Produced Water - Oilfield : RNA Seq Analysis of *Phaeodactylum tricornutum*

For:
SJSU CS 286

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https://github.com/codey-phoun/pw_oilfield

Project Introduction

Produced water (PW) is water produced as a byproduct during the extraction of oil and natural gases. The water quality of PW ranges from well to well, but most PW contains oils, heavy metals, and traces of naturally occurring radioactive material. PW can serve as an alternative water source if the pollutants found in the water are removed. For now, PW can be used in algae cultivation for biofuel production. In this study, we aim to examine the exponential growth and physiology of the diatom *Phaeodactylum tricornutum* under three types of conditions: no treatment (control), 10% PW, and 100% PW. Also, we will examine the stationary growth phase under no treatment and high treatment PW.

Oil and gas produced water as a growth medium for microalgae cultivation: A review and feasibility analysis - Graham 2017

Project Setup

Connect to the CoS HPC

You must first be connected to the SJSU VPN. See https://www.sjsu.edu/it/services/network/vpn/index.php

```
ssh cphoun@spartan01.sjsu.edu
```

Conda Environment

```
conda activate RNAseq_v2.0
```

Working Directory and File Structure on CoS HPC

```
~/pw_oilfield/assembly
~/pw_oilfield/annotation
~/pw_oilfield/data
~/pw_oilfield/fastqc
~/pw_oilfield/scripts
~/pw_oilfield/trimmed
```

Transferring to and from CoS HPC with rsync - Examples

From local to HPC

```
rsync -azhP *.sh cphoun@spartan01.sjsu.edu:/home/cphoun/pw_oilfield/scripts/
```

From HPC to local

```
rsync -azhP cphoun@spartan01.sjsu.edu:/home/cphoun/pw_oilfield/fastqc/ ./fastqc/
```

Submitting Jobs to CoS HPC - Example

```
sbatch job.sh # submit a job
squeue # check status of a job
```

See http://spartan01.sjsu.edu/dokuwiki/doku.php?id=hpc:intro:job-submission for more details.

Data Files

Sample Name example	Short Sample Description	Sample description
HQ_ST1	Normal Medium Stationary	Harvested in the stationary phase from cultures grown in normal medium (control)
PW_ST1	100% PW Stationary	Harvested in the stationary phase from cultures grown in pure oil-field produced water (treatment)
HQE1	Normal Exponential	Harvested in the exponential phase from cultures grown in normal medium (control)
HQ10E1	Normal + 10% PW Exponential	Harvested in the Exponential phase from cultures grown in normal medium to which 10% oil-field produced water was added (intermediate level of treatment)
PWE1	100% PW Exponential	Harvested in the Exponential phase from cultures grown in pure oil-field produced water (high level of treatment)

file	num_seqs	sum_len	min_len	avg_len	max_len
HQ10E1_1.fq.gz	23551403	3532710450	150	150.0	150
HQ10E1_2.fq.gz	23551403	3532710450	150	150.0	150
HQ10E2_1.fq.gz	26688235	4003235250	150	150.0	150
HQ10E2_2.fq.gz	26688235	4003235250	150	150.0	150
HQE1_1.fq.gz	26535998	3980399700	150	150.0	150
HQE1_2.fq.gz	26535998	3980399700	150	150.0	150
HQE2_1.fq.gz	19332203	2899830450	150	150.0	150
HQE2_2.fq.gz	19332203	2899830450	150	150.0	150
HQ_ST1_1.fq.gz	18270958	2740643700	150	150.0	150
HQ_ST1_2.fq.gz	18270958	2740643700	150	150.0	150
HQ_ST2_1.fq.gz	22481656	3372248400	150	150.0	150
HQ_ST2_2.fq.gz	22481656	3372248400	150	150.0	150
PWE1_1.fq.gz	22364529	3354679350	150	150.0	150
PWE1_2.fq.gz	22364529	3354679350	150	150.0	150
PWE2_1.fq.gz	22976366	3446454900	150	150.0	150
PWE2_2.fq.gz	22976366	3446454900	150	150.0	150
PW_ST1_1.fq.gz	21253694	3188054100	150	150.0	150
PW_ST1_2.fq.gz	21253694	3188054100	150	150.0	150
PW_ST2_1.fq.gz	26092202	3913830300	150	150.0	150
PW_ST2_2.fq.gz	26092202	3913830300	150	150.0	150

RNA integrity number (RIN) of samples HQ_ST2 and PW_ST2 was between 4.6-5.8. Moderate degradation observed.

Phaeodactylum tricornutum Files

Phaeodactylum tricornutum files can be accessed through Ensembl ftp

```
ftp
open ftp.ensemblgenomes.org
```

Downloading assembly files

https://protists.ensembl.org/Phaeodactylum_tricornutum/Info/Index/

```
wget ftp://ftp.ensemblgenomes.org/pub/protists/release-
50/fasta/phaeodactylum_tricornutum/dna/Phaeodactylum_tricornutum.ASM15095v2.dna.toplevel.fa.gz
```

Downloading annotation files

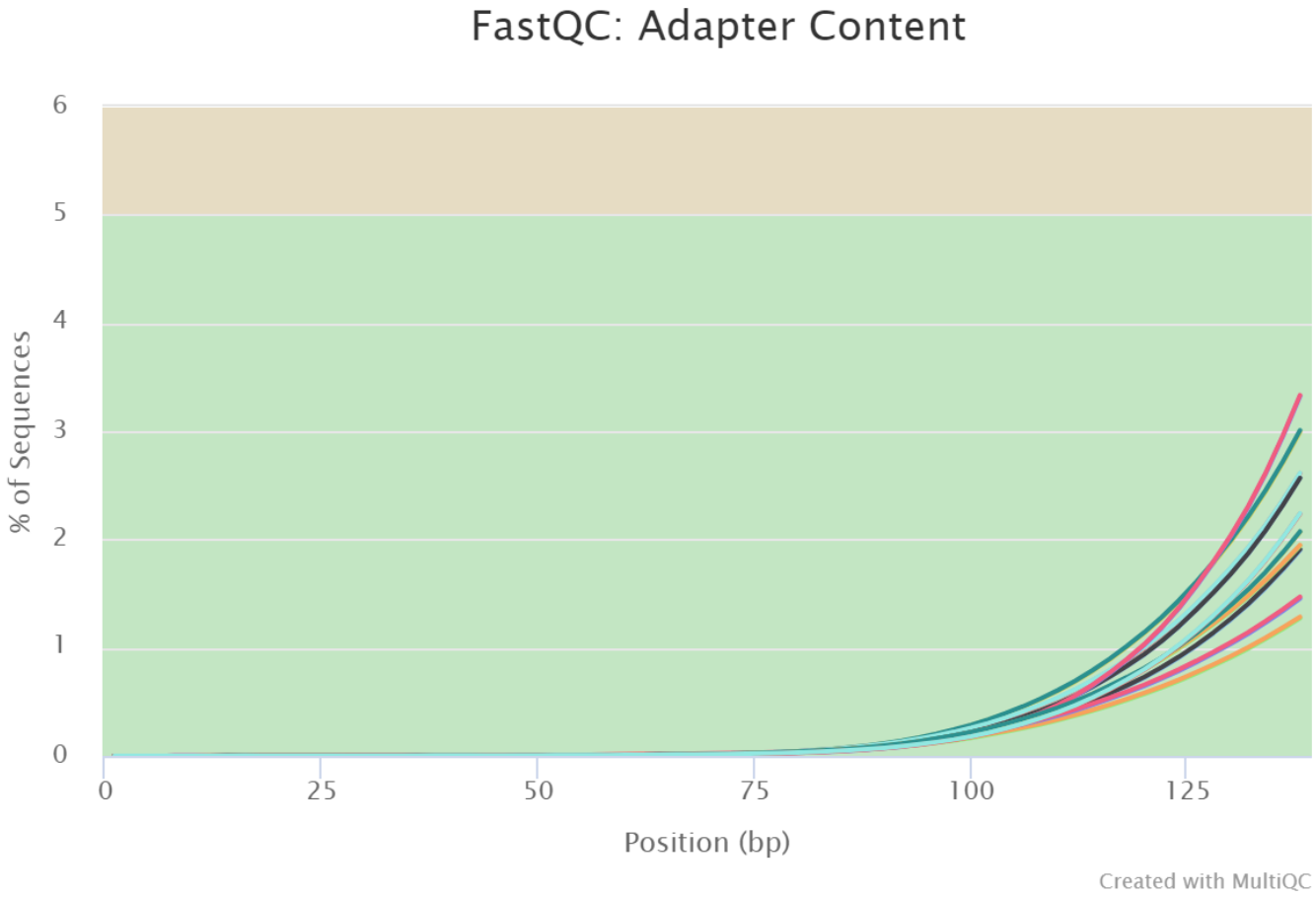
```
wget ftp://ftp.ensemblgenomes.org/pub/protists/release-
50/gff3/phaeodactylum_tricornutum/Phaeodactylum_tricornutum.ASM15095v2.50.gff3.gz
wget ftp://ftp.ensemblgenomes.org/pub/release-
50/protists/gtf/phaeodactylum_tricornutum/Phaeodactylum_tricornutum.ASM15095v2.50.gtf.gz
```

Checking quality of data files with FastQC

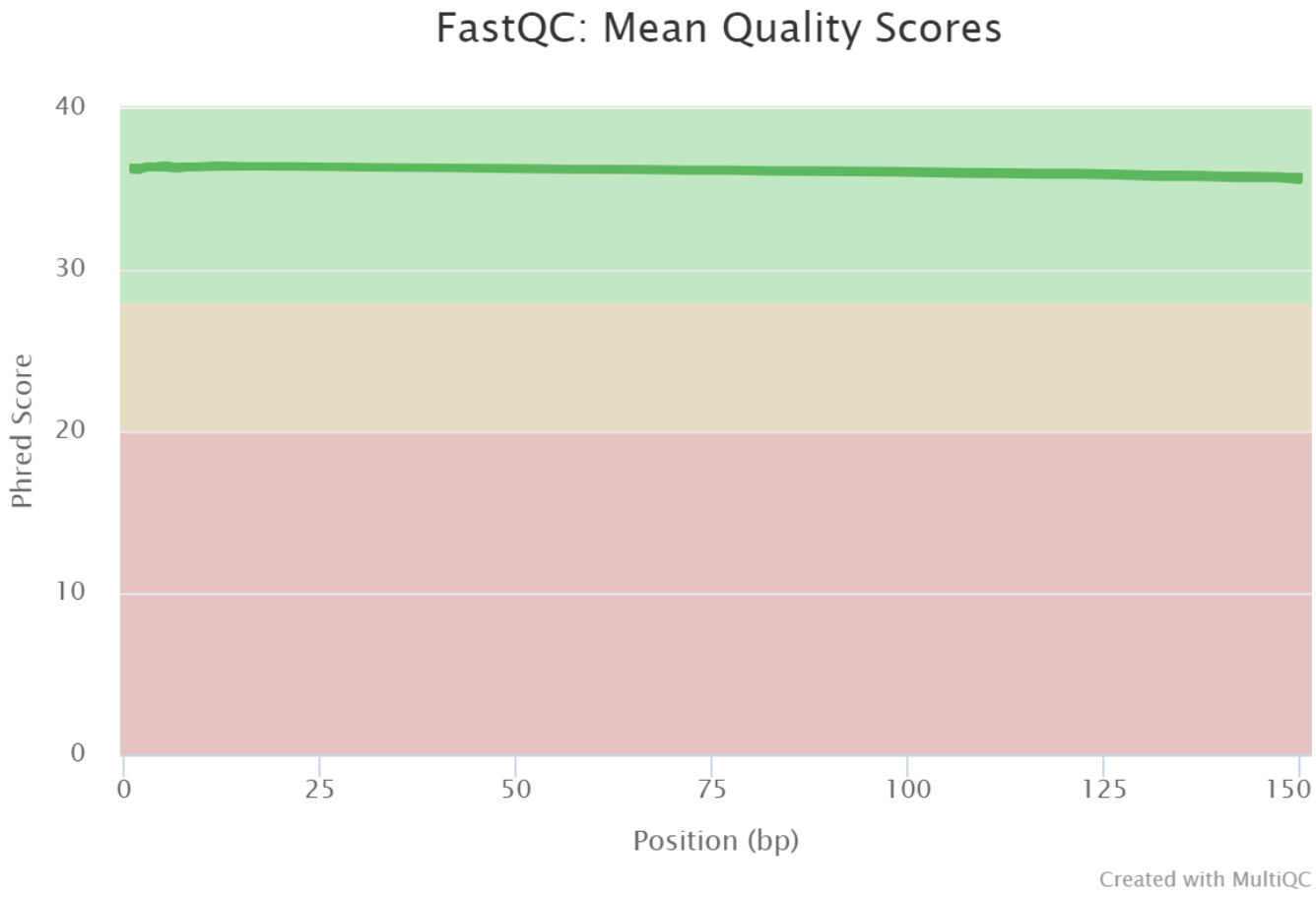
Run each data file through FastQC and consolidate reports with MultiQC

```
fastqc ~/pw_oilfield/data/* \
--threads 56 \
--outdir ~/pw_oilfield/fastqc

multiqc ~/pw_oilfield/fastqc \
--filename fastqc_multiqc.html \
--outdir ~/pw_oilfield/fastqc/multiqc
```

Illumina Universal Adapters are present at the ends of the reads.



Overall quality scores are high and consistent across the reads.



HQ10E2_2 shows presence of poly-T tail in reads

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	31507	0.11805576502155352	No Hit

Adapter Trimming with Cutadapt

Cutadapt script

```
for sample in HQ_ST1 HQ_ST2 PW_ST1 PW_ST2 HQE1 HQE2 HQ10E1 HQ10E2 PWE1 PWE2

do
    echo "Processing $sample"

    cutadapt \
    -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA `# TruSeq single index Read 1` \
    -A AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT `# # TruSeq single index Read 2` \
    --cores 0 `# auto-detect number of CPU cores to use` \
    --overlap 10 `# minimum length overlap between read and adapter` \
    --quality-cutoff 20,20 `# trim low-quality bases from 5' and 3' ends before adapter removal` \
    --minimum-length 50 `# minimum read length after trimming` \
    -o ~/pw_oilfield/trimmed/${sample}_1_trimmed.fq.gz `# Read 1 output` \
    -p ~/pw_oilfield/trimmed/${sample}_2_trimmed.fq.gz `# Read 2 output` \
    ~/pw_oilfield/data/${sample}_1.fq.gz `# Read 1 input` \
    ~/pw_oilfield/data/${sample}_2.fq.gz `# Read 2 input` \
    > ~/pw_oilfield/trimmed/${sample}_trim_log.txt

done
```

Cutadapt Results

Sample	pairs_processed	r1_with_adapters	r2_with_adapters	pairs_too_short	pairs_written	bp_processed	quality_trimmed	bp_written

Sample	pairs_processed	r1_with_adapters	r2_with_adapters	pairs_too_short	pairs_written	bp_processed	quality_trimmed	bp_written
HQ10E1_2	23551403	542058	540914	21374	23530029	7065420900	7096090	7026739438
HQ10E2_2	26688235	399295	399884	22288	26665947	8006470500	9661715	7970253086
HQE1_2	26535998	453925	455116	23560	26512438	7960799400	8824952	7922420846
HQE2_2	19332203	703448	700253	17660	19314543	5799660900	6389443	5754219764
HQ_ST1_2	18270958	575768	573495	19446	18251512	5481287400	7117969	5441467786
HQ_ST2_2	22481656	699219	696639	23800	22457856	6744496800	8536049	6696879626
PWE1_2	22364529	516687	516029	24157	22340372	6709358700	8904517	6668569658
PWE2_2	22976366	945548	942695	31648	22944718	6892909800	9762339	6834065958
PW_ST1_2	21253694	529637	527825	24462	21229232	6376108200	8041844	6336399004
PW_ST2_2	26092202	710715	709480	24598	26067604	7827660600	8576226	7779612587

Sample	percent_trimmed
HQ10E1_2	0.547
HQ10E2_2	0.452
HQE1_2	0.482
HQE2_2	0.784
HQ_ST1_2	0.726
HQ_ST2_2	0.706
PWE1_2	0.608
PWE2_2	0.854
PW_ST1_2	0.623
PW_ST2_2	0.614

Poly-T tails are also no longer present in HQ10E2_2.

Indexing Reference Genome and Annotation

Index the genome with STAR

```
STAR \
--runThreadN 56 \
--runMode genomeGenerate \
--genomeDir ~/pw_oilfield/assembly \
--genomeFastaFiles ~/pw_oilfield/assembly/Phaeodactylum_tricornutum.ASM15095v2.dna.toplevel.fa \
--sjdbGTFfile ~/pw_oilfield/annotation/Phaeodactylum_tricornutum.ASM15095v2.50.gtf \
--sjdbOverhang 149 \
--genomeSAindexNbases 11
```

Align Reads to Reference Genome with STAR

Map the sample reads to the reference genome with STAR. Run the script run_star_align_parallel.sh

```
for sample in $(cd ~/pw_oilfield/trimmed && ls *.fq | sed s/_[12]_trimmed.fq// | sort -u)
do

sbatch ~/pw_oilfield/scripts/star_align_parallel.sh ${sample}
echo "Submitted ${sample}"

done
```

run_star_align_parallel.sh submits a STAR job in SLURM for each sample.

```
STAR \
--runThreadN 12 \
--runMode alignReads \
--genomeDir ~/pw_oilfield/assembly \
--quantMode GeneCounts \
--outSAMtype BAM SortedByCoordinate \
--limitBAMsortRAM 32000000000 `#32GB - can be increased if needed`\
--readFilesIn ~/pw_oilfield/trimmed/${sample}_1_trimmed.fq ~/pw_oilfield/trimmed/${sample}_2_trimmed.fq \
--outFileNamePrefix ~/pw_oilfield/alignment_sorted/${sample}_
```

Create Count Table from STAR Alignment Results

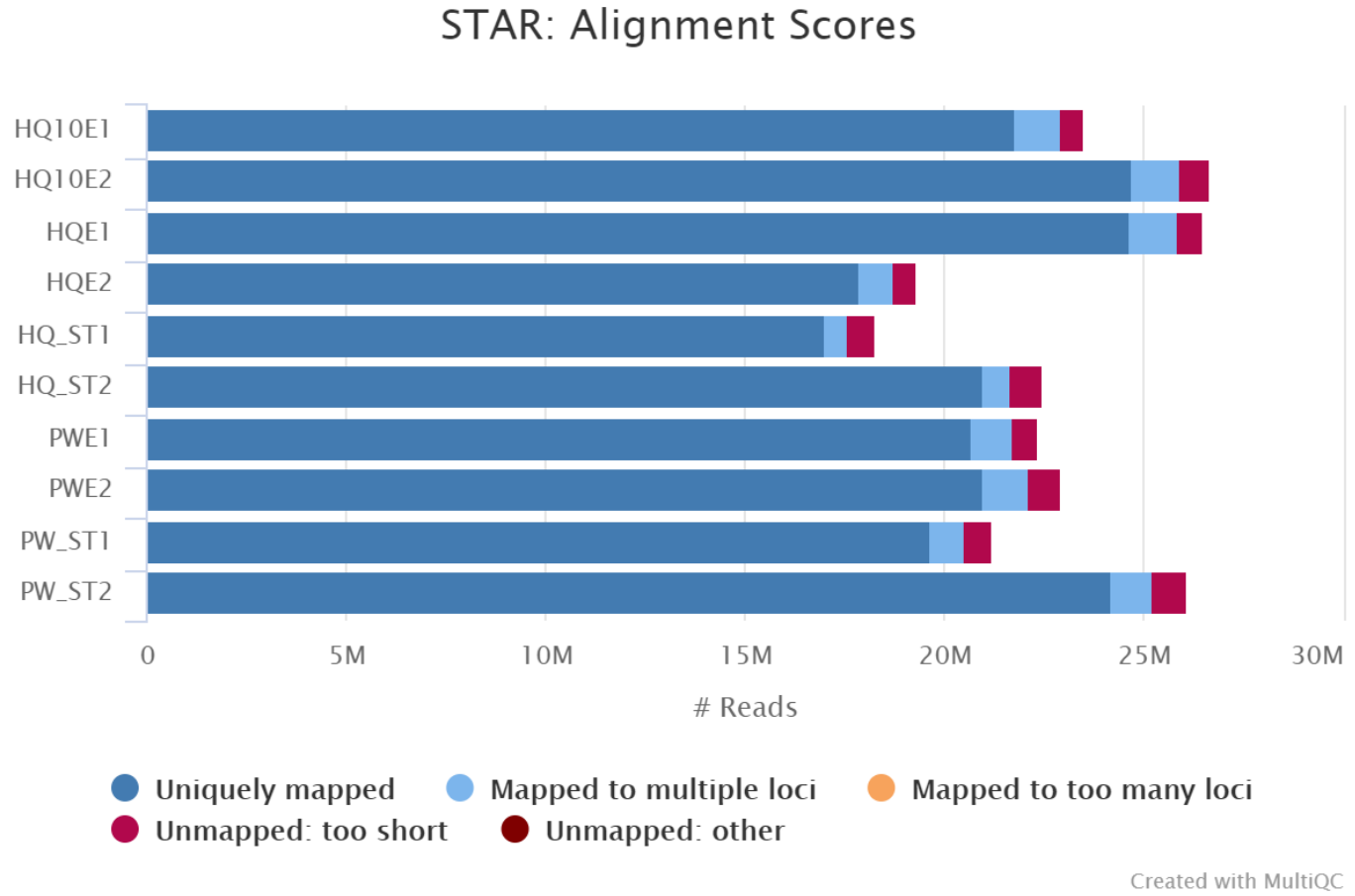
The merge_star.sh script creates a matrix of the gene counts for each sample.

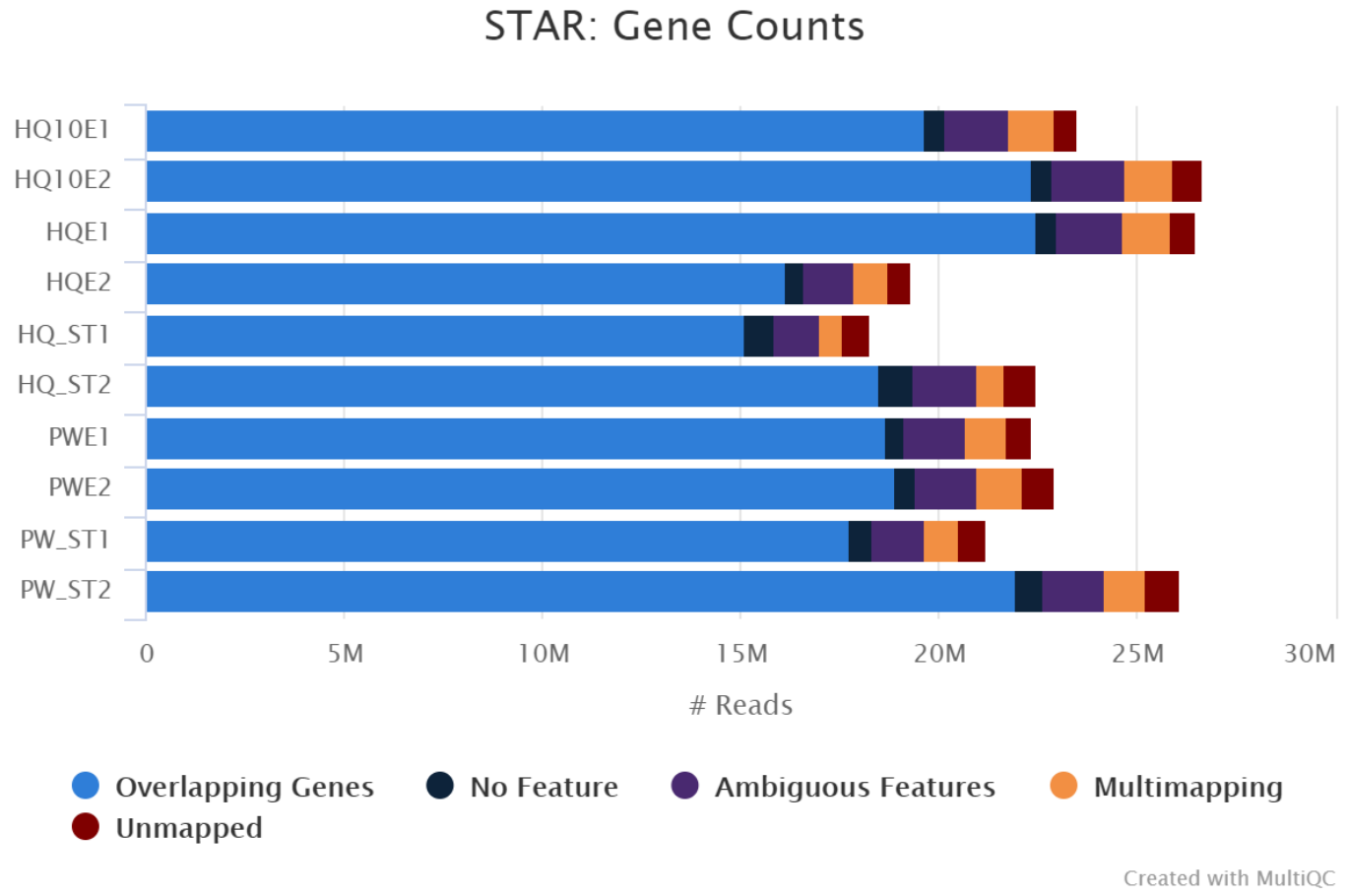
```
# modified from https://ucdavis-bioinformatics-training.github.io/2020-mRNA_Seq_Workshop/data_reduction/03-counts_mm

# create header file
echo gene_name $(cd ~/pw_oilfield/alignment_sorted && ls *_ReadsPerGene.out.tab | sed s/_ReadsPerGene.out.tab// | sort
-u) > ~/pw_oilfield/alignment_sorted/tmp/header.txt

# Place each sample's STAR gene count file - ReadsPerGene.out.tab in the tmp/ directory
# The 2nd column (-f2) of ReadsPerGene.out.tab contains the non-stranded counts
for sample in $(cd ~/pw_oilfield/alignment_sorted && ls *_ReadsPerGene.out.tab | sed s/_ReadsPerGene.out.tab// | sort
-u)
do
    echo ${sample}
    cat ~/pw_oilfield/alignment_sorted/${sample}_ReadsPerGene.out.tab | tail -n +5 | cut -f2 >
~/pw_oilfield/alignment_sorted/tmp/${sample}.count
done

# get a list of gene ids (-f1)
tail -n +5 ~/pw_oilfield/alignment_sorted/HQ10E1_ReadsPerGene.out.tab | cut -f1 >
~/pw_oilfield/alignment_sorted/tmp/geneids.txt

# combine all the columns of the count files
paste ~/pw_oilfield/alignment_sorted/tmp/geneids.txt ~/pw_oilfield/alignment_sorted/tmp/*.count >
~/pw_oilfield/alignment_sorted/tmp/tmp.out

# add the header
cat <(cat ~/pw_oilfield/alignment_sorted/tmp/header.txt | sed 's/ /\t/g') ~/pw_oilfield/alignment_sorted/tmp/tmp.out >
~/pw_oilfield/alignment_sorted/STAR_counts.txt

# remove the tmp folder
rm -rf ~/pw_oilfield/alignment_sorted/tmp
```

STAR Results

Overall, over 90% of the RNA seq reads uniquely mapped to the genome.

Sample Name	% Aligned	M Aligned
HQ10E1	92.6%	21.8
HQ10E2	92.7%	24.7
HQE1	93.1%	24.7
HQE2	92.6%	17.9
HQ_ST1	93.3%	17.0
HQ_ST2	93.4%	21.0
PWE1	92.6%	20.7
PWE2	91.5%	21.0
PW_ST1	92.6%	19.7
PW_ST2	92.9%	24.2





STAR_counts.txt head

gene_name	HQ10E1	HQ10E2	HQE1	HQE2	HQ_ST1	HQ_ST2	PWE1	PWE2	PW_ST1	PW_ST2
Phatr3_J31400	1	5	3	7	0	4	2	1	10	20

gene_name	HQ10E1	HQ10E2	HQE1	HQE2	HQ_ST1	HQ_ST2	PWE1	PWE2	PW_ST1	PW_ST2
Phatr3_J42422	144	172	214	153	402	451	117	101	203	253
Phatr3_J31402	0	0	0	0	0	0	0	0	0	0
Phatr3_J42423	27	44	77	42	557	508	46	39	110	304
Phatr3_J42424	1819	2172	1876	1254	1524	1841	1518	1508	1035	1562
Phatr3_J7430	455	510	620	443	467	513	333	456	337	364
Phatr3_J42426	8520	9594	8689	7289	5396	7052	6769	7244	5813	8494
Phatr3_EG02408	3806	4483	3948	2637	224	238	3580	3084	216	368
Phatr3_J31409	6448	7590	9257	5980	1008	1394	4863	4622	1381	1864

Differential Gene Expression and Gene Ontology analysis was then performed in R. See the R markdown script "Produced_water_RNA_Seq.rmd" and "Produced_water_RNA_Seq.html".