

Supporting Information

Complementarity of two Proteomic Data Analysis Tools in the Identification of Drug-Metabolising Enzymes and Transporters in Human Liver

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Table S1: Detailed parameters applied with MaxQuant.

Group specific Parameters	
1- Type Standard: none	Multiplicity: 1
2- Digestion mode: Specific	Enzyme: Trypsin/P
	Max. missed: 1
3- Modifications	Variable modifications: Oxidation (M) & Deamidation (NQ)
	Max number of modifications per peptide: 11
4- Instrument	Orbitrap
First search peptide tolerance 20	Main search peptide tolerance 5
Peptide tolerance unit ppm	Individual peptide mass tolerance (Checked)
Isotope match tolerance 2	Isotope match tolerance unit ppm
Centroid match tolerance 8	Centroid match tolerance unit ppm
Centroid half width 35	Centroid half width unit ppm
Time Valley Factor 1.4	Isotope Valley factor 1.2
Isotope time correlation 0.6	Theoretical isotope correlation 0.6
Recalibration unit ppm	Min Peak length 2
Max charge 7	Min score for recalibration 70
Cut peaks (checked)	Gap scans 1
Intensity threshold 0	Intensity determination Value at Maximum
5- Label free quantification: yes	
Global parameters	
1- Sequence	include contaminants (checked)
Fixed modifications Carbamidomethyl (C)	Min pep length 7
Max peptide mass [Da] 4500	Min peptide length for unspecific search 8
Max. peptide length for unspecific search 25	

2- Identification	PSM FDR 0.01
XPSM FDR 0.01	Protein FDR 0.01
Site decoy fraction 0.01	Min. peptides 1
Min.razor + unique peptides 1	Min. unique peptides 0
Min. score for unmodified peptides 0	Min. score for modified peptides 0
Min. delta score for unmodified peptides 0	Min. delta score for modified peptides 6
Main search max. combinations 200	Base FDR calculations on delta score (unchecked)
Razor protein FDR checked	
3- Protein Quantification	Label min. ratio count 2
Peptides for quantification Unique + razor	Use only unmodified peptides
Modifications used in protein quantification	Oxidation (M) & Deamidation (NQ)
Discard unmodified counterpart peptides (checked)	Advanced ratio estimation (checked)
4- Advanced	Calculate peak properties (unchecked)
Decoy mode Revert	Use for occupancies Normalized ratios
5- Label free quantification	Separate LFQ in parameter groups unchecked
Stabilize large LFQ ratios (checked)	Require MS/MS for LFQ comparisons (checked)
¡BAQ (unchecked)	Advanced site intensities (checked)
LFQ norm for sites and peptides (unchecked)	6- MS/MS FTMS
FTMS MS/MS match tolerance 20	FTMS MS/MS match tolerance unit ppm
FTMS MS/MS de novo tolerance 10	FTMS MS/MS de novo tolerance unit ppm
FTMS MS/MS deisotoping tolerance 7	FTMS MS/MS deisotoping tolerance unit ppm
FTMS top peaks per Da interval 12	FTMS top x mass window [Da] 100
FTMS de isotoping (checked)	FTMS higher charges (checked)
FTMS water loss (checked)	FTMS ammonia loss (checked)
FTMS dependent losses (checked)	FTMS recalibration (unchecked)

7- MS/MS ITMS	ITMS MS/MS match tolerance 0.5
ITMS MS/MS match tolerance unit Da	ITMS MS/MS de novo tolerance 0.25
ITMS MS/MS de novo tolerance unit Da	ITMS MS/MS deisotoping tolerance 0.15
ITMS MS/MS deisotoping tolerance unit Da	ITMS top peaks per Da interval 8
ITMStop x mass window [Da] 100	ITMS de-isotoping (unchecked)
ITMS higher charges (checked)	ITMS water loss (checked)
ITMS ammonia loss (checked)	ITMS dependent losses checked
ITMS recalibration (unchecked)	8- MS/MS- TOF
TOF MS/MS match tolerance 40	TOF MS/MS match tolerance unit ppm
TOF MS/MS de novo tolerance 0.02	TOF MS/MS de novo tolerance unit Da
TOF MS/MS deisotoping tolerance 0.01	TOF MS/MS deisotoping tolerance unit Da
TOF top peaks per Da interval 10	TOF top x mass window [Da] 100
TOF de isotoping (checked)	TOF higher charges (checked)
TOF water loss (checked)	TOF ammonia loss (checked)
TOF dependent losses (checked)	TOF recalibration (unchecked)
9- MS/MS unknown	Unknown MS/MS match tolerance 0.5
Unknown MS/MS match tolerance unit Da	Unknown MS/MS de novo tolerance 0.25
Unknown MS/MS de novo tolerance unit Da	Unknown MS/MS deisotoping tolerance 0.15
Unknown MS/MS deisotoping tolerance unit Da	Unknown top peaks per Da interval 8
Unknown top x mass window [Da] 100	Unknown de isotoping (unchecked)
Unknown higher charges (checked)	Unknown water loss (checked)
Unknown ammonia loss (checked)	Unknown dependent losses checked
Unknown recalibration (unchecked)	10- tables
Write msScans table (checked)	Write msms Scans table (checked)
Write ms3Scans table (checked)	Write all Peptides table (checked)
Write mzRange table (checked)	Write pasefMsms Scans table (checked)

Write accumulatedPasef Msms Scans table (checked)	
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Table S2. Range of cut-off scores in Progenesis. The cut-off scores for Progenesis are equivalent to a cut-off score of 40 in MaxQuant. The equation represents a mathematical relationship that allows conversion of cut-off scores from one software to the other.

Progenesis score is represented by y and MaxQuant score by x.

Sample	Equation to convert cut-off scores	R²	Cut-off score for Progenesis
HLM76	$y=0.3353x$	0.32	13.4
HLM02	$y=0.3445x$	0.34	13.8
HLM25	$y=0.3386x$	0.35	13.5
HLM48	$y=0.359x$	0.41	14.4
HLM73	$y=0.374x$	0.43	15.0
HLM80	$y=0.3539x$	0.34	14.2
HLM91	$y=0.3617x$	0.35	14.5
HLM06	$y=0.3439x$	0.32	13.8
HLM11	$y=0.3552x$	0.41	14.2
HLM38	$y=0.3673x$	0.40	14.7
HLM41	$y=0.3187x$	0.25	12.8
HLM72	$y=0.3489x$	0.41	14
HLM75	$y=0.3675x$	0.37	14.7
HLM90	$y=0.2964x$	0.30	11.9
HLM117	$y=0.3215x$	0.28	12.9
HLM08	$y = 0.4129x$	0.23	16.5
HLM71	$y = 0.3682x$	0.32	14.7
HLM77	$y = 0.3651x$	0.40	14.6
HLM78	$y = 0.3371x$	0.31	13.5
HLM74	$y = 0.3446x$	0.33	13.8
HLM100	$y = 0.3561x$	0.36	14.2
HLM89	$y = 0.3643x$	0.43	14.6
HLM01	$y = 0.363x$	0.35	14.5

Table S3. Regression equation for the relationship between Progenesis intensities (y) and MaxQuant intensities (x) and the coefficient of determination for each individual human liver microsome (HLM) sample.

Sample	Regression equation to convert intensities	R²
HLM01	y=0.0248x	0.68
HLM02	y=0.012x	0.62
HLM06	y=0.0123x	0.59
HLM08	y=0.0145x	0.62
HLM11	y=0.0162x	0.50
HLM25	y=0.0146x	0.60
HLM38	y=0.0118x	0.66
HLM41	y=0.01242x	0.59
HLM48	y=0.0144x	0.59
HLM71	y=0.0132x	0.65
HLM72	y=0.0171x	0.57
HLM73	y=0.0151x	0.59
HLM74	y=0.0123x	0.61
HLM75	y=0.0115x	0.65
HLM76	y=0.013x	0.74
HLM77	y=0.0147x	0.67
HLM78	y=0.0131x	0.56
HLM80	y=0.0144x	0.67
HLM89	y=0.0156x	0.57
HLM90	y=0.016x	0.57
HLM91	y=0.025x	0.63
HLM100	y=0.0127x	0.53
HLM117	y=0.0149x	0.49

Table S4. Comparison of the total number of peptides, peptides specific for a software and modified peptides identified by MaxQuant and Progenesis. This comparison was done before removing peptides with scores lower than 40 in MaxQuant or equivalent for Progenesis.

Sample	MaxQuant total peptides	Progenesis total peptides	MaxQuant only peptides		Progenesis only peptides		Overlap		MaxQuant modified		Progenesis modified	
			Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
HLM01	16366	20284	3280	14%	7198	31%	13086	56%	714	4%	5053	25%
HLM02	20584	19950	7485	27%	6851	25%	13099	48%	1259	6%	4919	24%
HLM06	18725	19236	6531	25%	7042	27%	12194	47%	937	5%	4784	25%
HLM08	18169	22645	3934	15%	8410	32%	14235	54%	872	5%	5801	26%
HLM11	17912	21733	4351	17%	8172	31%	13561	52%	946	5%	5534	26%
HLM25	18773	20558	5475	21%	7260	28%	13298	51%	1037	6%	5130	25%
HLM38	17909	22449	4212	16%	8752	33%	13697	51%	929	5%	5841	26%
HLM41	17789	21019	4580	18%	7810	31%	13209	52%	1470	8%	5656	27%
HLM48	17743	20701	4388	17%	7346	29%	13355	53%	917	6%	5258	25%
HLM71	16413	21986	2772	11%	8345	34%	13641	55%	847	5%	5990	27%
HLM72	17269	21027	4028	16%	7786	31%	13241	53%	936	5%	5305	25%
HLM73	17393	20847	3946	16%	7400	30%	13447	54%	3236	19%	7199	35%
HLM74	18170	19150	5741	23%	6721	27%	12429	50%	879	5%	4728	25%
HLM75	16809	20901	3957	16%	8049	32%	12852	52%	842	5%	5487	26%
HLM76	19091	19107	6554	26%	6570	26%	12537	49%	1103	6%	4673	24%
HLM77	17348	20466	3916	16%	7034	29%	13432	55%	1014	6%	5115	25%
HLM78	18794	18989	6467	25%	6662	26%	12327	48%	1113	6%	4790	25%
HLM80	17326	20734	4113	17%	7521	30%	13213	53%	839	5%	5366	26%
HLM89	17801	22059	3853	15%	8111	31%	13948	54%	1603	9%	6258	28%
HLM90	17497	21586	4142	16%	8231	32%	13355	52%	1030	6%	5693	26%
HLM91	17149	20650	4061	16%	7562	31%	13088	53%	836	5%	5224	25%
HLM100	18207	21220	4477	17%	7490	29%	13730	53%	988	5%	5242	25%
HLM117	19923	19628	7491	28%	7196	27%	12432	46%	1031	5%	4886	25%
Mean	17963	20736	4772	19%	7544	30%	13192	52%	1103	6%	5388	26%
SD	1021	1059	1311	5%	611	2%	531	3%	508	3%	581	2%
CV	6%	5%	27%	25%	8%	8%	4%	5%	46%	47%	11%	8%

Table S5. Statistical comparison between peptide characteristics for sample HLM76 in relation to peptide length, GRAVY score (hydrophobicity), isoelectric point (PI), and molecular weight of peptides detected by only one software tool MaxQuant (*Max.*) and Progenesis (*Pro.*).

	<i>Length</i>		<i>GRAVY score</i>		<i>PI</i>		<i>Molecular weight</i>	
	<i>Max.</i>	<i>Pro.</i>	<i>Max.</i>	<i>Pro.</i>	<i>Max.</i>	<i>Pro.</i>	<i>Max.</i>	<i>Pro.</i>
Median	13	13	-0.1667	-0.4692	5.848	6.613	1530.69	1450.72
Mode	11	7	-0.1	-0.3	9.831	9.831	1264.4	785.939
n	6447	2157	6447	2157	6447	2157	6447	2157
Mann-Whitney test P value	<0.0001		<0.001		<0.001		<0.001	

Table S6. Enzyme and transporter proteins detected in 23 human liver samples by the two software tools.

Gene name	Number of samples where unique/distinct peptides are identified			
	Progenesis only	MaxQuant only	Both	Neither
CYPs				
CYP1A1	5	0	2	16
CYP1A2	0	0	23	0
CYP17A1	0	0	1	22
CYP20A1	0	0	0	23
CYP26A1	1	3	1	18
CYP27A1	0	0	23	0
CYP39A1	0	6	13	4
CYP51A1	0	0	23	0
CYP3A4	0	0	23	0
CYP3A5	0	0	23	0
CYP2A6	0	0	23	0
CYP2A7	13	0	5	5
CYP3A7	3	0	14	6
CYP4A11	0	0	23	0
CYP2A13	1	0	0	22
CYP4A22	1	0	0	22
CYP1B1	0	0	2	21
CYP7B1	0	0	23	0
CYP8B1	0	0	23	0
CYP2B6	0	0	23	0
CYP2C8	0	0	23	0

CYP2C9	0	0	23	0
CYP2C18	0	2	20	1
CYP2C19	0	1	17	5
CYP2D6	0	0	23	0
CYP2E1	0	0	23	0
CYP2F1	22	0	0	1
CYP4F2	0	0	23	0
CYP4F3	0	2	21	0
CYP4F8	0	12	11	0
CYP4F11	0	0	23	0
CYP4F12	0	1	17	5
CYP4F22	6	5	10	2
CYP2J2	1	5	16	1
CYP2S1	2	7	8	6
CYP4V2	0	1	22	0
UGTs				
UGT1A1	0	0	23	0
UGT2A1	0	2	20	1
UGT3A1	1	3	2	17
UGT1A3	0	0	23	0
UGT2A3	0	0	23	0
UGT1A4	0	0	23	0
UGT1A5	1	1	0	21
UGT1A6	0	0	23	0
UGT1A9	0	0	23	0
UGT2B4	0	0	23	0

UGT2B7	0	0	23	0
UGT2B10	0	0	23	0
UGT2B11	1	3	0	19
UGT2B15	0	0	23	0
UGT2B17	0	2	17	4
SLC transporters				
SLC1A1	1	11	7	4
SLC2A1	6	1	4	12
SLC16A1	0	0	23	0
SLC22A1	0	1	22	0
SLC23A1	0	3	0	20
SLC26A1	0	6	7	10
SLC27A1	2	3	16	2
SLC28A1	0	1	0	22
SLC29A1	0	5	12	6
SLC30A1	0	8	9	6
SLC33A1	0	0	23	0
SLC40A1	0	6	3	14
SLC43A1	0	5	10	8
SLC44A1	0	5	18	0
SLC48A1	0	1	0	22
SLC1A2	0	5	1	17
SLC2A2	0	0	23	0
SLC7A2	0	3	18	2
SLC16A2	0	1	18	4
SLC20A2	0	3	0	20

SLC23A2	1	0	0	22
SLC25A2	1	0	0	22
SLC38A2	0	2	7	14
SLC43A2	5	0	0	18
SLC44A2	0	4	1	18
SLC1A3	1	11	2	9
SLC2A3	6	1	2	14
SLC7A3	0	1	0	22
SLC16A3	0	0	1	22
SLC19A3	0	9	8	6
SLC22A3	0	1	0	22
SLC29A3	7	1	4	11
SLC35A3	1	1	13	8
SLC38A3	0	1	22	0
SLC41A3	3	0	0	20
SLC43A3	0	0	23	0
SLC46A3	0	1	1	21
SLC1A4	0	3	13	7
SLC38A4	0	0	23	0
SLC7A5	0	0	1	22
SLC5A6	0	5	4	14
SLC16A7	0	4	2	17
SLC22A7	0	6	14	3
SLC26A7	0	2	0	21
SLC30A7	0	2	20	0
SLC38A7	0	1	0	22

SLC39A7	0	0	23	0
SLC7A8	4	1	2	16
SLC2A9	0	2	0	21
SLC22A9	0	4	16	3
SLC30A9	1	2	1	19
SLC39A9	0	4	18	1
SLC2A10	6	4	5	8
SLC38A10	0	13	9	1
SLC39A11	2	1	19	1
SLC2A12	2	0	0	21
SLC6A12	0	3	6	14
SLC16A12	0	2	0	21
SLC2A13	0	0	1	22
SLC39A14	0	0	23	0
SLC25A15	0	0	23	0
SLC22A18	0	0	23	0
SLC25A40	1	4	0	18
SLC25A42	0	0	23	0
SLC25A44	0	4	0	19
SLC25A46	0	15	2	6
SLC35B1	0	2	12	9
SLC35B2	0	3	4	16
SLC35C1	0	0	4	19
SLC35C2	0	1	0	22
SLC35D1	0	1	21	1
SLC35F6	0	3	18	2

SLCO1B1	0	0	23	0
SLCO1B3	0	0	23	0
SLCO2B1	0	2	20	1
ABC transporters				
ABCA1	0	6	16	1
ABCA2	1	13	3	6
ABCA6	0	0	23	0
ABCA8	0	3	14	6
ABCA10	1	0	0	22
ABCA13	1	0	0	22
ABCB1 (MDR1)	0	2	21	0
ABCB4 (MDR3)	0	3	3	17
ABCB5	4	2	0	17
ABCB6	0	4	17	2
ABCB7	0	1	22	0
ABCB8	0	0	23	0
ABCB10	0	0	23	0
ABCB11	0	2	21	0
ABCC1 (MRP1)	0	2	0	21
ABCC2 (MRP2)	0	9	12	2
ABCC3 (MRP3)	0	3	19	1
ABCC5 (MRP5)	1	0	0	22
ABCC6 (MRP6)	0	0	23	0
ABCD1	0	0	23	0
ABCD2	1	0	0	22
ABCD3	0	0	23	0

ABCD4	0	7	16	0
ABCE1	1	3	0	19
ABCG8	1	1	1	20

Table S7. Median and mode scores for software-specific peptides in each sample.

Sample	MaxQuant		Progenesis	
	Median score	Mode score	Median score (MaxQuant Equivalent)	Mode score (MaxQuant Equivalent)
HLM01	78.2	64.3	32.4 (89.3)	15.5 (42.6)
HLM02	123	114.9	51.4 (149)	88.6 (257.2)
HLM06	126.9	94.7	53.1 (154.2)	56.3 (163.7)
HLM08	74.6	60.5	23.9 (57.9)	17.7 (42.9)
HLM11	124.7	114.5	51.9 (146.1)	30.5 (85.9)
HLM25	92.8	91.6	30.6 (90.5)	17.5 (51.7)
HLM38	122.8	108.9	51.9 (141.4)	58.5 (159.2)
HLM41	123.7	101.6	49.8 (156.1)	61.9 (197.2)
HLM48	199.7	252.4	29.8 (83.1)	17.5 (48.7)
HLM71	79.6	58.4	31.7 (86.2)	14.9 (40.4)
HLM72	128.4	114.5	52.9 (151.6)	37.3 (106.9)
HLM73	77.7	50	32.3 (86.3)	18.6 (49.8)
HLM74	96.1	58.9	29.6 (85.9)	14.4 (41.8)
HLM75	122.6	122.1	52.5 (142.9)	35.3 (96.1)
HLM76	98.7	367.2	29.5 (87.9)	13.6 (40.4)
HLM77	79.2	46.5	34.5 (86.3)	16.2 (40.6)
HLM78	97.3	125.9	29.5 (87.5)	13.6 (40.5)
HLM80	87.4	47.6	29.8 (84.2)	15.3 (43.2)
HLM89	77.6	47.2	30.4 (83.5)	15.7 (42.9)
HLM90	123.1	108.9	45.5 (153.4)	23.2 (78.3)
HLM91	86.8	110.4	32.4 (89.6)	20.1 (55.5)
HLM100	86.1	62.5	32.2 (90.4)	14.9 (41.7)
HLM117	133.1	114.9	52.9 (164.7)	31.5 (98.1)

Table S8. Median and mode Gravy scores for software-specific peptides in each sample.

Sample	MaxQuant		Progenesis	
	Median Gravy score	Mode Gravy score	Median Gravy score	Mode Gravy score
HLM01	-0.20	-0.30	-0.49	-0.50
HLM02	-0.13	0.30	-0.52	-0.70
HLM06	-0.16	-0.30	-0.48	0.10
HLM08	-0.12	0.30	-0.49	-0.40
HLM11	0.09	0.20	-0.51	-0.70
HLM25	-0.14	-0.10	-0.51	-0.50
HLM38	-0.17	-0.40	-0.48	-0.52
HLM41	-0.35	-0.30	-0.50	-0.50
HLM48	-0.13	0.30	-0.52	-0.70
HLM71	-0.28	-0.70	-0.47	-0.50
HLM72	-0.14	-0.60	-0.48	-0.90
HLM73	-0.09	0.40	-0.47	-0.70
HLM74	-0.19	-0.20	-0.51	-0.50
HLM75	-0.21	-0.20	-0.43	-0.70
HLM76	-0.17	-0.10	-0.47	-0.30
HLM77	-0.14	0.10	-0.44	0.10
HLM78	-0.20	-0.20	-0.46	-0.30
HLM80	-0.20	-0.10	-0.50	-0.70
HLM89	-0.14	0.30	-0.47	-0.70
HLM90	-0.09	-0.1	-0.53	-0.50
HLM91	-0.20	-0.20	-0.43	-0.70
HLM100	-0.17	-0.10	-0.50	-0.70
HLM117	-0.16	-0.30	-0.47	-0.20

Table S9. Abundance of ABC transporters using MaxQuant and Progenesis. The abundance values have been quantified using the total protein approach and are expressed as parts per million (PPM).

ABC transporter (UniProt Accession)	Abundance in Progenesis (PPM)	Abundance in MaxQuant
ABCA1_HUMAN	43	11
ABCA2_HUMAN	38	7
ABCA6_HUMAN	109	112
ABCA8_HUMAN	80	11
ABCBA_HUMAN	14	23
ABCB5_HUMAN	11	5
ABCB6_HUMAN	22	13
ABCB7_HUMAN	21	38
ABCB8_HUMAN	18	28
ABCD1_HUMAN	17	14
ABCD2_HUMAN	55	4
ABCD4_HUMAN	222	5
ABCE1_HUMAN	5	1
ABCG8_HUMAN	13	3
ABCBB_HUMAN	35	32
MRP2_HUMAN	11	9
MDR1_HUMAN	41	42
MRP5_HUMAN	5	5
MRP6_HUMAN	40	41
MDR3_HUMAN	25	19
ABCD3_HUMAN	1217	842
MRP3_HUMAN	2520	15