Maastro LAB HIV test protocol

Testing of host cell lines before lenti-viral transduction.

To meet these requirements:

In een aantal IG vergunningen staat de aanvullende voorwaarde "*het te gebruiken gastheermateriaal moet vrij zijn van HIV-1, HIV-2, HTLV-1, HTLV-2, SIV en andere non-humane lentivirussen*.

PCR optimized by Prof dr. W Hendriks (Cell Biology, Radboudumc).

Positive controls (by A.J. Groot) to spike your genomic DNA:

The spike is a plasmid mix of TOPOpcr2.1 carrying these inserts:

HIV-1

TTTTGGTCCTTGTCTTATGTCCAGAATGCTGGTAGGGCTATACATTCTTACTATTTTATTTAATCCC
AGGATTATCCATCTTTTATAAATTTCTCCTACTGGGATAGGTGGATTAT

HIV-2

GGAGCACTCCGTCGTGGTTTGTTCCTGCCGCCCTTACTGCCTTCACTAAGCCGTGTCCCAAGACT
TCTCAGTCTTCTTCAAGTCCCTGTTCAGGCGCCAACCTGCTAGGGATTTTCCTGCCTCGGTTTCC
CAAAGCAAGAAGGGTCCTAACAGACCAGGGTCTTGTTACTCAGGTGAACACCGAATGACCAGGC
GGCGACTAGGAgAGATGGGAGCACACACTTAACTTGCTTCTAACTGGCAGCTTTATTAAGAGGTT
TTTAAGCAAGCAAGCGTGGGGCCGTCTGCCCAGCACCGGCCAAGTGCTGGTGAGAGTCTAGCA
GGGAACACCCAGGCTCTACCTGCTAGTGCTGGAGAGAACCTCCCAGG

SIV

TTGCAGTGCATGTAGCAGTGGATTCATATAAGCAGAGGTAATTCCACAAGAGACAGGAAGACAG
ACAGCACTATTTCTGTTAAAATTGGCAGGCAGATGGCCTATTACACATCTACACACAGATAATGG

HTLV

CCCTACAATCCAACCAGCTCAGGACTTGTARAACGCTCTAATGGCATTCTTAAAACCCTATTATAT
AAGTACTTTACTGACAAACCCGACCTACCCATGGATAATGCTCTATCCAGAGCCCTATGGACAAT
CAACCACCTGAATGTGTTAACCAACTGCCACAAAACCCGATGGCAAATCCATCAC

(30K copies per 5ul, since 100ng gDNA has 30K single gene copies)

Protocol for testing of 9 cell lines

In anatomy lab:
Sigma-Aldrich DNA gDNA isolation kit G1N350
Isolate DNA with kit from 2M cells, elute with elution buffer 200ul.

Prepare PCRs
Qiagen pcr taq polymerase 201205
Promega chemicals dNTP mix U1515

Control PCR after DNA isolation and input check:

hCARD15 (single copy gene)
Primerset:
Reference sequence: NT_010498 (amplicon = 123 bp; nucl. posities 4370686-4370809)
Annealingtemperature: 60°C

Dilute primer stock to 100ul work solution:

hCARD15-Fw : 5'- TCA GGT ACT CAC TGA CAC TGT CTG TT -3'	10ul
hCARD15-Rv: 5'- CAC CTC AAG CTC TGG TGA TCA C -3'	10ul
DEPC	80ul

10x reaction mix (50 µl per reaction):

50µl 10xPCR buffer
10ul primers
50µl 25 mM $MgCl_2$
12,5µl dNTPs (10 mM)
5 µl Taq Polymerase (5 U/µl)
0.5ul BSA (10mg/ml)
372.5µl DEPC

1 µl template gDNA

10x50ul per tube
Pipet neg control tube last (DEPC)
1µl gDNA per tube

PCR program hCARD15*:*
5' 94°C 39 x (50'' 94°C, 50'' 60°C, 45'' 72°C) 5' 72°C ∞ 4°C

Analyse:
3% agarosegel, so big casket)
Load at least 40ul of PCR products

HIV-1

Primerset:
Reference sequence: NC_001802 (amplicon = 114 bp; nucl. posities 1090-1204)
Annealingtemperature: 50°C

Dilute primer stock to 100ul work solution:

HIV1-Fw: 5'- ATA ATC CAC CTA TCC CAG TAG GAG AAA T -3'	10ul
HIV1-Rev: 5'- TTT GGT CCT TGT CTT ATG TCC AGA ATG C -3'	10ul
DEPC	80ul

20x reaction mix (50 µl per reaction):

100µl 10xPCR buffer
20µl primers
100µl 25 mM $MgCl_2$
25µl dNTPs (10 mM)
10µl Taq Polymerase (5 U/µl)
545µl DEPC
1ul BSA (10mg/ml)

Vortex and split

Samples	positive controls spiked
400ul	400ul
Add 100ul DEPC	
10x50ul per tube	10x40ul per tube

Pipet neg control tube last (DEPC)

1µl gDNA per tube 2x each sample (left and right) keep PCR strip tube separate.
In our lab add to the 9 samples 10ul per tube spike mix
Run
PCR program HIV_1:

5' 94°C	39 x (50'' 94°C, 50'' 50°C, 50'' 72°C)	5' 72°C	∞ 4°C

Analyse:
3-4% agarosegel (at least 3% run down all the way, so big casket)
Load at least 40ul of PCR products

HIV-2

Primerset:
Reference sequence: A05350 (amplicon = 369 bp; nucl. posities 33-402)
Annealingtemperature: 52°C

Dilute primer stock to 100ul work solution:

HIV2- Fw: 5'- CCT GRG AGG TTC TCT CCA GC -3'	20ul
HIV2-Rev: 5'- GGA GCW CTC CGT CGT GGT T -3'	20ul
DEPC	60ul

20x reaction mix (50 µl per reaction):

100µl 10xPCR buffer
20µl primers
100µl 25 mM $MgCl_2$
25µl dNTPs (10 mM)
10µl Taq Polymerase (5 U/µl)
645µl DEPC
1ul BSA (10mg/ml)

Vortex and split

Samples	positive controls spiked
450ul	450ul
Add 50ul DEPC	
10x50ul per tube	10x45ul per tube

Pipet neg control tube last (DEPC)

1µl gDNA per tube 2x each sample (left and right) keep PCR strip tube separate.
<u>In our lab</u> add to the 9 samples 5ul per tube spike mix

PCR program HIV_2:

5' 94°C	39 x (50'' 94°C, 50'' 52°C, 60'' 72°C)	5' 72°C	∞ 4°C

Analyse:
2-3% agarosegel (at least 2% run down all the way, so big casket)
Load at least 40ul of PCR products

HTLV

Primerset:
Reference sequence: NC_001436 (amplicon = 185 bp; nucl. posities 4384-4569)
Annealingtemperature: 60°C

Dilute primer stock to 100ul work solution:

HTLV-fw1: 5'- CCC TAC AAT CCA ACC AGC TCM G -3'	20ul
HTLV-fw2: 5'- CCT TAC AAT CCA ACC AGC TCA G -3'	10ul
HTLV-fw3: 5'- CCA TAC AAC CCC ACC AGC TCA G -3'	10ul
HTLV-rev1: 5'- GTG RTG GAT TTG CCA TCG GGT T -3'	20ul
HTLV-rev2: 5'- GTG GTG AAG CTG CCA TCG GGT T -3'	10ul
DEPC	30ul

20x reaction mix (50 µl per reaction):

100µl 10xPCR buffer
20µl primers
100µl 25 mM $MgCl_2$
25µl dNTPs (10 mM)
10µl Taq Polymerase (5 U/µl)
645µl DEPC
1ul BSA (10mg/ml)

Vortex and split

Samples	positive controls spiked
450ul	450ul

Add 50ul DEPC

10x50ul per tube	10x45ul per tube

Pipet neg control tube last (DEPC)

1µl gDNA per tube 2x each sample (left and right) keep PCR strip tube separate.
<u>In our lab</u> add to the 9 samples 5ul per tube spike mix

PCR programma HTLV*:*

5' 94°C	39 x (50'' 94°C, 50'' 60°C, 55'' 72°C)	5' 72°C	∞ 4°C

Analyse:
3-4% agarosegel (at least 3% run down all the way, so big casket)
Load at least 40ul of PCR products

SIV

Primerset:

Reference sequence: AY588945 (amplicon = 127 bp; nucl. posities 3856-3983), NC_001549, NC_004455

Annealingtemperature: 50°C

Dilute primer stock to 100ul work solution:

SIV-fw: 5'- GCA GT(A/C) CAT GTA GCI AGT (A/G)G -3'	40ul
SIV-rev: 5'- CCA TTA TC(T/A) GTG TGT AGA TG -3'	20ul
DEPC	40ul

20x reaction mix (50 µl per reaction):

100µl 10xPCR buffer
20µl primers
100µl 25 mM $MgCl_2$
25µl dNTPs (10 mM)
10µl Taq Polymerase (5 U/µl)
645µl DEPC
1ul BSA (10mg/ml)

Vortex and split

Samples	positive controls spiked
450ul	450ul
Add 50ul DEPC	
10x50ul per tube	10x45ul per tube

Pipet neg control tube last (DEPC)

1µl gDNA per tube 2x each sample (left and right) keep PCR strip tube separate.
In our lab add to the 9 samples 5ul per tube spike mix

PCR program SIV*:*

5' 94°C	39 x (50'' 94°C, 50'' 50°C, 45'' 72°C)	5' 72°C	∞ 4°C

Analyse:

3-4% agarosegel (at least 3% run down all the way, so big casket)
Load at least 40ul of PCR products