# ULTRA-DEEP SEQUENCING 454™ (Roche Life Sciences)

## PCR and sequencing Primers

Note 1: The fusion primers used to generate amplicon libraries are each composed of four parts fused together: adaptator (in blue), key (in red), Multiplex identifiers (MIDs, in green) and template-specific sequences (in black).
Roche has designed a set of 151 MIDs. These are described in TCB No. 005-2009.
Note 2: Reverse Transcriptase sequencing by UltraDeep Sequencing 454™ is performed in 2 fragments.

### •Reverse Transcriptase:

**Outer primers**
MJ3: 5'-AGTAGGACCTACACCTGTCA-3' (2480 to 2499)
PL1M: 5'- CCTGCTTCTGTATTTCTGCTATTAAGTCTTTTG-3' (3514 to 3546)

**Fusion primers** (2 fragments)
**RT1:**
RT1F: 5'-CGTATCGCCTCCCTCGCGCCATCAGMIDGTACCAGTAAAATTAAAGCCAGGAATG-3' (2571 to 2597)
RT1R: 5'-CTATGCGCCTTGCCAGCCCGCTCAGMIDAAGCACATTGTACTGATATCTAATCCC-3' (2970 to 2996)
**RT2:**
RT2F: 5'-CGTATCGCCTCCCTCGCGCCATCAGMIDTTCAGGAAGTATACTGCATTTACCATAC-3' (2919 to 2946)
RT2R: 5'-CTATGCGCCTTGCCAGCCCGCTCAGMIDATTGACAGTCCAGCTGTCTTTT-3' (3293 to 3314)

### •Protease:

**Outer primers**
5' prot 1: 5'-TAATTTTTTAGGGAAGATCTGGCCTTCC-3' (2082 to 2109)
3' prot 1: 5'-GCAAATACTGGAGTATTGTATGGATTTTCAGG-3' (2703 to 2734)

**Fusion primers**
PR1F: 5'-CGTATCGCCTCCCTCGCGCCATCAGMIDTTCAGAGCAGACCAGAGCCAACAG-3' (2136 to 2158)
PR1R: 5'-CTATGCGCCTTGCCAGCCCGCTCAGMIDTTACTGGTACAGTTTCAATAGGACTAAT-3' (2553 to 2580)

### •Integrase:

**Outer primers**
INPS1: 5'- TAGTAGCCAGCTGTGATAAATGTC- 3' (4336 to 4359)
INPR8: 5'- TTCCATGTTCTAATCCTCATCCTG- 3' ( 5082 to 5105)

**Fusion primers**
IN1F: 5'-CGTATCGCCTCCCTCGCGCCATCAGMIDGAAGCCATGCATGGACAAG-3' (4371 to 4389)
IN1R: 5'-CTATGCGCCTTGCCAGCCCGCTCAGMIDCTGCCATCTGTTTTCCATARTC-3' (5037 to 5058)

## PCR runs on Eppendorf Mastercycler® ep Gradient S thermocycler (times have to be adapted to the thermocycler and taq polymerases used)

**•Conditions for RT and prot:**

**- RT-PCR Step, using RT-PCR kit Titan (Roche): Outer primers**
60’ at 50°C
5’ at 94°C
then: (30’’ at 94°C; 30” at 55°C; 1’ at 68°C) x 40 cycles
**- Nested PCR step, using Q5® High-Fidelity DNA polymerase (New England Biolabs®): Fusion primers**
1’ at 98°C
then (10’’ at 98°C; 30” at 54°C; 45’’ at 72°C) x 40 cycles
7’ at 72°C

**•Conditions for integrase:**

**- RT-PCR Step, using RT-PCR kit Titan (Roche): Outer primers**
60’ at 50°C
5’ at 94°C
then: (30’’ at 94°C; 30” at 55°C; 1’ at 68°C) x 40 cycles
**- Nested PCR step, using Q5® High-Fidelity DNA polymerase (New England Biolabs®): Fusion primers**
1’ at 98°C
then (10’’ at 98°C; 30” at 55°C; 45’’ at 72°C) x 40 cycles
7’ at 72°C

## Sequencing

Library purification/quantitation, emPCR amplification, DNA library bead enrichment and sequencing run are performed according to manufacturer recommendations.