Creating the DeSeq2 object:

First, the user needs to set the design of the model:

```
dds <- DESeqDataSetFromMatrix(countData = counts, colData = metadata, design = ~ Treatment)
```

```
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in design formula are
## characters, converting to factors
```

Set contrasts

One then needs to set the contrasts as follows. Each comparison should be set to a variable (e.g., comparison_1), which equals a character vector, consisting of 3 character strings: 1. The first string needs to correspond to the factor in design formula 2. The name of the numerator level for the fold change 3. The name of the denominator level for the fold change.

```
comparison_1<-c("Treatment", "TGF", "Untreated")
comparison_2<-c("Treatment", "LPA_TGF", "TGF")

comparisons<-list(comparison_1,comparison_2)
```

Count normalization

To calculate normalized counts:

```
dds <- estimateSizeFactors(dds)
# show size factors for each sample:
sizeFactors(dds)
```

```
## Untreated_1 Untreated_2 Untreated_3       TGF_4       TGF_5       TGF_6   LPA_TGF_7   LPA_TGF_8
##   1.0260794   1.0994421   1.0785351   1.0791479   1.0432896   0.8962024   0.9828280   0.9811014
##   LPA_TGF_9
##   0.9032011
```