

Exercise sheet 9: Data Driven Life Sciences

Exercise 1

Question 1A Arrange the following terms into their correct order in the Illumina sequencing method and describe each of them briefly:

- bridge amplification
- deblocking
- library preparation
- annealing of template strands to flow cell
- fluorescence detection

Solution 1. Library preparation:

A sequencing *library* gets *prepared* from a sample by fragmenting the original DNA and adding Illumina-specific adapter sequences to both ends of the fragments. The *library* is what gets read during sequencing.

2. Template strand annealing

The single-stranded library fragments are used as *template strands* in the sequencing and are *annealed* to primer sequences, which are bound to the *flow cell* and are complementary to the adapter sequences of the fragments.

3. Bridge amplification

After complementary strands have been synthesized and the templates been washed off, the now flow cell-bound fragments are *amplified* in several cycles of so-called *bridge-amplification* to form fragment colonies, or *clusters* on the flow cell to guarantee a detectable fluorescence signal during sequencing.

4. Fluorescence detection

Illumina-sequencing is a form of *sequencing-by-synthesis* in which the nucleotides incorporated into the growing strand are detected via attached *fluorophores*. After the first 3 steps, the following steps are iterated to sequence the entire read:

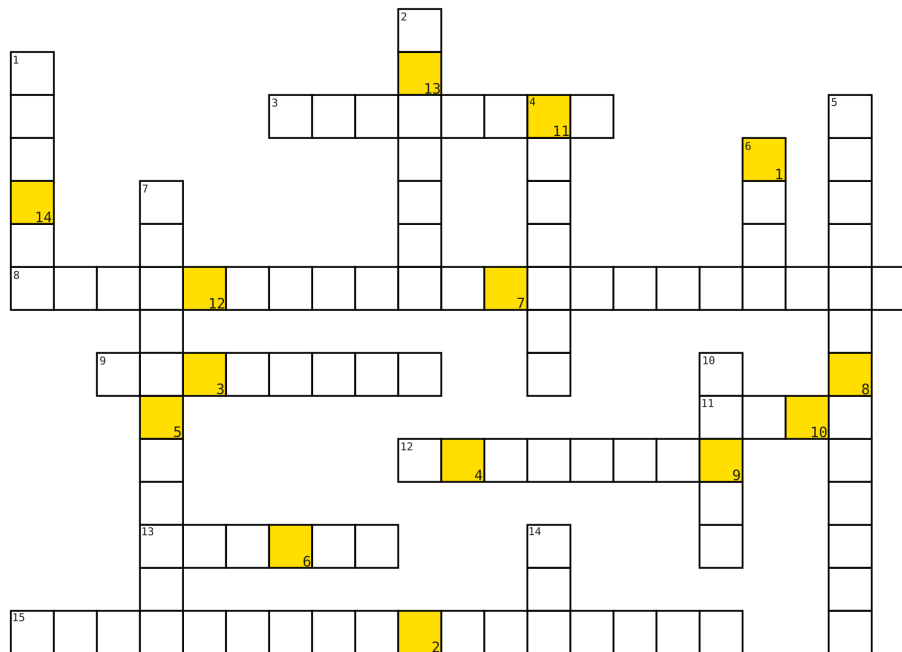
Modified nucleotides, containing a fluorescent group, are used to extend the strand, their blocking groups are cleaved from their 3'-OH groups.

5. Deblocking

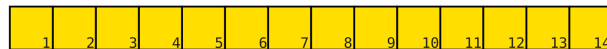
Deblocking is the removal of the fluorophore (blocking group). It is necessary before a new round of elongation by one nucleotide can begin.

More information about this topic can be found on the Illumina Webpage.

Exercise 2



Solution:



Question 2A Solve the crossword puzzle!

Horizontal:

- 3. Added to DNA fragments during library preparation.
- 8. Illumina way of determining the order of nucleotides in a DNA strand. (3 words)
- 9. ChIP-Seq can be used for sequencing DNA regions that are bound by these.
- 11. The alphabet of life.
- 12. Formed by bridge-amplification on Illumina flow-cells.
- 13. Flowcell surface filled with these 2 different DNA molecules.
- 15. Measure to assess the quality of the identification of nucleobases generated by automated DNA sequencing. (3 words)

Vertical:

- 1. Dideoxynucleosidetriphosphates (abbrev.)
- 2. Process of determining positions of reads on the reference genome.
- 4. Gene expression can be measured using this. (abbrev. hyp.)
- 5. The process of making many copies of a piece of DNA.
- 6. Found in pairs in DNA.

