

# Chapter 6

## Monitoring the Transcriptome

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### ABSTRACT

*The technologies monitoring the transcriptome are under a continuous status of development and implementation, producing high amounts of expression data which require reliable and well structured databases. This chapter provides a general overview and a reliable guide of these technologies, as developed from the early '90s until today, and of the most important available expression databases. The first part aims at introducing to the reader the fundamental functional aspects of these technologies, which are under a continuous development in order to obtain a more accurate description of the transcriptome. The second part offers the necessary information to those who are interested in further exploiting expression data in their research.*

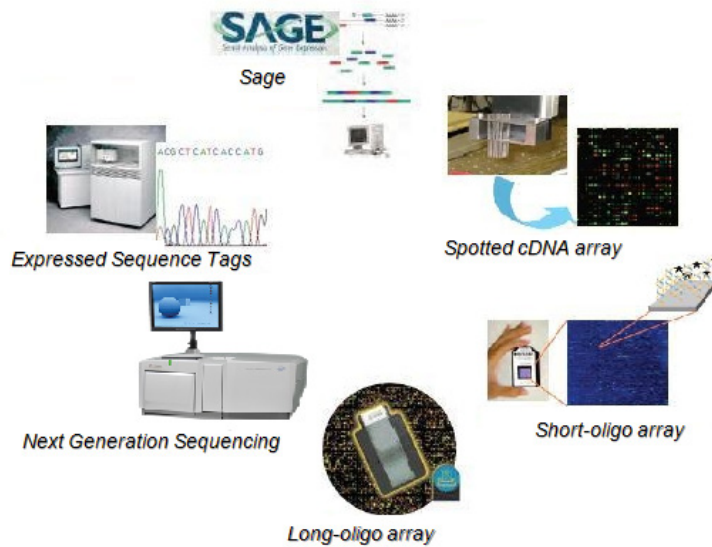
### INTRODUCTION: FROM THE GENOME TO THE TRANSCRIPTOME

After the complete sequencing of the human genome, and that of other organisms (Venter et al., 2001; Lander et al., 2001, 2002) there was an unavoidable increase of genomic information which allowed the development of different technologies, in order to achieve a complete use of this large information, and a faithful identification of the transcriptome, i.e. the combined variability

of all transcripts produced by the genome. Since the official announcement from the International Human Genome Sequence Consortium (IHGSC) in 2001 that there are only 32,000 genes in the human genome, new sequences are still being added into the chromosome maps and their relative locations are being adjusted (Kidd et al., 2008; The ENCODE project Consortium, 2007; Jobling et al., 2004). Furthermore, the overall gene fragmentation has rapidly decreased the number of genes originally identified. For example, the number of genes located by Ensembl genome annotation system (<http://www.ensembl.org/index>.

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Figure 1. Transcriptome techniques



html) decreased from the initial 29,700 to 22,800 in one year.

Unlike the ‘genome’, the profiles of the counts of the human genes’ transcripts will vary not only among cell types, but also among the state of a cell, and from the stimuli on that cell. Consequently, the transcriptome will therefore vary both spatially and temporally within an individual. Indeed, one could define ‘transcriptome’ as a dynamic entity which is spatially and temporally changing (formed by pooling transcripts from all cell types at all times), and an experiment would provide a snapshot of this dynamic entity. Alternatively, one might define the term ‘transcriptome’ as the transcripts (mRNA species and their levels) at the particular time and place in the history of the individual when the snapshot was taken (see, e.g., the National Human Genome Research Institute’s definition; <http://www.genome.gov/13014330>; Stearns et al., 2003). However, in both cases the snapshot is in practice *ex vivo* and often obtained only after experimental manipulation of the cells or tissue samples. In order to assess how such a picture corresponds to the *in vivo* situation, cautious samples manipulation, verified experimental procedures,

and reproducibility is necessary. This latter bias became the major force driving the development and improvement through time, of several technologies monitoring the transcriptome (Figure 1). These technologies can be divided mainly in two major types: sequencing- and hybridization-based techniques. The first include Expressed Sequence Tags (ESTs), Serial Analysis of Gene Expression (SAGE) (SAGE: Velculescu et al., 1995), the Massively Parallel Signature Sequencing (MPSS) (MPSS: Brenner et al., 2000a, 2000b; Meyers et al., 2004a, 2004b), and the Next Generation Sequencing techniques, i.e., 454 (<http://www.454.com>; Margulies et al., 2005) and the Solexa system (<http://www.illumina.com>). The second category, named hybridization-based techniques, is mainly referred to the microarray technology. Microarray technology can be classified based on different attributes such as, commercial or custom made arrays, probes’ length arrays, spotted or *in situ* synthesized, glass or membrane based, one- or two-colour arrays. Here, arrays will be classified in two major categories, the 3’-based arrays and the recently developed exon arrays. The former arrays will be examined in this chapter,

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