

The concept of replicate error

The idea is that if you have an experiment design that involve replicates of any type you want to remove spots that differ too much from the other spots in their replicate. To do this duplicates are created from each replicate and the ratio of the duplicates is studied. The ratio from a duplicate is calculated as ratio of the ratios of the two spots. Every duplicate that differ a number of standard deviations from the average is a bad duplicate and a spot that is only present in bad duplicates is a bad spot and is removed.

How is the replicates created?

A replicate consists of every spot with the same reporter. If the method is “within assay” each position is one spot in the replicate. For the method “across assays” the spots are considered merged on reporter for calculating purpose and the spots in the replicate is one reporter (merged on position) from each assay in assay group. The grouping is determined by the grouping parameters.

How is a replicate transformed to duplicate?

As mentioned before the calculations are done on duplicates so how is a replicate transformed to a duplicate. Well to be precise, a replicate is always transformed to one or more duplicates. Every spot in a replicate is paired with every other spot and this creates a set of one or more duplicates. For this purpose a duplicate is considered equal to another duplicate regardless of the order of the spots, $\langle s_1, s_2 \rangle = \langle s_2, s_1 \rangle$, and a spot cannot create a duplicate with itself.

$$\langle s_1, s_2, s_3 \rangle \rightarrow \langle s_1, s_2 \rangle, \langle s_1, s_3 \rangle, \langle s_2, s_3 \rangle$$

$$\langle s_1, s_2, s_3, s_4 \rangle \rightarrow \langle s_1, s_2 \rangle, \langle s_1, s_3 \rangle, \langle s_1, s_4 \rangle, \langle s_2, s_3 \rangle, \langle s_2, s_4 \rangle, \langle s_3, s_4 \rangle$$

What spots are removed?

- Every spot that can't create a ratio, i.e. one of the intensities is less than or equal to 0.
- Every spot that is present in a replicate that can't produce a valid duplicate. A valid duplicate is a duplicate that has a duplicate ratio.
- Every spot that isn't present in any duplicate that passes the filter.

Thesaurus

Singleton	A spot occurring once
Duplicate	A spot occurring twice
Triplicate	etc.
Replicate	A spot occurring more than once