How we use deepTools

You will find many examples from ChIP-seq analyses in this tutorial, but this does not mean that deepTools is restricted to ChIP-seq data analysis. However, some tools, such as *bamFingerprint* specifically address ChIP-seq-issues. (That being said, we do process quite a bit of RNA-seq, other -seq and genomic sequencing data using deepTools, too, but many normalization issues arose during handling of ChIP-seq data).

As shown in the flow chart above, our work usually begins with one or more FASTQ file(s) of deeply-sequenced samples. After a first quality control using FASTQC, we align the reads to the reference genome, e.g. using bowtie2 "bowtie, one of the most popular aligners").
We then use deepTools to assess the quality of the aligned reads:

1. **Correlation between BAM files** (*bamCorrelate*). This is a very basic test to see whether the sequenced and aligned reads meet your expectations. We use this check to assess the reproducibility - either between replicates and/or between different experiments that might have used the same antibody/the same cell type etc. For instance, replicates should correlate better than differently treated samples.
2. **GC bias check** (*computeGCbias*). Many sequencing protocols require several rounds of PCR-based amplification of the DNA to be sequenced. Unfortunately, most DNA polymerases used for PCR introduce significant GC biases as they prefer to amplify GC-rich templates. Depending on the sample (preparation), the GC bias can vary significantly and we routinely check its extent. In case we need to compare files with different GC biases, we use the *correctGCbias* module to match the GC bias. See the paper by Benjamini and Speed for many insights into this problem.
3. **Assessing the ChIP strength**. This is a QC we do to get a feeling for the signal-to-noise ratio in samples from ChIP-seq experiments. It is based on the insights published by Diaz et al..

Once we are satisfied by the basic quality checks, we normally **convert the large BAM files into a leaner data format, typically bigWig**. bigWig files have several advantages over BAM files that mainly stem from their significantly decreased size:

- useful for data sharing & storage
- intuitive visualization in Genome Browsers (e.g. IGV)
- more efficient downstream analyses are possible

The deepTools modules *bamCompare* and *bamCoverage* do not only allow the simple conversion from BAM to bigWig (or bedGraph for that matter), **the main reason why we developed those tools was that we wanted to be able to *normalize* the read coverages** so that we could compare different samples despite differences in sequencing depth, GC biases and so on.

Finally, once all the files have passed our visual inspections, the fun of downstream analyses with *heatmapper* and *profiler* can begin!