Sequencing protocol – in brief
test SarcomaFusion
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:
- ☐ **GEP-SP-001** primer
- ☐ **GEP-SP-002** primer
- ☐ Illumina sequencing reagents

Steps :

If only pool of SarcomaFusion libraries

- ☐ Dilute primers
  - **GEP-SP-001** (3 µL)
  - **GEP-SP-002** (3 µL)
  - HT1 Buffer (594 µL)
- ☐ Load 600 µL into reservoir #18 of the flowcell

If pool of SarcomaFusion libraries is associated with others libraries

- ☐ Pipet 600 µL from reservoir #12
- ☐ Add
  - **GEP-SP-001** (3 µL)
  - **GEP-SP-002** (3 µL)
- ☐ Load all volume into reservoir #18 of the flowcell

3. Preparation of the injection sheet

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

- If the GENEXPATH SarcomaFusion libraries are combined with other sequencing libraries, generate the injection sheet using the usual parameters, without entering the GENEXPATH SarcomaFusion 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.