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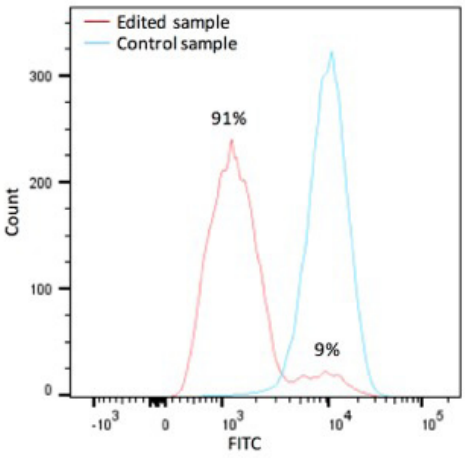
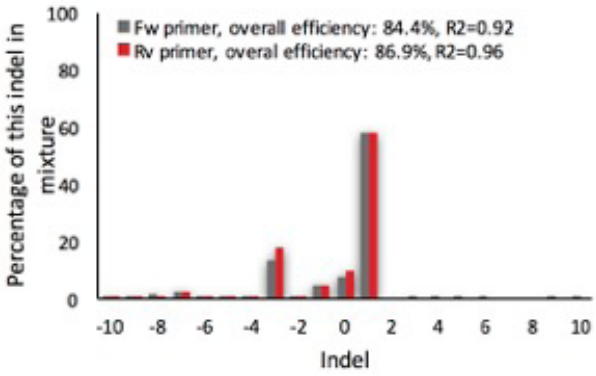
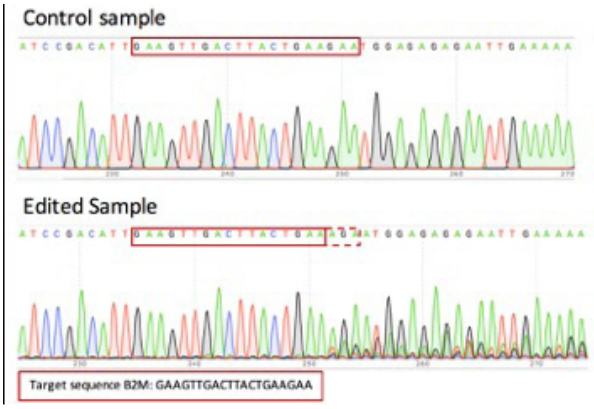
Results

We have developed sgRNA probes that target the B2M gene encoding Beta2-micro-globulin, which is part of the MHC class 1 cell surface complex (MHC1 Neefjes *et al.*, Nature Chemical Biology (2013). The expression of MHC1 can be measured by FACS (Flow Cytometry) using a fluorescent antibody that recognizes the MHC1 complex. When the B2M gene is edited by iTOP-mediated CRISPR/Cas9 editing, the Beta2-micro-globulin protein is no longer expressed, resulting in a lack of MHC1 expression at the cell surface.





The figure above is a result of FACS analysis, in which the expression of MHC1 is measured. It can be observed that more than 90% of cells have lost MHC1 expression.



iTOP mediated gene-editing of the B2M gene was confirmed by Sanger sequencing at the target sequence and subsequent analysis with TIDE: https://tide.deskgen.com (Brinkman *et al.*, Nucl. Acids Res. (2014). This showed that the mutation rate and the percentage of indels induced via the iTOP method were 84.4% and 86.9% respectively.

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