Table 1

The first single-cell method for measuring genome-wide 5mC used a reduced representation bisulfite sequencing (scRBBS) approach based on enrichment of CpG dense regions (such as CpG islands) via restriction digestion, and it allows the measurement of approximately 10 % of CpG sites [6]. scRRBS is powerful because it allows assessment of a large fraction of promoters with relatively low sequencing costs, but its limitation is poor coverage of many important regulatory regions such as enhancers.

To develop true whole-genome single-cell approaches [7, 8] technological developments have been based on a

post-bisulfite adapter-tagging (PBAT) approach in which bisulfite conversion is performed before library preparation so that DNA degradation does not destroy adaptor-tagged fragments [9]. As a result, methylation in up to 50 % of the CpG sites in a single cell can now be measured and this has allowed, for example, the detection of high variability between single cells in distal enhancer methylation (not usually captured by scRRBS) in mouse embryonic stem cells (ESCs) [7].

Building on this method has allowed BS-seq and RNA-seq in parallel from the same single cell (scM&T-seq) [10]. This was made possible by way of

a method for physical separation of poly-A mRNA from DNA (genome and transcriptome sequencing or G&T-seq [11]), and this now allows intricate investigations of links between epigenetic and transcriptional heterogeneity within a particular cell and tissue type.

Hydroxymethylated cytosine (5hmC) is also thought to have a role in epigenetic gene regulation and has been analyzed in bulk samples using modified bisulfite sequencing methods [12, 13], 5hmC-specific restriction

In NOMe-seq, a methyltransferase enzyme is used to methylate exposed GpC dinucleotides while DNA bound by nucleosomes is protected. Sequencing of the bisulfite-converted DNA can then be used to map nucleosome positions and this is particularly attractive for single-cell use since it will also give a readout of CpG methylation within the same single cell. Indeed nucleosome positioning has already been studied using locus-specific bisulfite PCR in the yeast *HO5* gene, which revealed significant variability between cells that correlated with gene expression [27]. Single-cell nuclei prepared according to this method should be compatible with scBS-seq.

In addition to defining the linear chromatin organization of single cells, it is now possible to assess chromosome conformation at the single-cell level using a HiC-based method [28, 29]. Single-cell HiC is currently limited in its resolution but still allows description of the individual chromosome organization and compartmentalization, as well as interchromosomal interactions. This is a good example of how single-cell approaches can really provide cutting-edge tools, as regular HiC was traditionally performed on millions of cells resulting in an average of all chromosome organization within the cell population and hence some ambiguity in interpretation of the results.

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Development of single-cell approaches is intimately linked to the development of physical equipment and devices. The first step in any single-cell analysis is the isolation and lysis of single cells from culture or dissociated tissue. This can be performed manually with a pipette and a microscope but such methods cannot

by bisulfite. Another important consideration is the effect of cell dissociation on downstr

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