In vitro protocol – in brief
test SarcomaFusion

1. Reverse Transcription

Reagents:

- ☐ 5X Vilo reaction mix
- ☐ 10X super script
- ☐ Nuclease free water
- ☐ RNA samples

Steps :

- ☐ Traw reagents
- ☐ Prepare reverse transcription mix
  - 5X Vilo reaction mix (1 µL)
  - Nuclease free water (1 µL)
  - 10X super script (0,5 µL)
- ☐ Deliver 2,5 µL of mix per tube
- ☐ Add 2,5 µL of RNA sample
- ☐ Vortex and centrifuge
- ☐ Put samples in thermocycler and start program 1 step 1
- ☐ Once the program is over and the block cool down at 4°C, put out tubes
- ☐ Centrifuge
- ☐ Put tubes on ice or cooling block

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

2. Hybridization of probes

Reagents:

- ☐ SALSA MLPA Buffer
- ☐ **GEP- SFPM** Probe Mix

Steps :

- ☐ Traw reagents
- ☐ Prepare hybridization mix
  - Salsa MLPA Buffer (1,5 µL)
  - **GEP- SFPM** 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

Steps :

- ☐ Traw 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 step 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 temperatures (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
- ☐ **GEP-BCC-xxx** GAPDH Barcodes
- ☐ Red'y' Star PCR Mix
- ☐ Nuclease free water

Steps :

- ☐ Traw reagents
- ☐ Prepare PCR mix
  - Red'y' Star PCR Mix (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** or **GEP-BCC-xxx** following the test) 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 temperatures (e.g. 4°C in the thermocycler or at room temperature).

5. Purification and dosage of sequencing libraries

Reagents:

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

Steps :

Ensure beads are completely re-suspended before use.

- ☐ Purify 25 µL of PCR products with 45 µL AMPure XP
- ☐ Add 50 µL of TE buffer to each well of the reaction plate

After purification, libraries can be stored between -30°C and -15°C before sequencing.

- ☐ Dose 10 µL of each library by fluorimetry

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