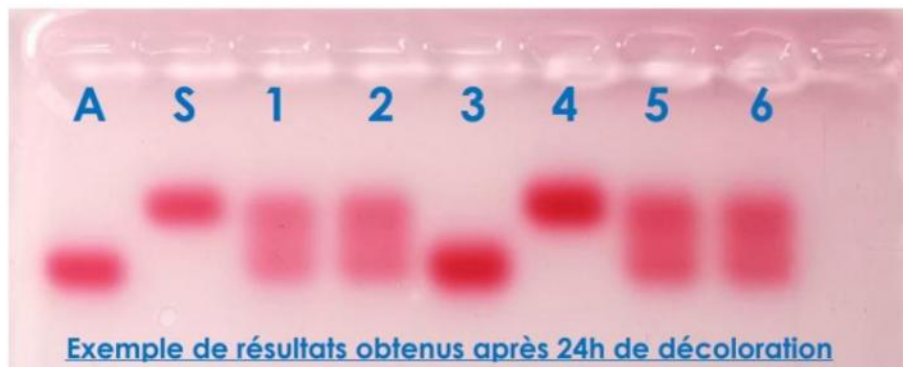


Reference : ELECPROT



Electrophoresis of proteins

Handling and results in 1h15

Comprendre les relations de dominance et de récessivité sur les gènes de la globine

Cognitive Objectives:

Hemoglobin electrophoresis from normal and sickle cell individuals is used to identify hemoglobins A and S which allows for genotyping and study of genotype-phenotype relationships.

Characterization of molecular phenotypes and identification of genotypes by hemoglobin electrophoresis.

Mutations that confer a selective advantage have a high probability of spreading in the population:

The approach is identical to that of the first year, but in the final year the aim is to link genetics and evolution.

Proposed experiment:

Agarose gel electrophoresis of hemoglobin A, hemoglobin S and a mixture of the two.

The abnormal hemoglobin, S (sickle), differs from the normal hemoglobin, A, by only one amino acid in the beta chain at position 6 (glutamic acid replaced by valine). The two alleles differ by only one codon.

The two hemoglobins can be distinguished by their electrophoretic mobility. By applying the technique to the hemoglobin extracted from the red blood cells of an individual, one can therefore determine which hemoglobin(s) he possesses and deduce his genotype: heterozygous, HbA/HbS or homozygous HbA/HbA or HbS/HbS.

Protein migration is visible even without staining, so students can see their result immediately at the end of the migration.

The visualization is clearer after staining and then decolourization with ponceau red (minimum total time: 30 min).

Electrophoresis on agarose gel: migration time: 40 min with the BLUEGEL cell; 30 min with standard cells at 160V

The electrophoresis is done during a 1H30 session.

Composition (for 20 pairs) :

- 1 g of agarose (QSP 100 ml of 1% gels of 3 mm thickness)
- 0.3 ml of hemoglobin A and S solution (2.5 mg/ml)
- 1 dose per 1 L of migration buffer (tris-glycine)
- 500 ml of ponceau red
- 5% ethanoic acid
- 10X deposition blue
- Technical and pedagogical manual available on our website.

Required equipment:

BLUEGEL electrophoresis tank (allows to make 3mm gels, and 2mm wells)

Micropipettes 2-20 μ L

Storage : 6 months

Hemoglobin solutions: freezer