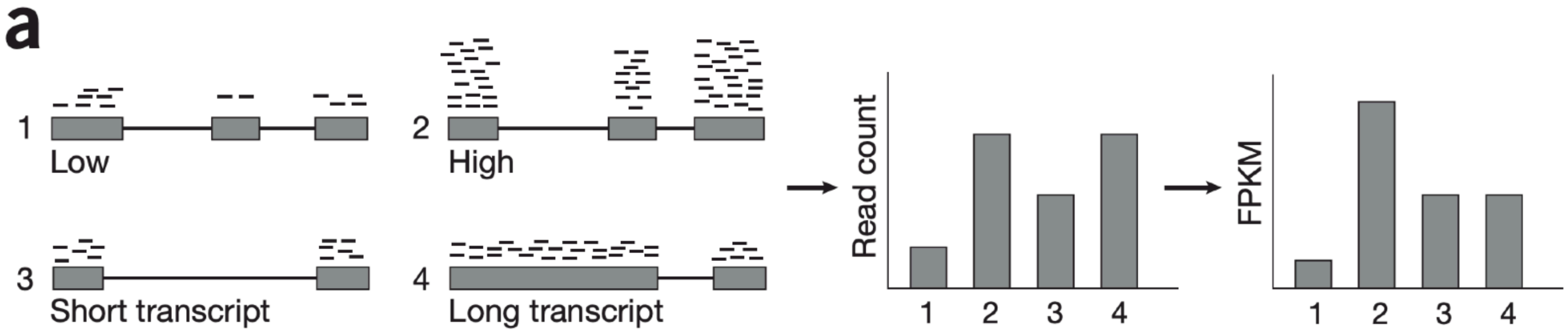
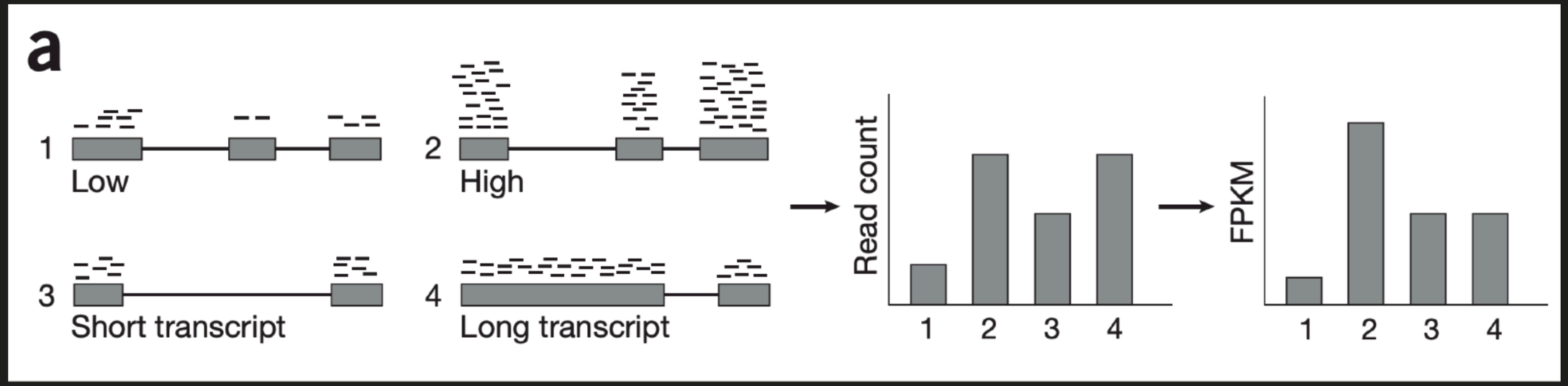

a
1
Low
2
High
3
Short transcript
4
Long transcript
Read count
1
2
3
4
FPKM
1
2
3
4

Measuring gene expression with NGS



The number of reads from a transcript is proportional to its abundance.

With random RT primers, you also need to correct for transcript length.

Garber, Grabherr, Guttman, & Trapnell, *Nature Methods* 2011

PRIMER

How to map billions of short reads onto genomes

Cole Trapnell & Steven L Salzberg

Mapping the vast quantities of short sequence fragments produced by next-generation sequencing platforms is a challenge. What programs are available and how do they work?

A new generation of DNA sequencers that can rapidly and inexpensively sequence billions of bases is transforming genomic science. These new machines are quickly becoming the technology of choice for whole-genome sequencing and for a variety of sequencing-based assays, including gene expression, DNA-protein interaction, human resequencing and RNA splicing studies[1–3]. For example, the RNA-Seq protocol, in which processed mRNA is converted to cDNA and sequenced, is enabling the identification of previously unknown genes and alter-

Table 1 A selection of short-read analysis software

Program	Website	Open source?	Handles ABI color space?	Maximum read length
Bowtie	http://bowtie.cbcb.umd.edu	Yes	No	None
BWA	http://maq.sourceforge.net/bwa-man.shtml	Yes	Yes	None
Maq	http://maq.sourceforge.net	Yes	Yes	127
Mosaik	http://bioinformatics.bc.edu/marthlab/Mosaik	No	Yes	None
Novoalign	http://www.novocraft.com	No	No	None
SOAP2	http://soap.genomics.org.cn	No	No	60
ZOOM	http://www.bioinfor.com	No	Yes	240