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🌐 CMV Resistance testing (UL54 and UL97)

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We use this protocol and it's working

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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_L EFT	TGCGCGCGGAAA GTCAG	UL97_4_ RIGHT	CGGCATAACAGATC TTGTGGC
	UL97_5_LE FT	CTCTGCGAGCTCT CTATCTCCT	UL97_5_ RIGHT	AGCAGACAGCAGCC CGT
	UL97_6_LE FT	TGGCGAGCAACA GCAGC	UL97_6_ RIGHT	GCGCGCATGATCTC GCT
	UL97_7_LE FT	TGCCACTTTGACA TTACACCCA	UL97_7_R IGHT	TCCGACATGCAATA ACGCCG
	UL97_8_L EFT	TTTCCGACCCATG CCGCT	UL97_8_ RIGHT	ATGCTCGCCCAGGA GACAG
	UL97_9_LE FT	CATGGGTACGGAG GCGTTG	UL97_9_ RIGHT	GGCCAACAGACGC TCCA

UL97 Primers

UL54 Primers



	UL54_1_L EFT	TGCAAAACTTGT CCTTGCGC	UL54_1_ RIGHT	ATTCTGTAACCACC GGCGTG
	UL54_2_L EFT	GTAGTTGCACACG GCCGAC	UL54_2_ RIGHT	CGTCAATCTAACCT GCCGCA
	UL54_3_L EFT	CGTAAAAGACCCG ATCCCCG	UL54_3_ RIGHT	TCTCGCTGCTCTTT GAGGATC
	UL54_4_L EFT	CTTCATCGAGTGAG AGGCGC	UL54_4_ RIGHT	AGGCTTTGGTGGC GCGT
	UL54_5_L EFT	GCTTGACGGGCTC CACAAAA	UL54_5_ RIGHT	GGCGCGGTTTCATCA AAGACA
	UL54_6_L EFT	TCCCGCGTTCCCA CTACATA	UL54_6_ RIGHT	CAACAAGTGGGTTT CGCAGC
	UL54_7_L EFT	ATACGGCGCACAG GGTCTT	UL54_7_ RIGHT	GTGTTTGAGCCCGA GGTGG
	UL54_8_L EFT	AGTAGCAGAGGTT GTGAGCCA	UL54_8_ RIGHT	GGTTCTGTGGCGG CTATGTT
	UL54_9_L EFT	AAACGCCGTCCTG ACTCGA	UL54_9_ RIGHT (V2)	CTTGCAATCTGCGC CGTC
	UL54_10_ LEFT	GGATCTGCTGTCC GTCAAAGA	UL54_10_ RIGHT	ATATTGCGGGTTCG GTGGTT
	UL54_11_L EFT	TGTTGAGCTTATAG TTGGGCCGA	UL54_11_ RIGHT	CGGCCTTTGTGACC GGTTAC

	UL54_12_ LEFT	CCTTATACAGGTAC TCGAGGCG	UL54_12_ _RIGHT	GTGCTACGAGACGG GAGGA
	UL54_13_ LEFT	AAGTGCAGCCCCG ACCAT	UL54_13_ _RIGHT	GGATCACCACGTTC GGCTG
	UL54_14_ LEFT	CCTCGATATCACAA GTCGACGC	UL54_14_ _RIGHT	GGCGAACTAGTGC CCGAAC
	UL54_15_ LEFT	CCGTACCCGTAGAT GGAGGT	UL54_15_ _RIGHT	GGGACCTATTCGTT TTCACACCTA
	UL54_16_ LEFT	ACGATAGCGCGGC GACA	UL54_16_ _RIGHT	CGGCGTCAGCGTTT GCA

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 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 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 ¹⁰⁰⁰ Polymerase	0,25
Q5 Buffer	5
H2O	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 approximately 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.