MASTR Workflow

Plan run

1

Multiplex PCR

2

Quality Control

3

Universal PCR

4

Dilution step & Universal PCR

5

Quality Control

6

Equimolar pooling

7

Purification

8

NGS

9

Data analysis

10

Microfluidic-based method

2 % Agarose

/microfluidic-based method

DNA sample

Sequencing sample

GeneScan pattern on ABI capillary sequencer

CNV Calculator

CNV

MASTR Reporter

CNV

Tagged amplicon library

Purified tagged amplicon library

Purification

DNA sample

NGS (Sequencer)

Equimolar pooling

Purification

NGS

Data analysis

Plan run

Mixing plexes of single sample

Tagged amplicon library

Purified tagged amplicon library

Sequencing sample

NGS

Mixing plexes of single sample

Purification

Purified tagged amplicon library

Sequencing sample

NGS

Mixing plexes of single sample

Purification

Purified tagged amplicon library

Sequencing sample

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NGS

Mixing plexes of single sample

Purification

Purified tagged amplicon library

Sequencing sample

NGS
<table>
<thead>
<tr>
<th>N°</th>
<th>Information</th>
<th>Turn-around time (* hands-on time)</th>
<th>SFP</th>
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</thead>
</table>
| 1  | • Quality Control extracted DNA  
• DQC Calculator: advised for FFPE-derived DNA, based on outcome Multiplicom QC Plex  
• Sequencing Calculator: schedule samples to combine in one sequencing run | ~30 min*  
2h 15 min |  |
| 2  | • Prepare Multiplex master mix per plex (n plexes) using MASTR kit  
• Add DNA: 20-50 ng per plex (HMW) or according to QC Plex output (FFPE)  
• Run PCR | ~30 min*  
2h 15 min | Yes |
| 3  | • Recommended for germline MASTR assays only | ~30 min* |  |
| 4  | • Prepare one Universal master mix per sample (n plexes) using MID kit  
• Add diluted n plexes per sample  
• Run PCR | ~30 min*  
~15 min*  
2h 15 min | Yes |
| 5  | • Recommended for all MASTR assays | ~30 min* |  |
| 6  | • Mix all plexes per sample (predefined mixing scheme) to obtain complete single-tube tagged amplicon library  
• Tagged amplicon library still contains small residual DNA fragments (dNTPs, primers/primer dimer) | ~15 min* | Yes |
| 7  | • Add Agencourt AMPure XP magnetic beads to tagged amplicon library  
• Purify and elute with water | ~45 min  
(30 min*) | Yes |
| 8  | • Measure concentration of each purified tagged amplicon library  
• Note that for assays utilizing Amplification Reagent 3 (AR3) spectrophotometry needs to be used  
• Dilute libraries in TE and pool equimolarly to obtain single-tube sequencing sample | ~30 min* |  |
| 9  | • Prepare sequencing template according to NGS system manufacturer’s instructions  
• Sequence |  |  |
| 10 | • Multiplicom MASTR Reporter  
• Multiplicom CNV Calculator  
• Other |  |  |