

Supplementary Information

Accurate and ultra-fast *de novo* HLA-I immunopeptide sequencing with robustness evaluation

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Supplementary materials

Contents

<u>Supplementary Table 1. Collected HLA-I immunopeptidomics project IDs and numbers of raw files.</u>	<u>2</u>
<u>Supplementary Table 2. FoxNovo identification and ncORF mapping of 42 validated non-canonical peptides.</u>	<u>5</u>
<u>Supplementary Figure 1. Peptide-charge pair distributions before and after weighted sampling.</u>	<u>7</u>
<u>Supplementary Figure 2. Analysis of database search (pFind3) on the peak removal benchmark.</u>	<u>8</u>
<u>Supplementary Figure 3. Determining the QC score cutoff for FoxNovo.</u>	<u>9</u>
<u>Supplementary Figure 4. FoxNovo performance on database search-missed spectra.</u>	<u>10</u>
<u>Supplementary Figure 5. Benchmarking de novo AI models on peak-removal-with-refill test sets.</u>	<u>11</u>

Supplementary Table 1. Collected HLA-I immunopeptidomics project IDs and numbers of raw files.

Project ID	Raw files	Reference
PXD000394	26	Ref ¹
PXD004023	186	Ref ²
PXD005231	41	https://www.ebi.ac.uk/pride/archive/projects/PXD005231
PXD006455	28	Ref ³
PXD006939	52	Ref ⁴
PXD007596	16	Ref ⁵
PXD007679	15	Ref ⁶
PXD007756	68	Ref ⁷
PXD008500	15	Ref ⁸
PXD008937	10	Ref ⁹
PXD011257	1	Ref ¹⁰
PXD011723	30	Ref ¹¹
PXD012348	18	Ref ¹²
PXD012498	6	Ref ¹³
PXD012797	21	Ref ¹⁴
PXD013064	8	Ref ¹⁵
PXD013831	81	Ref ¹⁶
PXD014397	83	Ref ¹⁷
PXD014804	15	Ref ¹⁸
PXD015039	14	Ref ¹⁹
PXD015957	30	Ref ²⁰
PXD018539	23	Ref ²¹
PXD019466	32	https://www.ebi.ac.uk/pride/archive/projects/PXD019466
PXD019643	576	Ref ²²
PXD020186	9	Ref ²²
PXD020750	24	Ref ²³
PXD021177	2	Ref ²⁴
PXD021398	34	https://www.ebi.ac.uk/pride/archive/projects/PXD021398
PXD021755	9	https://www.ebi.ac.uk/pride/archive/projects/PXD021755
PXD022020	7	Ref ²⁵
PXD022150	36	Ref ²⁶
PXD022323	1	https://www.ebi.ac.uk/pride/archive/projects/PXD022323
PXD022884	72	https://www.ebi.ac.uk/pride/archive/projects/PXD022884
PXD022949	7	Ref ²⁷
PXD022950	13	Ref ²⁸
PXD023044	47	Ref ²⁹
PXD023064	7	https://www.ebi.ac.uk/pride/archive/projects/PXD023064
PXD023143	67	Ref ³⁰
PXD023545	65	https://www.ebi.ac.uk/pride/archive/projects/PXD023545
PXD024109	28	Ref ³¹
PXD024562	4	https://www.ebi.ac.uk/pride/archive/projects/PXD024562
PXD025499	73	Ref ³²
PXD025655	11	Ref ³³
PXD026184	20	Ref ³⁴

Project ID	Raw files	Reference
PXD027182	26	https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PX027182
PXD028088	4	https://www.ebi.ac.uk/pride/archive/projects/PXD028088
PXD028633	29	Ref ³⁵
PXD028862	36	Ref ³⁶
PXD028985	62	Ref ³⁷
PXD029567	60	Ref ³⁸
PXD029594	11	https://www.ebi.ac.uk/pride/archive/projects/PXD029594
PXD029613	16	https://www.ebi.ac.uk/pride/archive/projects/PXD029613
PXD029860	3	Ref ³⁹
PXD029882	42	https://www.ebi.ac.uk/pride/archive/projects/PXD029882
PXD029895	3	Ref ⁴⁰
PXD031451	16	https://www.ebi.ac.uk/pride/archive/projects/PXD031451
PXD031709	9	https://www.ebi.ac.uk/pride/archive/projects/PXD031709
PXD033685	3	https://www.ebi.ac.uk/pride/archive/projects/PXD033685
PXD033868	15	Ref ⁴¹
PXD034017	1	Ref ⁴²
PXD034429	13	https://www.ebi.ac.uk/pride/archive/projects/PXD034429
PXD034772	62	Ref ⁴³
PXD034820	6	Ref ⁴⁴
PXD036277	23	https://www.ebi.ac.uk/pride/archive/projects/PXD036277
PXD037365	6	https://www.ebi.ac.uk/pride/archive/projects/PXD037365
PXD037627	8	Ref ⁴⁵
PXD037655	107	Ref ⁴⁶
PXD037837	72	Ref ⁴⁷
PXD040651	196	https://www.ebi.ac.uk/pride/archive/projects/PXD040651
PXD041893	301	Ref ⁴⁸
PXD043057	7	Ref ⁴⁹
PXD043542	16	Ref ⁵⁰
PXD043984	6	https://www.ebi.ac.uk/pride/archive/projects/PXD043984
PXD043989	16	Ref ⁵¹
PXD044085	6	Ref ⁵²
PXD046803	19	Ref ⁵³
PXD046825	9	Ref ⁵⁴
PXD046857	1	Ref ⁵⁵
PXD049996	13	https://www.ebi.ac.uk/pride/archive/projects/PXD049996
PXD051190	1	Ref ⁵⁶
PXD051538	8	Ref ⁵⁷
PXD051734	8	Ref ⁵⁸
PXD052187	9	Ref ⁵⁹
PXD053264	13	Ref ⁶⁰
PXD055042	4	https://www.ebi.ac.uk/pride/archive/projects/PXD055042
PXD055609	5	https://www.ebi.ac.uk/pride/archive/projects/PXD055609
PXD055726	23	Ref ⁶¹
PXD056426	17	Ref ⁶²
PXD059824	63	https://www.ebi.ac.uk/pride/archive/projects/PXD059824
PXD066255	4	https://www.ebi.ac.uk/pride/archive/projects/PXD066255
PXD066752	1	Ref ⁶³

Project ID	Raw files	Reference
PXD003790	17	https://www.ebi.ac.uk/pride/archive/projects/PXD003790
PXD008127	61	Ref ⁶⁴
PXD009738	26	Ref ⁶⁵
PXD009925	4	https://www.ebi.ac.uk/pride/archive/projects/PXD009925
PXD012083	10	Ref ⁶⁶
PXD013649	52	Ref ⁶⁷
PXD014062	8	Ref ⁶⁸
PXD015646	23	Ref ⁶⁹
PXD016060	10	Ref ⁷⁰
PXD016582	14	Ref ⁷¹
PXD020011	96	Ref ⁷²
PXD028364	6	https://www.ebi.ac.uk/pride/archive/projects/PXD028364
PXD031108	11	Ref ⁷³
PXD037270	45	Ref ⁷⁴
PXD037845	9	Ref ⁷⁵
PXD038271	9	Ref ⁷⁶
PXD038371	6	Ref ⁷⁷
PXD044235	18	Ref ⁷⁸
PXD045000	106	Ref ⁷⁹
PXD045265	15	Ref ⁸⁰
PXD046182	33	Ref ⁸¹
PXD046891	2	Ref ⁸²
PXD051490	20	Ref ⁸³
PXD052031	14	Ref ⁸⁴
PXD024533	9	Ref ⁸⁵
PXD027830	9	Ref ⁸⁶
PXD040740	5	http://central.proteomexchange.org/cgi/GetDataset?ID=PXD040740
PXD047303	5	Ref ⁸⁷
PXD065200	2	Ref ⁸⁸
PXD055841	11	https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PX055841-1
MSV000084442	46	Ref ⁸⁹
MSV000080527	72	Ref ⁹⁰
MSV000084172	339	Ref ⁸⁹

Supplementary Table 2. FoxNovo identification and ncORF mapping of 42 validated non-canonical peptides.

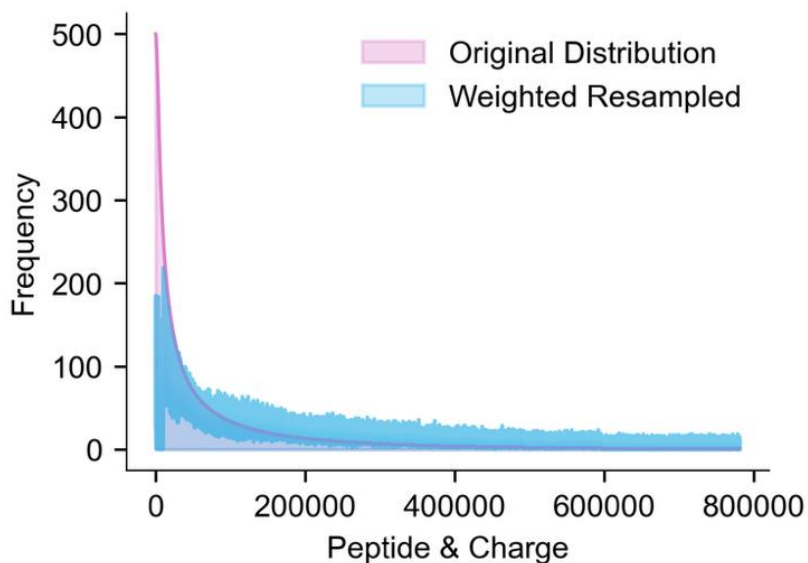
HLA peptide origin	Sequence	Recovered by FoxNovo	Mapped to ncORF database	ncORF match
lncRNA	RFTDVRVQF	Yes	Yes	c8norep34
lncRNA	VTDAQASHIY	Yes	No	N/A
lncRNA	ATEHDKNSTNIY	Yes	Yes	c1riboseqorf140
lncRNA	FIETESQLY	Yes	Yes	c2norep199
lncRNA	RVSHLTSSV	Yes	No	N/A
lncRNA	IFNPVSKNF	Yes	Yes	c2norep207; c2norep212
lncRNA	IWNGKGHFSF	Yes	No	N/A
lncRNA	SYLEPQVTF	Yes	No	N/A
lncRNA	ANRPPTQAL	Yes	No	N/A
lncRNA	RPGPGTRKSL	Yes	Yes	c22riboseqorf33
lncRNA	SYLRRHLDF	Yes	No	N/A
lncRNA	SPREPAGGGQV	Yes	No	N/A
lncRNA	FSDFPGGDKGKFY	Yes	Yes	c1riboseqorf116
lncRNA	SPAQRDRVVM	Yes	Yes	c6riboseqorf130
lncRNA	AAEPDRAVL	Yes	No	N/A
lncRNA	RQFLEPSGF	Yes	Yes	c2riboseqorf65
lncRNA	TPNSLAKPL	Yes	No	N/A
lncRNA	KSDLKPLSY	Yes	No	N/A
lncRNA	NPRNDGIAL	Yes	Yes	c4norep80; c4riboseqorf44; c4riboseqorf46
lncRNA	LPQETRIPL	Yes	No	N/A
lncRNA	DVDIHKDLY	Yes	No	N/A
lncRNA/TEs	SPRSPPSL	No	No	N/A
lncRNA	RIHDSRSSL	Yes	No	N/A
lncRNA	ASKGAGVSF	Yes	No	N/A
lncRNA	KMEETTVTY	Yes	No	N/A
lncRNA	EYNNIFHTF	Yes	No	N/A
lncRNA	ETDIOMETRY	Yes	Yes	c8riboseqorf79
lncRNA	YLDPAQQNLY	Yes	No	N/A
lncRNA	VPHQPAPPF	Yes	Yes	c20riboseqorf37; c20riboseqorf38
lncRNA	TFQKKTkem	Yes	No	N/A
lncRNA	APKLMVRPF	Yes	Yes	cXriboseqorf15
lncRNA	QTEKENSky	Yes	No	N/A
lncRNA/TEs	KPRHQGPAL	Yes	No	N/A
lncRNA	APKSSSGFSL	Yes	No	N/A
lncRNA	YSETLSNGGKY	Yes	No	N/A

lncRNA	RPPHPRPSL	Yes	No	N/A
lncRNA	KFLVQNNVF	Yes	No	N/A
lncRNA	HIKTQDTGY	Yes	No	N/A
lncRNA	VMKGINQGY	Yes	No	N/A
TEs annotated	KVFKNGNAF	Yes	No	N/A
TEs	NMDGPGGHY	Yes	No	N/A
TEs	AAFDRAVHF	Yes	No	N/A

Note:

This table lists the 42 PRM-validated non-canonical melanoma HLA-I peptides from Chong et al⁶⁷. and indicates whether each peptide was recovered by FoxNovo de novo sequencing and whether it mapped to the public ncORF database^{91,92}.

Supplementary Figure 1. Peptide-charge pair distributions before and after weighted sampling.



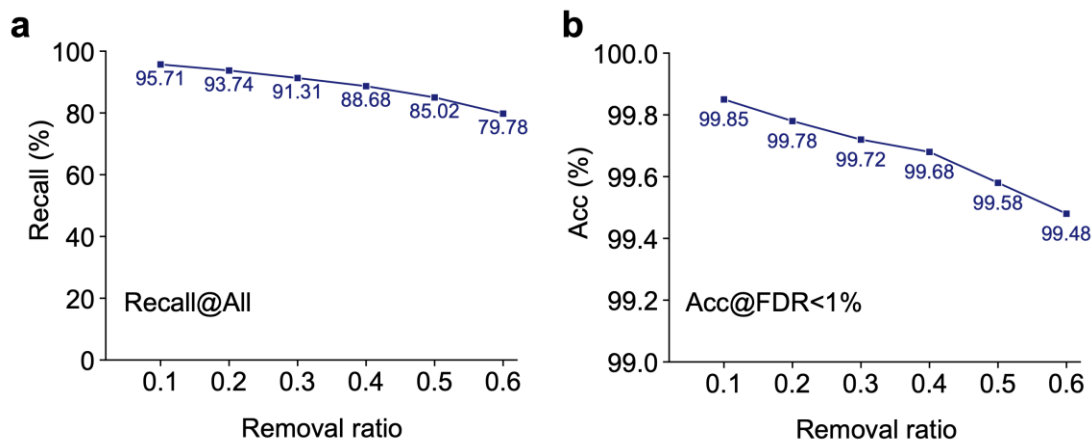
Note:

The training datasets contain 781,588 peptide-charge pairs.

Sampling statistics are shown for one training epoch.

Peptide-charge-weighted sampling reduces the dominance of high-frequency pairs while increasing the sampling frequency of most low-frequency pairs.

Supplementary Figure 2. Analysis of database search (pFind3) on the peak removal benchmark.



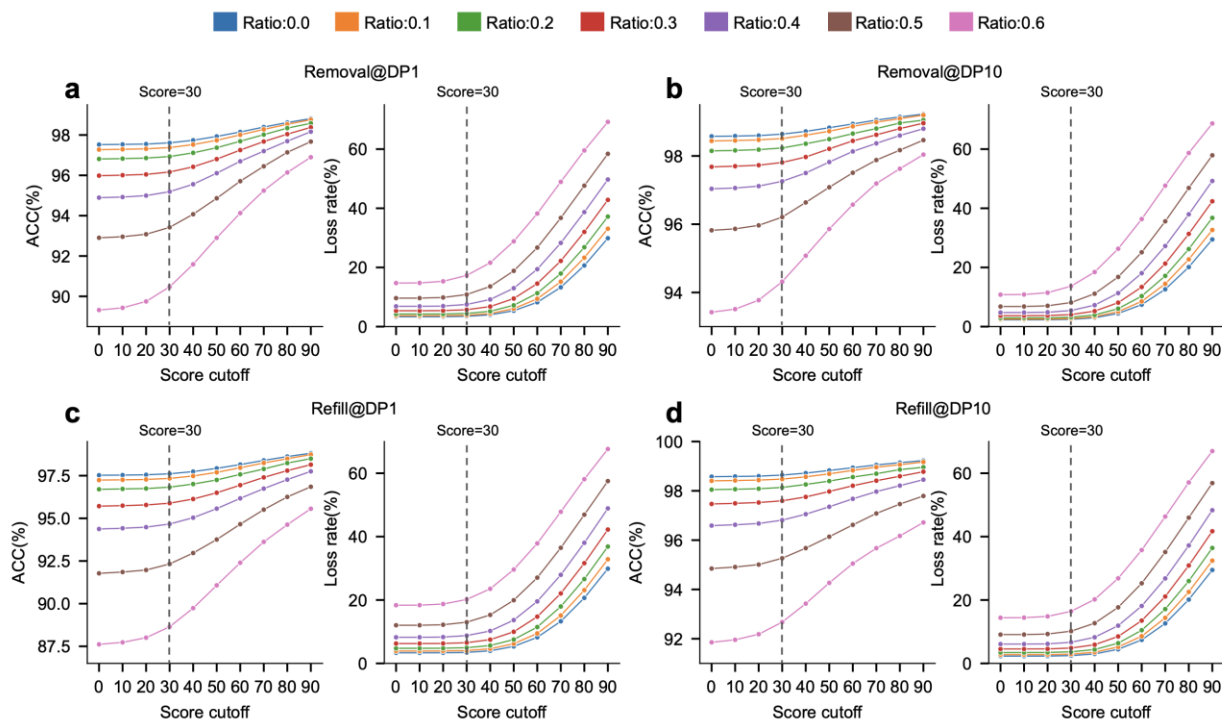
- (a) Recall of pFind3 across all spectra at different peak removal ratios, without applying FDR filtering.
(b) Accuracy of pFind3 at different peak removal levels after applying a <1% PSM-level FDR threshold.

Note:

Database search recall decreased as the peak removal ratio increased, reaching 85.02% and 79.78% at 50% and 60% removal, respectively.

FDR control effectively maintained high identification accuracy, with accuracy remaining above 99% across all peak removal ratios.

Supplementary Figure 3. Determining the QC score cutoff for FoxNovo.



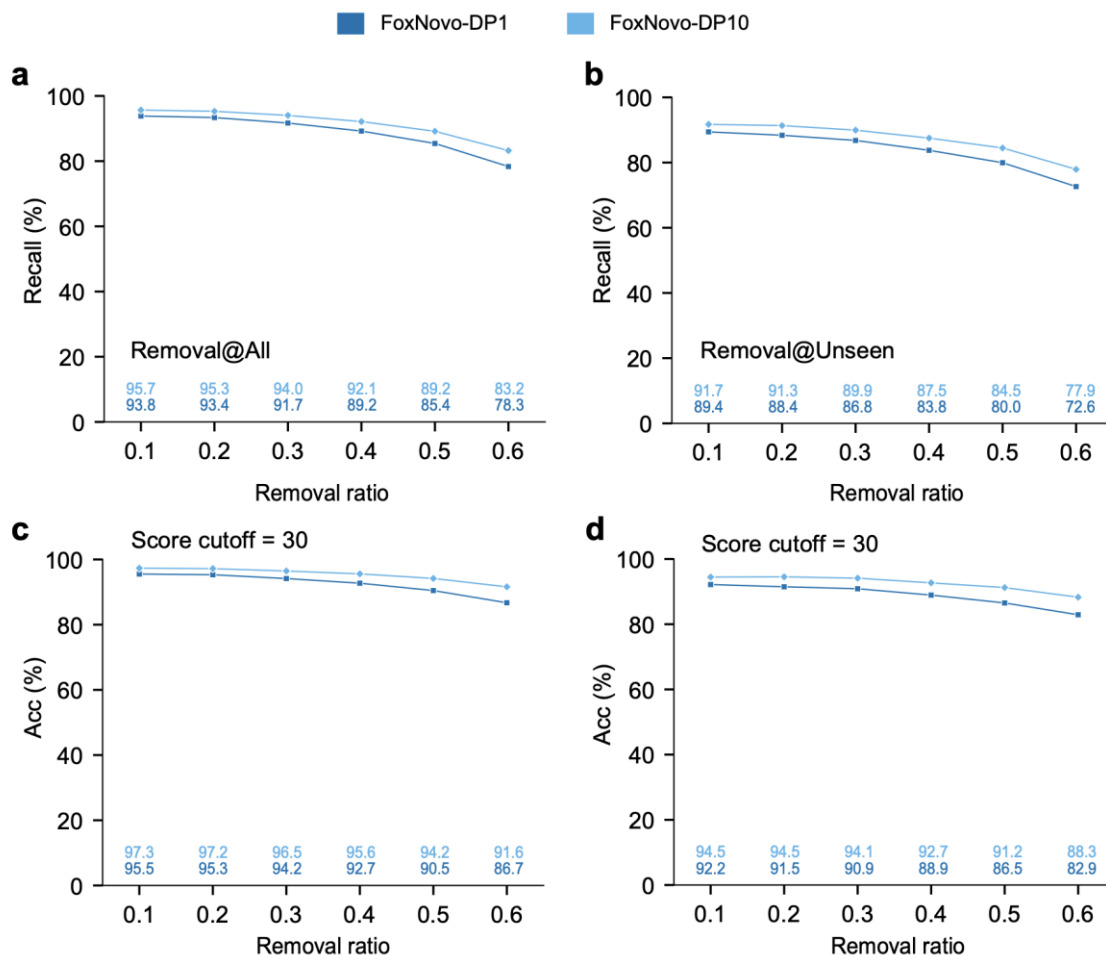
- (a) Accuracy and loss rate at different cutoffs on the peak removal test dataset for FoxNovo-DP1.
(b) Accuracy and loss rate at different cutoffs on the peak removal test dataset for FoxNovo-DP10.
(c) Accuracy and loss rate at different cutoffs on the peak-removal-with-refill test dataset for FoxNovo-DP1.
(d) Accuracy and loss rate at different cutoffs on the peak-removal-with-refill test dataset for FoxNovo-DP10.

Note:

The loss rate is defined as one minus recall after score filtering. The refill process refers to peak-removal-with-refill setting.

The final QC score threshold was set to 30, which provides a good balance between accuracy and loss rate.

Supplementary Figure 4. FoxNovo performance on database search-missed spectra.



(a) Recall of FoxNovo-DP1 and FoxNovo-DP10 on database-search-missed peak-removal spectra across different peak-removal ratios.

(b) Recall of FoxNovo-DP1 and FoxNovo-DP10 on database-search-missed spectra from the unseen-peptide subset across different peak-removal ratios.

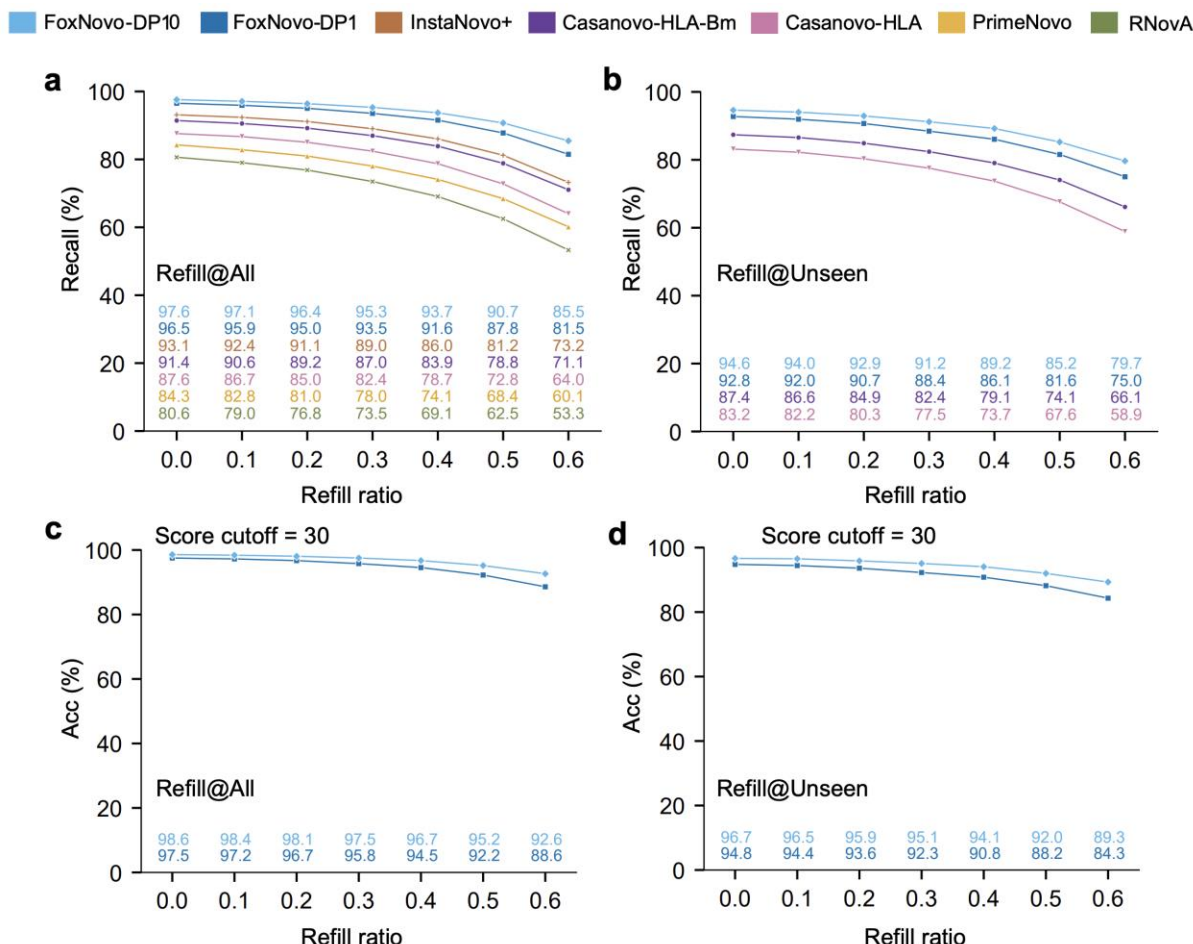
(c) Accuracy of FoxNovo-DP1 and FoxNovo-DP10 with QC filtering (score cutoff) on database-search-missed peak-removal spectra across different peak-removal ratios.

(d) Accuracy of FoxNovo-DP1 and FoxNovo-DP10 with QC filtering on database-search-missed spectra from the unseen-peptide subset across different peak-removal ratios.

Note:

FoxNovo-DP1 achieved recall of 85.4% and 78.3% on database-search-missed perturbed spectra at peak-removal ratios of 50% and 60%, respectively.

Supplementary Figure 5. Benchmarking de novo AI models on peak-removal-with-refill test sets.



(a) Recall of all de novo models on all spectra in the peak-removal-with-refill benchmark across different refill ratios.

(b) Recall of FoxNovo-DP1 and FoxNovo-DP10 on unseen-peptide spectra in the peak-removal-with-refill benchmark across different refill ratios.

(c) Accuracy of FoxNovo-DP1 and FoxNovo-DP10 with QC filtering (score cutoff) on all spectra in the peak-removal-with-refill benchmark across different refill ratios.

(d) Accuracy of FoxNovo-DP1 and FoxNovo-DP10 with QC filtering on unseen-peptide spectra in the peak-removal-with-refill benchmark across different refill ratios.

Note:

FoxNovo-DP1 achieved recall of 87.8% and 81.5% on perturbed spectra with refill ratios of 50% and 60%, respectively.

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