**Sequencing analysis of SkinCom with varied chemicals (*in vitro*)**

Paired end sequencing. Commands run as scripts on remote server with Bash shell.

### Concatenate forward reads and reverse reads from lanes 1 and 2. FASTQ files are in folder </home/SkinCom\_expt2/>

# Forward reads

cd /home/SkinCom\_expt2/ list1=($(find . -name '\*L001\_R1\_001.fastq.gz' | sort)) list2=($(find . -name '\*L002\_R1\_001.fastq.gz' | sort)) for i in {0..72};

do

cat ${list1[i]} ${list2[i]} > ../fastq\_cat/${list1[i]}\_cat.fastq.gz

done

# Reverse reads

list3=($(find . -name '\*L001\_R2\_001.fastq.gz' | sort)) list4=($(find . -name '\*L002\_R2\_001.fastq.gz' | sort))

for i in {0..72};

do

cat ${list3[i]} ${list4[i]} > ../fastq\_cat/${list3[i]}\_cat.fastq.gz

Done

### Check sequence quality using FastQC and MultiQC

set -uex

cd /home/SkinCom\_expt2/fastq\_cat/

# FastQC /opt/fastqc\_0.11.5/FastQC/fastqc \*fastq.gz

# MultiQC

source /home/jkcoker/miniconda3/bin/activate # Activate conda conda activate python3.7 # Activate python3.7 environment with MultiQC multiqc .

### Remove adapters with Trimmomatic. Forward and reverse reads were trimmed at different times and therefore analyzed with single-end commands, but the reads could also be analyzed with the paired-end command.

cd /home/SkinCom\_expt2/fastq\_cat/

# Forward reads list1=($(find . -name '\*\_R1\_001.fastq.gz\_cat.fastq.gz' | sort)) for i in {0..72};

do

java -jar /opt/trimmomatic/0.36/trimmomatic-0.36.jar SE -phred33 ${list1[i]} ${list1[i]}\_forward\_trim.fq ILLUMINACLIP:/opt/trimmomatic/0.36/adapters/NexteraPE-PE.fa:2:30:10 LEADING:10 TRAILING:10 SLIDINGWINDOW:4:15 MINLEN:36

done

mkdir ../forward\_trim

mv \*\_forward\_trim.fq ../forward\_trim/

# Reverse reads

list2=($(find . -name '\*\_R2\_001.fastq.gz\_cat.fastq.gz' | sort)) for i in {0..72};

do

java -jar /opt/trimmomatic/0.36/trimmomatic-0.36.jar SE -phred33 ${list2[i]} ${list2[i]}\_reverse\_trim.fq ILLUMINACLIP:/opt/trimmomatic/0.36/adapters/NexteraPE-PE.fa:2:30:10 LEADING:10 TRAILING:10 SLIDINGWINDOW:4:15 MINLEN:36

done

mkdir ../reverse\_trim

mv \*\_reverse\_trim.fq ../reverse\_trim/

### Run FastQC and MultiQC again after Trimmomatic to confirm adapter removal and sequence quality

set -uex

source /home/jkcoker/miniconda3/bin/activate

conda activate python3.7

### Unpaired reads (post-trimmomatic)

source /home/jkcoker/miniconda3/bin/activate

conda activate python3.7

# Forward reads

cd /home/SkinCom\_expt2/forward\_trim/

/opt/fastqc\_0.11.5/FastQC/fastqc \*\_forward\_trim.fq multiqc .

# Reverse reads

cd /home/SkinCom\_expt2/reverse\_trim/

/opt/fastqc\_0.11.5/FastQC/fastqc \*\_reverse\_trim.fq multiqc .

### Align paired reads to custom Bowtie2 index with genome sequences of the community members

module load bowtie2\_bowtie2-2.2.3

cd /home/SkinCom\_expt2/forward\_trim/

list1=($(find . -name '\*\_forward\_trim.fq' | sort))

cd /home/SkinCom\_expt2/reverse\_trim/

list2=($(find . -name '\*\_reverse\_trim.fq' | sort))

for i in {0..72};

do

bowtie2 -p 10 -x /home/bowtie2\_index\_skincomm/skincomm\_index -1 ${list1[i]} -2 ${list2[i]} -S /home/SkinCom\_expt2/bowtie\_mapping\_merged/${list1[i]}.bowtie.sam

done

### Process SAM files to make a counts table for each sample

cd /home/SkinCom\_expt2/bowtie\_mapping\_merged/ for i in `ls \*bowtie.sam`;

do

grep -v "^@" ${i} | cut -f 3 | sort | uniq -c > /home/SkinCom\_expt2/bowtie\_counts/${i}\_output.txt done

#### Counts table processed further in R in Jupyter notebook SkinCom\_expt2.ipynb