

## Sequencing protocol – In brief test LymphoSign Illumina MiSeq system

### 1. Dilutions and denaturation

#### Reagents:

- Illumina sequencing reagents

#### Steps :

- Dilute each library at 2-4 nM concentration
  - Pool libraries in equivolume
- If other libraries are sequenced, adjust pool concentrations and then combine them
- Denature and dilute final pool at 8-10 pM loading concentration

### 2. Preparation of sequence primers

#### Reagents:

- Primers **GEP-SP-001**
- Illumina sequencing reagents

If only pool of LymphoSign libraries

- Dilute primers
    - Amorce **GEP-SP-001** (3 µL)
    - HT1 Buffer (597 µL)
  - Load 600 µL into reservoir #18 of the flowcell
- If pool of LymphoSign libraries is associated with other libraries
- Pipet 600 µL from reservoir #12
  - Add 3 µL of primer (**GEP-SP-001**)
  - Load all volume into reservoir #18 of the flowcell

### 3. Preparation of the injection sheet

- If the GENEXPATH LymphoSign library is sequenced alone, create the injection sheet to generate the FASTQs, providing for 120 cycles in read 1.
- If the GENEXPATH LymphoSign libraries are combined with other sequencing libraries, generate the injection sheet using the usual parameters, without entering the GENEXPATH LymphoSign samples.
- Specify the use of custom during run setup (With Local Run Manager, on the Create Run page. In manual run mode, on the Run Setup screen).



**In all cases, ensure that reading in read 1 is done with a minimum of 120 cycles and that the Custom Primer for Read 1 box is selected.**

### 4. Sequencing start

Initiate sequencing following the procedure described in the MiSeq System Illumina Guide.



**This rapid protocol completes the notice. It does not dispense with the complete reading of the notice.**