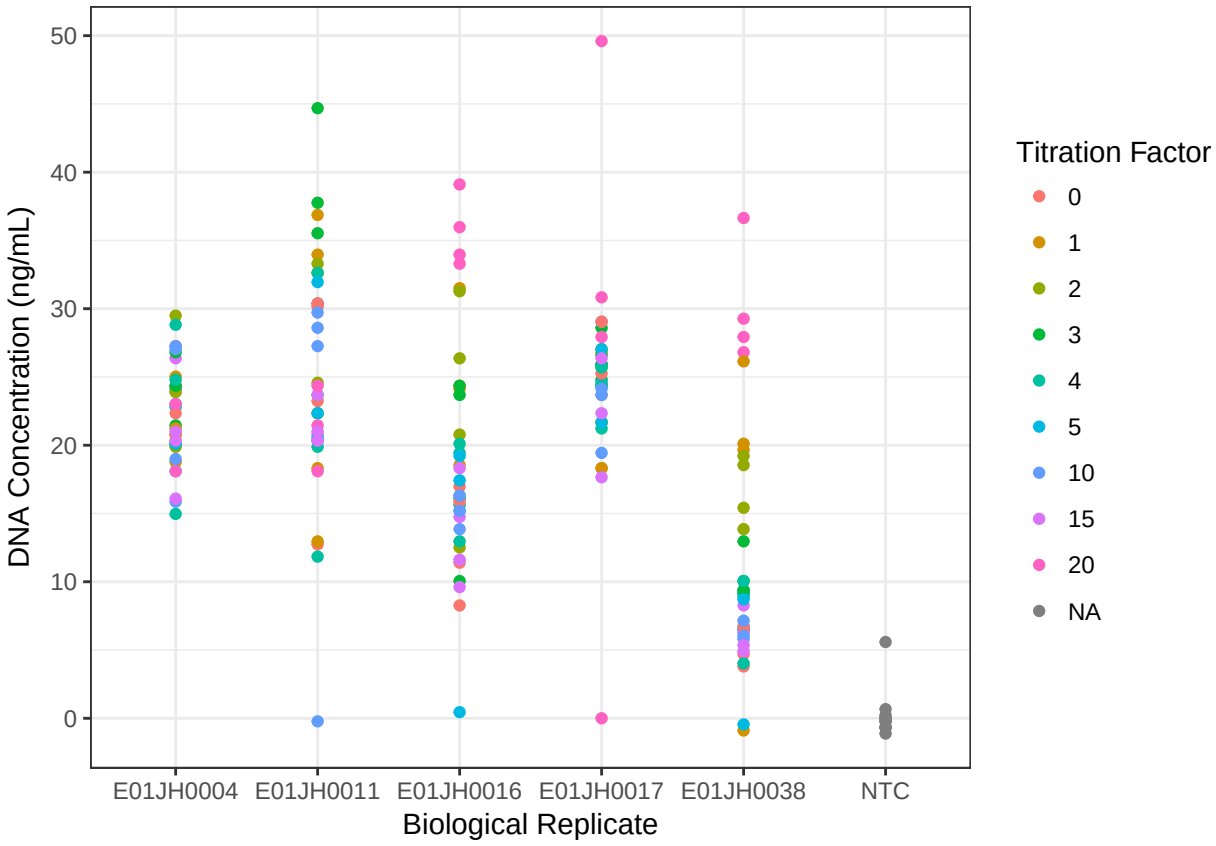

Figure 2: Picogreen DNA concentration measurements of 16S PCR product after clean-up.

- Picogreen concentration **TODO** Script to extract concentration information excel file 16032_NIST_norm_15uL.xlsx

Library Pooling

- Assay methods
- QC
  - methods
  - results