**Borrelia burgdorferi** is the causal agent of the Lyme disease or Lyme borreliosis, which is the most common tick-borne disease in North America and Europe. Nowadays, there is not an effective vaccine to prevent the infection. Without using antibiotics and even with them, sometimes the spirochetes can spread into the body and cause a late phase where organs as brain, eyes, articulations, among others, can be affected.

In this project it is described a method for the generation of B. burgdorferi attenuated strains for using as potential vaccine candidates.

### Objectives

To design a method to develop an attenuated *B. burgdorferi* strain for being used as a vaccine against the Lyme disease.

### Benefits

- The development of a vaccine against the Lyme borreliosis is, for itself, a benefit since there is not an effective vaccine.
- Attenuated vaccines can frequently produce a better immunogenic response.
- It has been considered the type of immune response produced by the attenuated mutants.

### Materials and methods

#### Transposon mutagenesis

The suicide vector pMarGent (Fig. 2) used by Stewart et al. [1] can be used in this project. In this vector, the transposase Himar1 and a hyperactive C9 transposase (to increase the frequency of transposition) are under control of the borreliial promoter fgly, which is fused with a gentamicin marker (fglyaac3C). Thanks to transpositions in different sites at the genome of Borella, some of the mutants obtained will have deleted essential genes for virulence.

As it is shown in Figure 3, the strains can be transfected by electroporation and then the transfectants should be plated in presence of gentamicin. The presence of the vector in the B. burgdorferi colonies grown in selective media can be confirmed by PCR of the aacC1 cassette region. Those positive colonies should be cultured and then it would be necessary to identify the precise insertion sites of the transposon in different mutants to identify the missing genes.

It would be interesting to analyze if isolated strains after transposon mutagenesis have lost some plasmids required for full infectivity. Those mutants which have lost some of these plasmids will be discarded for the next phases of the project.

### Expected results

#### Infectivity study

- **Attenuated:** Present in 0/4 or in 1/4 tissues
- **Infectious:** Mutants present in 2/4, 3/4 or 4/4 tissues

#### ELISA

- **Presence of antibodies in blood mice:** Predomination of the type 2 cytokine IL-4
- **Absence of antibodies in blood mice:** Predomination of cytokines IL-17 and IFN-γ

#### ELISpot

- **Presence of antibodies in blood mice:** Predomination of the type 2 cytokine IL-4
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4. Botkin et al. [1] can be used in this project. In this vector, the transposase Himar1 and a hyperactive C9 transposase (to increase the frequency of transposition) are under control of the borreliial promoter fgly, which is fused with a gentamicin marker (fglyaac3C).
5. The presence of the vector in the B. burgdorferi colonies grown in selective media can be confirmed by PCR of the aacC1 cassette region. Those positive colonies should be cultured and then it would be necessary to identify the precise insertion sites of the transposon in different mutants to identify the missing genes.
6. The transposon mutagenesis system for *B. burgdorferi* Suicide vector pMarGent is electroporated into competent *B. burgdorferi* cells allowing transient expression of the Himar1 transposase (1). After transposition, the Himar1 gene remains on a DNA fragment that is presumably degraded by intracellular nucleases (2). Mutants are selected in the presence of gentamicin, and those wild-derived phenotypes were transferred to liquid culture for DNA isolation. The *B. burgdorferi* DNA flanking the transposon insertion site is recovered by digestion with HindIII, an enzyme that does not cut within the transposon (3 and 4), self-ligation, and transformation into *E. coli* (5). Purified plasmid DNA was then isolated from *E. coli* clones and sequenced.
7. The transposon mutants can be divided into different infectivity phenotypes according to spirochete isolation from the tissues examined.
8. The transposon mutagenesis system for *B. burgdorferi* Suicide vector pMarGent is electroporated into competent *B. burgdorferi* cells allowing transient expression of the Himar1 transposase (1). After transposition, the Himar1 gene remains on a DNA fragment that is presumably degraded by intracellular nucleases (2). Mutants are selected in the presence of gentamicin, and those wild-derived phenotypes were transferred to liquid culture for DNA isolation. The *B. burgdorferi* DNA flanking the transposon insertion site is recovered by digestion with HindIII, an enzyme that does not cut within the transposon (3 and 4), self-ligation, and transformation into *E. coli* (5). Purified plasmid DNA was then isolated from *E. coli* clones and sequenced.
9. The transposon mutants can be divided into different infectivity phenotypes according to spirochete isolation from the tissues examined.