2. which genes respond to stimulation in mutant cells, and

3. which genes respond differently in mutant compared to wild-type cells.

as these are the questions which are most usually relevant in a molecular biology context. The first of these questions relates to the `WT.S` vs `WT.U` comparison and the second to `Mu.S` vs `Mu.U`. The third relates to the difference of differences, i.e., `(Mu.S-Mu.U)-(WT.S-WT.U)`, which is called the *interaction* term.

9.5.2 Analysing as for a Single Factor

We describe first a simple way to analyze this experiment using limma commands in a similar way to that in which two-sample designs were analyzed. Then we will go on to describe the more classical statistical approaches using factorial model formulas. All the approaches considered are equivalent and yield identical bottom-line results. The most basic approach is to fit a model with a coefficient for each of the four factor combinations and then to extract the comparisons of interest as contrasts:

```
> TS <- factor(TS, levels=c("WT.U","WT.S","Mu.U","Mu.S"))
> design <- model.matrix(~0+TS)
> colnames(design) <- levels(TS)
> fit <- lmFit(eset, design)
```

This fits a model with four coefficients corresponding to `WT.U`, `WT.S`, `Mu.U` and `Mu.S` respectively. Our three contrasts of interest can be extracted by

```
> cont.matrix <- makeContrasts(
+     SvsUinWT=WT.S-WT.U,
+     SvsUinMu=Mu.S-Mu.U,
+     Diff=(Mu.S-Mu.U)-(WT.S-WT.U),
+     levels=design)
> fit2 <- contrasts.fit(fit, cont.matrix)
> fit2 <- eBayes(fit2)
```

We can use `topTable()` to look at lists of differentially expressed genes for each of three contrasts, or else

```
> results <- decideTests(fit2)
> vennDiagram(results)
```

to look at all three contrasts simultaneously.

This approach is recommended for most users, because the contrasts that are being tested are formed explicitly.