Detection of low viral contamination on CHO cells culture using Next Generation Sequencing

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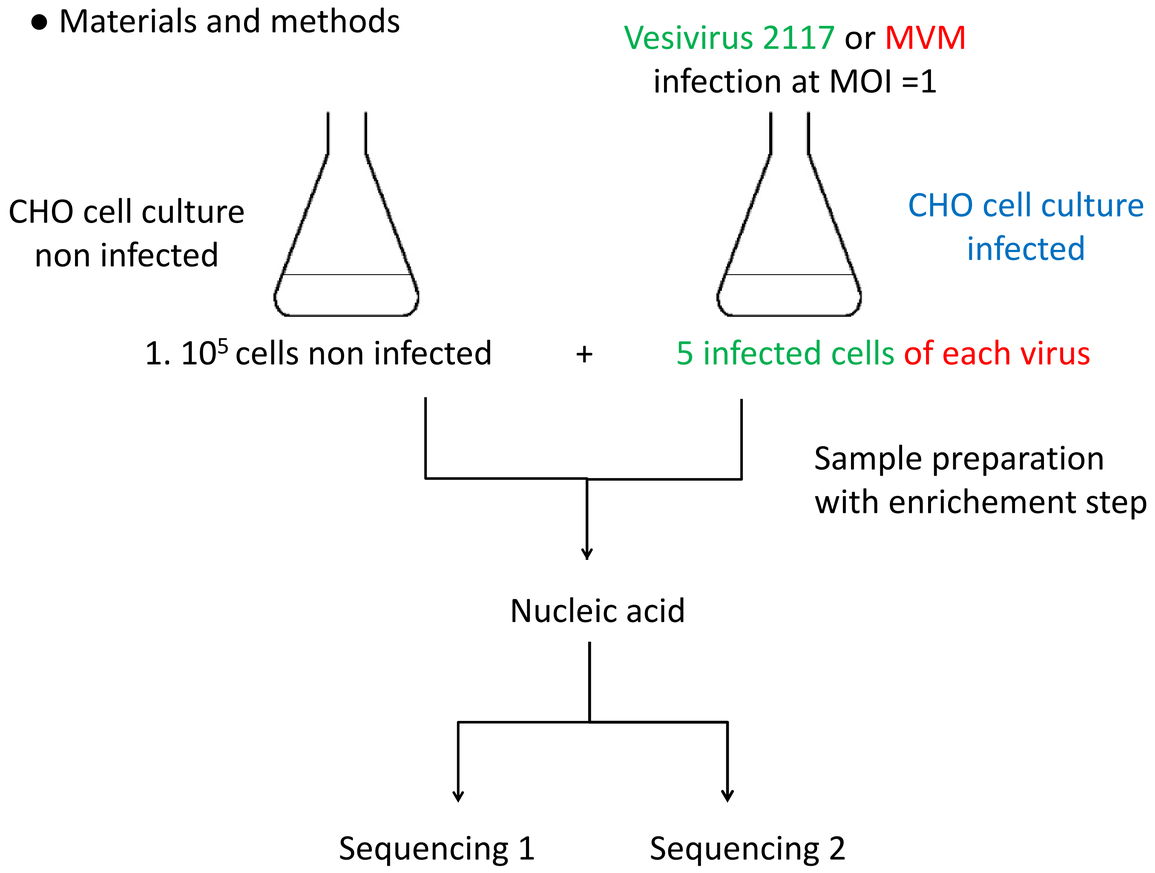
● Introduction

Adventitious agents are a major concern to pharmaceutical / biotechnological companies mainly because of cell culture use in their process. The contamination of biologicals manufacturing process by adventitious agents can have multiple causes: cells, raw materials, errors made by operator, etc. Recent examples of viral contamination have been reported in vaccine and biotech product like Genzyme with Vesivirus 2117 in bioreactor CHO cells, or GSK with PCV1 in Rotavirus vaccine.
Next-Generation Sequencing (NGS) has the capacity to explore a wide spectrum of micro-organisms without an “a priori” testing. Texcell developed a patented sample preparation method to perform NGS. This sample preparation allows to be as sensitive as qPCR (0,1*qPCR LOD).
We present a model which mimic a low CHO viral contamination with Minute Mice Virus (MVM) and Vesivirus 2117 to evaluate the viral detection by NGS. Five infected CHO cells spiked on 1.10^5 CHO cells total were analyzed by two different NGS treatments after the same sample preparation.

● Materials and methods



CHO cells was cultivated in normal conditions to obtain a cell pellet (1.10^5 cells) of non infected cells. In parallel, the CHO cells was infected with Vesivirus 2117 or MVM in a titration plaque with cascade dilution. The cells contained on dilution next to the cytopathogenic effect dilution were counted and 5 cells of this dilution for each virus were added to the cell pellet. Then, nucleic extraction, reverse transcription, and patented treatment were realized on this model.
qPCR on Vesivirus 2117 (commercial) and on MVM (in house) were realized on samples just after the extraction and also after the total sample preparation. Nucleic acid obtained after this sample preparation was sequenced by two different facilities and their own bioinformatics treatment.

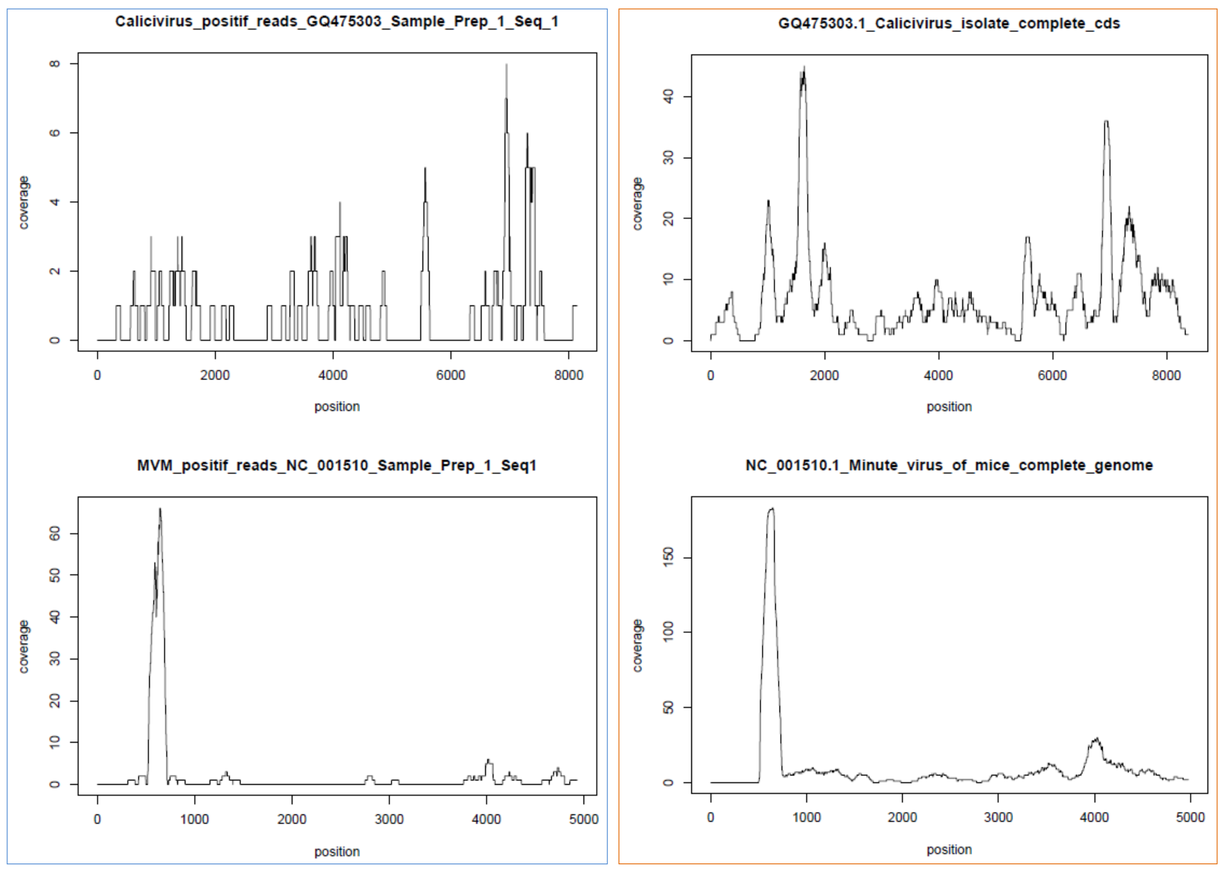
● Results

	Cp values in qPCR	
	MVM	Vesivirus 2117
After nucleic acid extraction*	31,9	28,52
After end of sample preparation**	27,73	22,59

Volume proportion evaluated in qPCR : *1/25 and **1/140

➔ qPCR performed on two virus shown a significant enhance of genome viral sequence.

	Sequencing 1	Sequencing 2
Sequencer	Illumina HiSeq2000	Illumina HiSeq2500
Lenght	2 x 75 bp	2 x 150 bp
Number of reads	143’244’050	215’294’660



➔ Genome viral are detected by the two differents NGS and their own bioinformatics treatment with the same sample preparation

● Conclusion

Sample preparation realized by Texcell allows genome viral detection by NGS with an increase of viral sequence presence and a diminution of background DNA genomic.