GGHH On Campus Laboratory report 2021. ***Word Length is 2000 words: this includes all figure legends and tables but does not include the reference list.***			
Section	**Key points from the marking rubric**	**Figures and Analysis**	**Links and ideas**
Introduction *Constructing a narrative or story line*	The rationale and/or hypothesis and the aims for the study are concisely described and supported by appropriate references; the results section clearly addresses the aims of the study and the there is a conclusion or summary that clearly restates the rationale or hypothesis and the aims in the context of the results obtained	**There are no figures or tables in the introduction**, but it is essential you explain why the study was done (this puts the report in context, and it is here you will need to cite references); key results are summarised in words (no numbers); what are your main conclusions? **The TaqMan Assay** analyses gene copy number of the EGFR gene encodes a receptor tyrosine kinase – consider what the effects of an increased copy number of such a gene might be in the context of cancer. Note that **the results section will include a figure that shows the methylation of two genes (*TAL1* and *CDH1*)** in two different cell lines – you need to consider how DNA methylation provides information on gene expression and how different genes are expressed in different cell types	Write concisely, probably no more than 400 words; this report describes the TaqMan copy number assay and the construction of a Next Generation DNA sequencing library for DNA methylation analysis.
Methods *Concise and clear description of a method*	Methods are concisely and completely described, with all units of measure and time included. **No dot points or tables are permitted in the methods section**	The methods section should use sub-headings to describe all details of the TaqMan assay and the use of ligation-mediated PCR to construct the sequencing library; using your own notes and the laboratory manual, combine information to provide a logical description of each protocol. Be aware of the type of DNA used: **the TaqMan assay** was done using three samples: genomic DNA from the white blood cells of a healthy individual and HCC827 genomic DNA from a lung cancer sample. **Clearly describe** what was in each of the three DNA samples analysed (what was the rationale for this?); the ligation-mediated PCR HELP assay was done using genomic DNA from the white blood cells of a healthy individual	It is essential that you clearly articulate DNA concentration; primer concentration; units of enzyme, final volumes; temperature and time. both methods have multiple steps – ensure you clearly link each step in a protocol. As a starting point, sketch out a flow chart for each method and then use this to guide you in writing the methods

Results *Analytical Skills: A clear description of data shown in a figure or a table must be accompanied by an analysis of that data*	Figure legends clearly and concisely describe the content of the figure (but do not interpret the figure); Tables have titles that clearly identify the content of the table; the text accompanying the figures and tables clearly and concisely identifies and describes key findings. The Mann-Whitney test has been applied to the analysis of differences in Ct values between EGFR and RNaseP for DNA samples 1, 2 and 3; an example of the statistical calculation appears in an Appendix;	**TaqMan Assay:** you will include the amplification plot; a graph that shows individual data points (Ct values) for EGFR and RNaseP for DNA samples 1, 2 and 3, with measures of average and standard deviation overlaying the individual data points; results of Mann-Whitney statistical analysis will be clearly displayed on the graph by use of brackets and symbols (NS = not significant; * is $p < 0.05$; ** $p<0.01$; *** $p<0.001$); **Construction of DNA Sequencing Library:** you will include the agarose DNA gene that shows the digestion of genomic DNA (undigested, *Hpa*II, *Msp*I) and the amplified products; you will include the figure that shows the DNA methylation of *TAL1* and *CDH1* in leukemia and lung cancer cell lines. **I have provided a pdf file of this figure that is in the practical class folder on vUWS (DNA Methylation Figure Practical Report GGHH 2021). Use this file for your report (you will need to write the figure legend)**	The important part of a results section in a scientific paper is to understand the roles of the figure legend and the text accompanying a figure. The figure legend tells the reader what is in the figure – this can be listing the contents of each lane in an agarose gel, the meaning of symbols (e.g. NS, *, **, ***); colours and markings (e.g. the use of different colours to indicate different percentages of DNA methylation). In this way, the figure legend allows the reader to understand the contents of the figure – it does not interpret the figure. It is the main text that accompanies the figure that identifies and describes key findings. These key findings are then analysed and placed in context of published papers in the discussion.
Discussion *Analytical Skills: an analysis of results in the context of other published work*	The summary or conclusion of the report interprets the results in the context of appropriate published studies	**TaqMan analysis:** Summarise key results from samples 1, 2 and 3 and relate this to published studies – what are the effects of amplification of the *EGFR* gene? **The ligation-mediated PCR HELP assay: consider the importance of DNA methylation**; from the published literature find examples that show the importance of studying DNA methylation (hint: see tumour suppressors and cancer, remember that DNA methylation inhibits gene transcription). Note and discuss the tissue-specific expression of genes *TAL1* and *CDH1*	A good discussion will begin by briefly summarising what was done and the key results that were obtained. Building on from this, the discussion will consider the results in the context of published papers and will finish with a conclusion of the significance of the results. I have posted the original paper that described the HELP assay on vUWS – be sure to compare your results with the results shown in Figure 1 of that paper.
Bibliography *Reference List*	References follow the Vancouver style; all references clearly support information presented in the report; there is minimal use of review articles and good use of research articles; all references are peer-reviewed		When citing papers, always make sure you have read and understand the papers you cite; a report of this length would usually have between 6-8 references, and certainly no more than 10.

Example of figure formatting.

Note the labelling of y- and x-axes; data is shown as individual observations and the mean and standard deviation is superimposed on the individual data points. Significance is indicated by asterisks

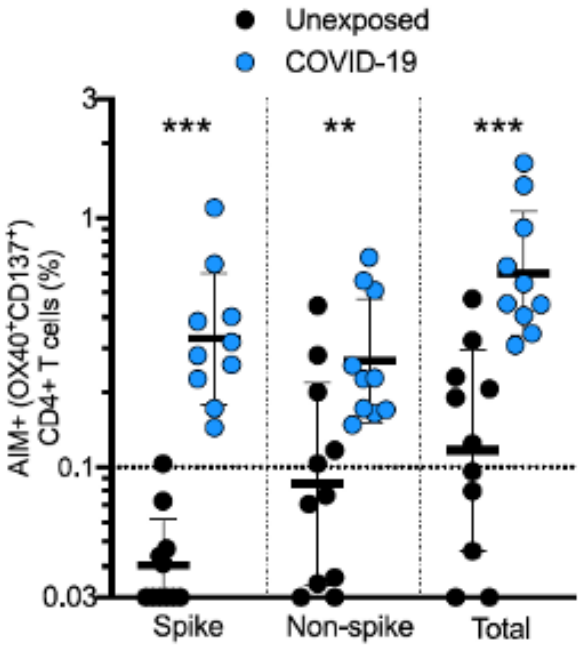


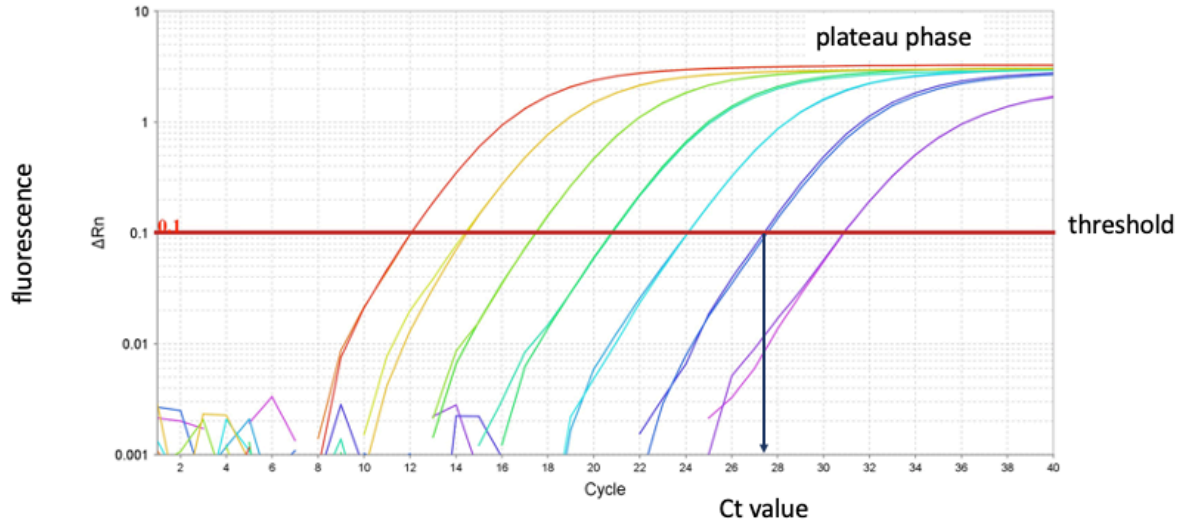
Figure 2. SARS-CoV-2-Specific CD4+ T Cell Responses of Recovered COVID-19 Patients. SARS-CoV-2-specific CD4+ T cells measured as percentage of AIM+ (OX40+CD137+) CD4+ T cells after stimulation of PBMCs with peptide pools encompassing spike only (Spike) MP or the CD4_R MP representing all the proteome without spike (Non-spike). Data were background subtracted against DMSO negative control and are shown with geometric mean and geometric standard deviation. Samples were from unexposed donors (Unexposed, n = 11) and recovered COVID-19 patients (COVID-19, n = 10). Statistical comparisons across cohorts were performed with the Mann-Whitney test. **p < 0.01; ***p < 0.001.

Important points

1. The figure has a number and title
2. There is a concise description of what is being shown
3. The number of samples is indicated
4. The type of statistical test performed, and the results of the test, are indicated

The text that accompanies the figure will interpret the figure, for example: "To determine whether infection with the virus that causes COVID19, SARS-CoV-2, leads to an immune response in infected individuals, we isolated circulating white blood cells from infected and uninfected individuals and measured immune cell activation in each group following stimulation with the spike and non-spike proteins. Relative to uninfected individuals, we found a significant increase of approximately 10-fold in the percentage of activated T-cells in infected individuals when stimulated with the spike (Fig. 2, Spike, $p < 0.001$); a smaller but significant response was also observed after stimulation with the non-spike protein (Fig. 2, Non-spike, $p < 0.01$)."

Example of an amplification plot



The figure legend will describe the y- and x-axes, noting the position of the threshold value and the calculation of the Ct value.

DNA Methylation Result (a follow-on from the ligation-mediated PCR HELP assay)

I have provided a pdf file of this figure that is in the practical class folder on vUWS (DNA Methylation Figure Practical Report GGHH 2021). Use this file for your report (you will need to write the figure legend)

The figure shows:

1. The 5' ends of the CDH1 and TAL1 genes, including the location of the transcription start sites (TSS)
2. Shown are methylation results at the 5' end of these genes from leukeamia and lung cancer cells
3. The figure below is based on the use of a library much like the one you created in the practical class. The percent methylation is calculated by comparing the sequencing depth of MspI and HpaII fragments between samples. At loci showing equal sequencing depth (both MspI and HpaII cut the DNA), there is no methylation (green); at loci where the MspI sequencing depth is more abundant that the HpaII sequencing depth, there is either full methylation (red) or partial methylation (yellow)
4. The figure is shown below (copy paste the pdf version of this figure), note the scale at the bottom that is used to interpret the methylation marks