### Set contrast groups

In the meta-data table of experimental design, if multiple factors are selected, they will be merged to create a single column of grouping labels (Figure B). 3D RNA-seq App provides a flexible way where users can generate contrast groups of simple pair-wise analyses and complex experimental design such as time-series, development series and multiple conditions from grouping labels. For example, in Supplemental Figure 4B, contrast groups can be set as WT.B-WT.A (WT.B vs WT.A) and MU.B-MU.A (MU.B vs MU.A) to compare expression of condition WT.B to WT.A and MU.B to MU.A, respectively. Contrast group settings of pair-wise conditions suit to most experimental design in RNA-seq studies. In addition, users can set contrast group (MU.B+WT.B)/2-(MU.A+WT.A)/2 to compare the mean of multiple conditions.

**Note**: for time-series RNA-seq study, the time-points can be treated as different conditions to set contrast groups.

**Figure**: Generate group labels of factor levels of experimental design and set contrast groups for expression comparisons. Meta-table of single factor (A) and multiple factors (B).