Supplementary Information

Kotli et al. A Peptidomics Method for Assessing Sex from Modern and Ancient Bovine Tooth Enamel.

SI 1: Proteomics

PrintMapR plots- AmelX

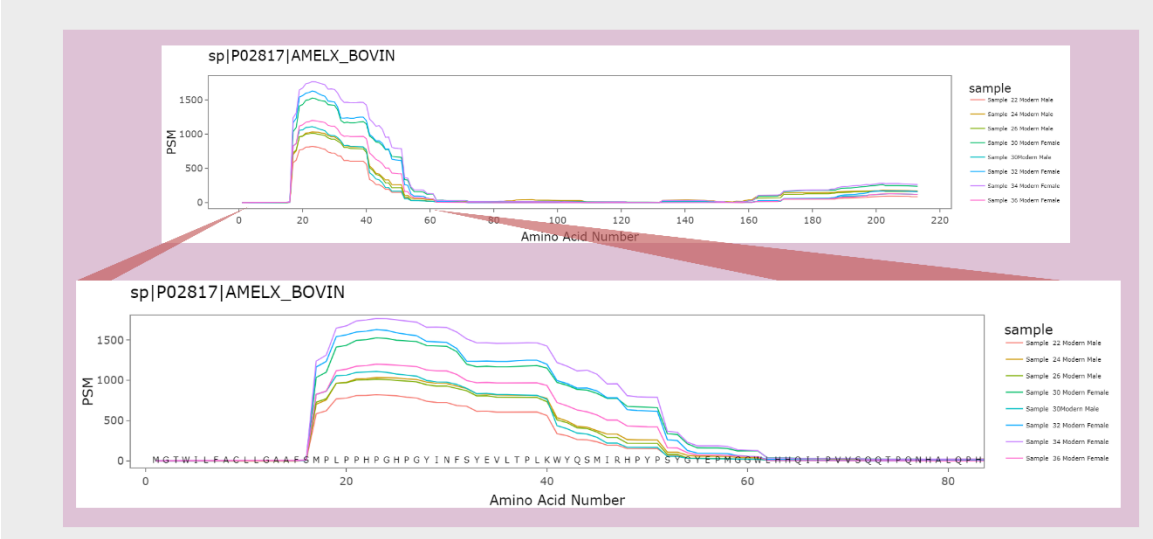


Figure 1. Spectral counting-based coverage of AmelX protein in peptidomics data from eight modern samples - four males and four females, y-axis presents the number of ID spectra per amino acid position in the protein in linear scale. Note, there are not difference between male and female samples in this region.

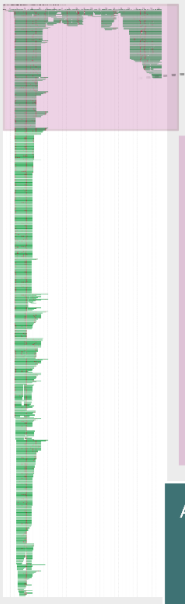
PSM coverage of AmelX and AmelY. Including examples after data filter were applied. More info- excel raw data files.

Sample 22 - Male

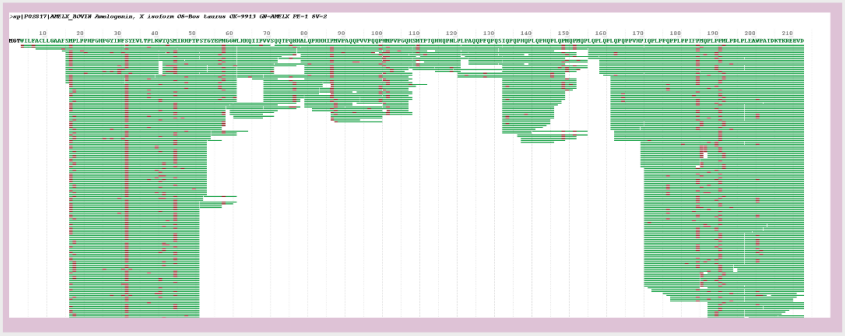
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	|Log Prob|	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	3416.45	23.54	1341.9	2529	1032	834	98.6	213	2.44E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	579	19.31	1136.6	310	197	157	88	192	1.35E+09
3 | 3	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	2.08	332	12	10	6	38	213	4.26E+07
4 | 4	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.78	165.1	2	2	2	9.9	192	3.63E+06

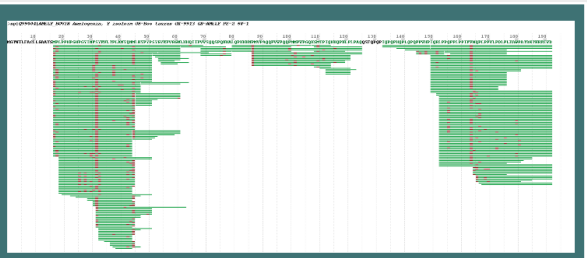
AmelX



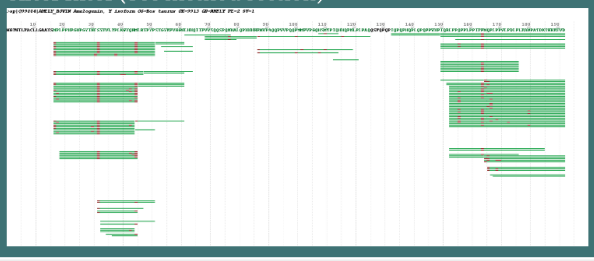
AmelX coverage - close up



AmelY coverage



After filter (See method section)

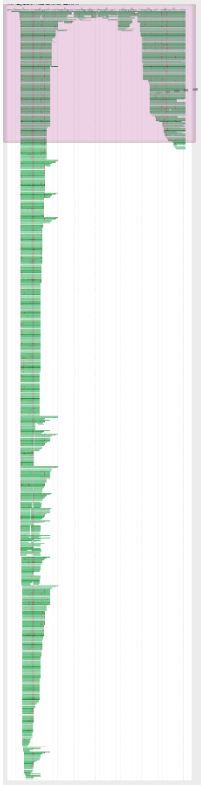


Sample 24 - Male

Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

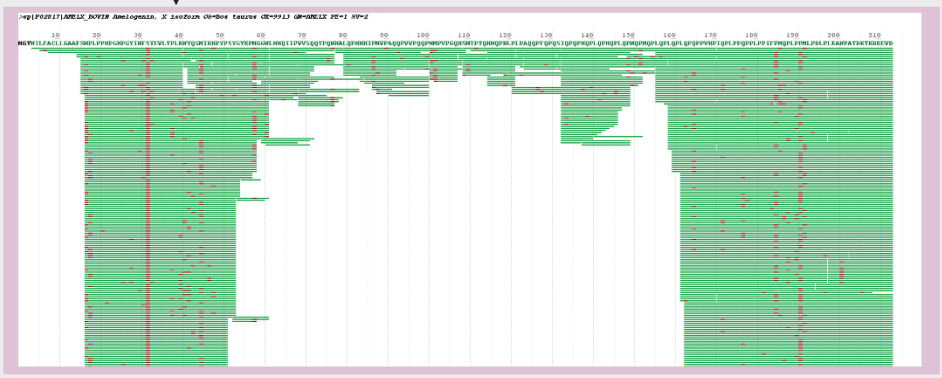
Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	3896.52	20.57	1189	3038	1304	1141	98.6	213	4.96E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	745.36	22.52	1383.1	426	302	274	99.5	192	3.81E+09
3 | 3	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	2.25	93.2	6	6	6	46.9	192	5.36E+07
4 | 4	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	1.94	409.5	14	12	11	42.7	213	5.69E+07

AmelX

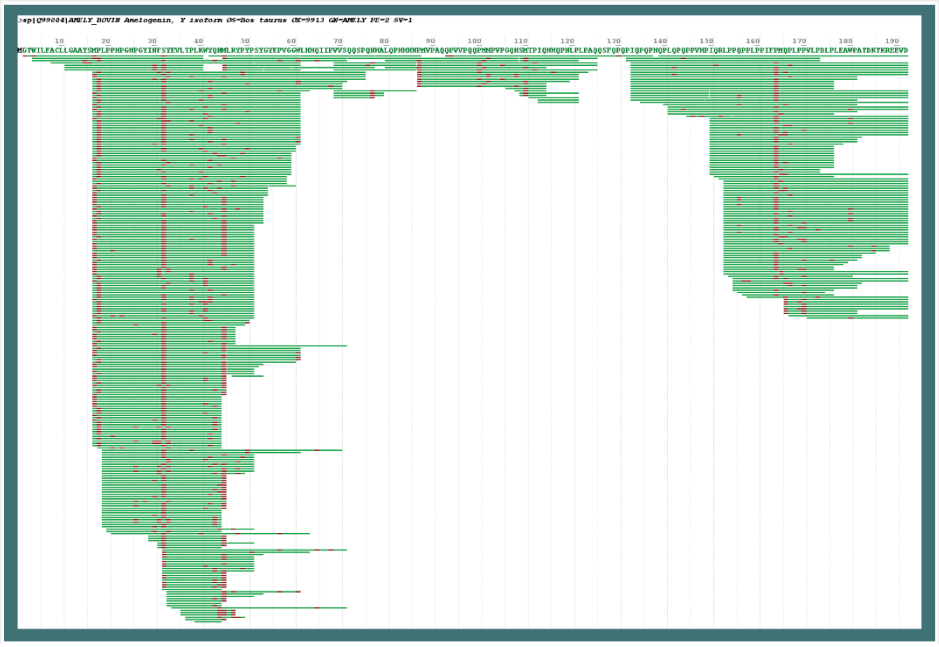


AmelX coverage

AmelX coverage - close up



AmelY coverage

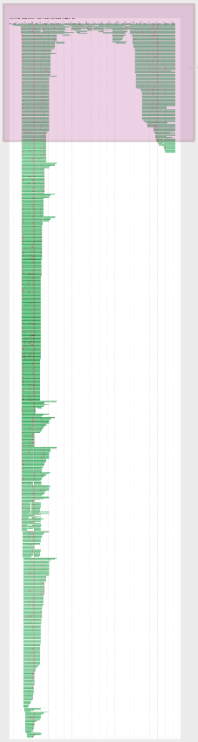


Sample 26 - Male

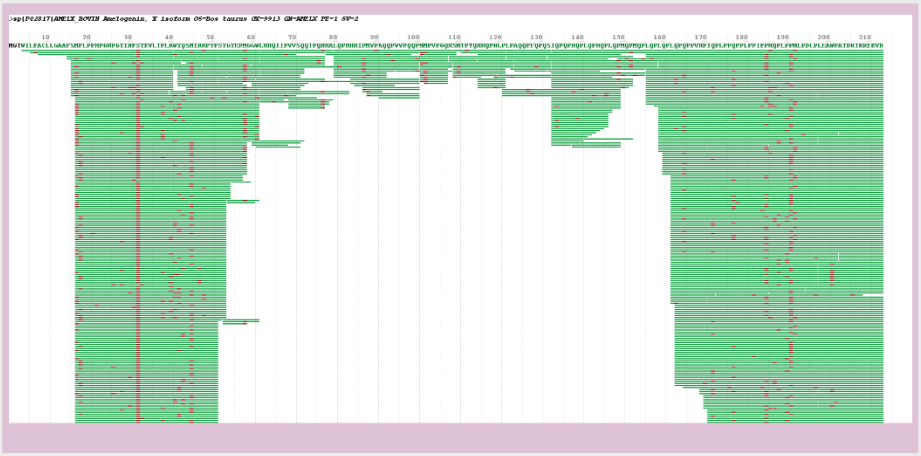
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	3874.41	26.56	1363	2818	1206	970	98.6	213	3.42E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	686.31	19.42	1111.4	366	235	192	94.3	192	2.48E+09
3 | 3	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	2.02	347	12	11	6	45.5	213	9.38E+07
4 | 4	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.87	84.7	3	3	3	40.6	192	6.41E+06

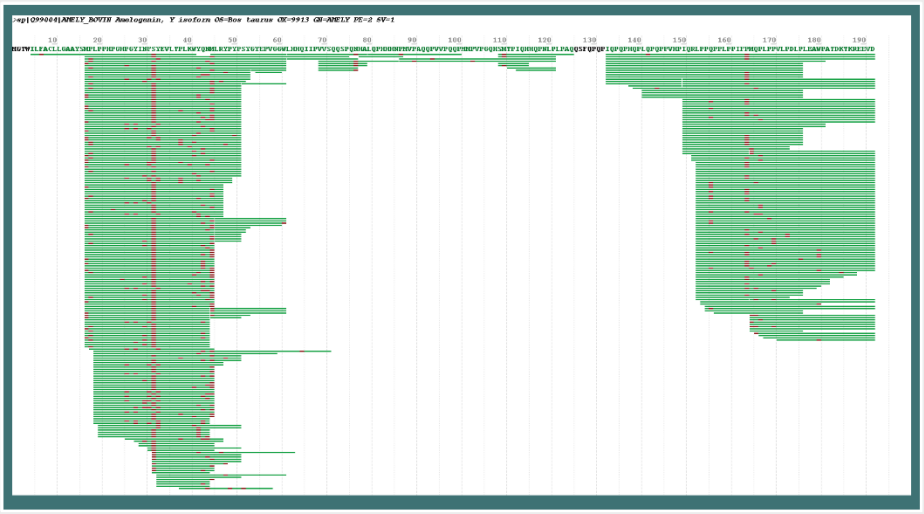
AmelX



AmelX coverage - close up



AmelY coverage

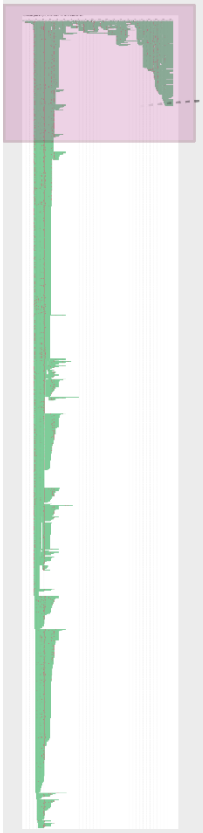


Sample 28 - Male

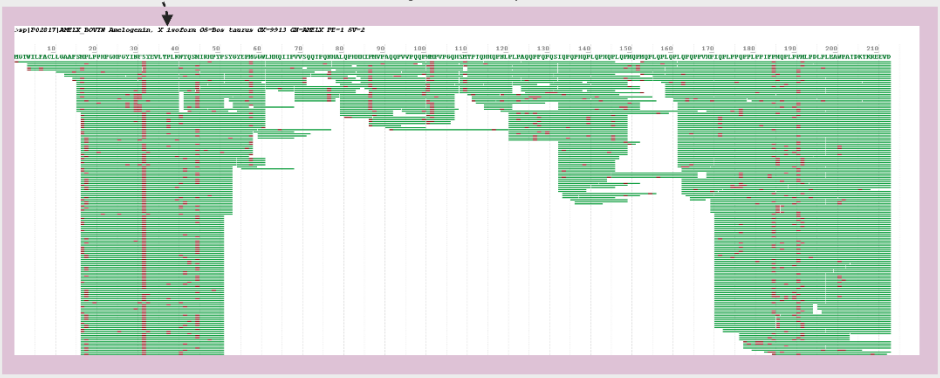
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	3661.01	24.69	1300.4	3458	1278	1025	100	213	3.89E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	557.05	19.85	1068.9	389	272	232	96.9	192	2.48E+09
3 | 3	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	1.83	329.5	72	68	50	92.5	213	3.73E+08
4 | 4	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.95	259.1	35	30	27	93.2	192	3.83E+08

AmelX



AmelX coverage - close up



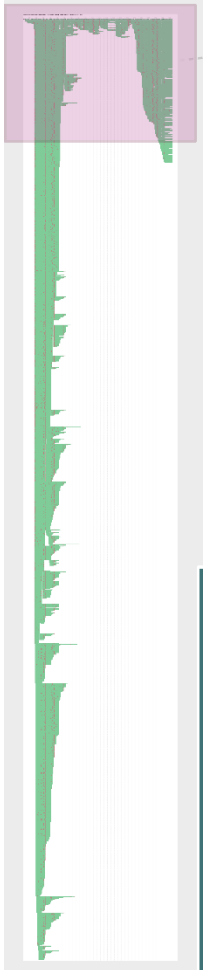
AmelY coverage

Sample 30 - Female

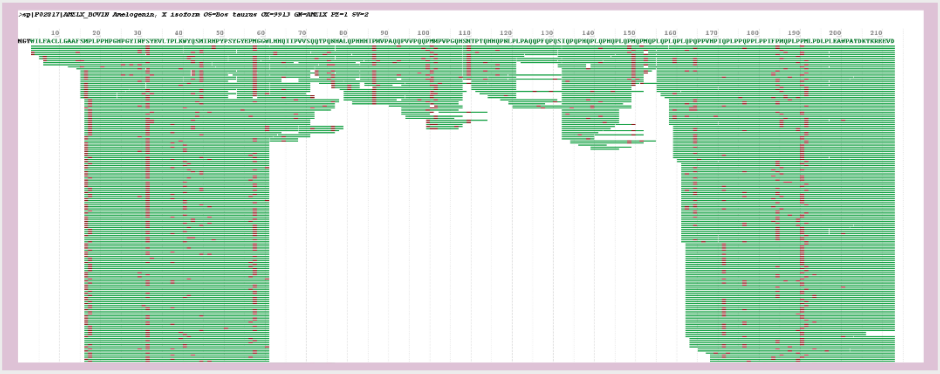
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	5090.04	21.47	1308.4	4163	1703	1519	98.6	213	1.24E+11
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	101.88	10.28	603.5	107	93	91	71.9	192	1.66E+09
3 | 3	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	2.43	452.6	6	6	5	59.9	192	7.25E+07
4 | 4	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	2.47	314.2	14	13	11	68.1	213	1.77E+08

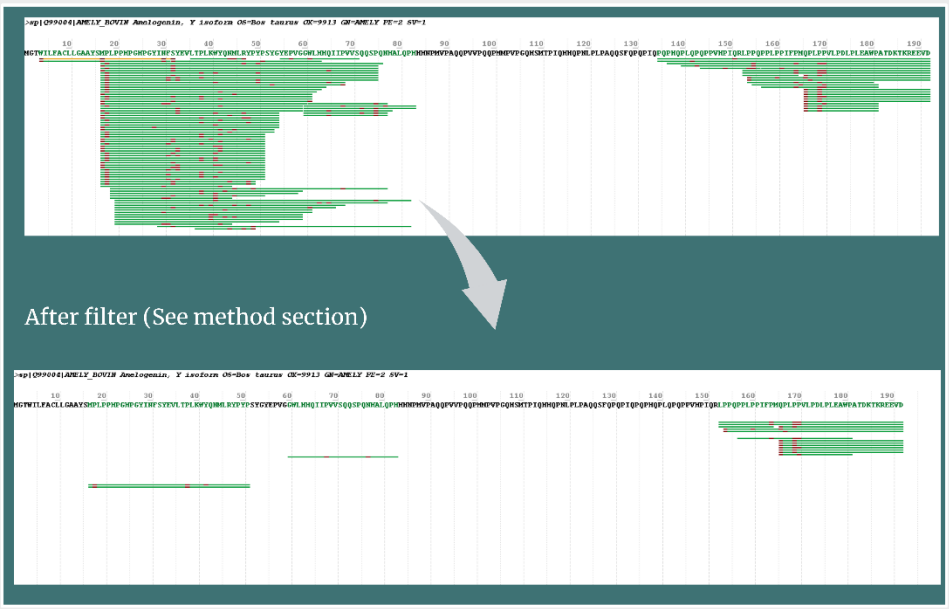
AmelX



AmelX coverage - close up



AmelY coverage

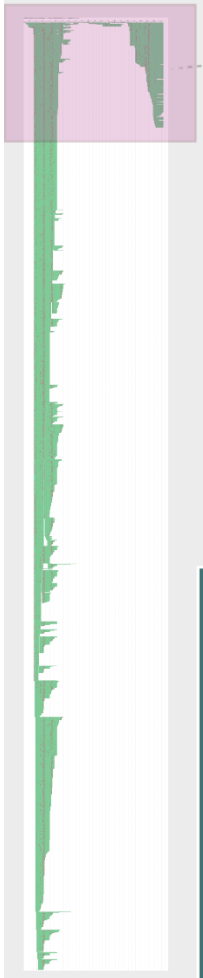


Sample 32 - Female

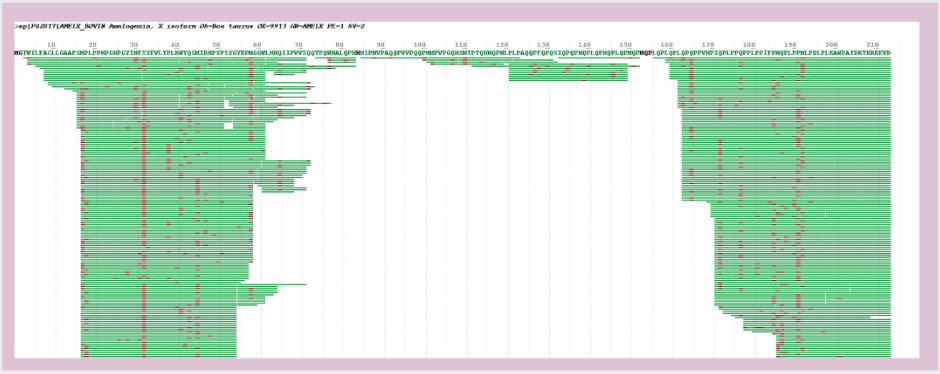
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	5134.25	25.33	1375.2	4285	1651	1428	97.2	213	9.17E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	32.67	9.86	591.9	75	66	64	65.1	192	8.84E+08
3 | 3	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	2.06	319.3	13	12	7	42.3	213	1.56E+08
4 | 4	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.87	172.8	4	4	4	28.1	192	3.32E+07

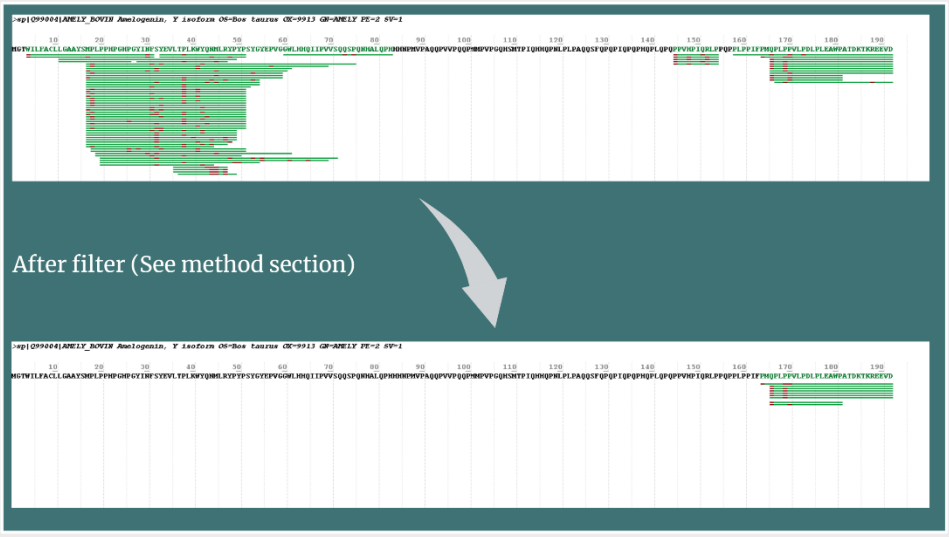
AmelX



AmelX coverage - close up

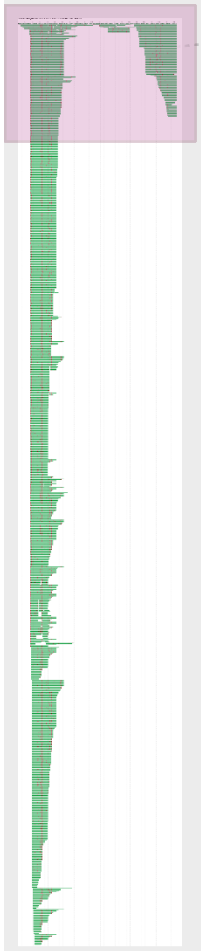


AmelY coverage



Sample 36 - Female

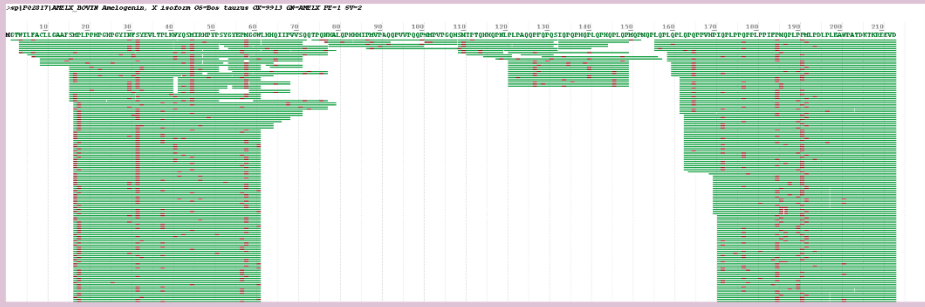
AmelX



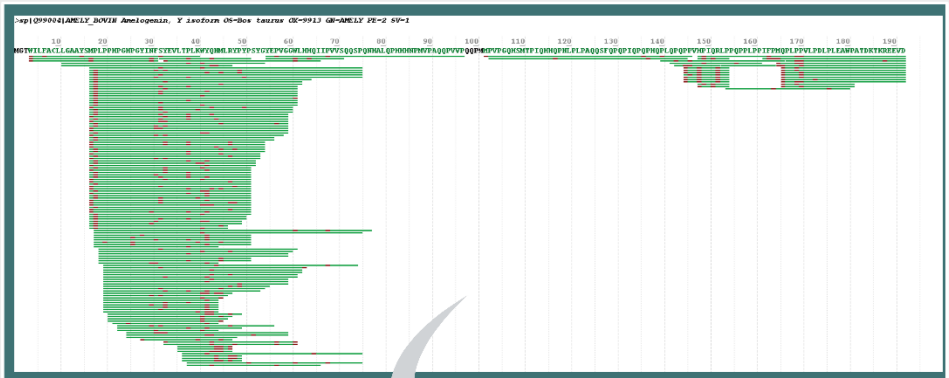
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	3813.54	22.62	1317.2	3446	1509	1371	99.5	213	4.76E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	120.53	9.89	595.9	169	151	147	96.4	192	1.27E+09
3 | 3	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.92	118.6	6	6	6	40.6	192	2.30E+07
4 | 4	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	1.84	268.1	14	12	9	52.6	213	1.35E+08

AmelX coverage - close up



AmelY coverage



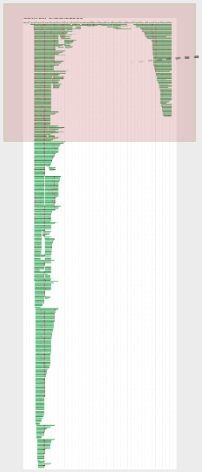
After filter (See method section)

Sample 81 - Male

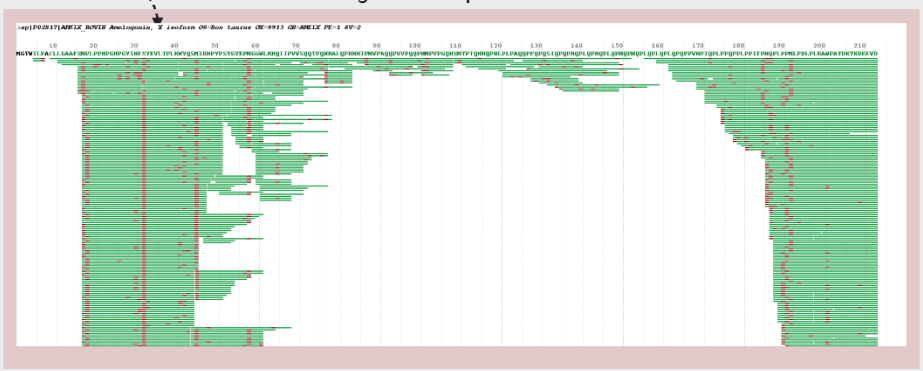
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	3081.99	19.88	1150.4	2288	1046	888	97.7	213	1.78E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	361.51	18.59	1114.7	191	147	119	91.7	192	1.44E+09
3 | 3	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.95	282.3	56	53	44	81.2	192	2.24E+08
4 | 4	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	1.72	209.3	33	32	26	72.8	213	1.19E+08

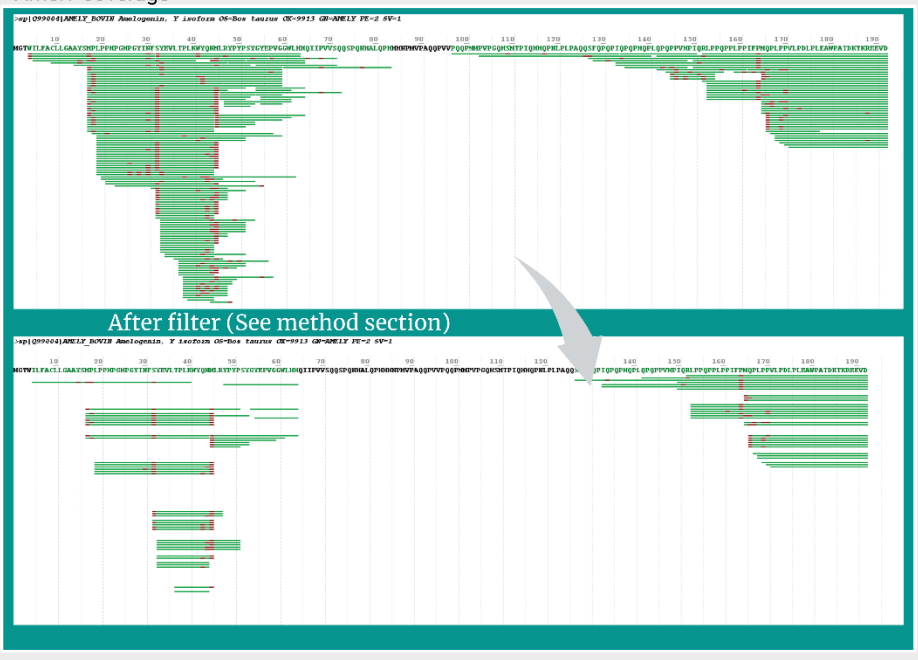
AmelX



AmelX coverage - close up



AmelY coverage

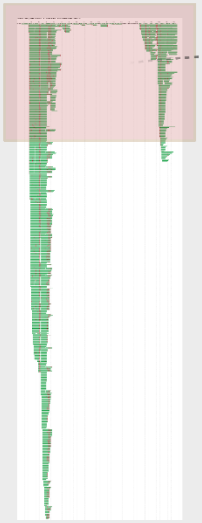


Sample 82 - Unknown

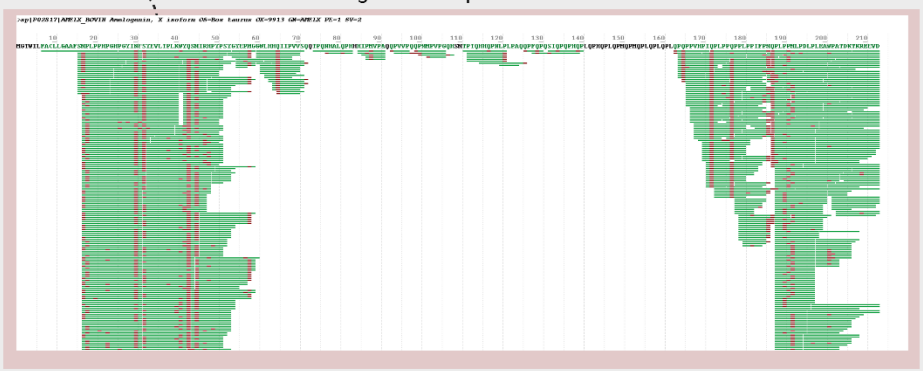
Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	3589.34	18.49	1059.8	3226	1125	925	84.5	213	2.24E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	377.17	8.47	637	312	196	144	66.1	192	8.69E+08
3 | 3	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	2.85	343.9	104	79	62	56.3	213	3.60E+08
4 | 4	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.39	258.4	20	17	14	38	192	4.20E+07

AmelX



AmelX coverage - close up



AmelY coverage

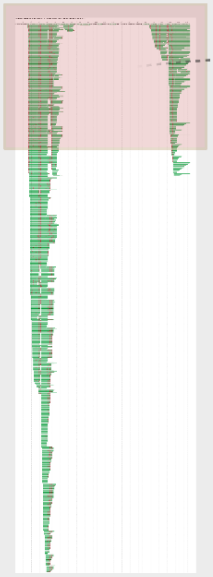
After filter (See method section)

Sample 84 - Unknown

Byonic PSMs recovery (raw) data before filters on bovine amelogenin peptides.

Prot. Rank	Protein Name	Log Prob	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	4230.89	19.81	1119.4	3075	1104	926	68.1	213	3.01E+10
2 | 2	>sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	5.44	595.4	24	19	18	40.1	192	1.12E+08
3 | 3	>Reverse >sp|P02817|AMELX_BOVIN Amelogenin, X isoform OS=Bos taurus OX=9913 GN=AMELX PE=1 SV=2	0.01	2.36	322.4	18	15	14	32.4	213	7.40E+07
4 | 4	>Reverse >sp|Q99004|AMELY_BOVIN Amelogenin, Y isoform OS=Bos taurus OX=9913 GN=AMELY PE=2 SV=1	0.01	1.62	146.6	1	1	1	2.6	192	1.56E+06

AmelX



AmelX coverage - close up

AmelY coverage

SI 1: Photos of modern and Neolithic teeth sampling procedure

Modern cattle (*Bos taurus)*, In total 8 samples (4 Females, 4 Males), obtained from the abattoir Tira Ltd., Israel

Figure 2. Four complete male cattle mandibles photographed when they were first received from the abattoir.

Figure 3. Process of tooth identification and selection (a and b); incisors I3 and I4 extracted for further cleaning and enamel peptide extraction (c).

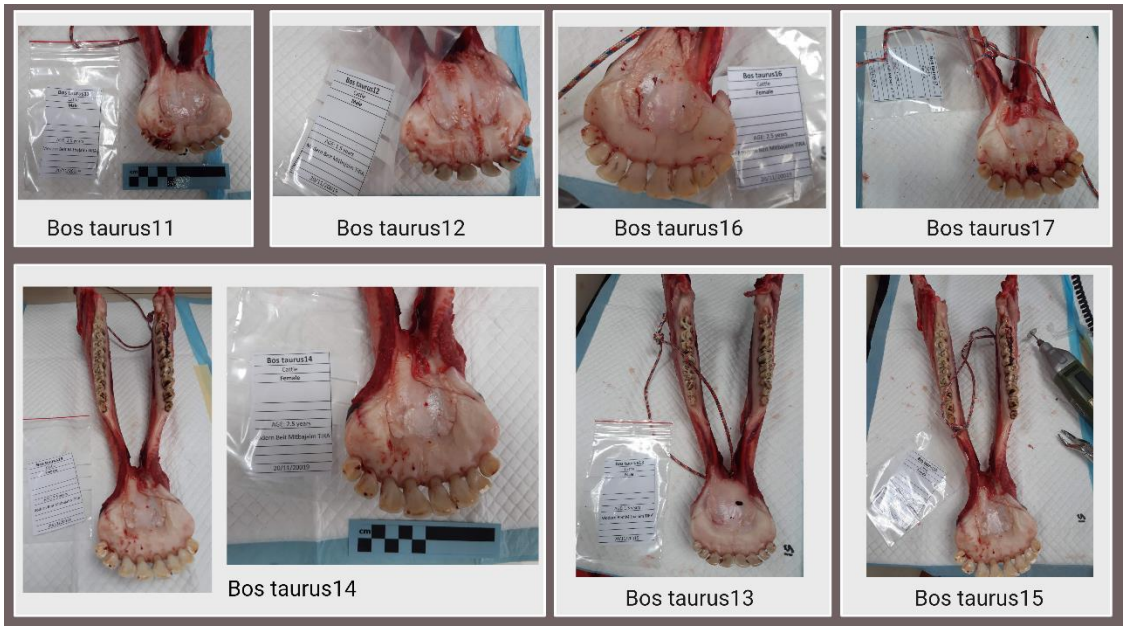


Figure 4. Figure 5. Modern mandibles with their specimen IDs: *Bos taurus* 10 to 17

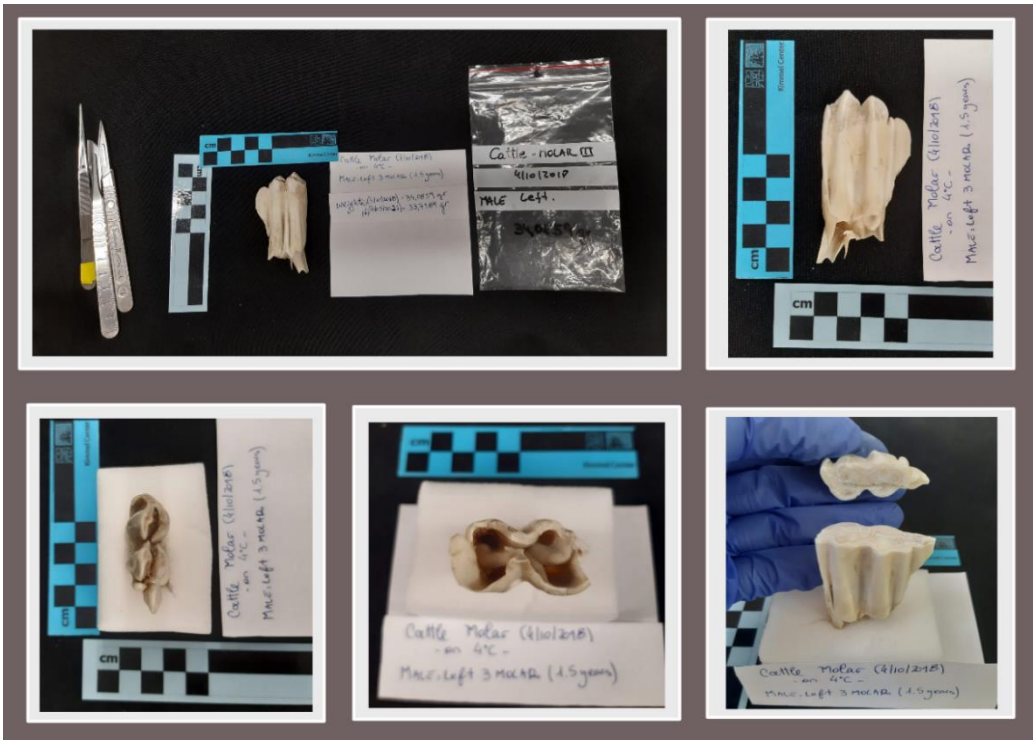
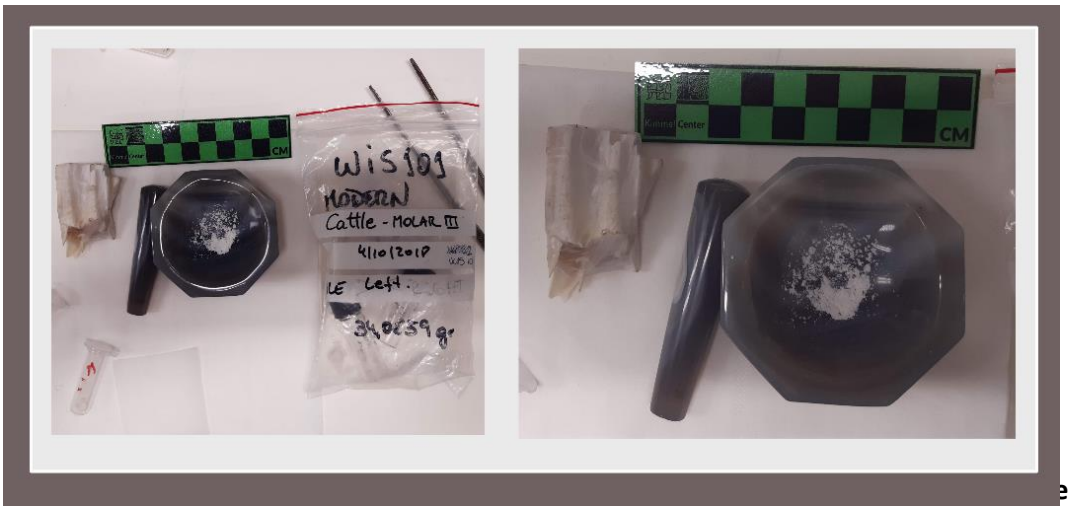


Figure 6 - Photos show modern cattle lower third molar Sample 81 (WIS 101) before enamel extraction.



etching or demineralizing process with HCl 5%.

Figure 8. Work conditions for enamel peptide extraction under a chemical hood.

Figure 7. Sample 82 – Beisamoun, Neolithic period, *Bos* sp. Molar, unknown sex. The sample was enclosed in a "cement" matrix that was only possible to remove with mechanical tools.

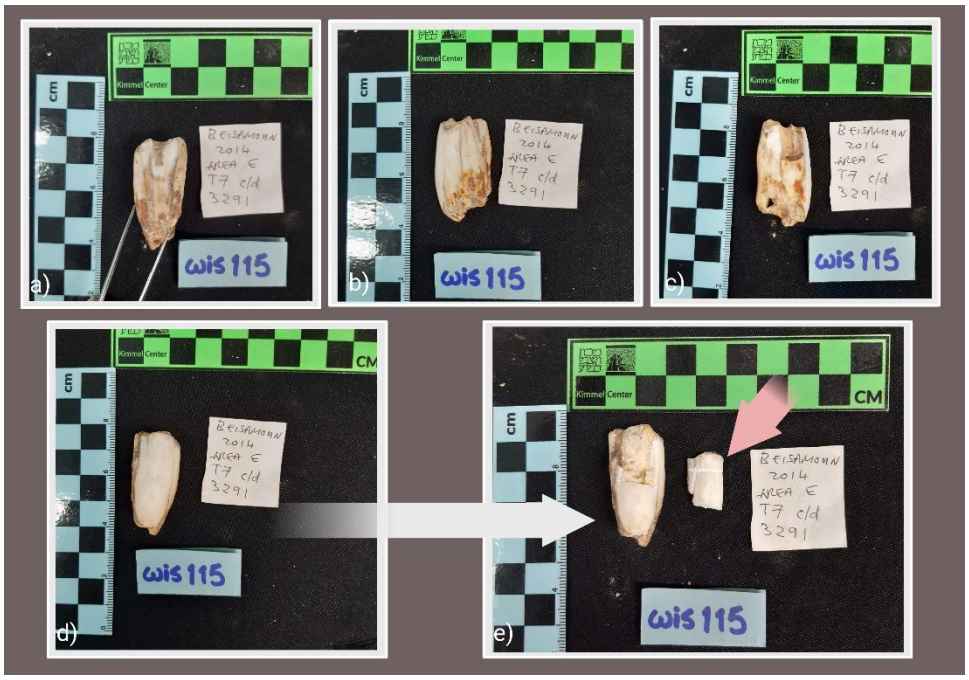


Figure 8 a-d show sample 82 (Wis115) after sediment has been removed with mechanical tools. 8e shows the enamel fragment after extraction from the molar, the pink arrow show the enamel fragment.

Figure 9. Sample 84 – Abu Gosh, Neolithic. *Bos* sp. incisor. The pink arrow shows the enamel fragment after its been cut from the tooth.