Cube

Computational Systems Biology

Quantitative Methoden in der Molekularbiologie

10. Whole transcriptome analysis

WS 2015/16

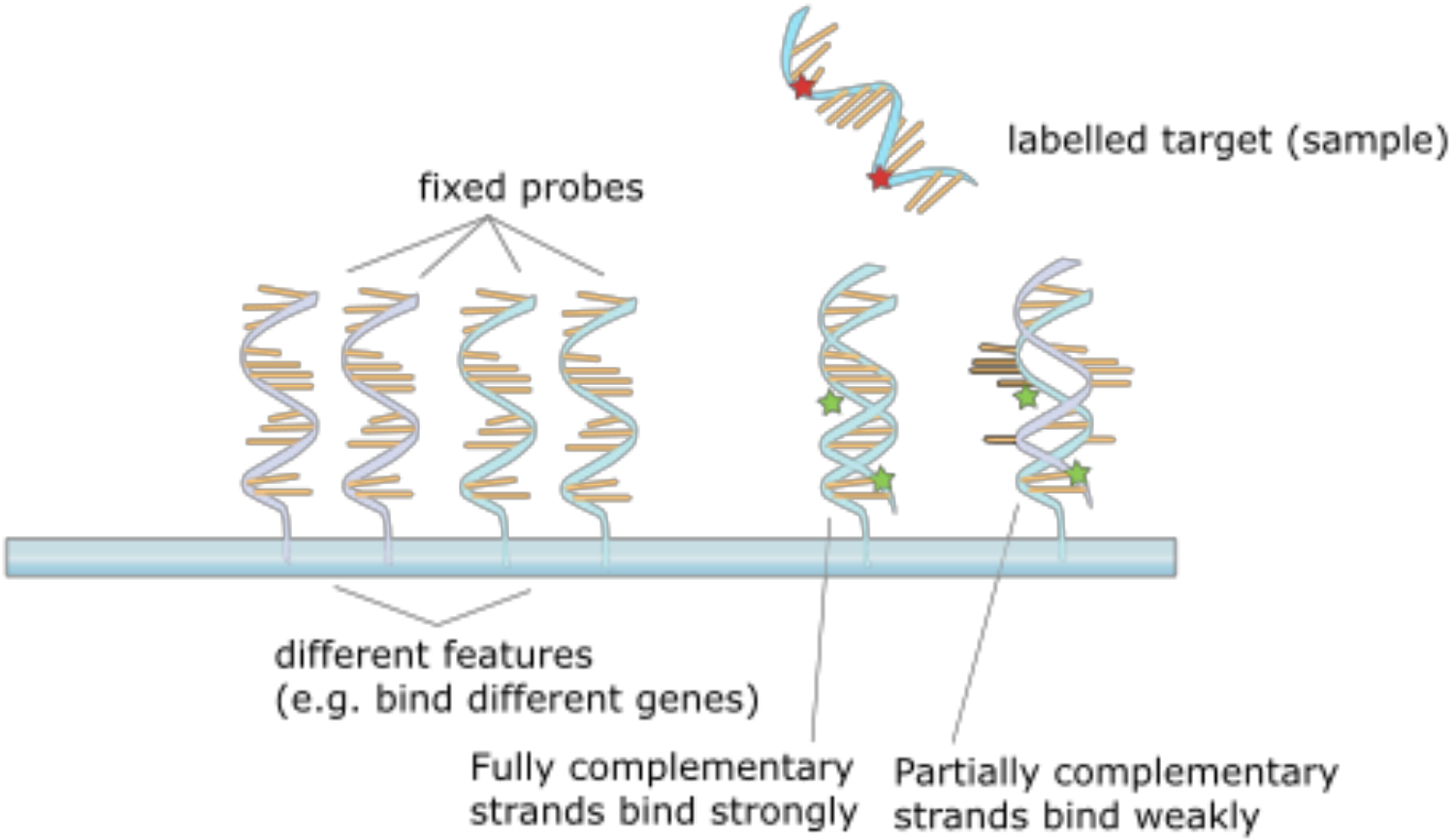
Outline

1. Expression microarrays

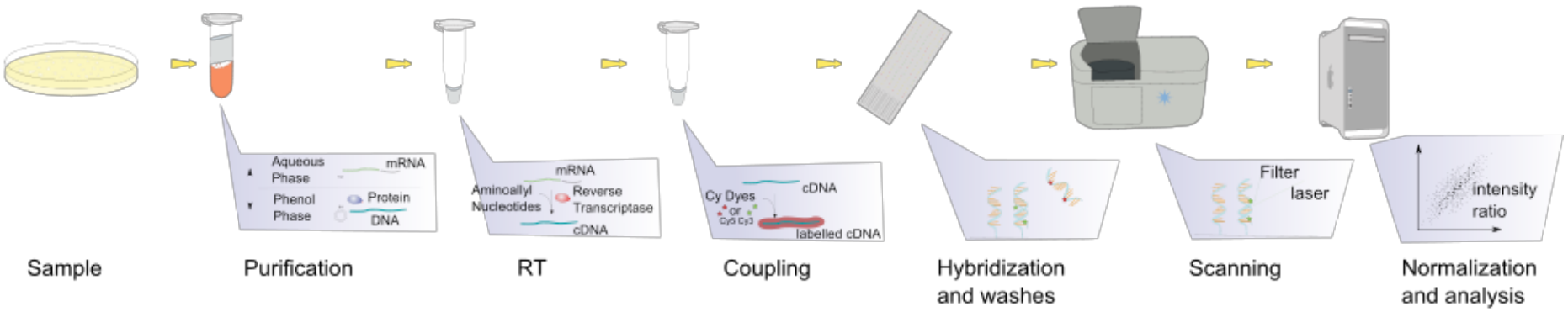
2. RNA-seq

3. Classification

Principle

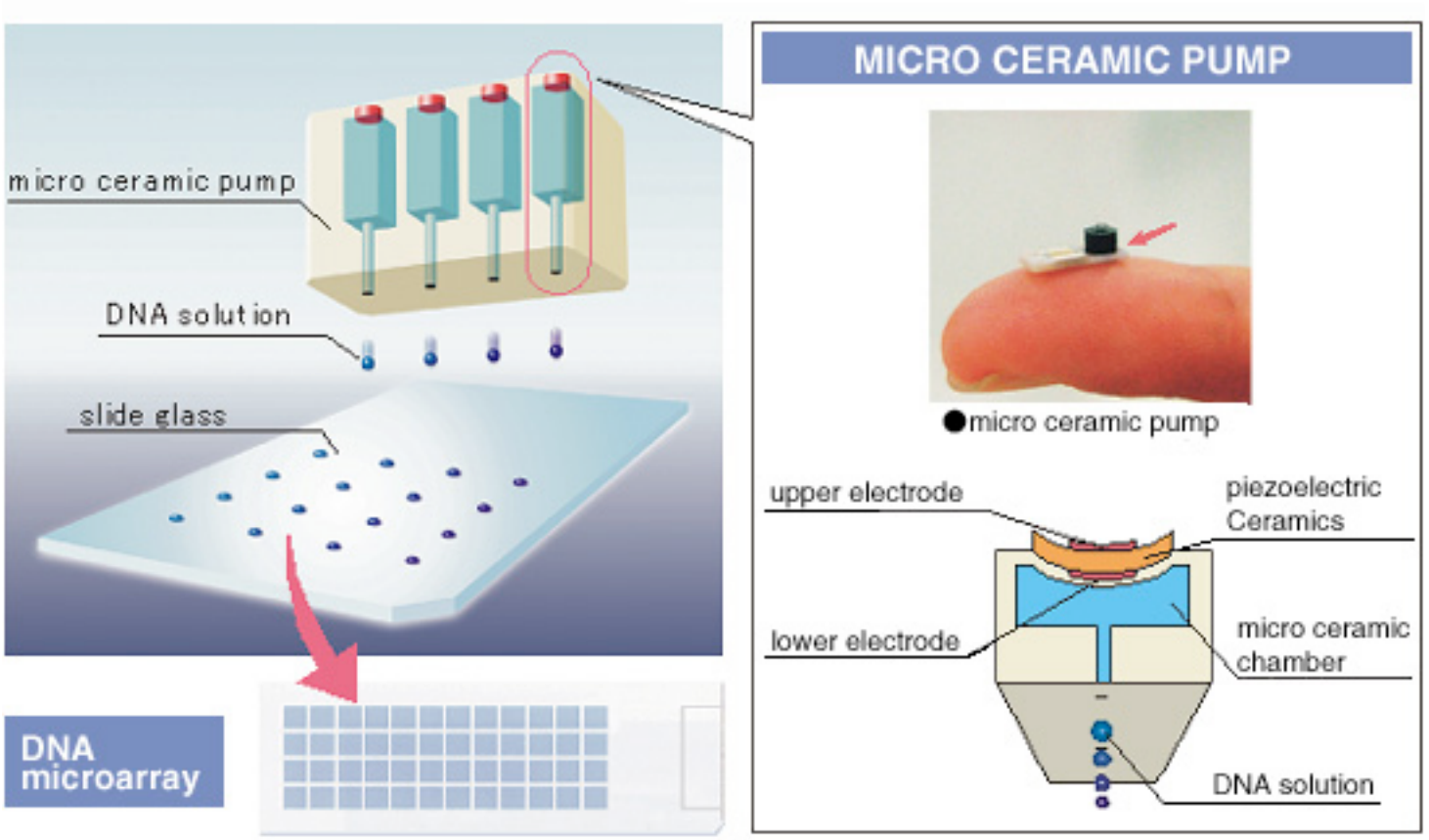

labelled target (sample)
fixed probes
different features
(e.g. bind different genes)
Fully complementary
strands bind strongly
Partially complementary
strands bind weakly

Microarray analysis workflow

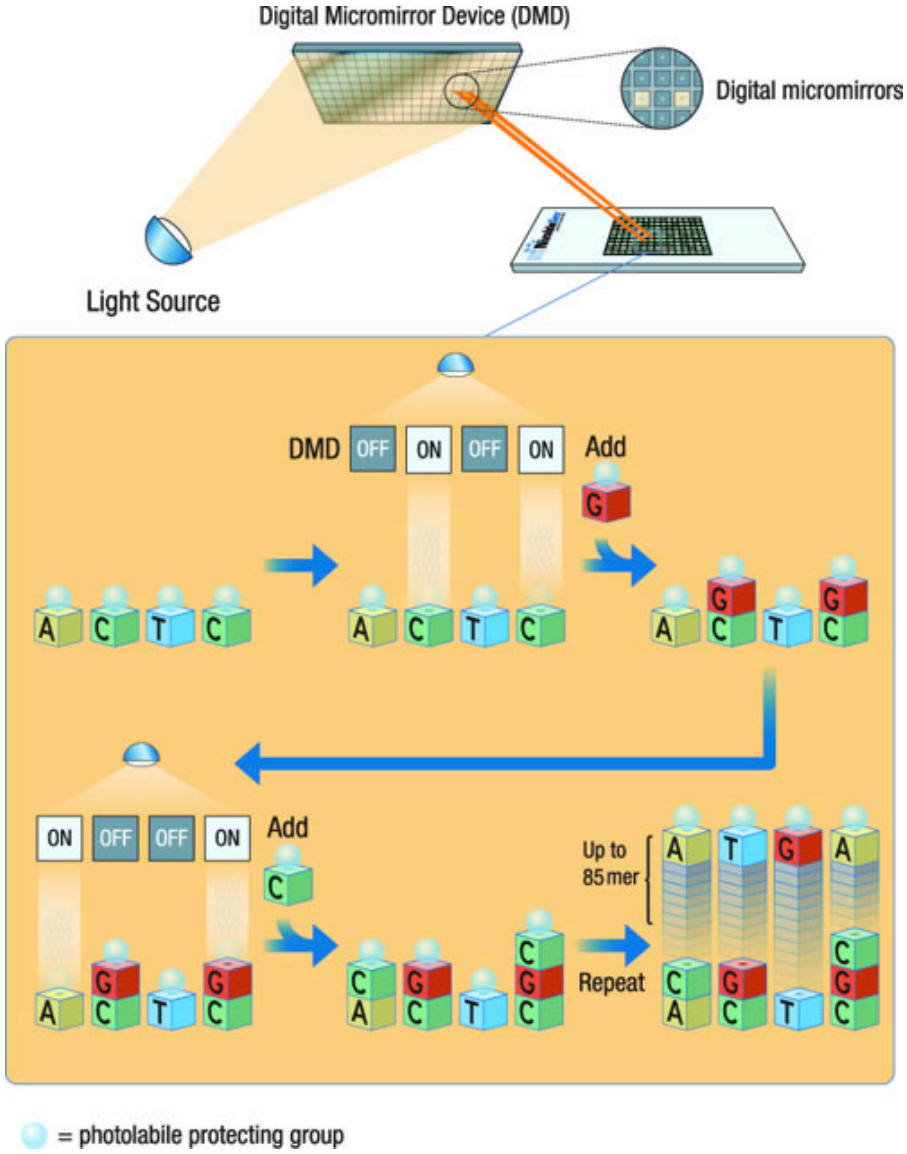


Affymetrix example

Fabrication: spotting



Fabrication: on-chip synthesis



NimbleGen example

Outline

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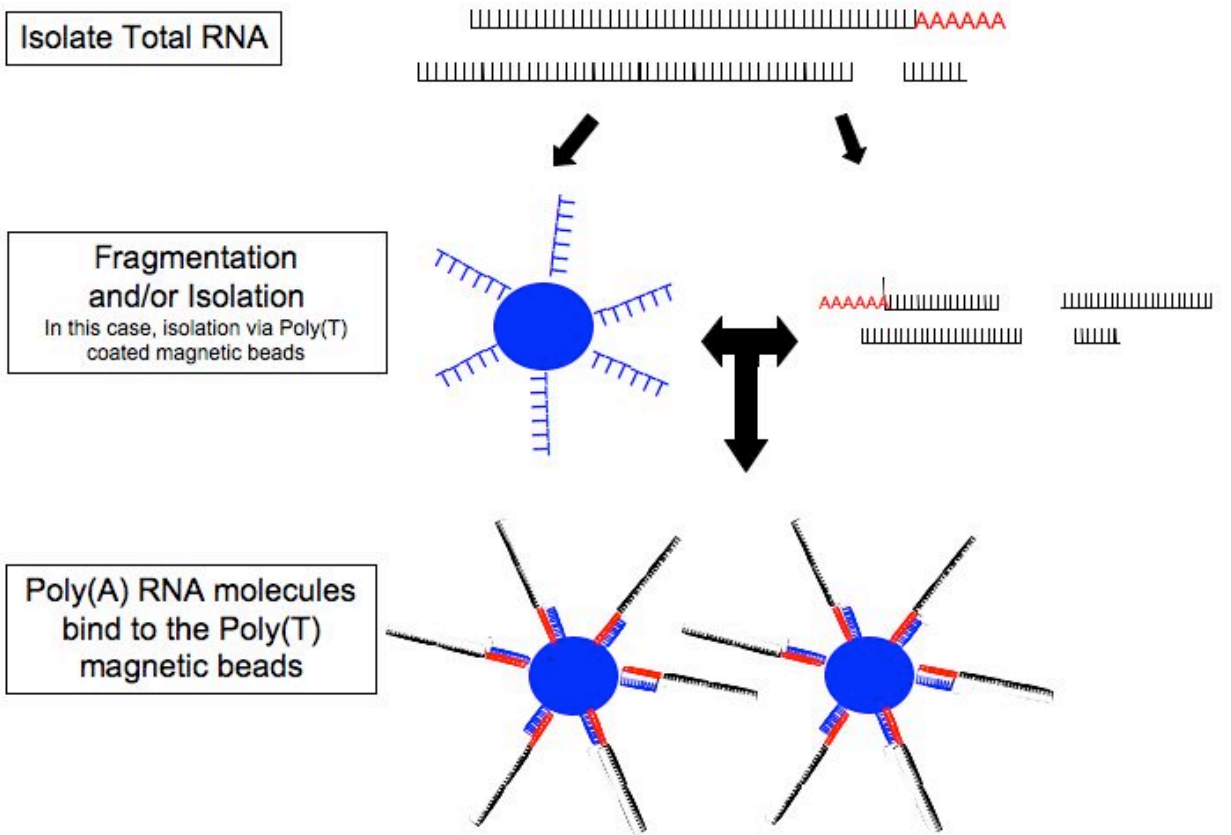
3. Classification

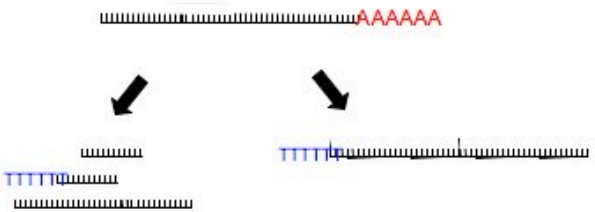
mRNA isolation/enrichment



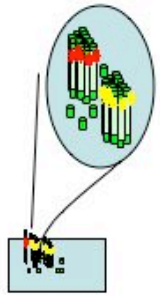
RNA seq

Magnetically isolate and wash beads

Fragment and/or Reverse Transcribe

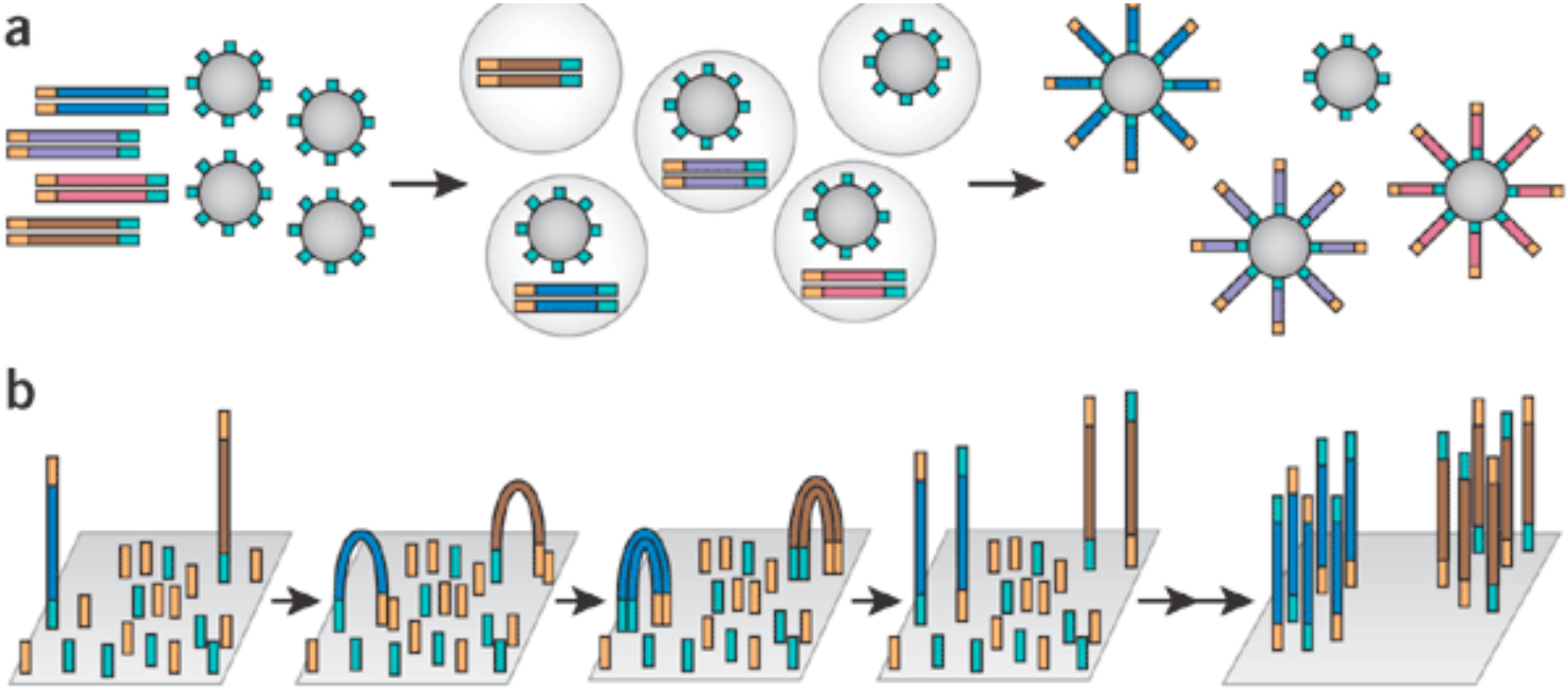


Fragmentation (if not done already), size selection, and sequence



Illumina Solexa, Roche 454, or ABI SOLiD
Graphic shown here is Illumina

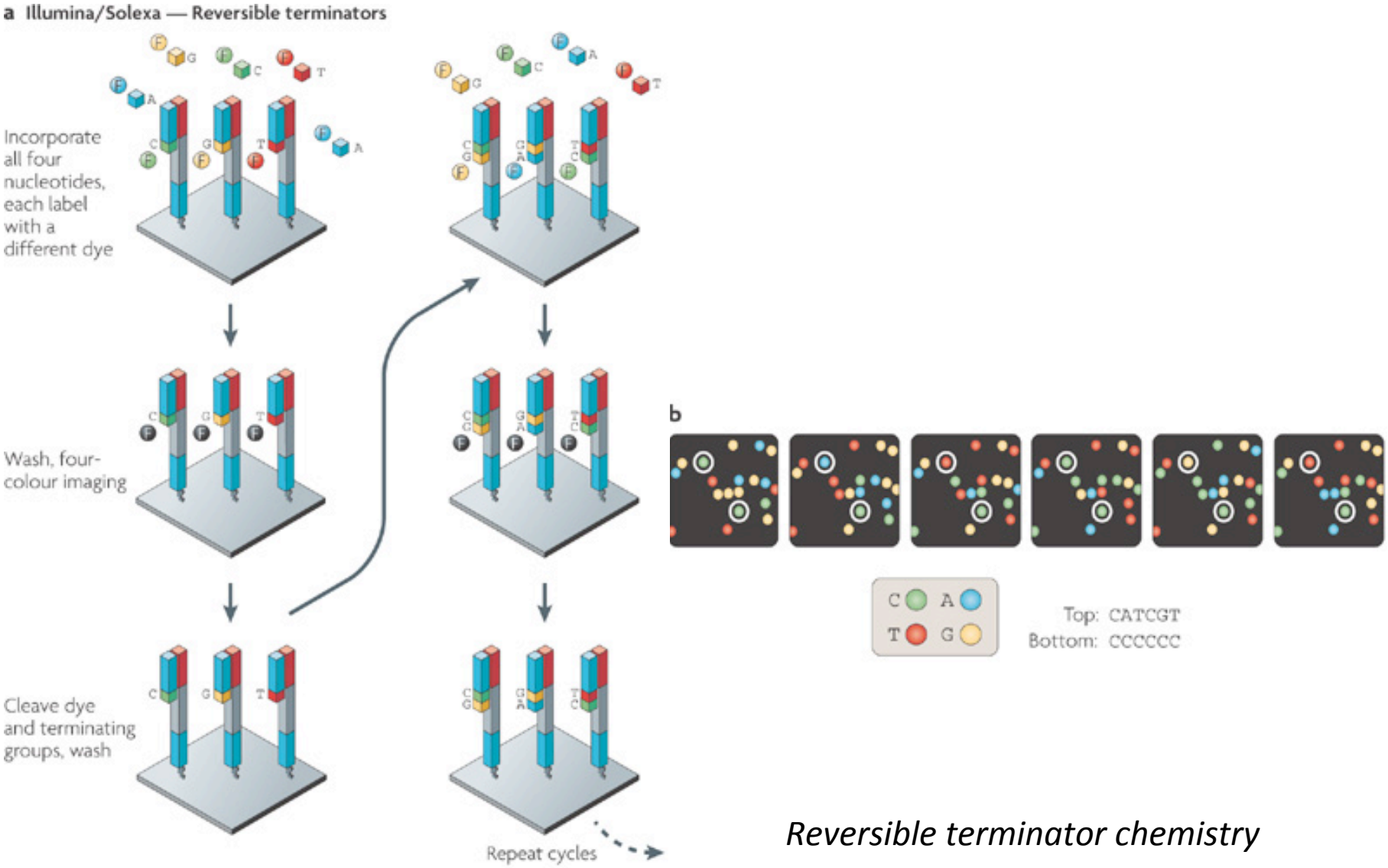
In vitro clonal amplification



Adapters may include sample specific tags.

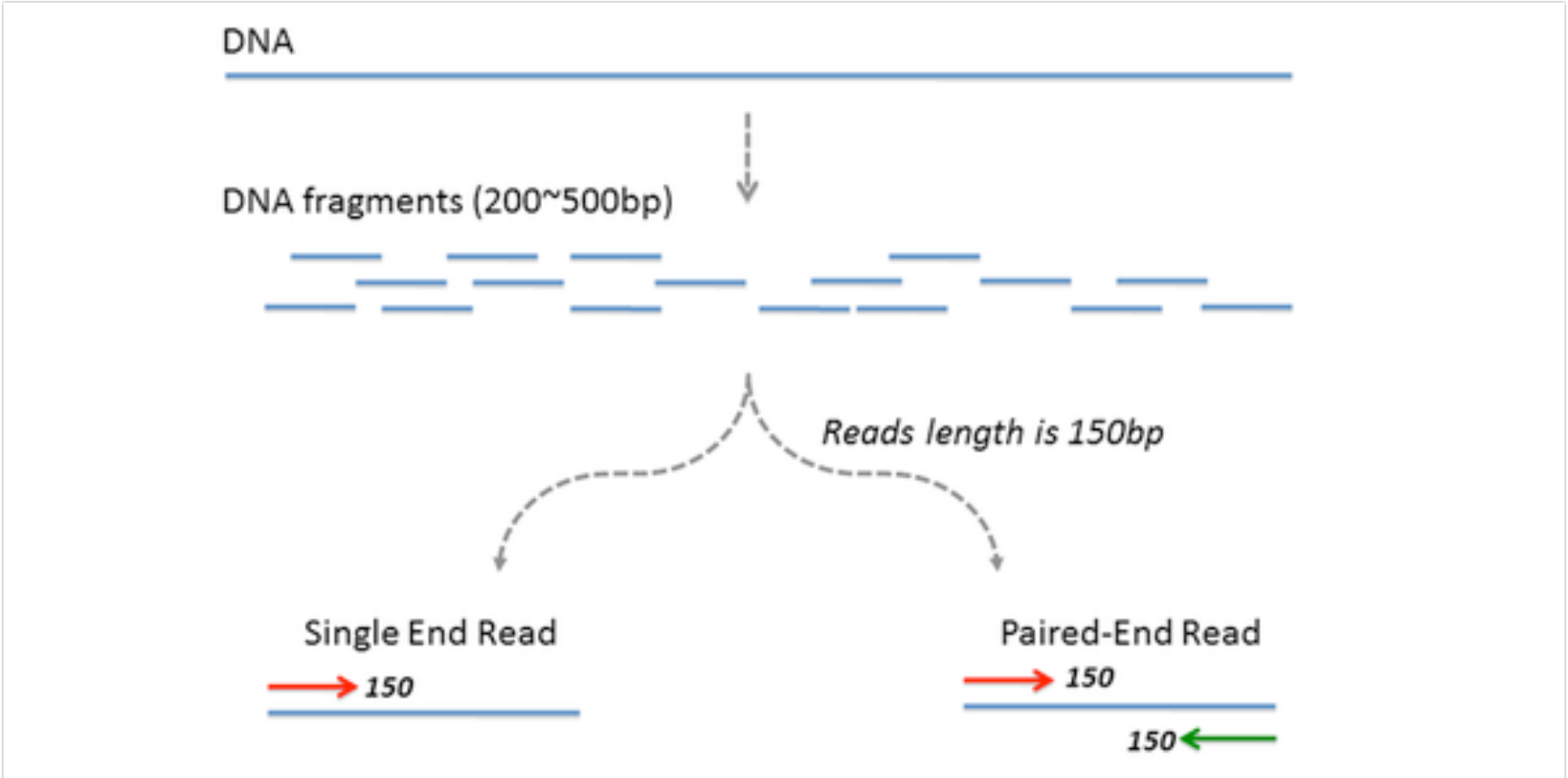
Shendure J & Ji H. Nature Biotech 26, 2008, 1135 – 1145.

Next generation sequencing: Illumina

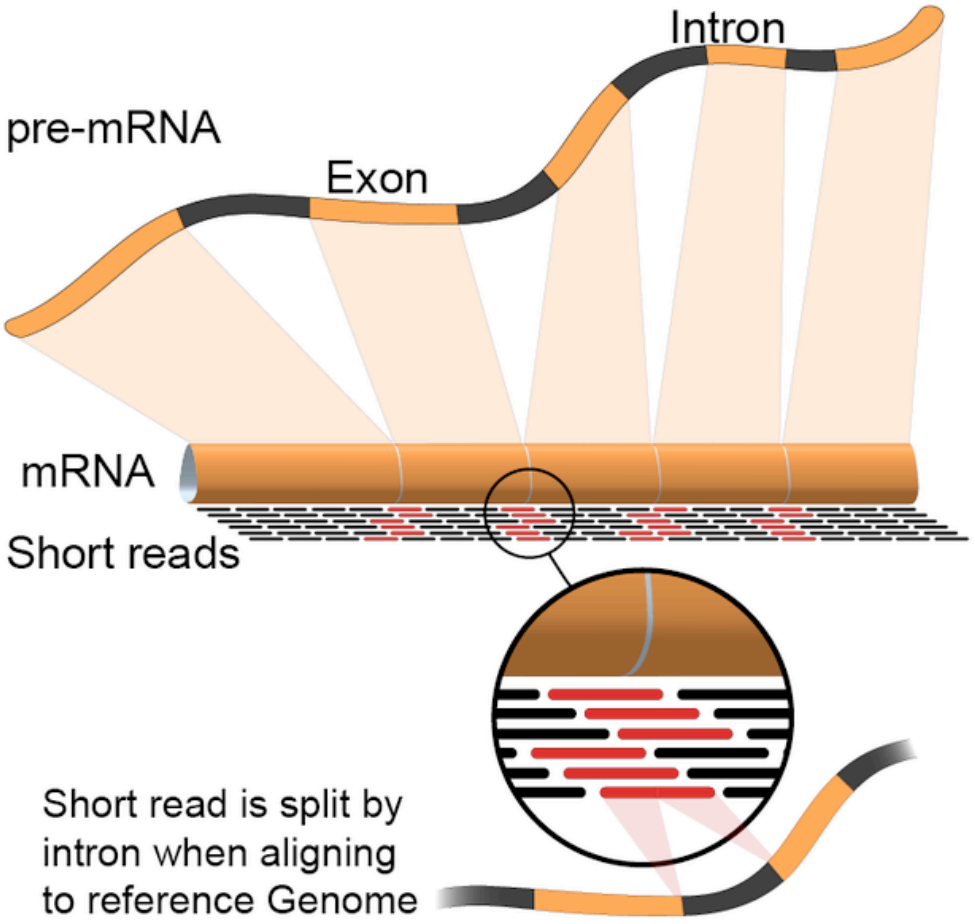


Reversible terminator chemistry

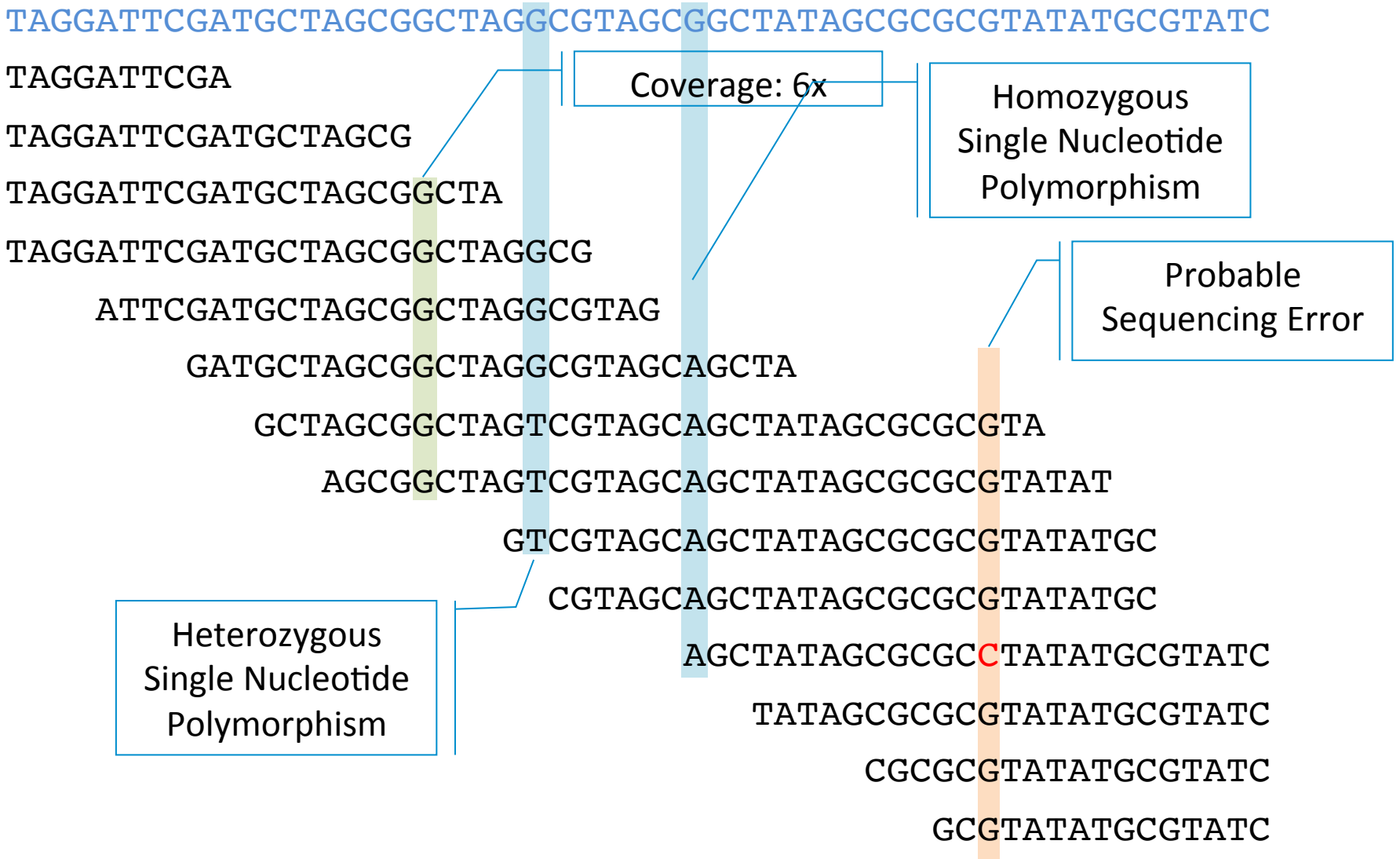
Single-end vs. Paired-end sequencing



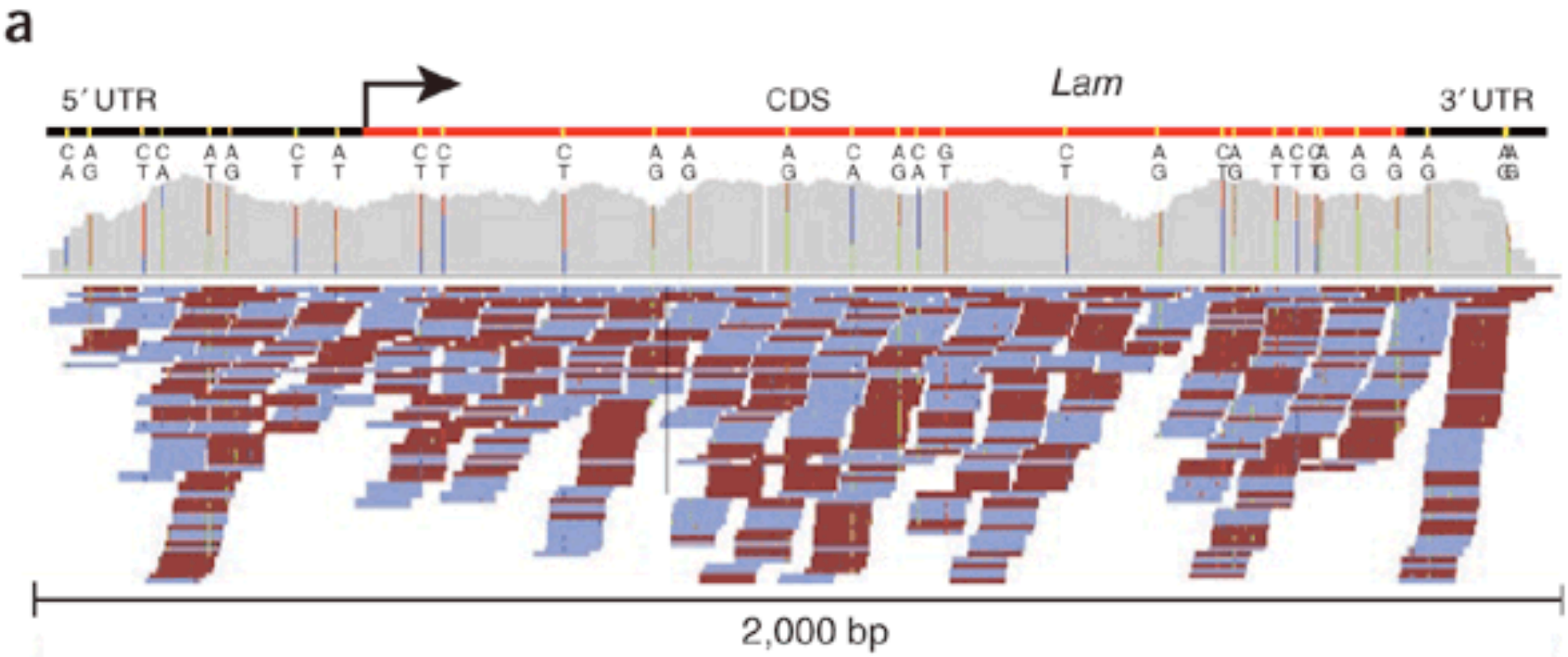
Mapping to reference genome



Assembly by mapping



Quantification



Normalization of coverage

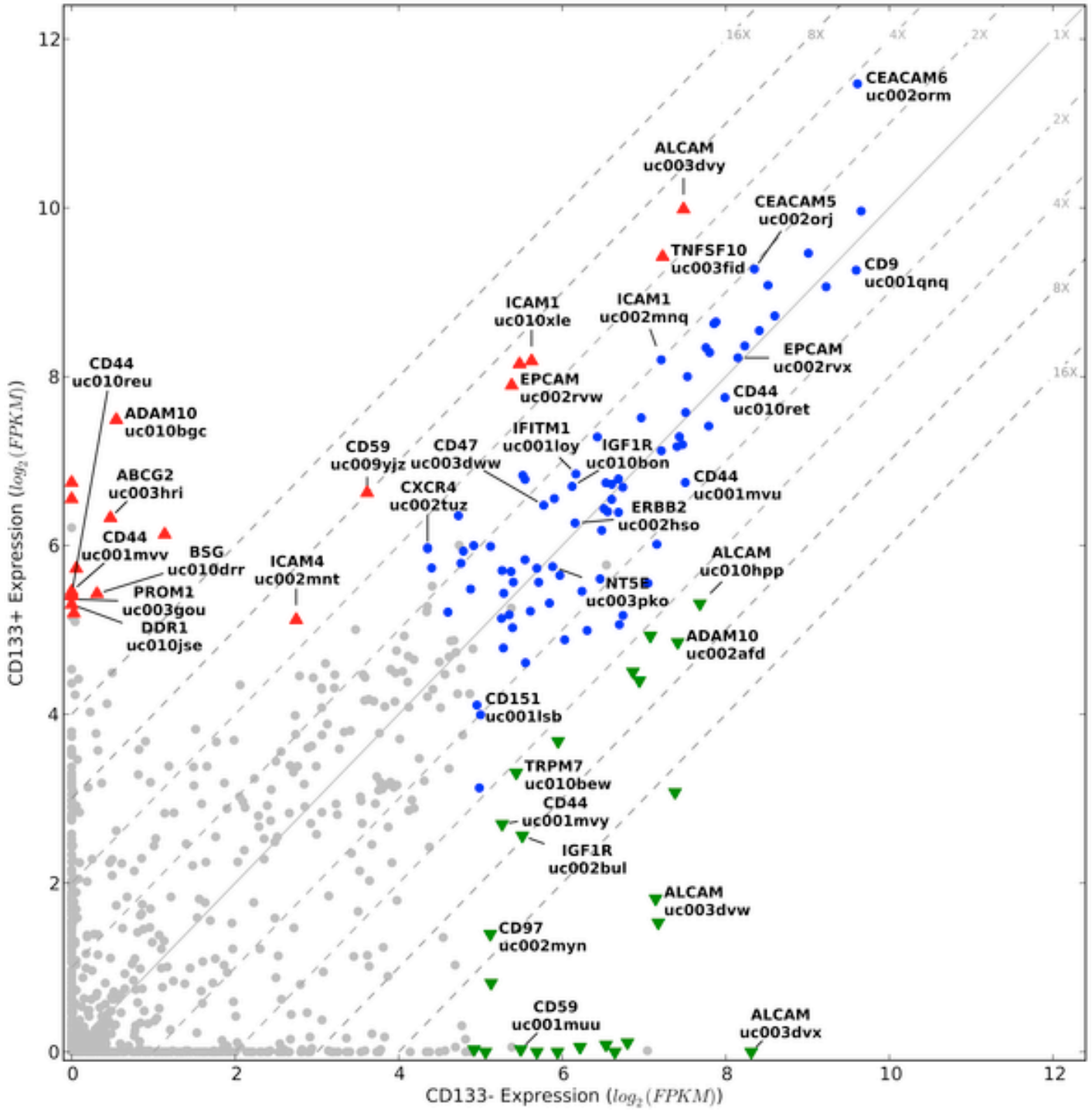
Per position or for fixed sequence lengths (e.g. amplicons):

RPM = Number of mapped reads (per position or per sequence)/Total number of mapped reads (in million)

For features of differing lengths (e.g. mRNAs):

RPKM = Number of mapped single-end reads (per feature)/Length of feature (in kbp)/Total number of mapped reads (in million)

FPKM = Number of mapped paired-end sequenced fragments (per feature)/Length of feature (in kbp)/ Total number of mapped reads (in million)



Transcriptome Sequencing of Tumor Subpopulations Reveals a Spectrum of Therapeutic Options for Squamous Cell Lung Cancer

Barrett et al., PLOS ONE 2013

Alzheimer's and miRNA

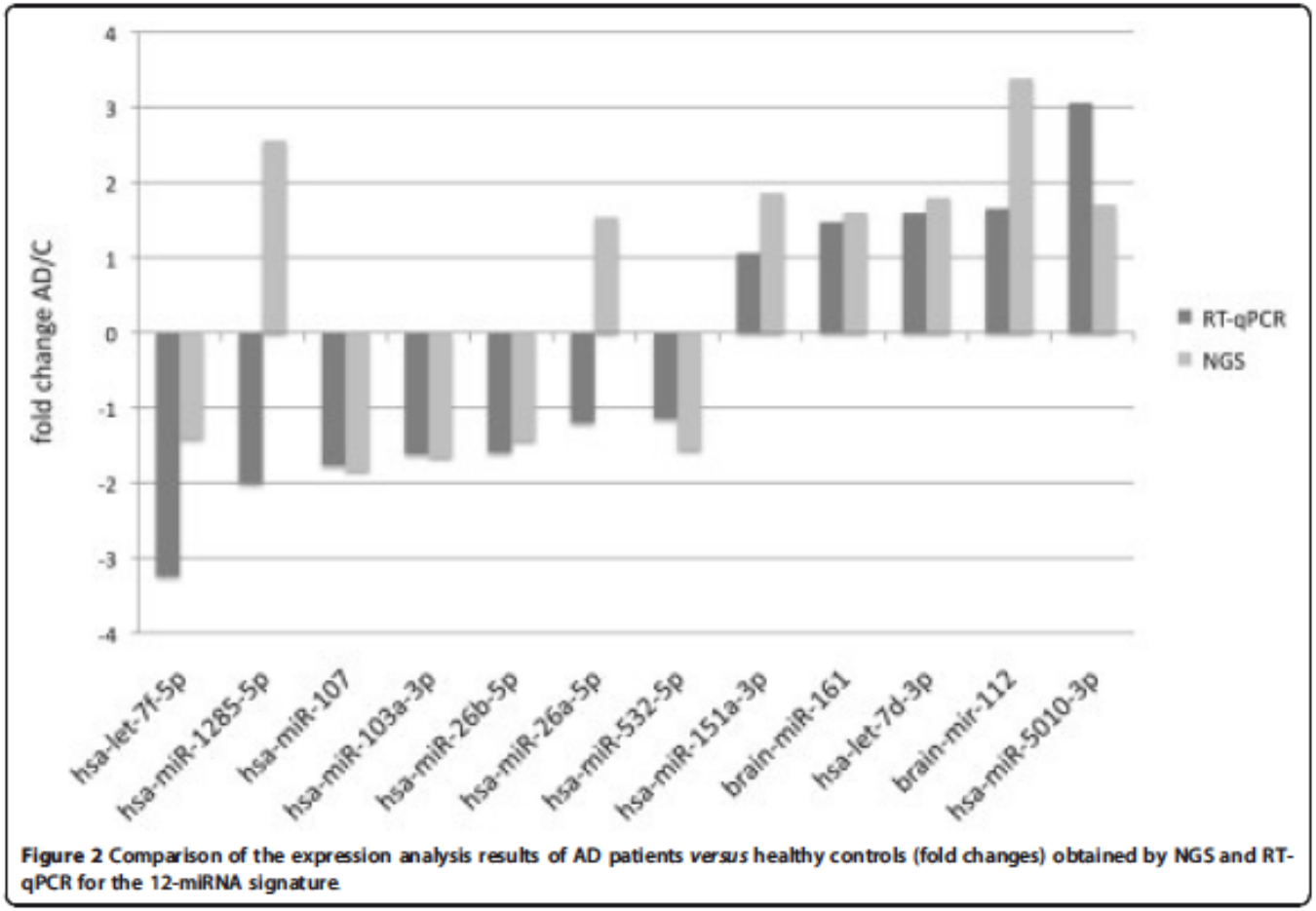


Figure 2 Comparison of the expression analysis results of AD patients *versus* healthy controls (fold changes) obtained by NGS and RT-qPCR for the 12-miRNA signature.

Leidinger et al., Genome Biol 2013