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CMV Resistance testing (UL54 and UL97)

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This protocol is a procedure for the study of antiviral resistance in Cytomegalovirus by NGS techniques.
The primers have been designed using **https://primalscheme.com/** with the intention of covering the most relevant regions of the UL54 and UL97 genes.

Troubleshooting

Before start

Take into account that the quality of the results is greatly affected by the time from sample extraction to DNA amplification.
The protocol may fail if the protocol is performed from refrigerated samples or DNA.

Prepare Reagents

1
- Q5® High-Fidelity DNA Polymerase (New England)
- Agarose gel 1%
- Ethanol 70%
- Mag-Bind® TotalPure NGS (omega)
- Elution Buffer

Set up primer pools (UL97 and UL54)

2 **UL97 Primers**

	UL97_4_LEFT	TGCGCGCGGAAAGTCAG	UL97_4_RIGHT	CGGCATAACAGATCTTGTGGC
	UL97_5_LEFT	CTCTGCGAGCTCTCTATCTCCT	UL97_5_RIGHT	AGCAGACAGCAGCCCGT
	UL97_6_LEFT	TGGCGAGCAACAGCAGC	UL97_6_RIGHT	GCGCGCATGATCTCGCT
	UL97_7_LEFT	TGCCACTTTGACATTACACCCA	UL97_7_RIGHT	TCCGACATGCAATAACGCCG
	UL97_8_LEFT	TTTCCGACCCATGCCGCT	UL97_8_RIGHT	ATGCTCGCCCAGGAGACAG
	UL97_9_LEFT	CATGGGTACGGAGGCGTTG	UL97_9_RIGHT	GGCCAACAGACGCTCCA

UL97 Primers

UL54 Primers

	UL54_1_LEFT	TGCAAAAACTTGTCCTTGCGC	UL54_1_RIGHT	ATTCTGTAACCACCGGCGTG
	UL54_2_LEFT	GTAGTTGCACACGGCCGAC	UL54_2_RIGHT	CGTCAATCTAACCTGCCGCA
	UL54_3_LEFT	CGTAAAAGACCCGATCCCCG	UL54_3_RIGHT	TCTCGCTGCTCTTTGAGGATC
	UL54_4_LEFT	CTTCATCGAGTGAGAGGCGC	UL54_4_RIGHT	AGGCTTTGGTGGCGCGT
	UL54_5_LEFT	GCTTGACGGGCTCCACAAAA	UL54_5_RIGHT	GGCGCGGTTCATCAAAGACA
	UL54_6_LEFT	TCCCGCGTTCCCACTACATA	UL54_6_RIGHT	CAACAAGTGGGTTTCGCAGC
	UL54_7_LEFT	ATACGGCGCACAGGGTCTT	UL54_7_RIGHT	GTGTTTGAGCCCGAGGTGG
	UL54_8_LEFT	AGTAGCAGAGGTTGTGAGCCA	UL54_8_RIGHT	GGTTCTGTGGCGGCTATGTT
	UL54_9_LEFT	AAACGCCGTCCTGACTCGA	UL54_9_RIGHT (V2)	CTTGCAATCTGCGCCGTC
	UL54_10_LEFT	GGATCTGCTGTCCGTCAAAGA	UL54_10_RIGHT	ATATTGCGGGTTCGGTGGTT
	UL54_11_LEFT	TGTTGAGCTTATAGTTGGGCGA	UL54_11_RIGHT	CGGCCTTTGTGACCGGTTAC

	UL54_12_LEFT	CCTTATACAGGTACTCGAGGCG	UL54_12_RIGHT	GTGCTACGAGACGGGAGGA
	UL54_13_LEFT	AAGTGCAGCCCCGACCAT	UL54_13_RIGHT	GGATCACCACGTTCGGCTG
	UL54_14_LEFT	CCTCGATATCACAAGTCGACGC	UL54_14_RIGHT	GGCGAACTAGTGCCCGAAC
	UL54_15_LEFT	CCGTACCCGTAGATGGAGGT	UL54_15_RIGHT	GGGACCTATTCGTTTTCACACCTA
	UL54_16_LEFT	ACGATAGCGCGGCGACA	UL54_16_RIGHT	CGGCGTCAGCGTTTGCA

UL54 Primers

2.1 Pool 1 UL97 (odd primers LEFT and RIGHT)
Pool 2 UL97(even primers LEFT and RIGHT)
Use 10 µL for each primer at [M] 10 micromolar (µM)

2.2 Pool 1 UL54 (odd primers LEFT and RIGHT)
Pool 2 UL54 (even primers LEFT and RIGHT)
Use 10 µL for each primer [M] 10 micromolar (µM) except:

- UL54_14_LEFT/RIGHT 14: 2,5 µl
- UL54_5_LEFT/RIGHT: 5 µl
- UL54_13_LEFT/RIGHT: 5 µl
- UL54_15_LEFT/RIGHT: 20 µl

DNA extraction

3 Perform DNA extraction with your method of choice. Preferably from a **plasma sample** collected on **the same day**.Perform DNA extraction with your method of choice. Preferably from a plasma sample collected on the same day.

The quality of the results is greatly affected by the time from sample extraction to DNA amplification.
The protocol may fail if the protocol is performed from frozen samples or DNA.

DNA amplification

4 For each pool and sample, mix the following reagents (two reactions per sample):

Reagent	Volume / sample (µl)
Primer Pool	2
Q5‰ Polymerase	0,25
Q5 Buffer	5
H20	5
dNTP	0.5
Sample DNA	12,5

PCR Mix for Pool 1 and 2

Set up the PCR with the following program

A	B	C
Cycles	Temperature	Time
1	98°C	30''
5	98°C	10''

	A	B	C
		65°C	3′
	30	98°C	10′′
		65°C	30′′
		72°C	2′
	1	72°C	2′
	1	4°C	∞

PCR program

Confirm amplification of aproximately 300bp fragments by **1% agarose gel**.

Product cleaning with Mag-Bind TotalPure NGS (omega)

5 Add 40 uL of beads to 20 uL of amplicon. Mix.

6 Incubate the mixture for 5 minutes at room temperature.

7 Place the tube in the magnet until the solution becomes clear.

8 Gently remove the supernatant by pipette.

9 Add 180 uL of 70% ethanol. Mix without breaking the pellet.

10 Gently discard the ethanol by pipette.

11 Incubate for 2 minutes at room temperature.

12 Remove any remaining ethanol. Note: The pellet must not be allowed to dry excessively. If it does occur, the pellet will appear black and cracked.

13 Remove the tube from the magnet.

14 Add 25 uL of EB. Mix. Note: do not break the pellet, just peel it away from the wall of the tube.

15 Incubate for 2 minutes at room temperature.

16 Place the tube back in the magnet until the solution clears.

17 Transfer 20 uL of the supernatant to a new tube.

NGS sequencing

18 Sequence the amplicons using the sequencer of choice according to the manufacturer's instructions.

Antiviral resistance

19 Generate consensus sequence from the amplicons using the bioinformatics procedure of choice.
Take into account that in cases of previous exposure to antivirals or prolonged treatment, minority variants may appear.
The website **http://cmv-resistance.ucl.ac.uk/herpesdrg/** is helpful for the study of resistance mutations.