In Vitro protocol in brief
test LymphoSign

1. Reverse transcription

Reagents:

- ☐ Kit M-MLV RT
- ☐ dNTPs (10mM)
- ☐ Hexamers (100µM)
- ☐ ARN to be tested

Steps :

- ☐ Thaw the reagents
- ☐ Prepare reverse transcription mix
  - RT buffer (1,25 µL)
  - DTT (0,5 µL)
  - dNTPs (1 µL)
  - Hexamers (1 µL)
- ☐ Deliver 3,75 µL mix per tube
- ☐ Add 2 µL of RNA sample
- ☐ Vortex and centrifuge
- ☐ Put samples in thermocycler and start program 1 step 1a
- ☐ Add 0,5 µL of M-MLV RT
- ☐ Centrifuge
- ☐ Put samples in thermocycler and start program 1 step 1b
- ☐ Once the program is over and the block cool down at 4°C, put out tubes
- ☐ Put tubes on ice or cooling block

Then proceed to step 2 or store cDNA products between -30°C et -15°C.

2. Hybridization of probes

Reagents:

- ☐ SALSA MLPA Buffer
- ☐ **GEP- LSPM** probe Mix

- ☐ Thaw reagents
- ☐ Prepare Hybridization mix
  - Salsa MLPA Buffer (1,5 µL)
  - **GEP-LSPM** Probe Mix (1,5 µL)
- ☐ Vortex and centrifuge
- ☐ Deliver 3 µL of mix per cDNA tubes
- ☐ Centrifuge
- ☐ Put samples in thermocycler and start program 1 step 2

3. Ligation

Reagents:

- ☐ SALSA Ligase Buffer A
- ☐ SALSA Ligase Buffer B
- ☐ SALSA Ligase 65
- ☐ Nuclease free water

- ☐ Thaw reagents
- ☐ Prepare ligation mix
  - Nuclease free water (25 µL)
  - Salsa Ligase Buffer A (3 µL)
  - Salsa Ligase Buffer B (3 µL)
- ☐ Vortex and centrifuge
  - Salsa Ligase 65 (1 µL)
- ☐ After 1h of hybridization, deliver 32 µL of mix per cDNA tubes
- ☐ Start program 1 étape 3

Then proceed directly to step 4 or store the ligation products between -30°C and -15°C.

After this step, do not store the products at high temperature (e.g. 4°C or at room temperature) in order to avoid non-specific ligations which could result from a residual activity of the enzyme.

4. Amplification and incorporation of barcodes and adaptators

Reagents:

- ☐ **GEP-BC-xxx** Barcodes
- ☐ Q5 MasterMix
- ☐ Nuclease free water

- ☐ Thaw the reagents
- ☐ Prepare PCR mix
  - Q5 MasterMix (12,5 µL)
  - Nuclease free water (5,5 µL)
- ☐ Vortex and centrifuge
- ☐ Deliver 18 µL of mix per wells of PCR plate
- ☐ Add 5 µL of ligation product in each well
- ☐ Add 2 µL of barcode (**GEP-BC-xxx**) in each well

Use different barcodes BEP-BC-xxx for each tested samples.

- ☐ Put samples in thermocycler and start program 2

Then proceed directly to step 5 or store the PCR products between -30°C and-15°C.

Do not store these products for prolonged periods at high temperature (e.g. 4°C in the thermocycler or at room temperature).

5. Purification and dosage sequencing librairies

Reagents:

- ☐ 100% Ethanol
- ☐ Nuclease free water
- ☐ AMPure XP Magnetic beads
- ☐ TE Buffer
- ☐ Qubit dsDNA HS Assay

Steps :

Ensure beads are complety re-suspended before use.

- ☐ Purify 25 µL products with 45 µL AMPure XP
- ☐ Elute PCR products in 50 µL of TE buffer

After purification, librairies can be stored between -30°C et -15°C before sequencing.

- ☐ Dose 10 µL of each library by fluorimetry

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

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.

Sequencing protocol – in brief
Test LymphoSign
Illumina NextSeq System

1. Dilutions et denaturation

Reagents:
- ☐ Illumina sequencing reagents

Steps :
- ☐ Dilute each library at 0.5-2 nM concentration
- ☐ Pool libraries in equivolume

If other libraries are sequenced, adjust pool concentrations and then combine them
- ☐ Denature and dilute final pool at 0.8-1 pM loading concentration

2. Preparation of the sequence primers

Reagents:
- ☐ **GEP-SP-001** primer
- ☐ Illumina sequencing reagents

Steps :

If only pool of LymphoSign libraries
- ☐ Dilute primers
  - **GEP-SP-001** primer (6 µL)
  - HT1 Buffer (1994 µL)
  - Load 2000 µL into reservoir #7 of the flowcell

If pool of LymphoSign libraries is associated with other libraries
- ☐ Pipet 2000 µL from reservoir #20
- ☐ Add 6 µL of primer (**GEP-SP-001**)
- ☐ Load all volume into reservoir #7 of the flowcell

3. Préparation of the injection sheet

4. If the GENEXPATH LymphoSign library is sequenced alone, create the injection sheet to generate the FASTQs, providing for 120 cycles in read 1.

5. 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.

6. 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.

7. Sequencing start

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

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