In Vitro protocol in brief
CustomPanel/ThemaPanel tests

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- CPPM** probe Mix

- ☐ Thaw reagents
- ☐ Prepare Hybridization mix

  Salsa MLPA Buffer (1,5 µL)
  GEP-CPPM Probe Mix (1,5 µL)
- ☐ Vortex and centrifuge
- ☐ Deliver 3 µL of mix per cDNA tubes
- ☐ Centrifuge
- ☐ Put samples in thermocycler and start program1 step2

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 the complete reading of the notice.