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Supplemental information

Systematic discovery and validation of T cell targets directed against oncogenic KRAS mutations

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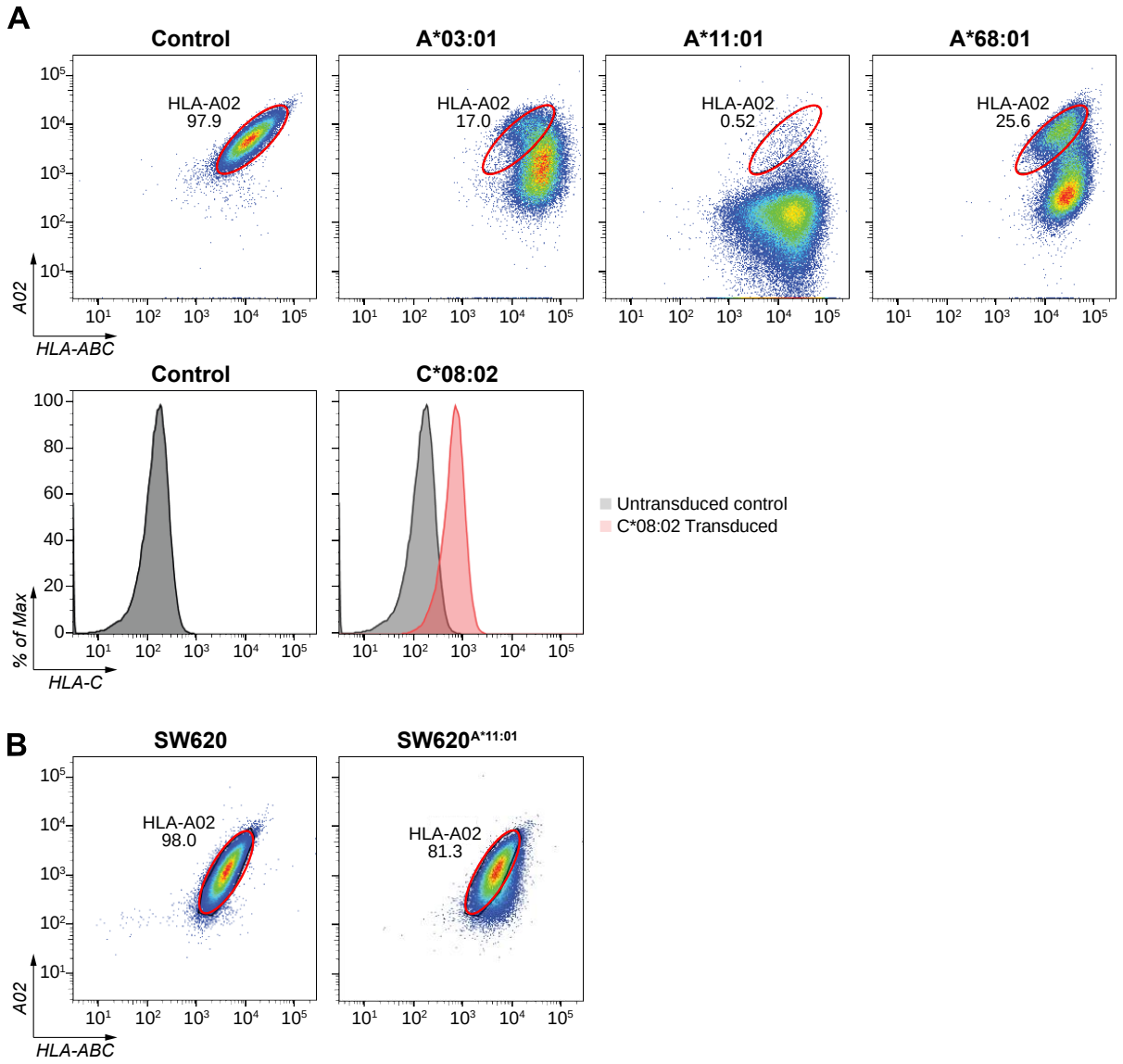


Figure S1. HLA-I expression after transduction. (A) In the absence of antibodies for specific HLA-I molecules, A375 cells engineered to express these molecules were evaluated for transduction and selection efficiency in multiple ways. Cells transduced with HLA-A molecules were evaluated for competitive downregulation of HLA-A*02, which is naturally expressed by A375 cells (top row). Cells transduced with HLA-C*08:02 were evaluated for total expression of HLA-C molecules relative to control (bottom row). **(B)** SW620 cells transduced with A*11:01 (SW620A*11:01) were evaluated for competitive downregulation of HLA-A*02, which is also expressed by SW620 cells. Related to Figure 3.

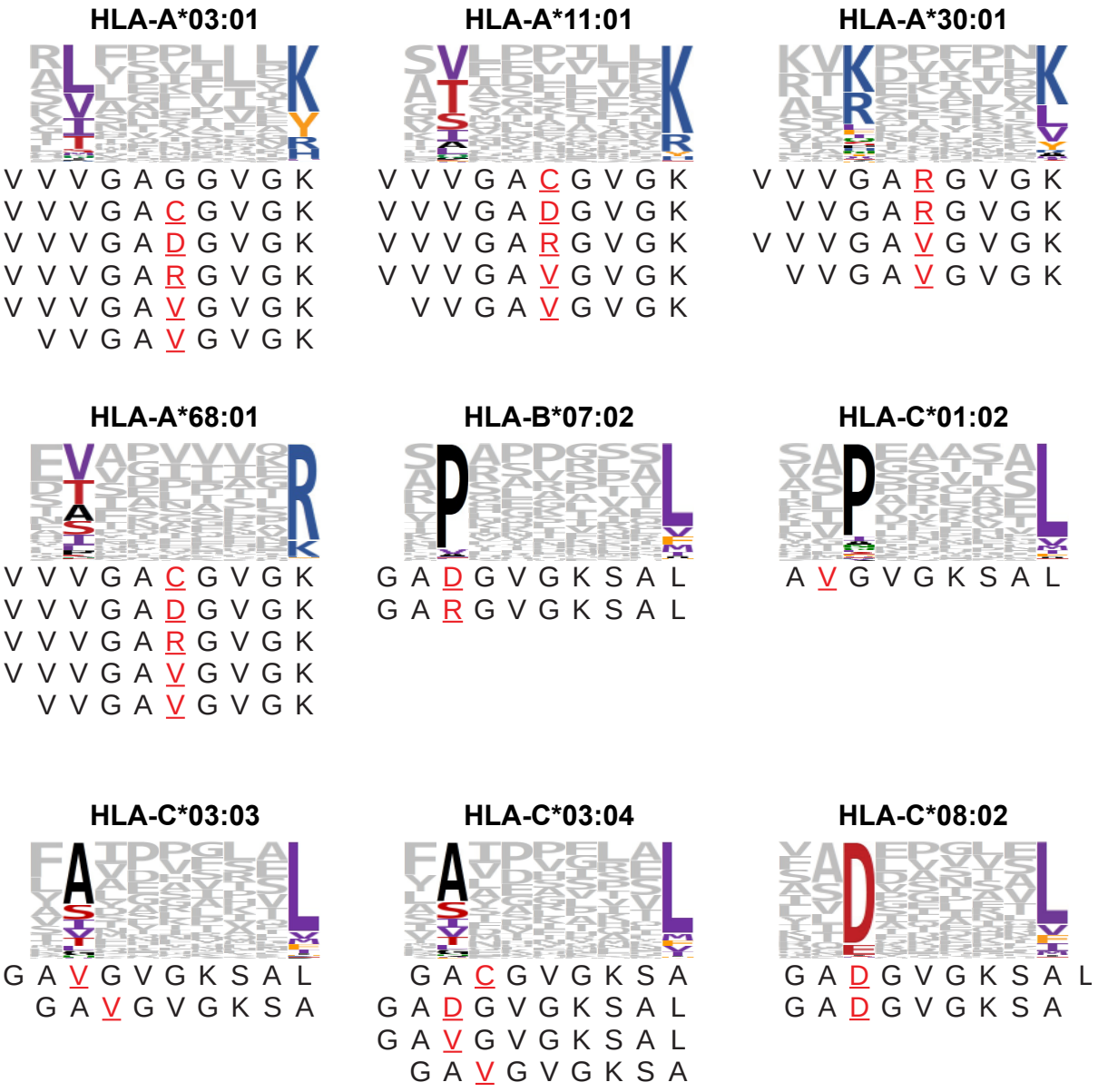


Figure S2. HLA-I sequence logos and MS detected neoantigens. Sequence logos reveal the anchor residues (large letters) for the nine HLA-I molecules where mutant KRAS peptides were detected by targeted MS. The detected neoantigens are listed under each sequence logo. Related to Table 1.

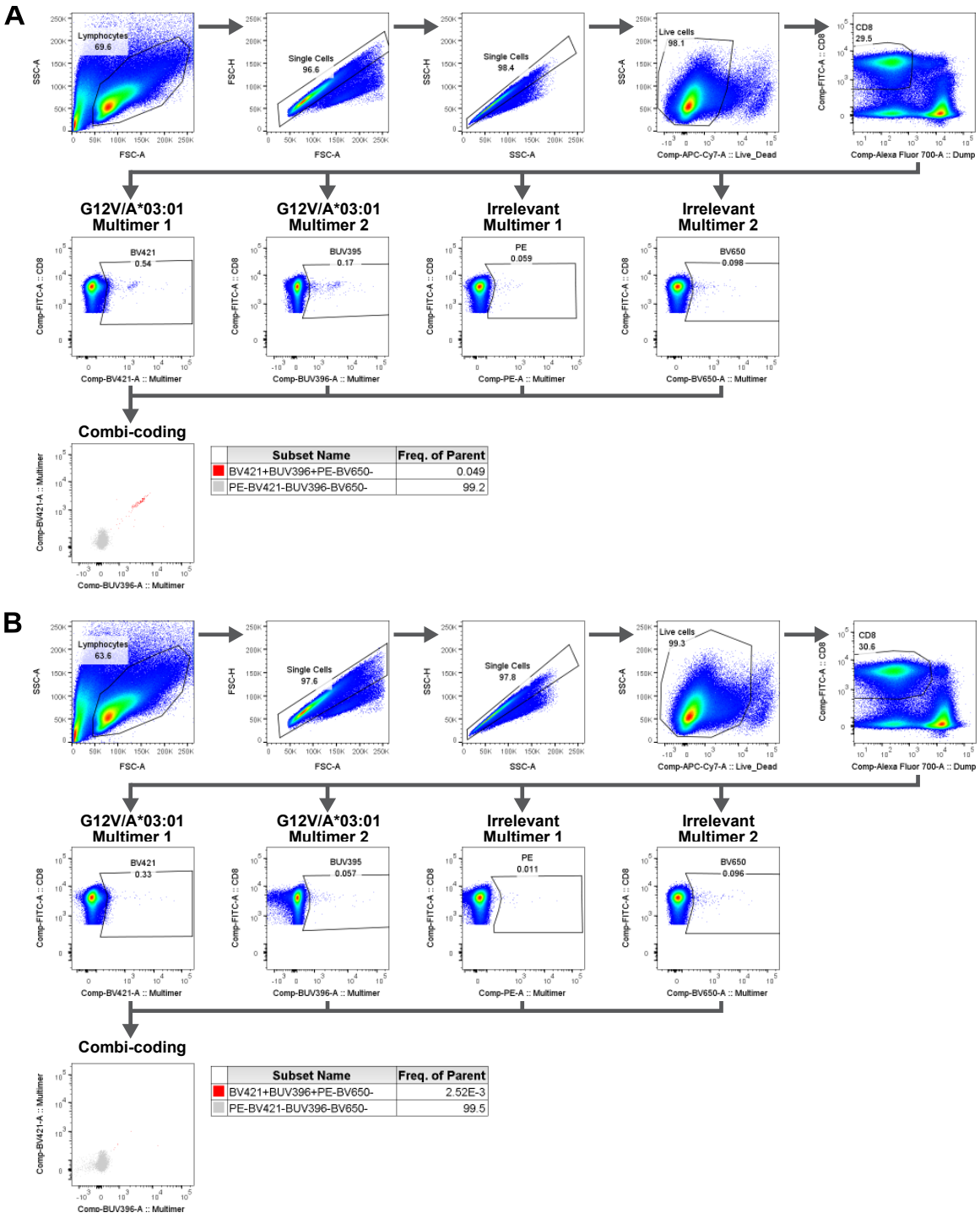


Figure S3. Combinatorial coding gating strategy for pHLA multimers. T cell induction cultures were evaluated for presence of mutant KRAS-specific CD8+ T cells using combinatorial coding after gating out dead cells and a lineage dump gate (composed of CD4, CD11b, CD16, and CD19). In this representative example, mutant KRAS peptide $HLA^{A*03:01}$ multimers were conjugated to BV421 and BUV395, and irrelevant pHLA multimers were conjugated to PE and BV650. Mutant KRAS specific CD8+ T cells were defined as BV421+BUV395+PE-BV650-. Positive responses were defined as greater than events on the diagonal comprising greater than 0.05% of CD8+ T cells. **(A)** Representative positive induction. **(B)** Representative negative induction from the same experiment. Related to Figure 3.

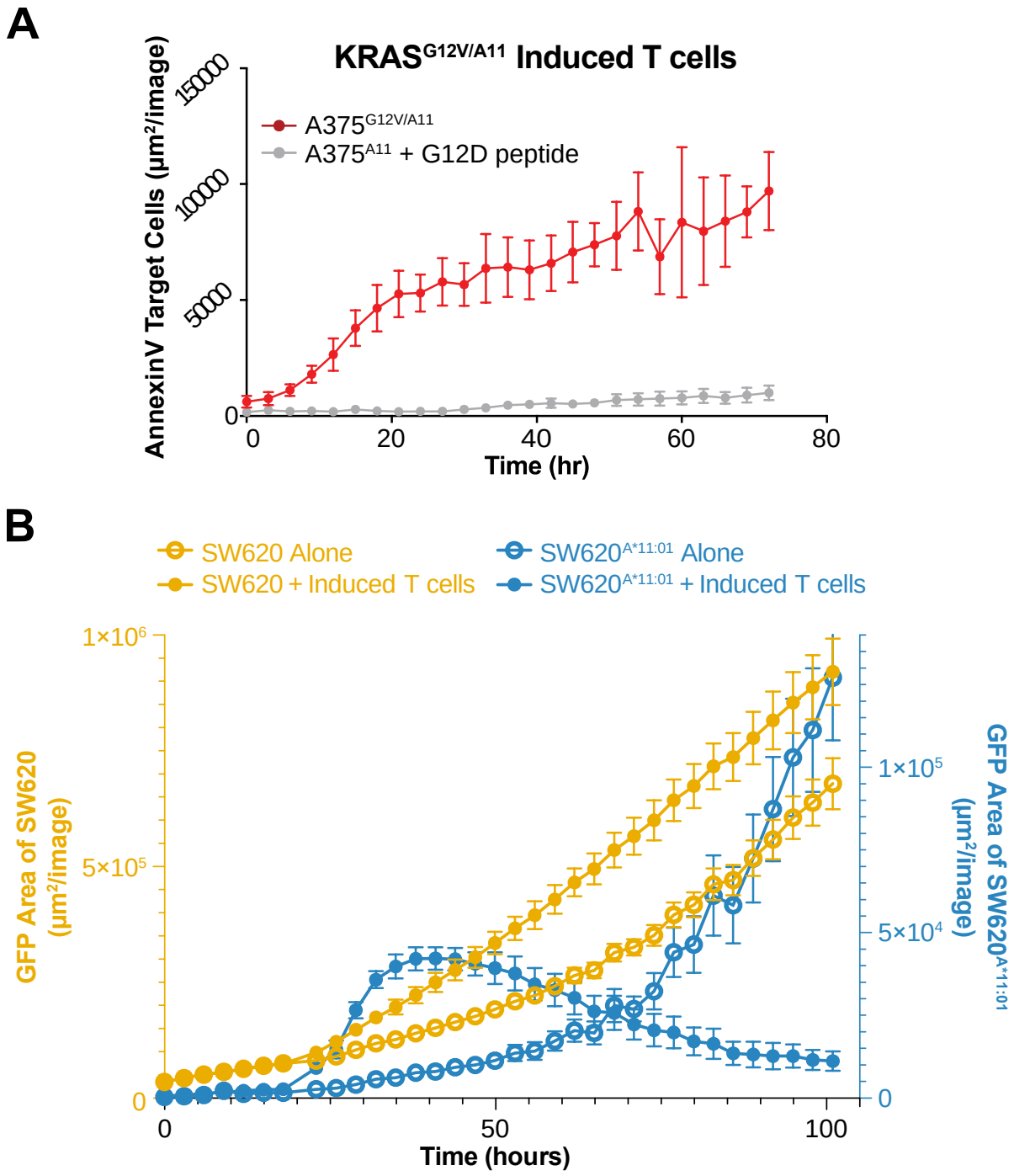


Figure S4. Specificity of induced T cells. (A) A cytotoxicity assay was performed with the same representative T cell culture used in Figure 3D to evaluate the specificity of these T cells. The T cells induced against the KRAS^{G12V}/HLA-A*11:01 neoantigens were co-cultured with A375$^{A11:01}$ cells that were either also transduced with KRAS^{G12V} (red) or loaded with KRAS^{G12D}/HLA-A*11:01 neoantigens (gray). Data are presented as mean ± SD. **(B)** A cytotoxicity assay was performed with a representative T cell culture induced against KRAS^{G12V}/HLA*11:01. The T cells were co-cultured with SW620$^{A11:01}$ cells (black) or unmodified parental SW620 cells (grey). Data are presented as mean ± SD. Related to Figure 3.

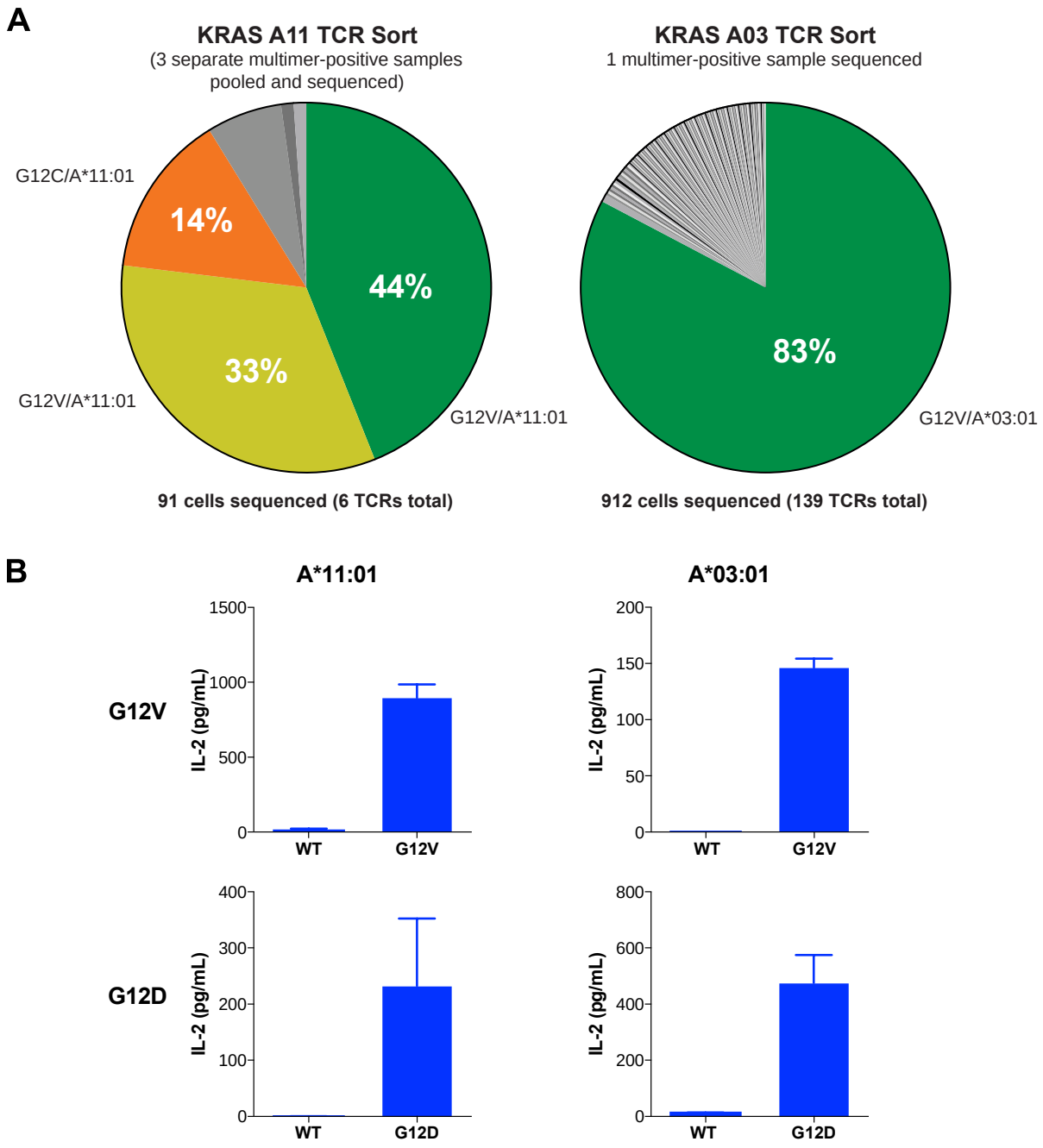


Figure S5. Clonality and specificity of sequenced TCRs. (A) Mutant KRAS specific T cells were sorted using pHLA multimers and single-cell TCR sequencing was performed using the 10X Genomics platform. The clonality of each TCR was determined by the number of sequenced cells that contained that TCR. (Left) Clonality of TCRs specific for mutant KRAS peptides on $HLA^{A*11:01}$ after pooling sorted T cells from three separate cultures, two of which were specific for $KRAS^{G12V}$ and one of which was specific for $KRAS^{G12C}$. (Right) Clonality of a TCR specific for $KRAS^{G12V}$ on $HLA^{A*03:01}$. **(B)** A co-culture was performed with the same TCR-transduced Jurkat cells used in Figures 4 A-D. TCR specificity was evaluated by co-culture with A375 cells transduced to express the cognate HLA-I molecule and loading of 10 mM of the cognate neoantigen or the wild-type peptide. TCR stimulation was assessed by IL-2 secretion. Data are presented as mean ± SD. Related to Figure 4.

HLA-I	Mutation	Peptide	Peptide Length	% Rank	Detected by Mass Spectrometry?
A*01:01	-	-	-	Not Applicable	-
A*02:01	G12C	KLVVVGACGV	10	1.6	No
	G12V	KLVVVGAVGV	10	0.8	No
A*03:01	G12C	VVVGACGVGK	10	1.2	Yes
	G12D	VVVGADGVGK	10	0.9	Yes
	G12D	VVGADGVGK	9	1.9	No
	G12R	VVVGARGVGK	10	0.7	Yes
	G12R	VVGARGVGK	9	0.6	**No
	G12V	*VVVGAVGVGK	10	0.3	Yes
	G12V	*VVGAVGVGK	9	0.3	Yes
A*11:01	G12C	VVVGACGVGK	10	0.3	Yes
	G12C	VVGACGVGK	9	0.3	No
	G12D	VVVGADGVGK	10	0.1	Yes
	G12D	VVGADGVGK	9	0.3	No
	G12R	VVVGARGVGK	10	0.2	Yes
	G12R	VVGARGVGK	9	0.2	**No
	G12V	^VVVGAVGVGK	10	0.1	Yes
	G12V	VVGAVGVGK	9	0.1	Yes
A*23:01	-	-	-	Not Applicable	-
A*24:02	-	-	-	Not Applicable	-
A*26:01	-	-	-	Not Applicable	-
A*29:02	-	-	-	Not Applicable	-
A*30:01	G12R	VVVGARGVGK	10	2.0	Yes
	G12R	VVGARGVGK	9	1.9	Yes
	G12V	VVVGAVGVGK	10	2.2 (Extrapolated)	Yes
	G12V	VVGAVGVGK	9	1.5	Yes
A*31:01	-	-	-	Not Applicable	-
A*32:01	-	-	-	Not Applicable	-
A*68:01	G12C	VVVGACGVGK	10	0.2	Yes
	G12C	VVGACGVGK	9	1.3	No
	G12D	VVVGADGVGK	10	0.1	Yes
	G12D	VVGADGVGK	9	1.3	No
	G12R	VVVGARGVGK	10	0.1	Yes
	G12R	VVGARGVGK	9	1.3	No
	G12V	VVVGAVGVGK	10	0.0	Yes
	G12V	VVGAVGVGK	9	0.2	Yes
B*07:02	G12D	GADGVGKSAL	10	1.3	Yes
	G12R	GARGVGKSAL	10	0.5	Yes

	G12V	AVGVGKSAL	9	1.5	No
B*08:01	-	-	-	Not Applicable	-
B*14:02	G12R	ARGVGKSAL	9	0.7	Not Targeted
B*15:01	-	-	-	Not Applicable	-
B*18:01	-	-	-	Not Applicable	-
B*27:05	-	-	-	Not Applicable	-
B*35:01	-	-	-	Not Applicable	-
B*40:01	G12D	ADGVGKSAL	9	1.6	Not Targeted
	G12V	GAVGVGKSAL	10	1.4	Not Targeted
B*44:02	-	-	-	Not Applicable	-
B*44:03	-	-	-	Not Applicable	-
B*51:01	G12C	CGVGKSALTI	10	1.8	Not Targeted
	G12D	DGVGKSALTI	10	1.2	Not Targeted
B*57:01	-	-	-	Not Applicable	-
C*01:02	G12C	GACGVGKSAL	10	0.7	No
	G12C	ACGVGKSAL	9	1.8	No
	G12D	GADGVGKSAL	10	0.3	No
	G12R	GARGVGKSAL	10	0.6	No
	G12V	GAVGVGKSAL	10	1.2	No
	G12V	AVGVGKSAL	9	0.2	Yes
C*02:02	-	-	-	Not Applicable	-
C*03:03	G12C	GACGVGKSAL	10	0.6	No
	G12D	GADGVGKSAL	10	0.3	No
	G12R	GARGVGKSAL	10	1.7	No
	G12V	GAVGVGKSAL	10	0.3	Yes
	G12V	GAVGVGKSA	9	1.7	Yes
C*03:04	G12C	GACGVGKSAL	10	1.9	No
	G12C	GACGVGKSA	9	6.7 (Extrapolated)	Yes
	G12D	GADGVGKSAL	10	1.6	Yes
	G12V	GAVGVGKSAL	10	1.0	Yes
	G12V	GAVGVGKSA	9	2.4 (Extrapolated)	Yes
C*04:01	G12D	GADGVGKSAL	10	0.9	Not Targeted
C*05:01	G12C	GACGVGKSAL	10	1.0	Not Targeted
	G12D	GADGVGKSAL	10	0.1	Not Targeted
	G12R	GARGVGKSAL	10	0.8	Not Targeted
	G12V	GAVGVGKSAL	10	1.8	Not Targeted
C*06:02	-	-	-	Not Applicable	-
C*07:01	-	-	-	-	-
C*07:02	G12R	ARGVGKSAL	9	1.5	Not Targeted

C*08:02	G12C	GA<u>C</u>GVGKSAL	10	0.6	No
	G12D	GA<u>D</u>GVGKSAL	10	0.0	Yes
	G12D	GA<u>D</u>GVGKSA	9	0.3	Yes
	G12R	GA<u>R</u>GVGKSAL	10	1.4	No
	G12V	GA<u>V</u>GVGKSAL	10	0.9	No
C*12:03	G12C	GA<u>C</u>GVGKSAL	10	1.9	Not Targeted
	G12D	GA<u>D</u>GVGKSAL	10	1.7	Not Targeted
	G12R	GA<u>R</u>GVGKSAL	10	2.0	Not Targeted
	G12V	GA<u>V</u>GVGKSAL	10	0.9	Not Targeted
C*16:01	-	-	-	Not Applicable	-

Table S1. Summary of KRAS neoantigens targeted by MS. KRAS $G12^{C/D/R/V}$ neoantigens targeted by PRM in this manuscript. Detected peptides are highlighted in green. Peptides that were detected in the NCH-H44I cell line are marked with (*), and those detected in the SW620 cell line are marked with (^). Peptides where targeted MS was performed without TMT labeling are marked with (**). Related to Table 1.

HLA-I	KRAS Mutation	# HDs Tested	% HDs with Positive Induction
A*03:01	G12C	7	43%
A*03:01	G12D	10	20%
A*03:01	G12R	3	33%
A*03:01	G12V	11	55%
A*11:01	G12C	2	50%
A*11:01	G12D	4	50%
A*11:01	G12R	2	100%
A*11:01	G12V	8	100%
A*68:01	G12C	1	0%
A*68:01	G12D	2	100%
A*68:01	G12R	2	100%
A*68:01	G12V	2	100%
B*07:02	G12D	2	100%
B*07:02	G12R	4	50%
C*01:02	G12V	1	100%
C*03:04	G12D	3	67%
C*08:02	G12D	3	67%

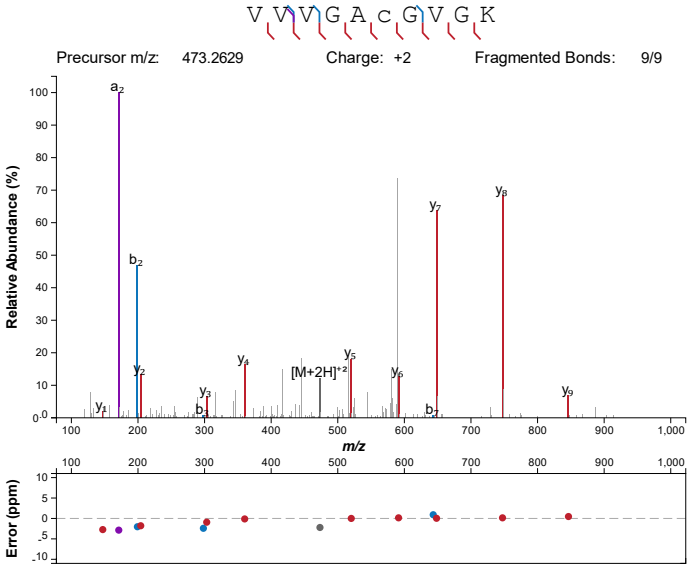
Table S2. Summary of KRAS neoantigen immunogenicity. Fraction of donors with an induced T cell response specific for each KRAS neoantigen. Related to Figure 3.

HLA-I	KRAS Mutation	Peptide	Peptide Length	Affinity (nM)	Stability (hr)
A*03:01	G12C	VVGAC<u>G</u>VGK	9	4.1	5.0
A*03:01	G12D	VVGA<u>D</u>GVGK	9	518.7	Not Stable
A*03:01	G12V	VVGA<u>V</u>GVGK	9	1.9	1.2
A*03:01	Wild-type	VVGA<u>G</u>GVGK	9	125.1	1.5
A*03:01	G12C	VVVGAC<u>G</u>VGK	10	1.6	2.5
A*03:01	G12D	VVVGA<u>D</u>GVGK	10	314.9	2.3
A*03:01	G12V	VVVGA<u>V</u>GVGK	10	44.2	6.7
A*03:01	Wild-type	VVVGA<u>G</u>GVGK	10	82.0	3.2
A*11:01	G12C	VVGAC<u>G</u>VGK	9	43.2	10.0
A*11:01	G12D	VVGA<u>D</u>GVGK	9	203.9	3.4
A*11:01	G12V	VVGA<u>V</u>GVGK	9	7.7	16.9
A*11:01	Wild-type	VVGA<u>G</u>GVGK	9	90.6	4.3
A*11:01	G12C	VVVGAC<u>G</u>VGK	10	69.3	15.7
A*11:01	G12D	VVVGA<u>D</u>GVGK	10	33.1	13.0
A*11:01	G12V	VVVGA<u>V</u>GVGK	10	26.1	24.3
A*11:01	Wild-type	VVVGA<u>G</u>GVGK	10	12.4	11.6

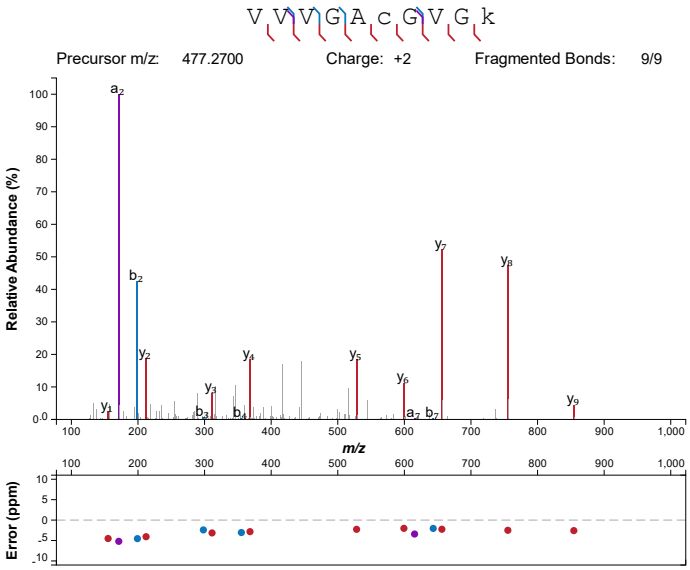
Table S3. Peptide affinity and stability. Affinity and stability measurement for mutant and wildtype peptides were assessed for HLA-A*03:01 and HLA-A*11:01. Related to Figure 3.

Data S2. Mass spectra for KRAS peptides detected by targeted MS. Mass spectra for all the KRAS mutant and synthetic peptides that were detected by PRM in engineered and natural cell lines. In some instances, the same heavy isotope-labeled synthetic peptide spectra were used to confirm endogenous peptide detections. Related to Table 1.

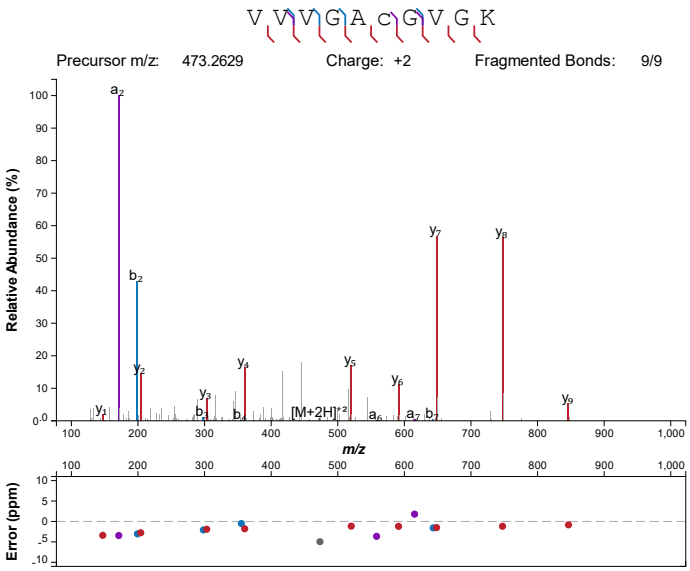
G12C
A*03:01
Mutant, 10mer



Synthetic



G12C
A*11:01
Mutant, 10mer



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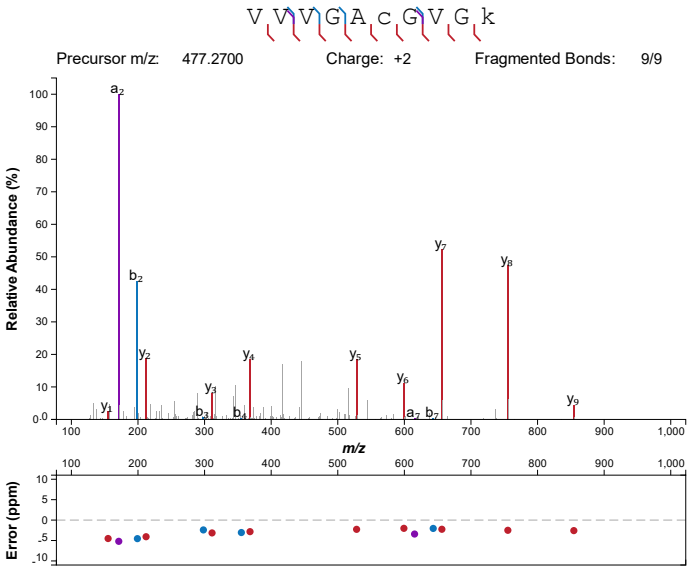
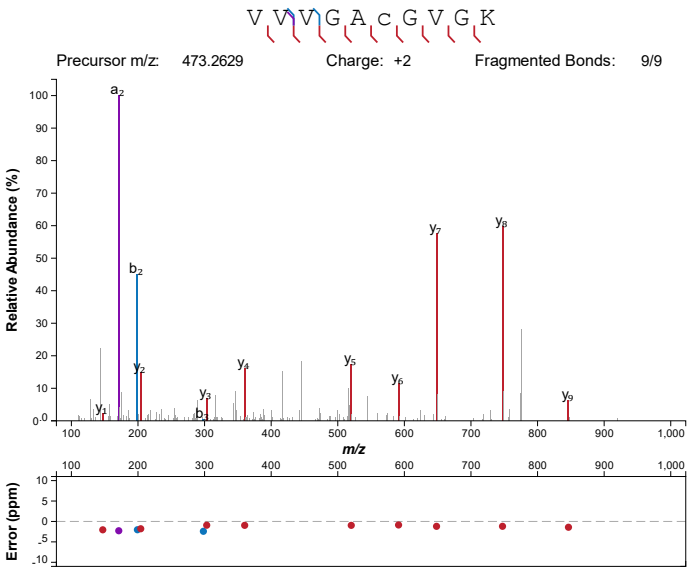


G12C
A*68:01
Mutant, 10mer



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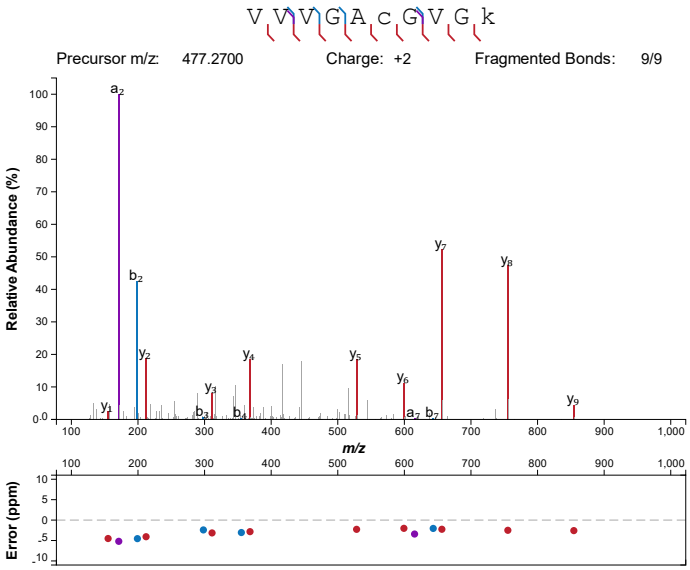
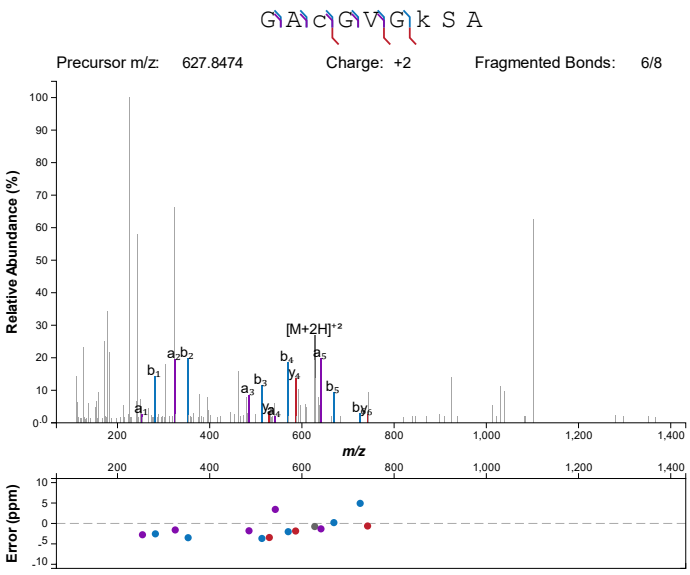


G12C
C*03:04
Mutant, 9mer



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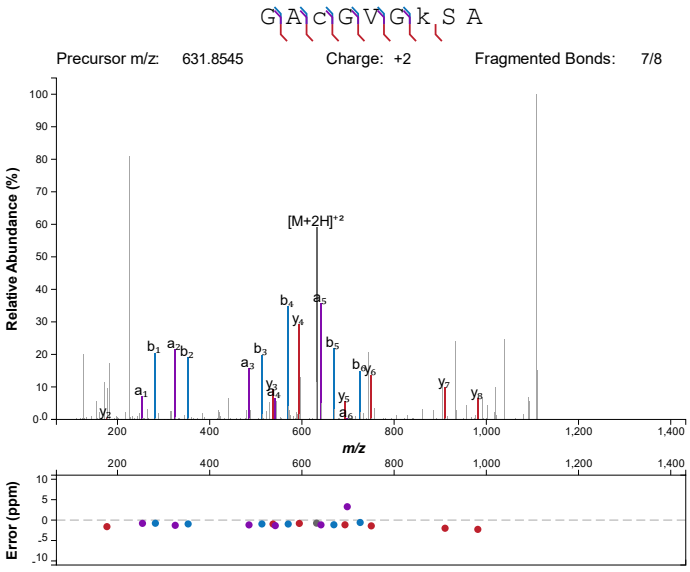
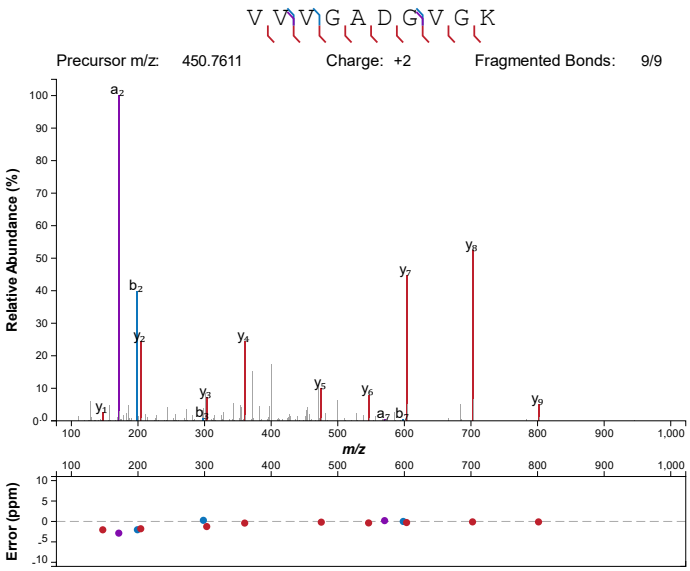


G12D
A*03:01
Mutant, 10mer



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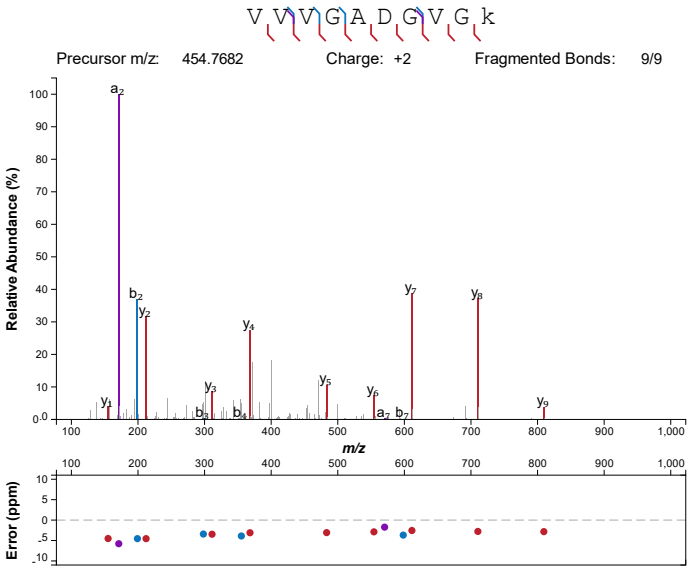
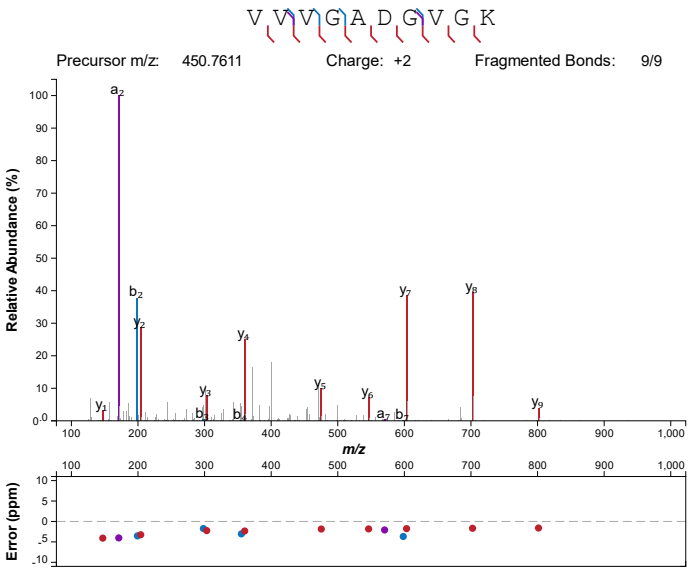


G12D
A*11:01
Mutant, 10mer



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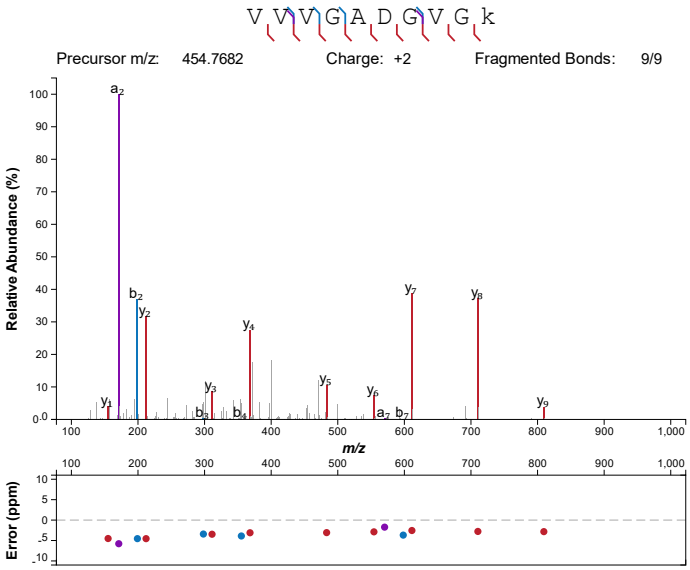
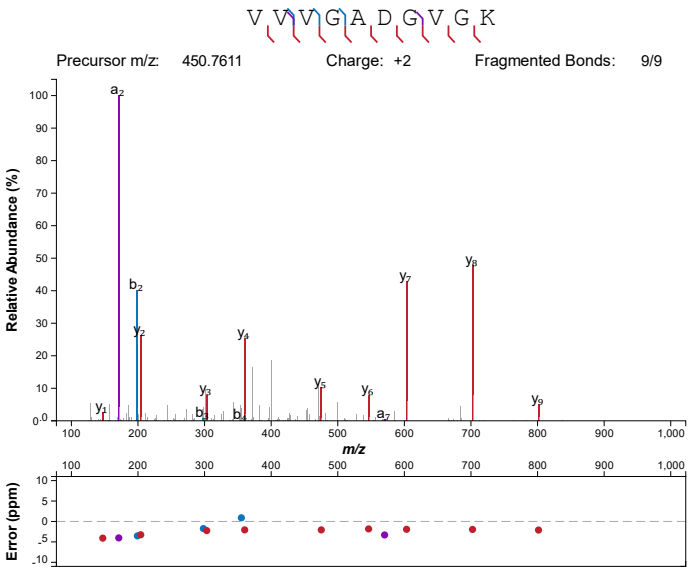


G12D
A*68:01
Mutant, 10mer



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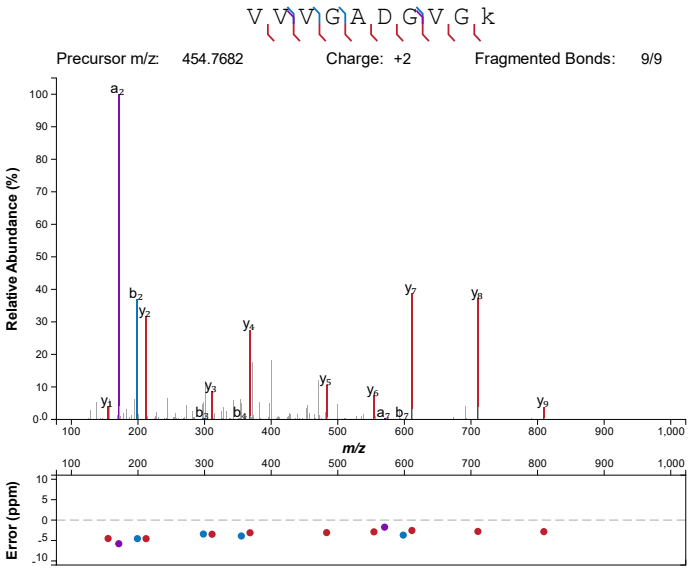
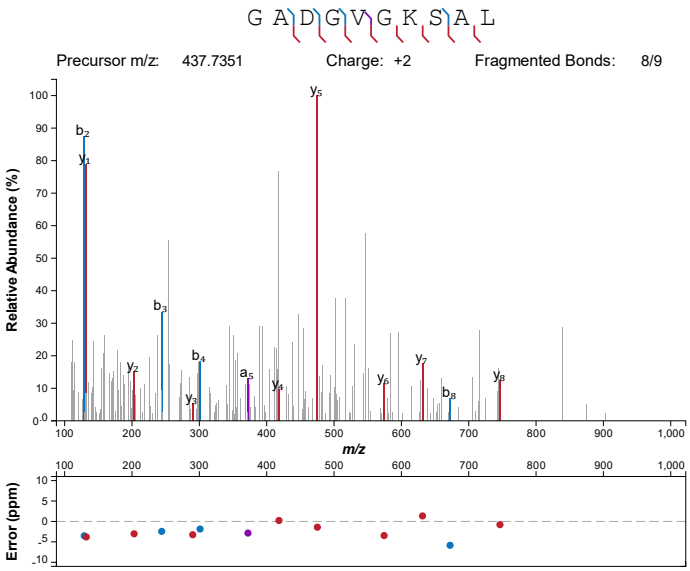


G12D
B*07:02
Mutant, 10mer



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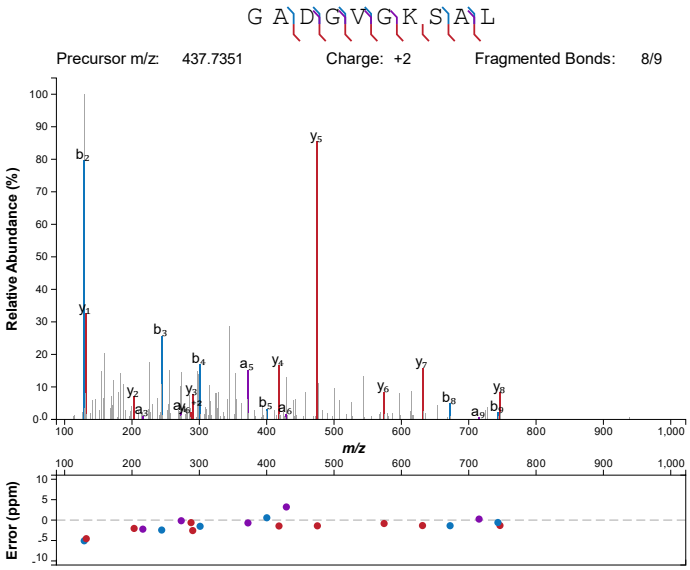
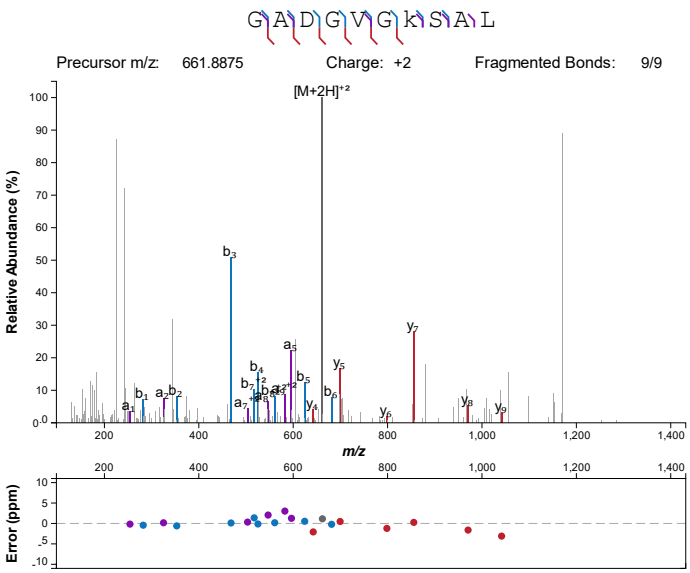


G12D
C*03:04
Mutant, 10mer



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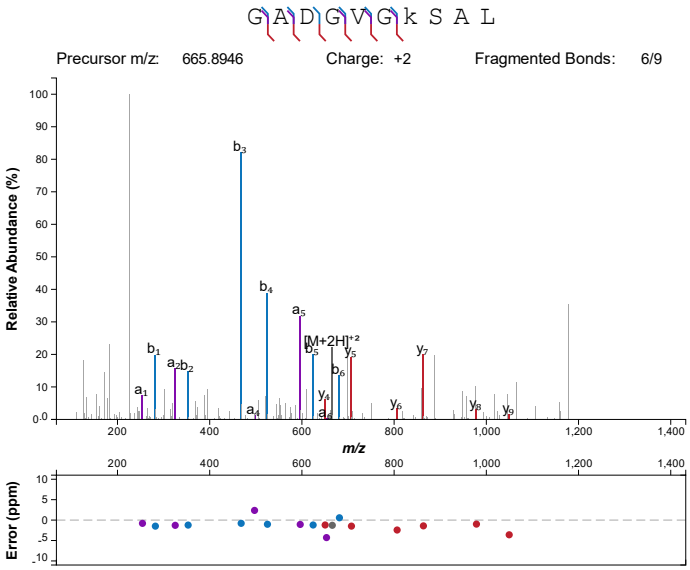
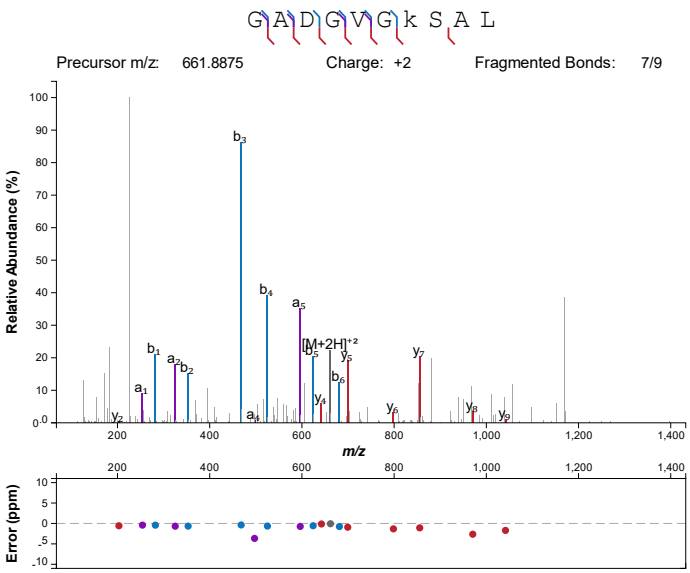


G12D
C*08:02
Mutant, 10mer



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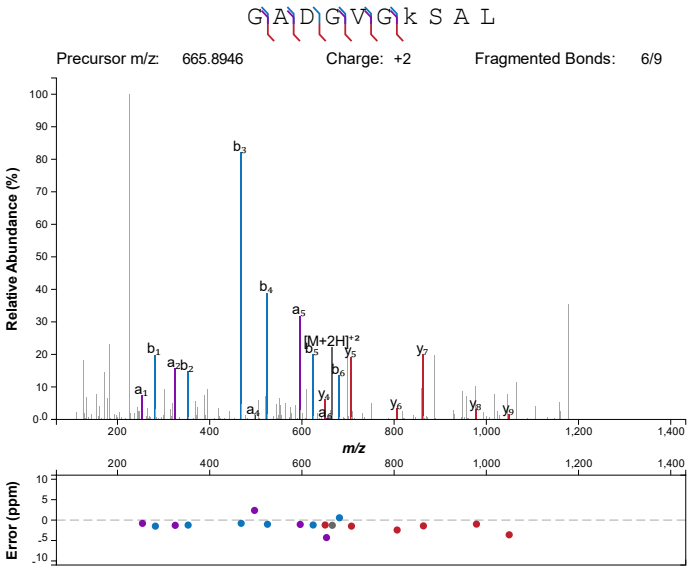
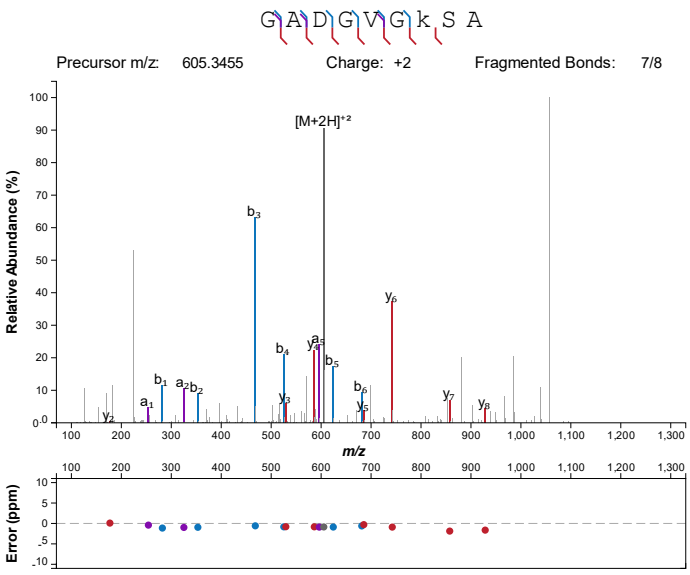


G12D
C*08:02
Mutant, 9mer



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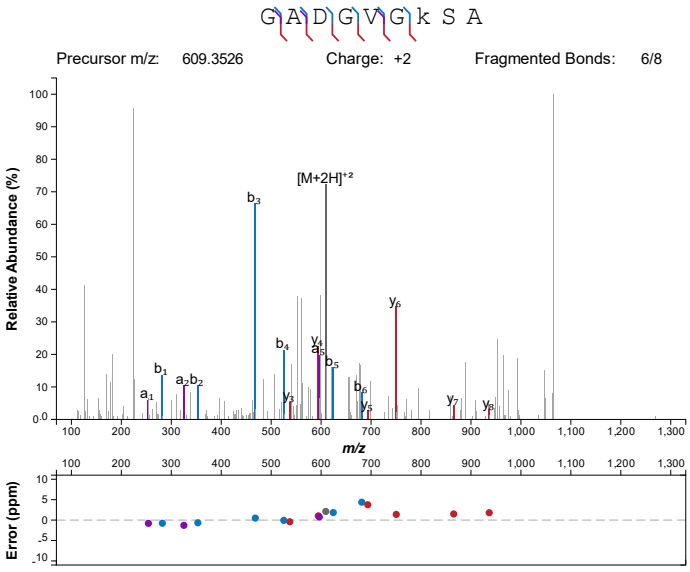
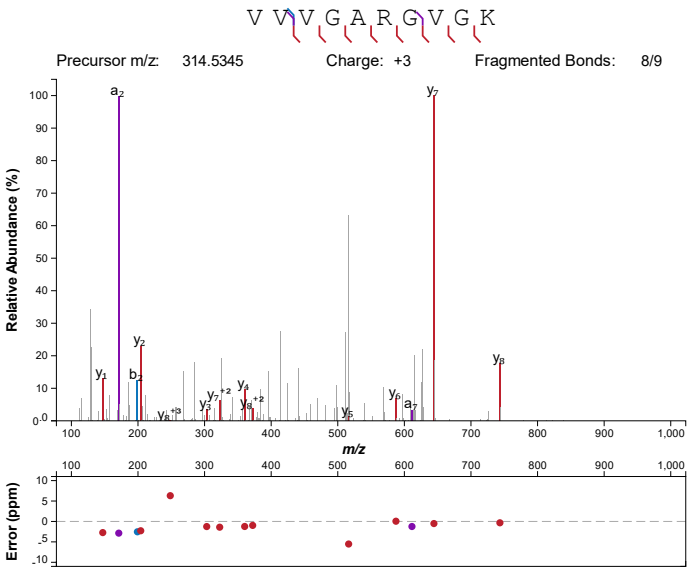


G12R
A*03:01
Mutant, 10mer



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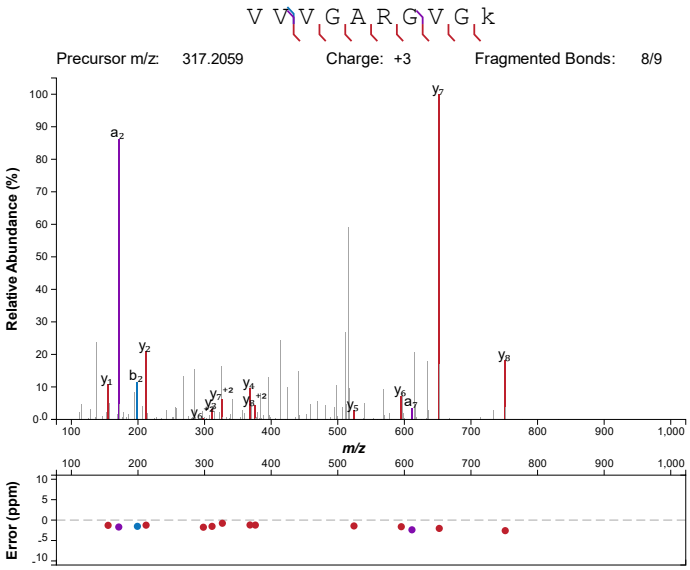
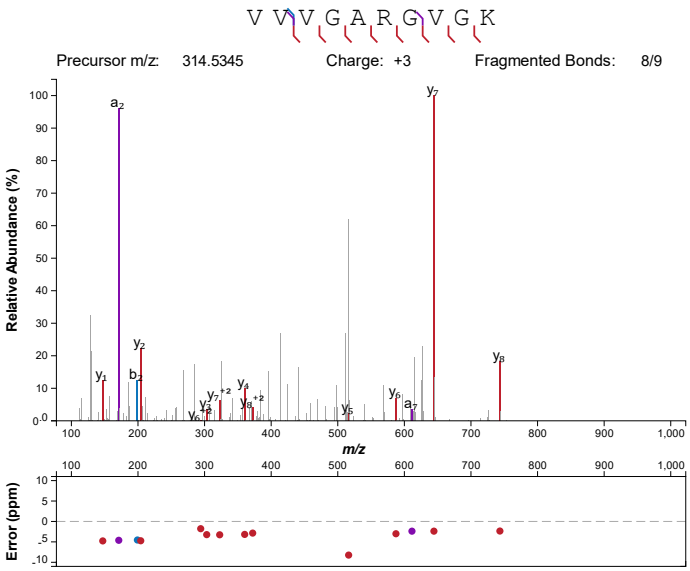


G12R
A*11:01
Mutant, 10mer



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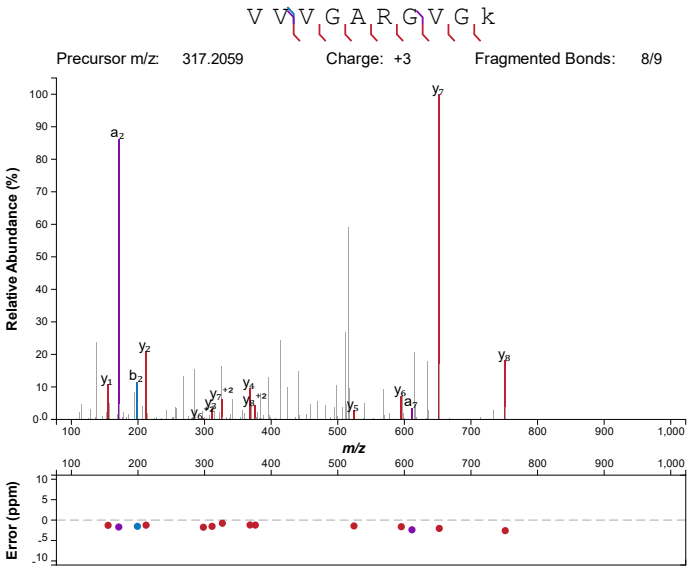
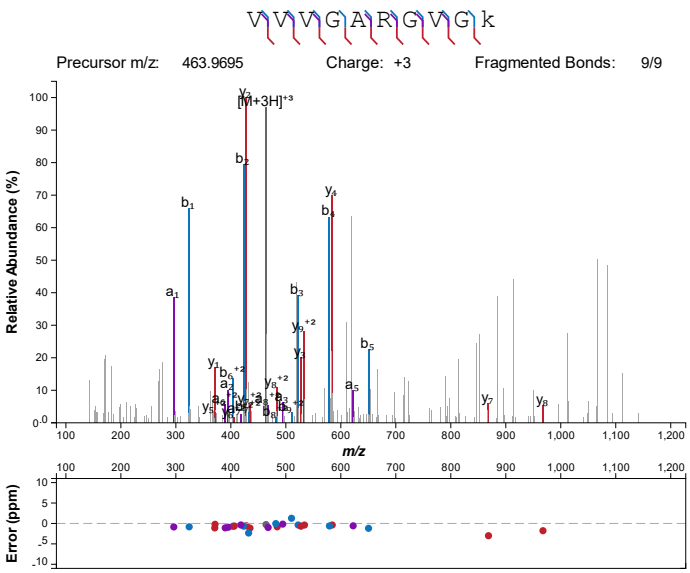


G12R
A*30:01
Mutant, 10mer



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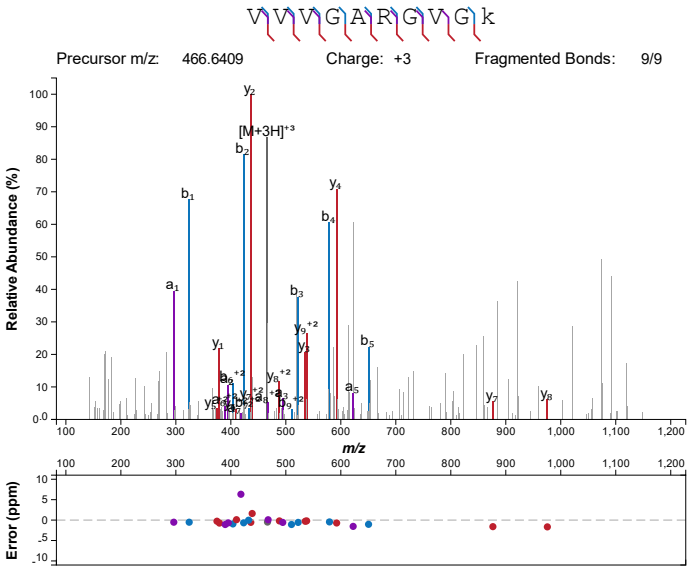
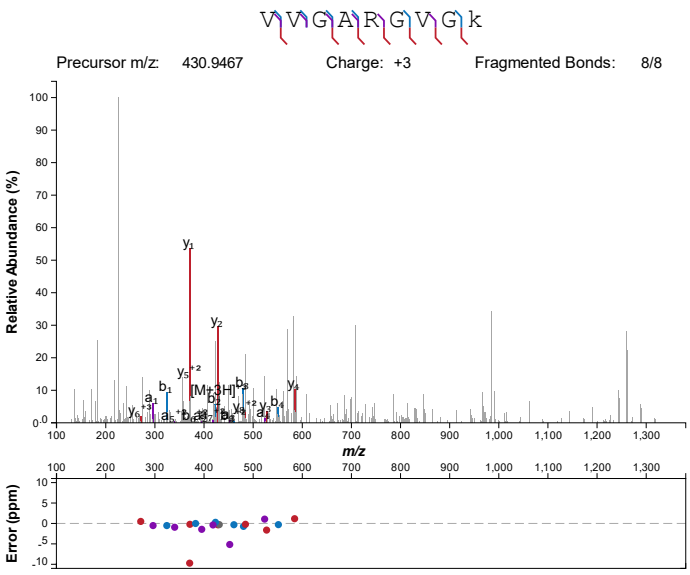


G12R
A*30:01
Mutant, 9mer



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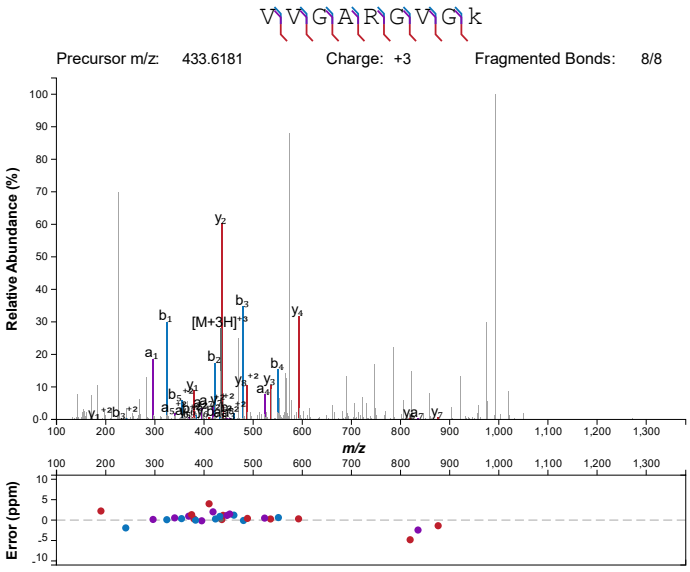
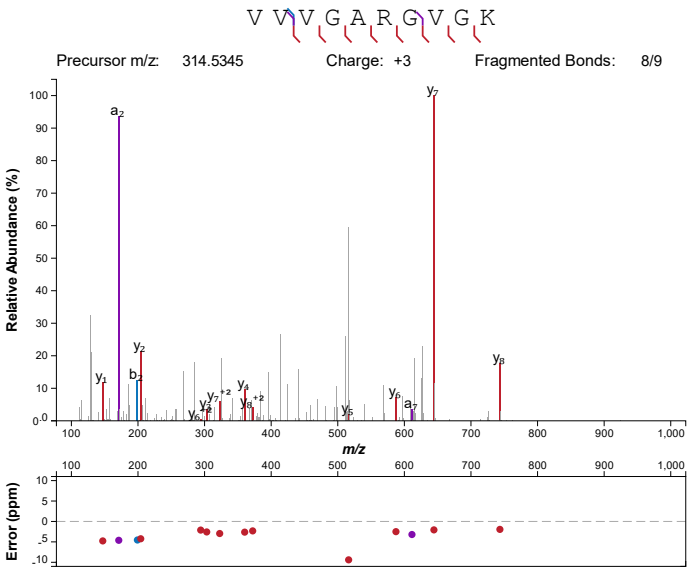


G12R
A*68:01
Mutant, 10mer



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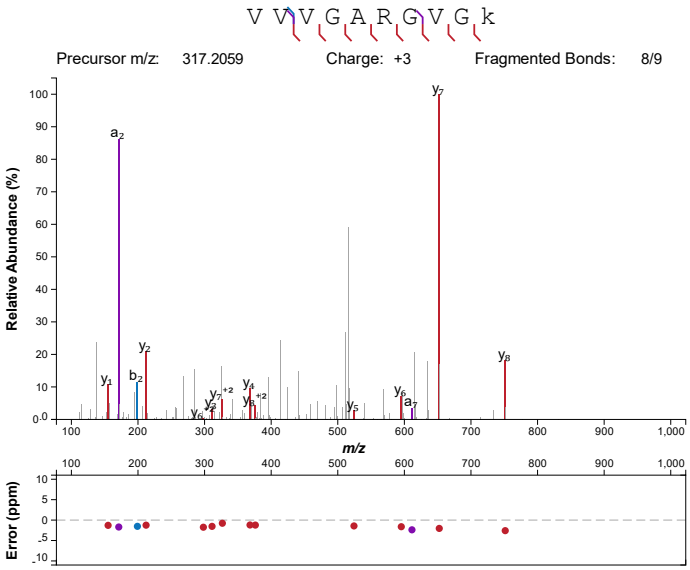
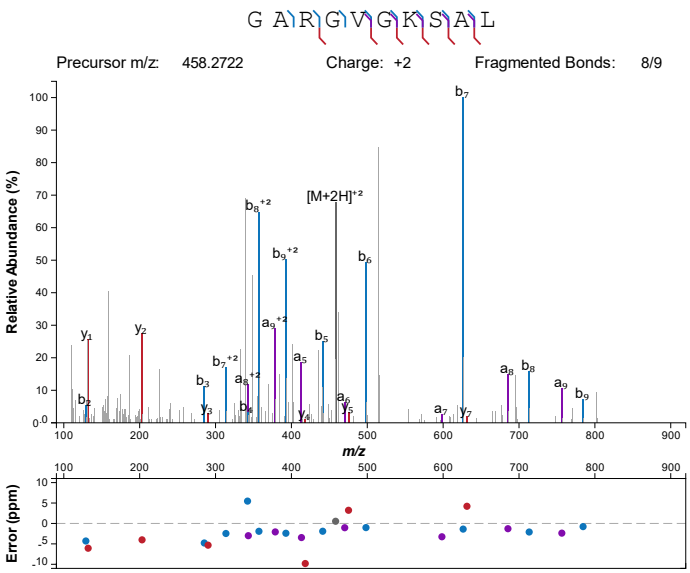


G12R
B*07:02
Mutant, 10mer



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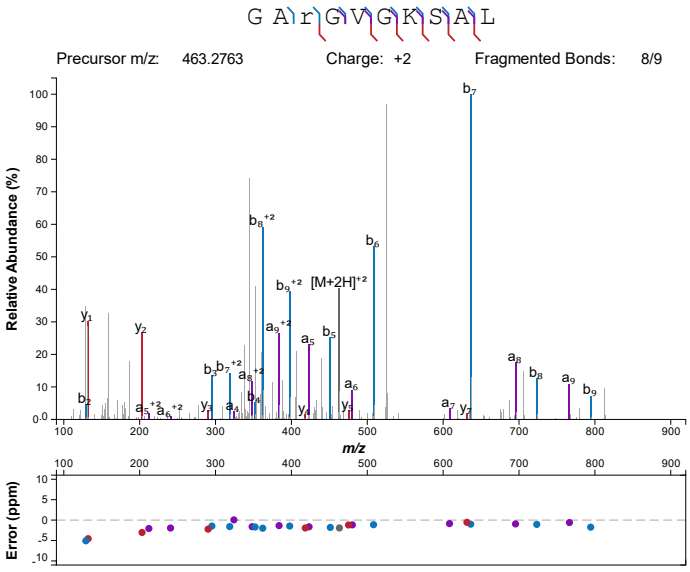
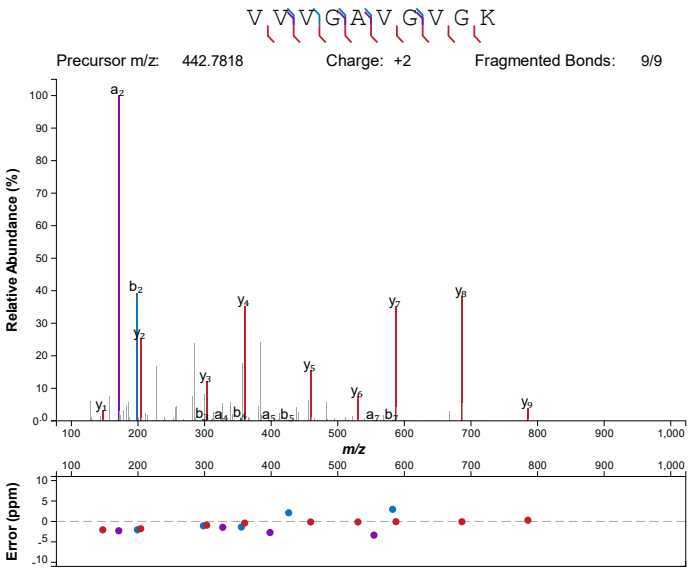


G12V
A*03:01
Mutant, 10mer



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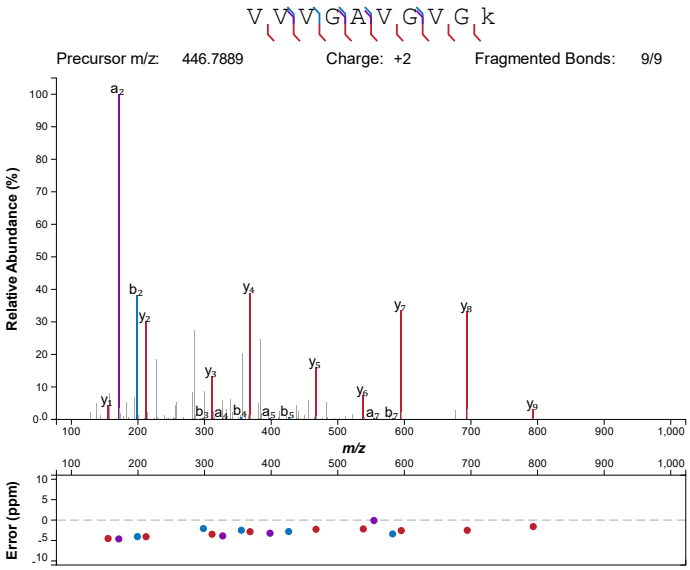
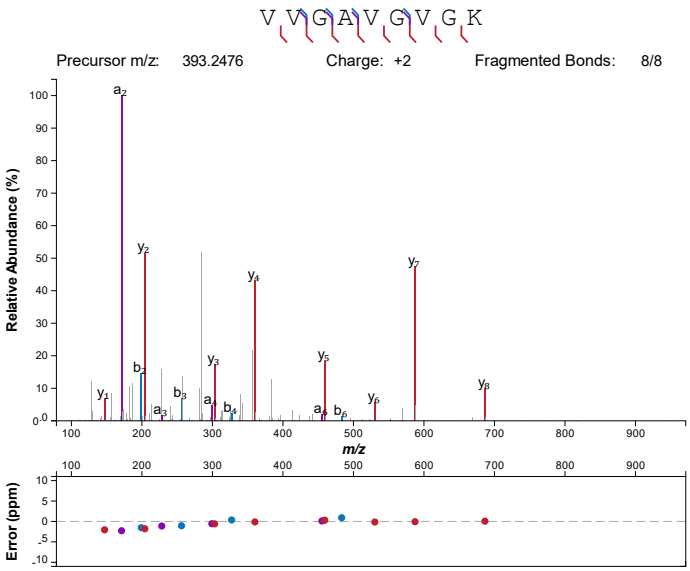


G12V
A*03:01
Mutant, 9mer



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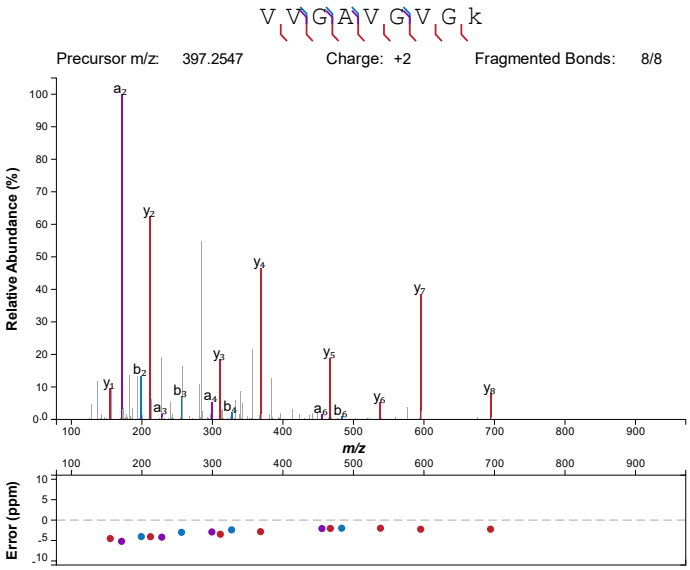
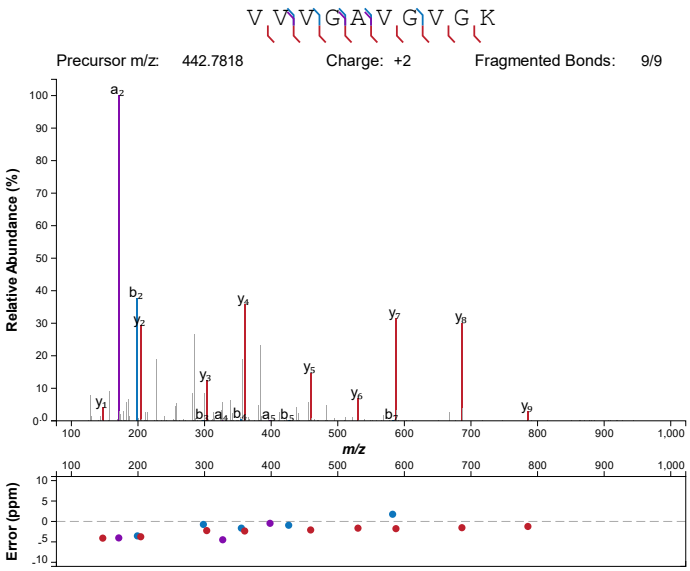


G12V
A*11:01
Mutant, 10mer



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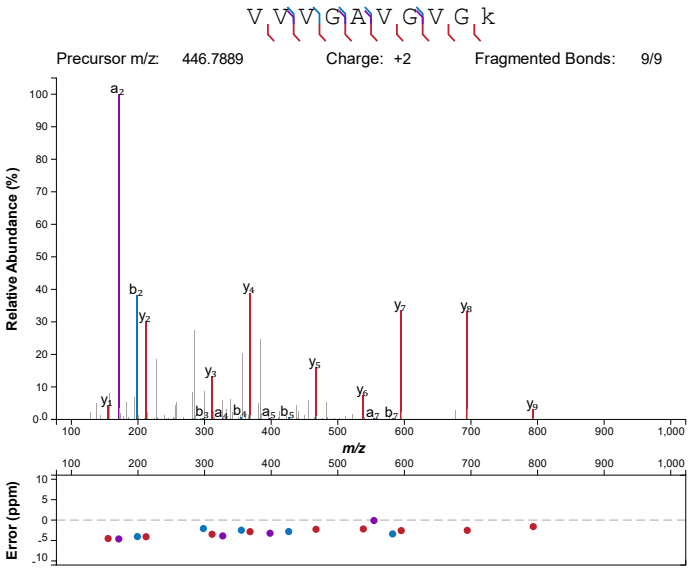
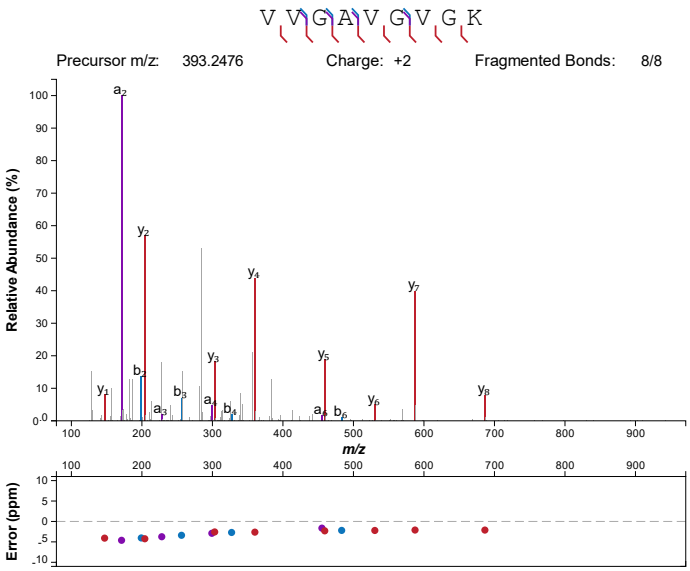


G12V
A*11:01
Mutant, 9mer



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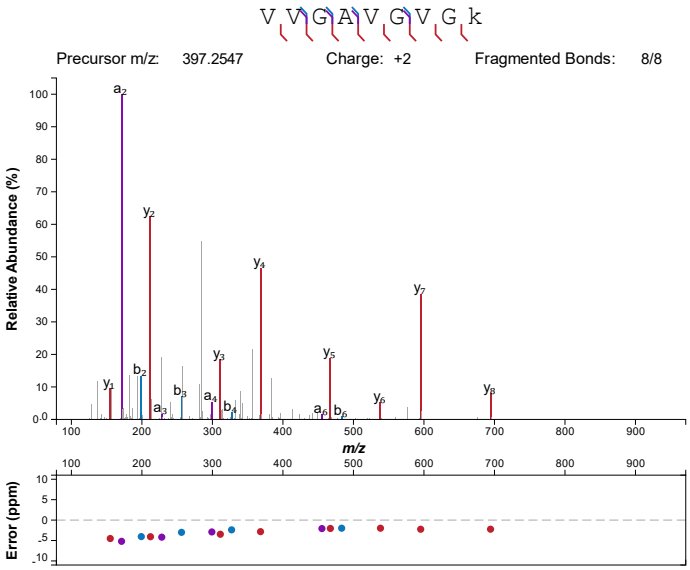
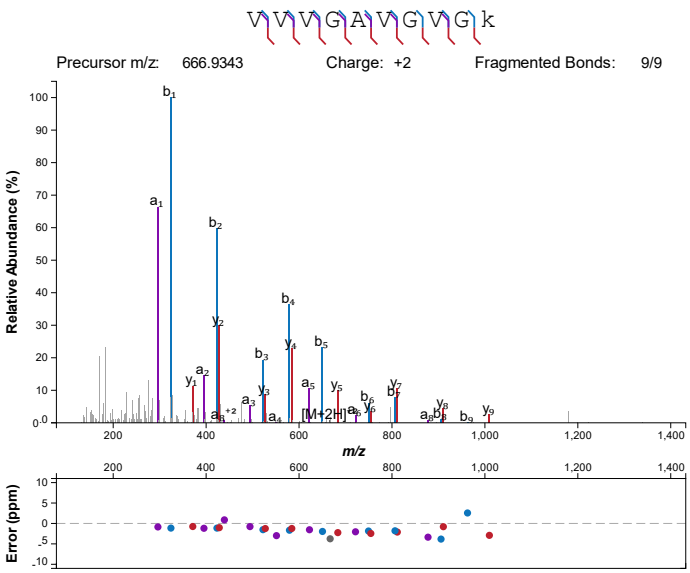


G12V
A*30:01
Mutant, 10mer



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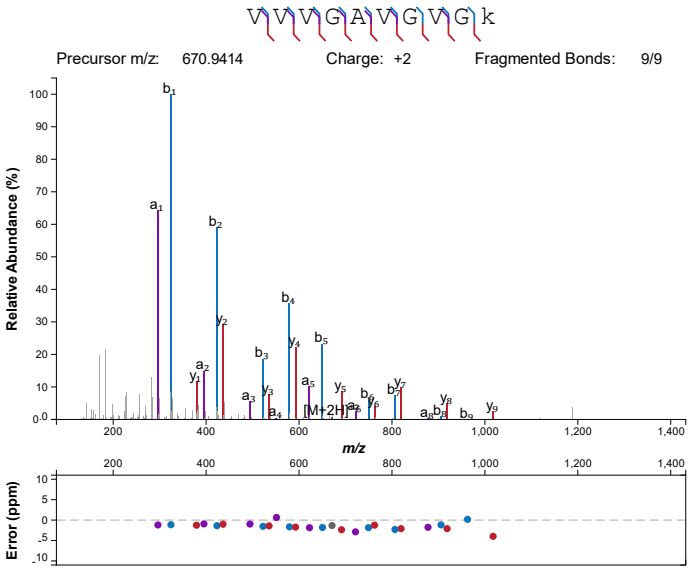
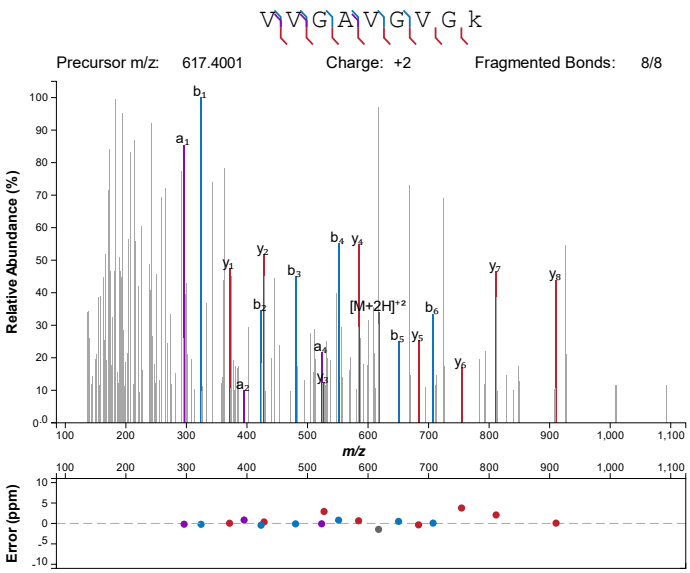


G12V
A*30:01
Mutant, 9mer



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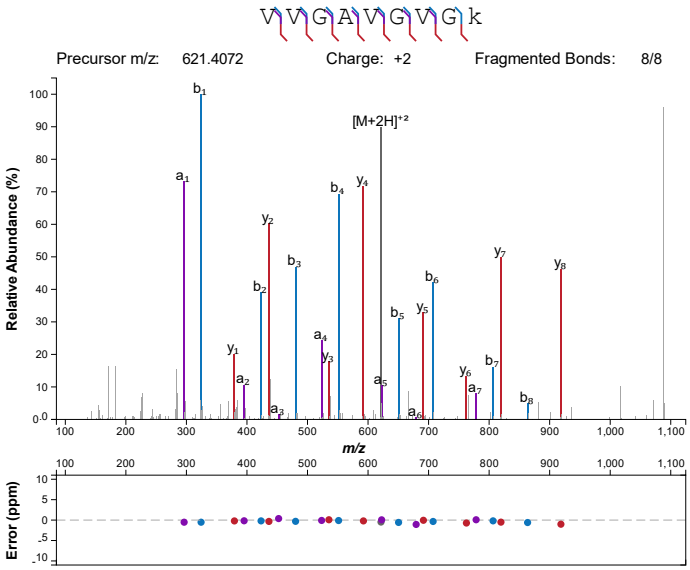
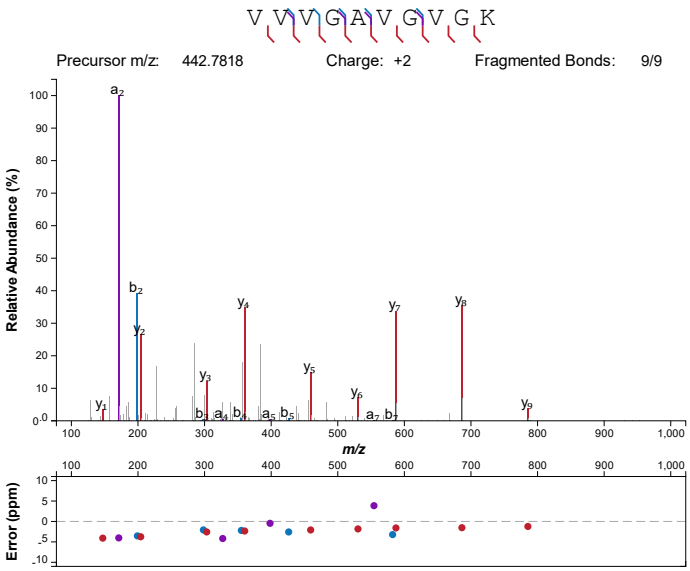


G12V
A*68:01
Mutant, 10mer



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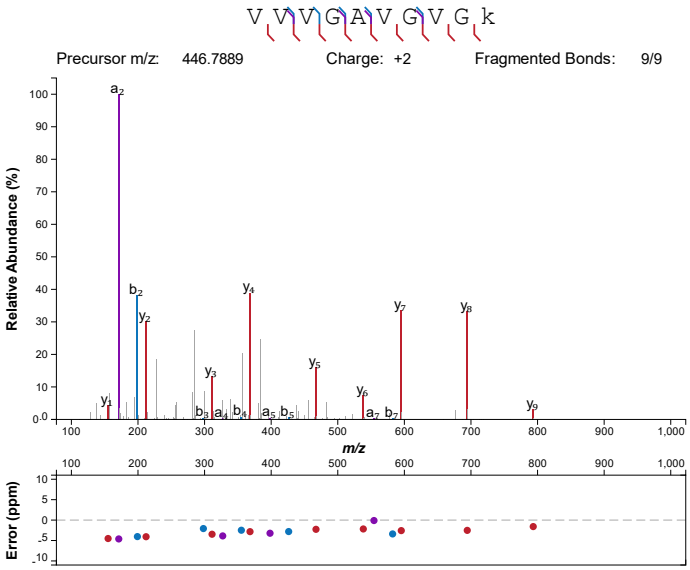
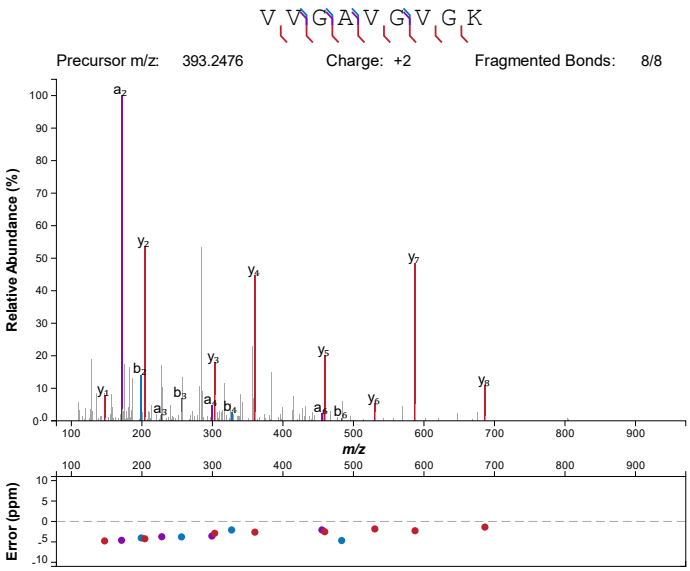


G12V
A*68:01
Mutant, 9mer



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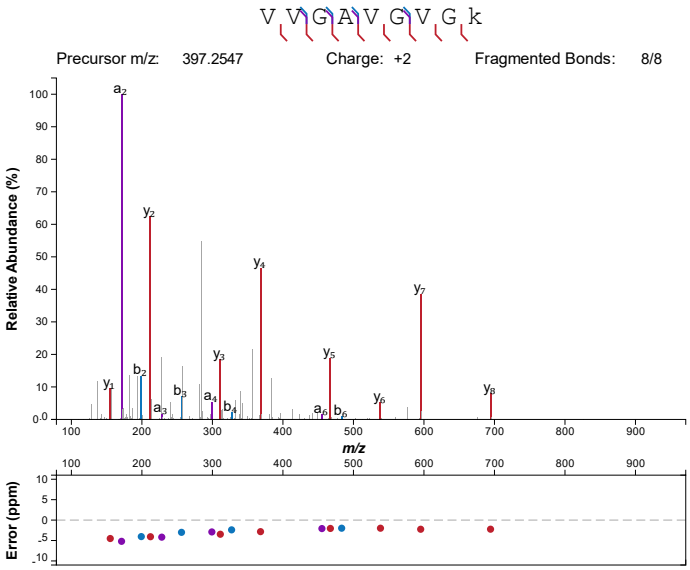
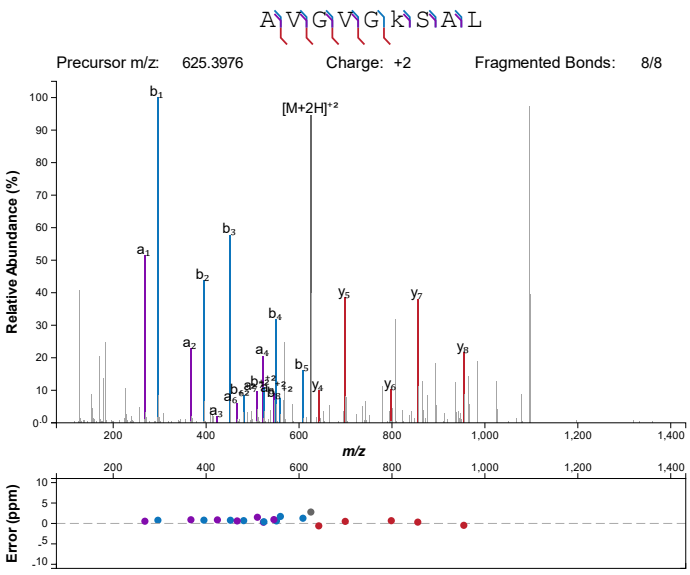


G12V
C*01:02
Mutant, 9mer



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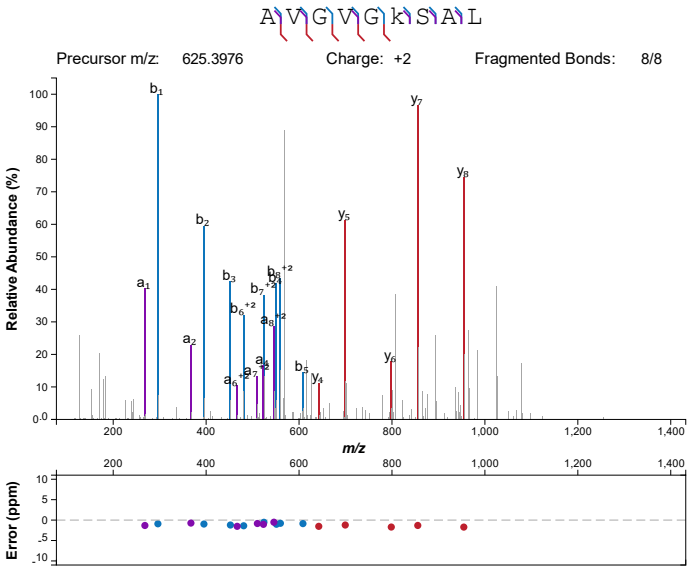
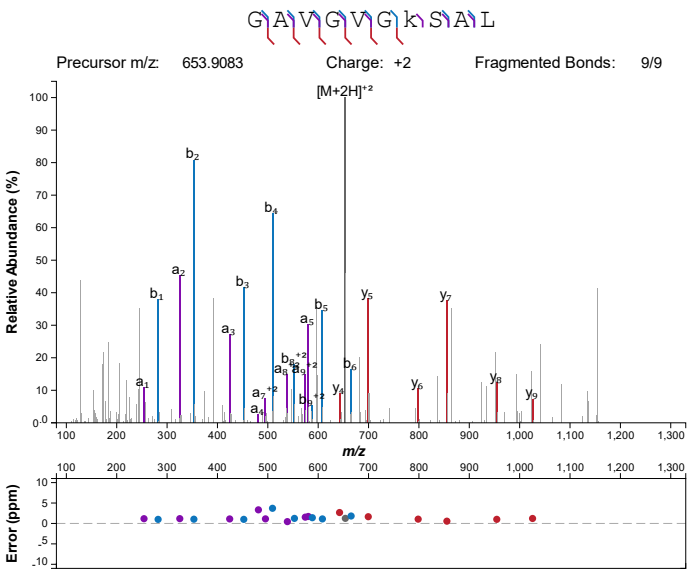


G12V
C*03:03
Mutant, 10mer



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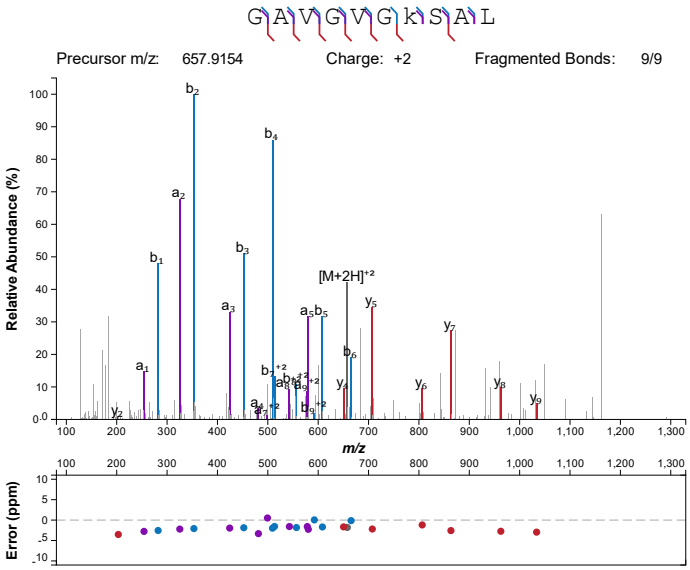
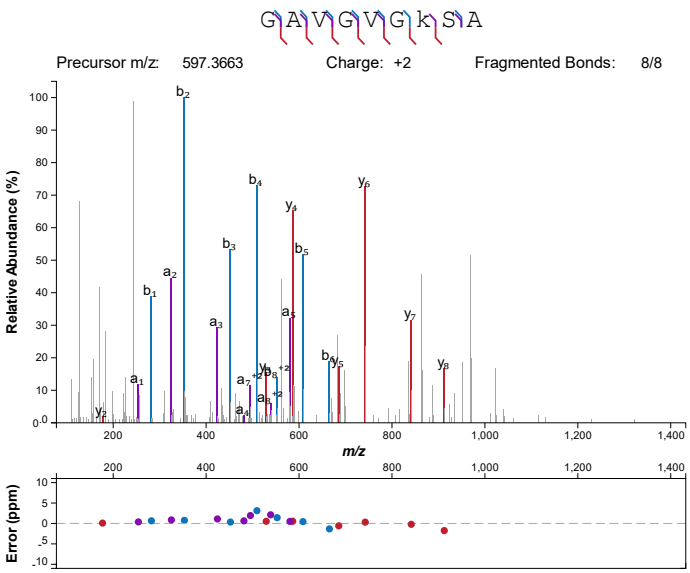


G12V
C*03:03
Mutant, 9mer



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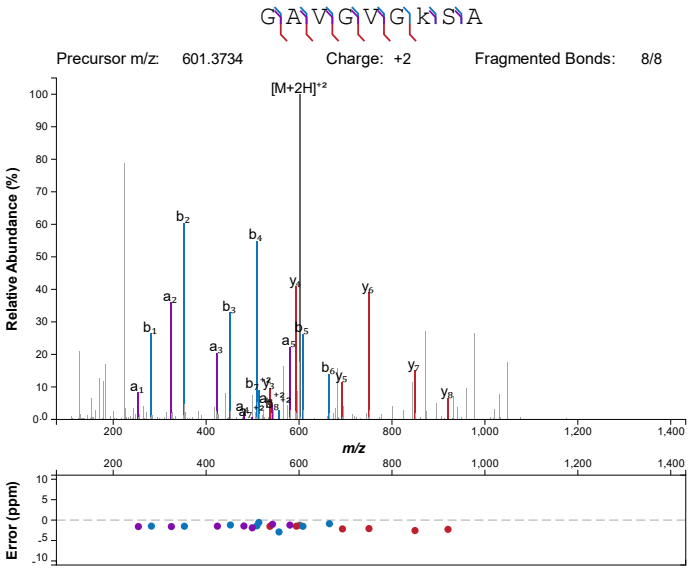
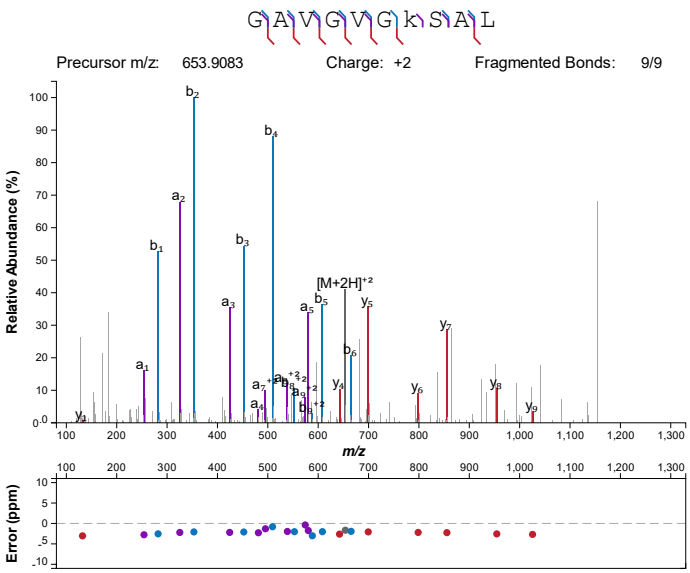


G12V
C*03:04
Mutant, 10mer



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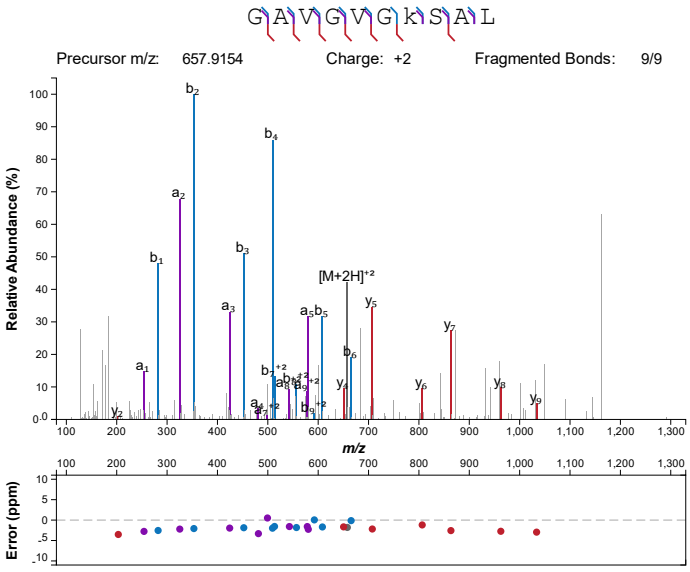
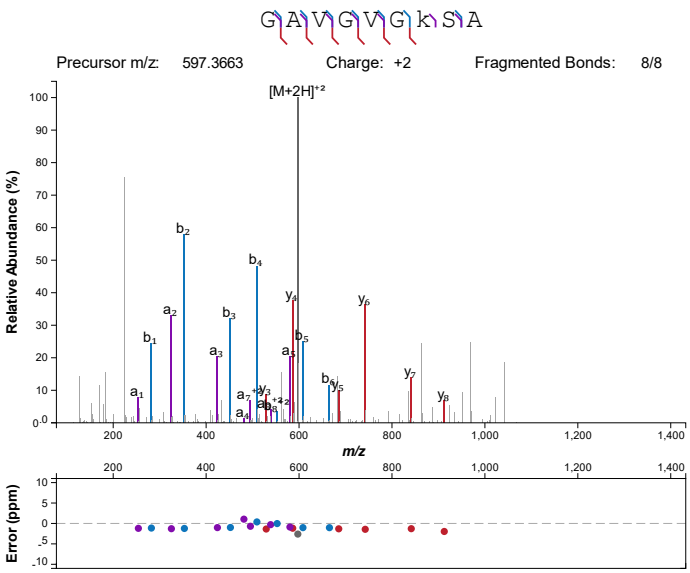


G12V
C*03:04
Mutant, 9mer



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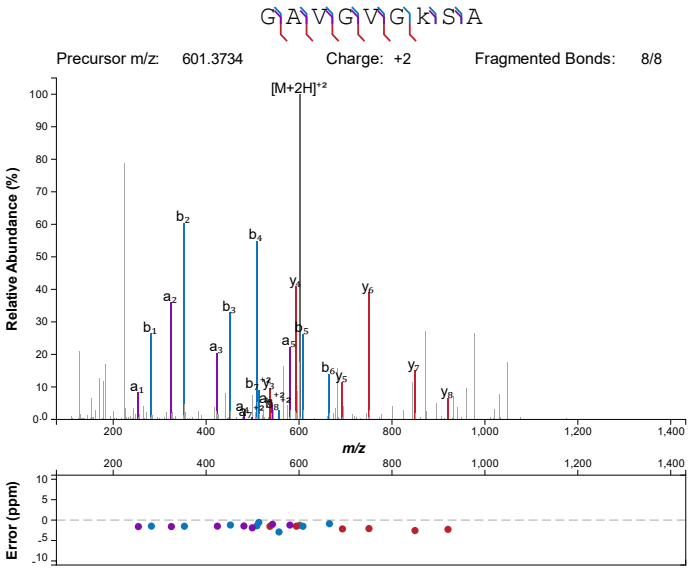
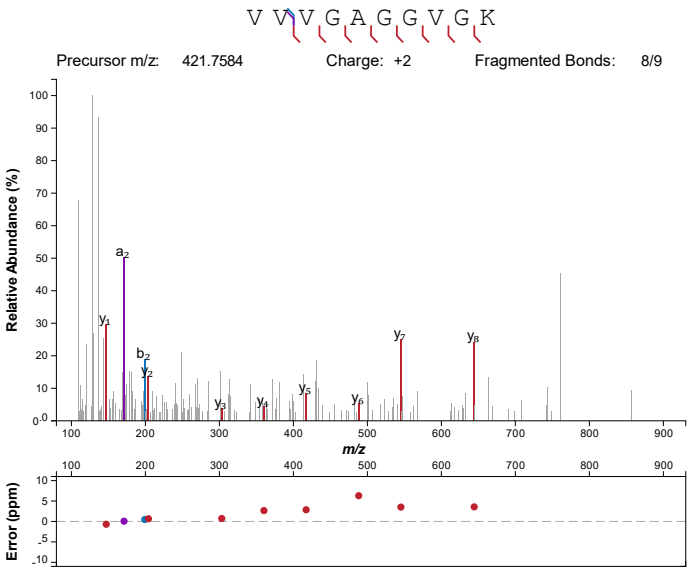


Wild-type, 10mer
A*03:01
NCI-H441



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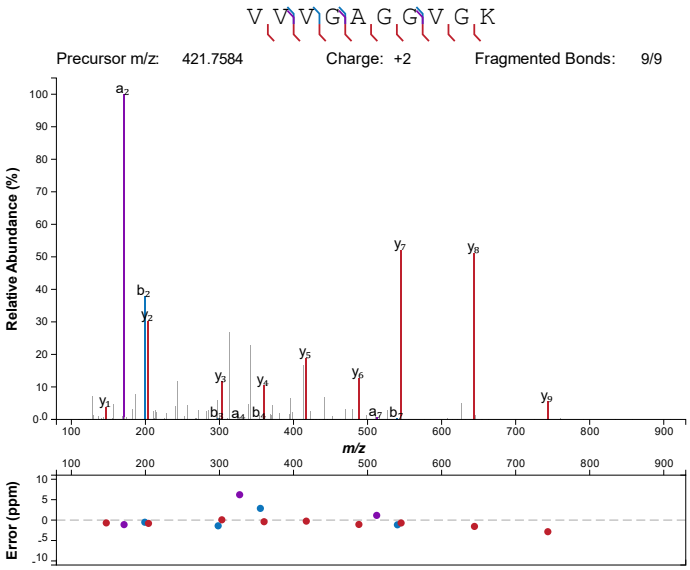
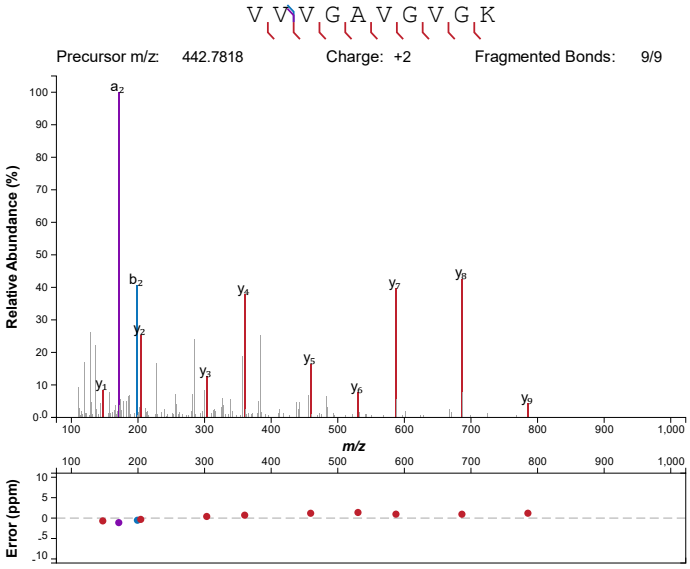


G12V
A*03:01
NCI-H441
Mutant, 10mer



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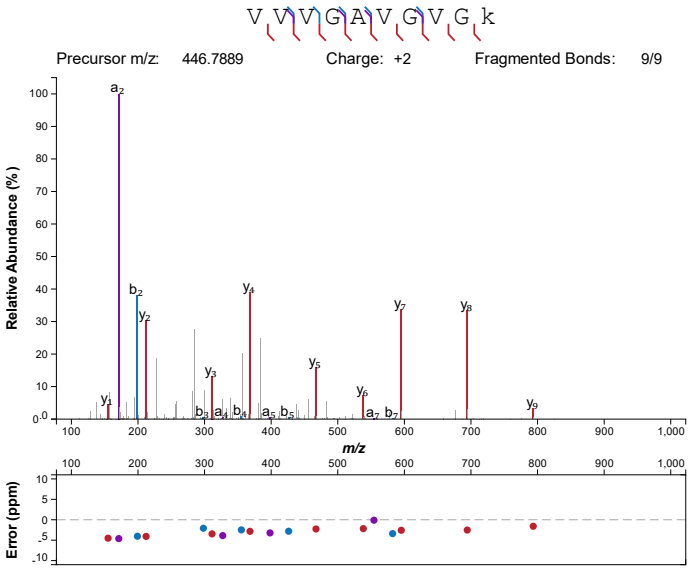
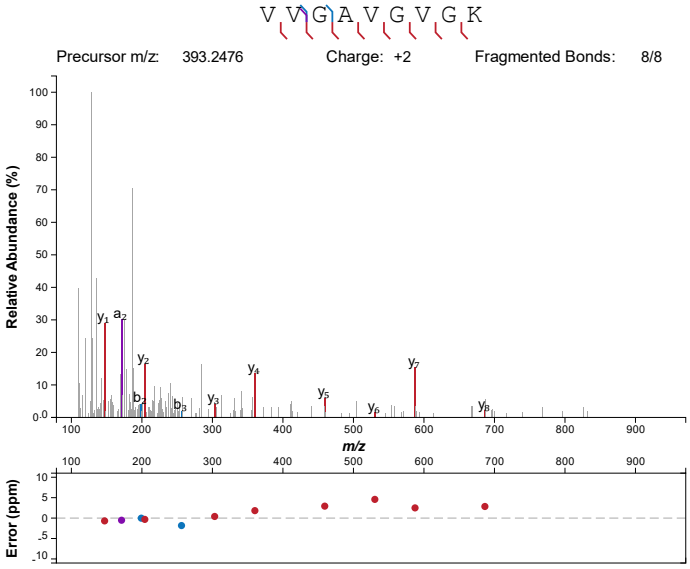


G12V
A*03:01
NCI-H441
Mutant, 9mer



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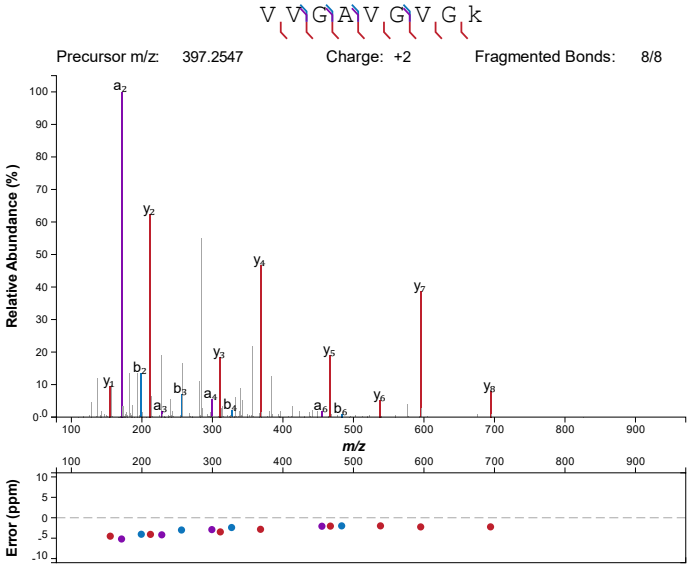
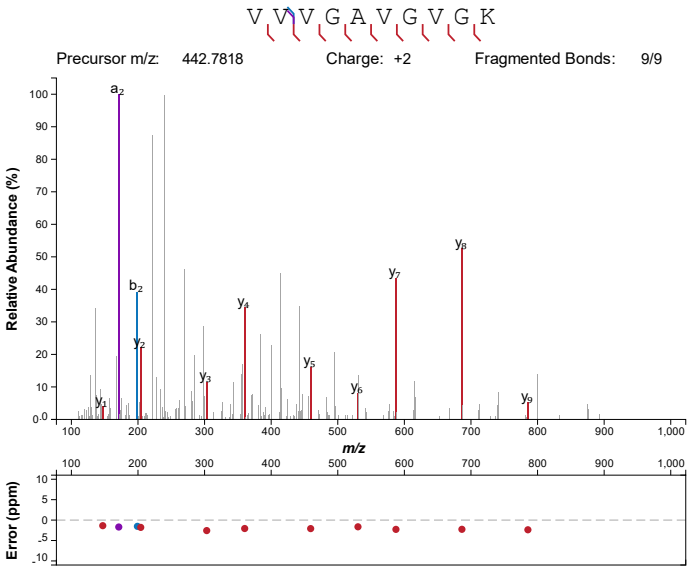


G12V
A*03:01
SW620
Mutant, 10mer



Synthetic