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* iswhat 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:

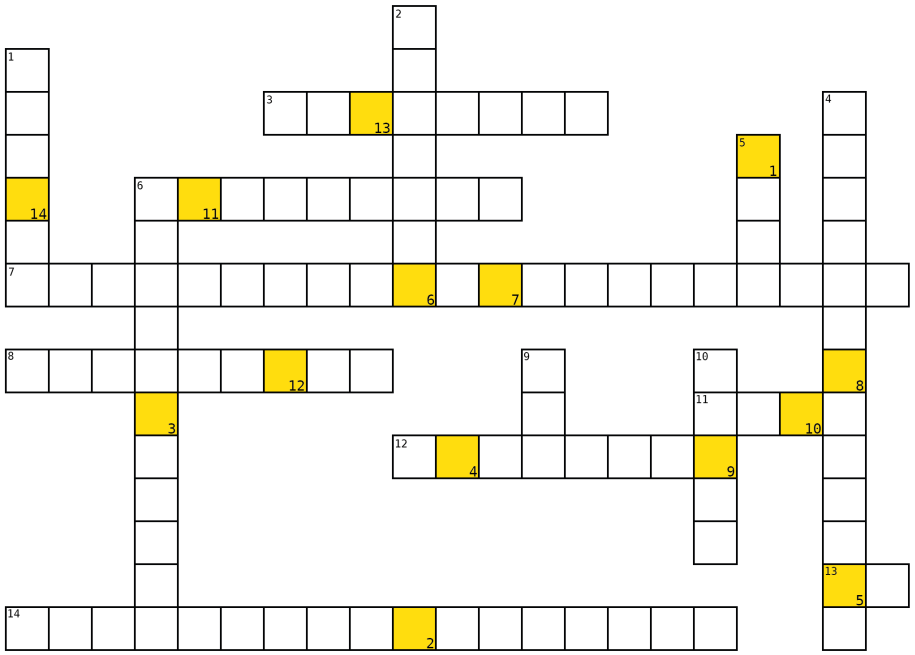
Modified nucleotides, containing a fluorescent group, are used to extend the strand, their blocking groups are cleaved from their 3‘-OH groups.

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:

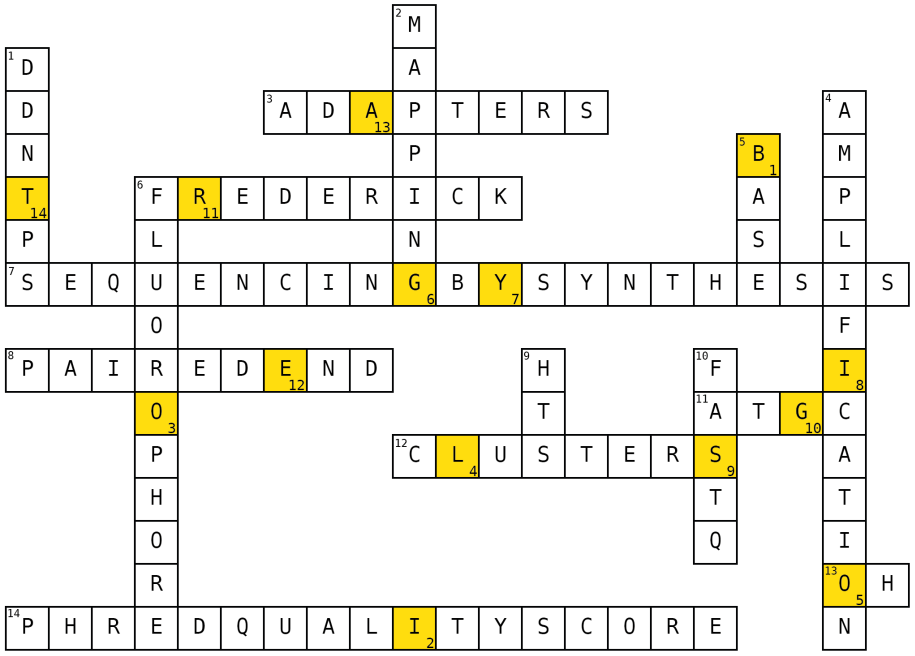
3. Added to DNA fragments during library preparation.
4. Has a sequencing method named after himself (first name)
5. Illumina/Pacbio way of determining the order of nucleotides in a DNA strand. (3 words)
6. More than just single-end.
7. The alphabet of life.
8. Formed by bridge-amplification on Illumina flow-cells.
9. Keeps nucleic acid synthesis going.
10. Measure to asses 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.
3. The process of making many copies of a piece of DNA.
4. Found in pairs in DNA.

5. Chemical group attached to nucleotides to monitor incorporation into DNA.
6. Breakthrough sequencing method (abbrev.)
7. File format used to store sequence information.

Solution



Solution:

Exercise 3

Question 3A You want to determine how many reads N are needed to achieve a coverage depth C of 20X when sequencing reads for *Escherichia coli*.

The length of the reads L is 30nt and the *E. coli* genome G is approximately 4.6 million bases long.

Formula

$$N = \frac{C \times G}{L}$$

Formula

$$N = \frac{20 \times 4600000}{30} \approx 3066667 \text{ reads}$$