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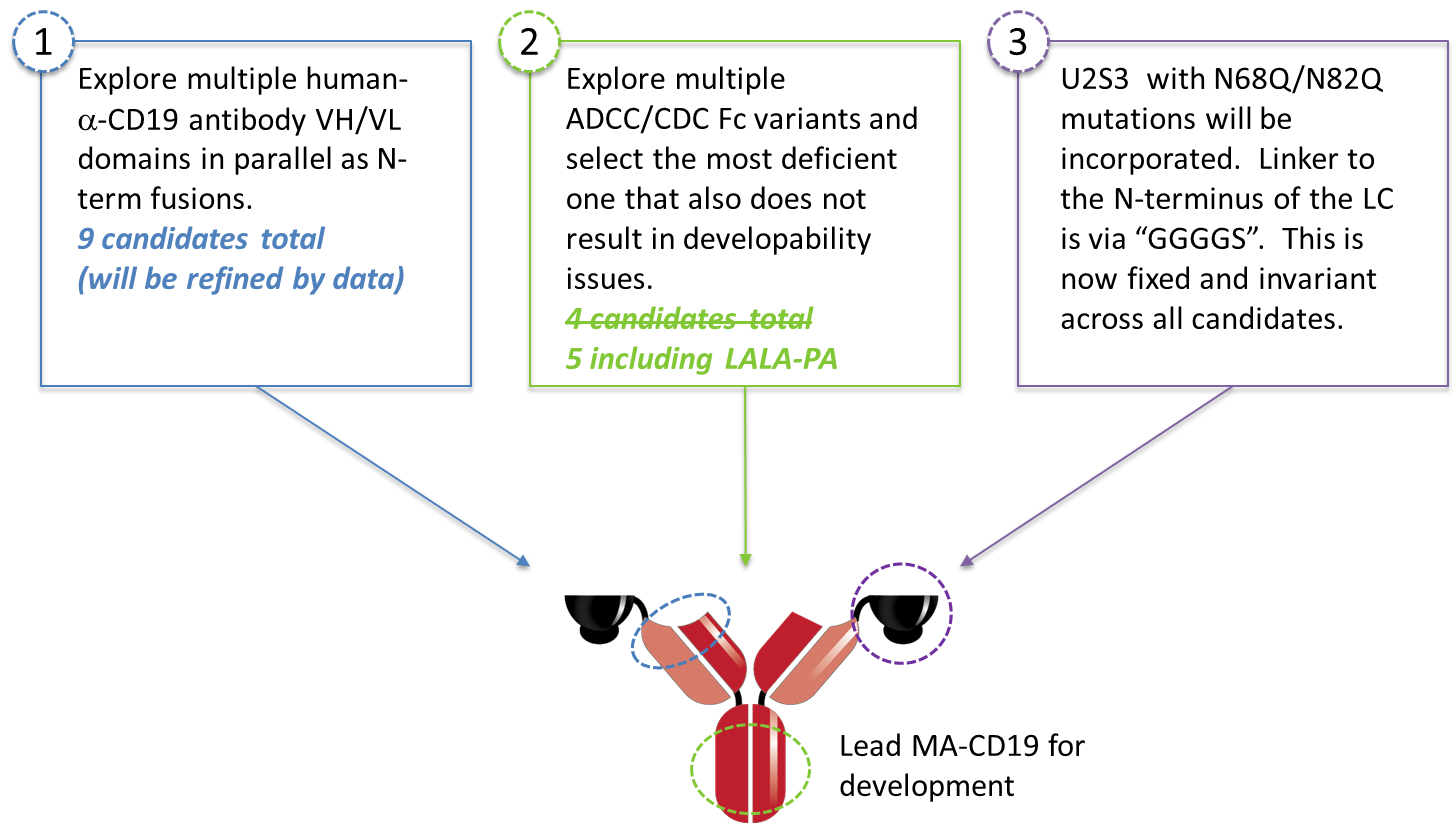
# Version history

* Original document dated 11.25.2020
* Current version updated 12.16.2020
  + Emphasis of D265A/N297A mutations information in the Fc
    - This would replace the LALA-Fc variant
  + Emphasizing removal of LALA-Fc variant

# Background Information

## Introduction

The purpose of this project is to take advantage of Absci’s high throughput and parallelization capabilities to assess a combinatorial library for potential developability issues. This will then better inform lead optimization for further development in Absci’s platform as well as potentially factor into decisions for preclinical progression. The overall library strategy is schematized below with a starting point of **45 MicAbody variants** comprised of different VH/VL and Fc-variant combinations.



## Sequence information for “Reference Material”

(see 201119\_CD19\_for\_Absci.pptx for copy of details provided below)

>KK989\_CD19\_HC\_DANA

EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWIGWVRQMPGKGLEWMGIIYPDDSDTRYSPSFQGQVTISADKSIRTAYLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

* VH = blue
* IgG1-Fc = green with D265A/N297A mutations for ADCC deficiency 🡪 *this will be swapped out for one of four other candidate ADCC/CDC deficient Fc’s per diagram above.*

>KK1425\_CD19\_ S3m\_LC

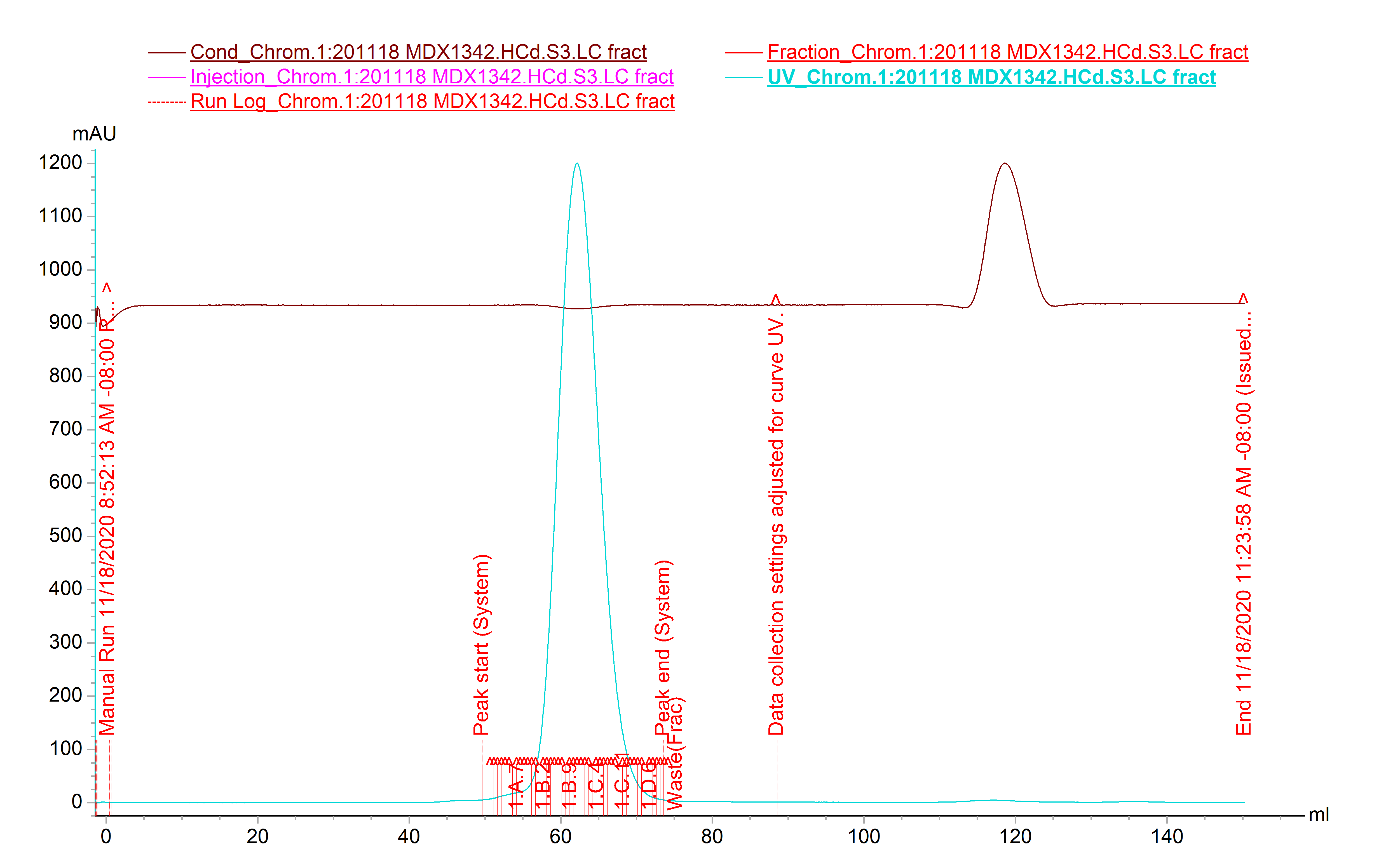
EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKAQNPVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVATKLYLWSMGDCIGWLEDFLMGMDSTLEPSAPTSSSGGGGSAIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

* U2S3 = purple 🡪 *this will be swapped out for U2S3 with N68Q/N82Q mutations and fixed in Design Cycle 0*
* Medium APTSSSGGGGS linker 🡪 *This will be shortened and fixed as a GGGGS in all constructs for Design Cycle 0*
* VL = blue
* Remainder of sequence is human-LC-kappa

## Additional Reference Material information

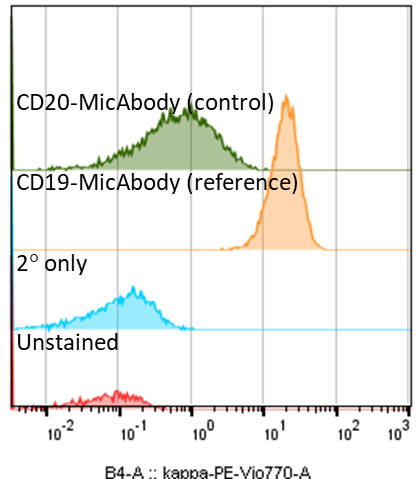
Lot/batch 11/20/2012, Akta SEC fractionated with 1B4-1D7 collected, in PBS @ 1.6 mg/mL (8.2 uM)

### SEC analysis

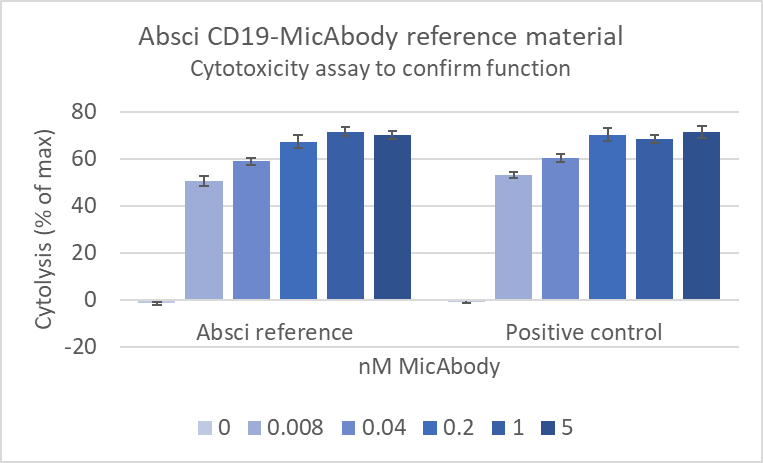


### Flow cytometry confirmation of target cell binding

CD19-based ELISAs have not been robust in our team’s hand. We’ve tried a couple of different commercially available recombinant CD19 antibodies from different sources without success. As a consequence, we rely on cell-based binding assays.



### Activity confirmation by cytotoxicity assay



# Components

## Anti-CD19 VH/VL domains

Table 1: VH+VL pairings for each antibody clone – note some redundant sequences

|  |  |  |
| --- | --- | --- |
| Clone | VH sequences | VL sequences |
| CD19A | >**VH1**  EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWIGWVRQMPGKGLEWMGIIYPDDSDTRYSPSFQGQVTISADKSIRTAYLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGQGTLVTVSS | >**VL1**  AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPYTFGQGTKLEIK |
| CD19B | Same as “**VH1**” | >**VL2**  AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK |
| CD19C | >**VH2**  QVQLVQSGAEVKKPGSSVKVSCKDSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTTNYAQQFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREAVAADWLDPWGQGTLVTVSS | >**VL3**  EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSRFTFGPGTKVDIK |
| CD19D | >**VH3**  EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQGYSSGWDSYYGMGVWGQGTTVTVSS | Same as “**VL1**” |
| CD19E | >**VH4**  QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYTINWVRQAPGQGLEWMGGIIPIFGIPNYAQKFQGRVTITADESTNTAYMELSSLRAEDTAVYYCARASGGSADYSYGMDVWGQGTAVTVSS | >**VL4**  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYKRYPYTFGQGTKLEIK |
| CD19F | >**VH5**  EVQLVQSGAEVKKPGESLQISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSINTAYLQWSSLKASDTAMYYCARGVSMIWGVIMDVWGQGTTVTVSS | >**VL5**  AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPWTFGQGTKVEIK |
| CD19G | >**VH6**  EVQLVQSGAEVKKPGESLQISCKGSGYTFTNYWIAWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSISTAYLQWSGLKASDTAMYYCARQGYSSGWRSYYGMGVWGQGTTVTVSS | >**VL6**  AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPHTFGQGTKLEIK |
| CD19H | Same as “**VH1**” | Same as “**VL5**” |
| CD19I | Same as “**VH1**” | Same as “**VL6**” |

## Fc-variants

Table 2: The four variants to be implemented – differences from wild-type human IgG1-Fc indicated in red.

|  |  |
| --- | --- |
| Variant | Sequence |
| ~~LALA~~  Drop from campaign | ~~ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK~~ |
| LALAKA | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| LALANG | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| LALAPG | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| LALAPA | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| DANA | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| wt hIgG1-Fc (for information purposes) | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

## U2S3 N68Q/N82Q sequence (with linker)

EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGQKTVTPVSPLGKKLQVTTAWKAQNPVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVATKLYLWSMGDCIGWLEDFLMGMDSTLEPSGGGGS

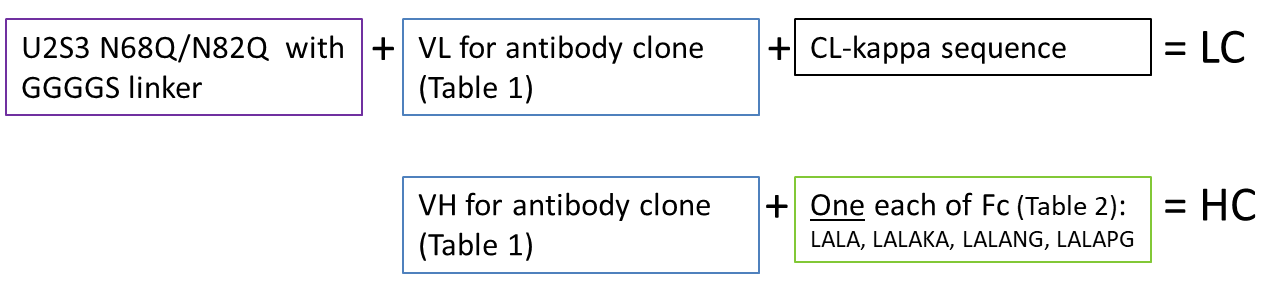
* U2S3 N68Q/N82Q
* GGGGS linker

## CL-kappa sequence

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

# Example strategy

If everything is populated correctly in this document, combinations can readily be generated by directly copy/pasting to assemble in the following order:



Example for CD19A candidates (resulting in the expression of four variants for each antibody clone) – this exercise is mostly for my benefit to make sure there aren’t HUGE errors in my population of information.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Component 1 | Component 2 | Component 3 |
| LC | U2S3 N68Q/N82Q | VL1 | CL-kappa |
| HC |  | ~~VH1~~ | ~~LALA~~ |
|  |  | VH1 | LALAKA |
|  |  | VH1 | LALANG |
|  |  | VH1 | LALAPG |
|  |  | VH1 | LALAPA |
|  |  | VH1 | DANA |

>CD19A\_LC

EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGQKTVTPVSPLGKKLQVTTAWKAQNPVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVATKLYLWSMGDCIGWLEDFLMGMDSTLEPSGGGGSAIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>CD19A\_HC\_LALAKA

EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWIGWVRQMPGKGLEWMGIIYPDDSDTRYSPSFQGQVTISADKSIRTAYLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

>CD19A\_HC\_LALANG

EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWIGWVRQMPGKGLEWMGIIYPDDSDTRYSPSFQGQVTISADKSIRTAYLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

>CD19A\_HC\_LALAPG

EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWIGWVRQMPGKGLEWMGIIYPDDSDTRYSPSFQGQVTISADKSIRTAYLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

>CD19A\_HC\_LALAPA

EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWIGWVRQMPGKGLEWMGIIYPDDSDTRYSPSFQGQVTISADKSIRTAYLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

>CD19A\_HC\_DANA

EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWIGWVRQMPGKGLEWMGIIYPDDSDTRYSPSFQGQVTISADKSIRTAYLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK