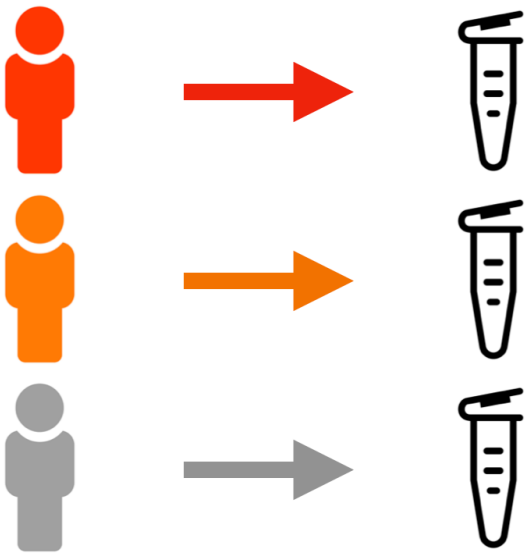
Biological vs technical replicates

What is a biological replicate?

Biological replicates are samples taken from different sources/individuals; for instance, blood samples taken from different individuals which are subsequently used for genetic analysis.

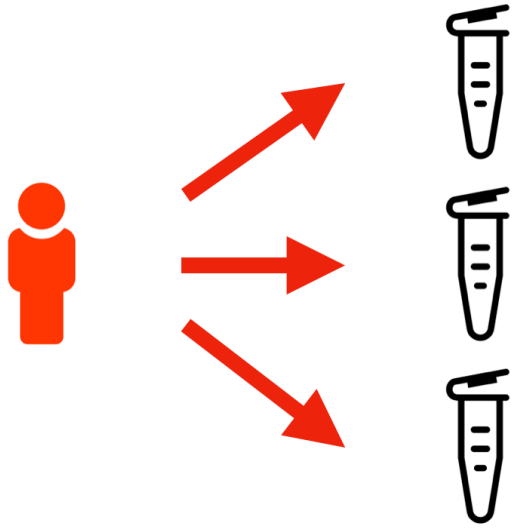
Biological replicates are used in gene expression studies, so conclusions can be generalised to groups represented by the various individuals/sources that the samples were taken from.



Variation evident between biological samples is likely due to random biological differences present in individuals, which may well be the focus of the research. Biological replicates increase the robustness of the data analysed and conclusions inferred.

What is a technical replicate?

Technical replicates are repeated analyses on a single sample; for example, a blood sample taken from one person which is then analysed multiple times.



Technical replicates are beneficial when the aim is to conclude results for one individual, or when proving a method. Generally, when technical replicates are performed the results are averaged, aiming to identify and remove 'noise' from small technical anomalies or environmental influences.

Which is best?

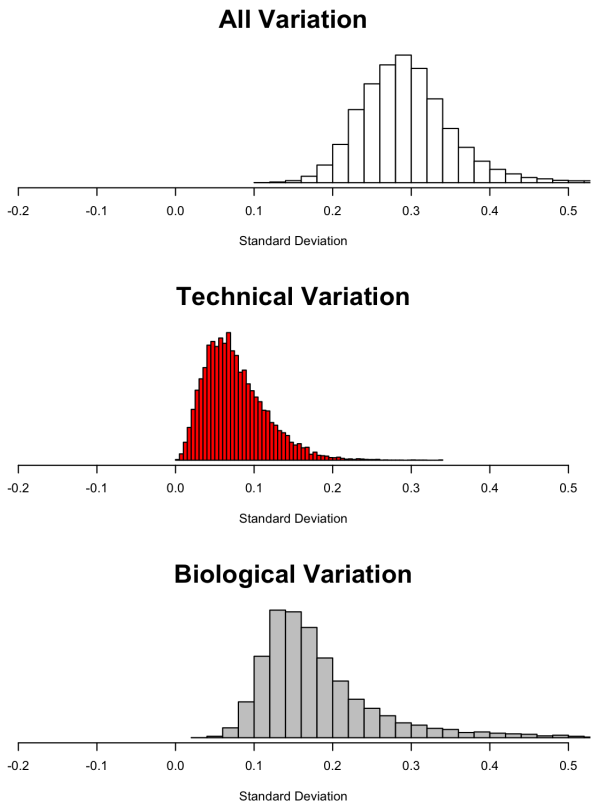
If the focus of your research is on method development or assessing one sample/individual in depth, then you will likely benefit from running technical replicates. However, if you aim to generalise your conclusions to a defined group of individuals then biological replicates will benefit your conclusion power.

Technical replicates do not add any value to certain experimental designs, such as RNASeq studies, in which case only biological replicates should be used. (Blainey et al. 2014)

Does the type of replicate matter?

If we're designing an experiment to determine transcription differences between sample groups, we have to be minded that differences in the results will comprise a combination of technical variation (e.g. pipetting differences, temperature changes, measurement differences) and biological variation.

Using a publically available microarray dataset (maPooling) we can extract real information about the variability in gene expression at the technical and biological level. In this example, showing that the biological variability is around twice that from the technical artefacts.



For the majority of experiments, it's the biological variation we're interested in, so we focus on good experimental design and good laboratory practice to ensure that the technical variability is kept to a minimum to allow the biological signal to be seen.

What about cell lines?

Bjorn (2016) outlines the issues researchers are faced with when distinguishing biological replicates from technical replicates in the case where a cell line is split into different colonies and left to grow either untreated (control) or with treatment.

They conclude "even if for each replicate a new frozen cell stock was used, ultimately all cells originated from the same starting material, therefore no biological replicates can possibly be achieved.

This problem can only be solved by generating several independent cell lines from several different human/animal tissue or blood samples, which demonstrates that reality often places constraints on what is statistically optimal."

Other considerations

It is extremely difficult to undertake an experimental design with optimal independence present between biological replicates to maximise statistical power and inference capability. **Three biological replicates should be the absolute minimum included in an experiment, whilst aiming for six or more (Schurch et al. 2016) is desirable.**

Limitations such as sourcing and using completely independent cell lines and time constraints will force certain decisions to be made: these limitations should be duly noted and subsequently communicated.

References

Blainey, P. et al. 2014. Replication. Nature Methods 11(9), pp. 879-880. doi: 10.1038/nmeth.3091

Bjorn. 2016. Accurate design of in-vitro experiments: why does it matter? Available at https://paasp.net/accurate-design-of-in-vitro-experiments-why-does-it-matter/ [Accessed 24th September 2020]

Schurch, N. J. et al. 2016. How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? *RNA (New York, N.Y.)* 22(6), pp. 839-851. doi: 10.1261/rna.053959.115