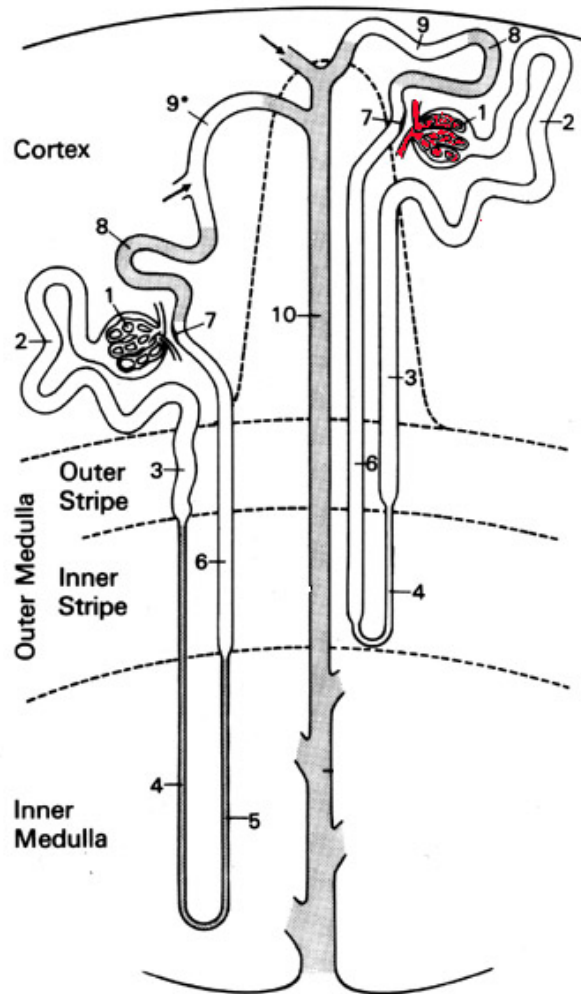


Human Kidney Glomerulus Proteome and Proposition of a Method for Native Protein Profiling

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Schematic Structure of Nephron



1	Glomerulus
2	Proximal convoluted tubule
3	Proximal straight tubule
4	Thin descending loop of Henle
5	Thin ascending loop of Henle
6	Thick ascending loop of Henle
7	Juxtaglomerular apparatus
8	Distal convoluted tubule
9	Distal straight tubule
10	Collecting tubule

Preparation of 2-DE Samples

Kidney cortex with normal appearance from patients under nephrectomy due to renal tumor

↓ A standard sieving method

Partially purified glomeruli

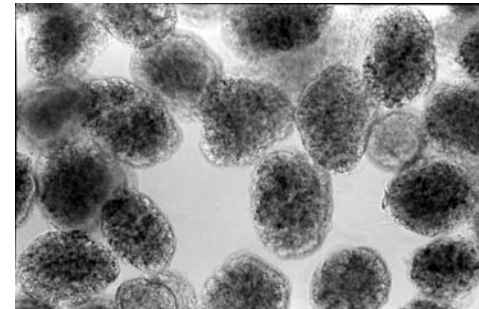
↓ Picking-up under a phase-contrast microscopy

Highly purified glomeruli

↓ Solubilized with lysis solution

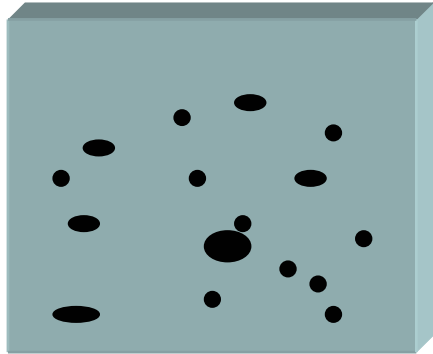
Lysis solution: 9.8 M urea, 2 % NP-40, 2 % Pharmalyte, pH 3-10, 100 mM DTT, 0.5 µg/ml E-64, 0.5 mM PMSF, 40 µg/ml TLCK, 1 µg/ml aprotinin, 10 µg/ml cyclostatin, 0.5 mM EDTA

↓ **2-DE sample**



Protein identification

Silver stain without glutaraldehyde



Pick out spots



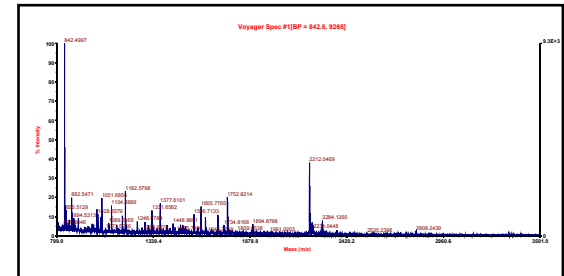
In gel digestion (Trypsin)



MALDI-TOF MS



PMF database search



Subject

Serial no.	Subject <i>anonymous</i> code	Sex	Age	Pathologic diagnosis
1	OQBfN779	male	50	No glomerular lesions except for slight age-related changes*
2	apXME367	male	68	No glomerular lesions except for slight age-related changes*
3	NMXFp768	male	50	No glomerular lesions except for slight age-related changes*
4	VpxZu206	male	38	Normal (no apparent abnormal lesions)

***Slight age-related changes:** a slight widening of mesangium; a small number of collapsed glomeruli; focal interstitial infiltration of lymphocytes; tubular atrophy ; hyaline degeneration

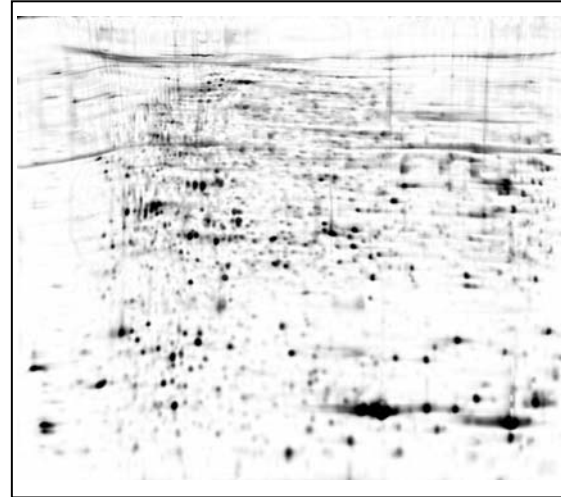
2-DE gel images of highly purified glomeruli from 4 subjects

1



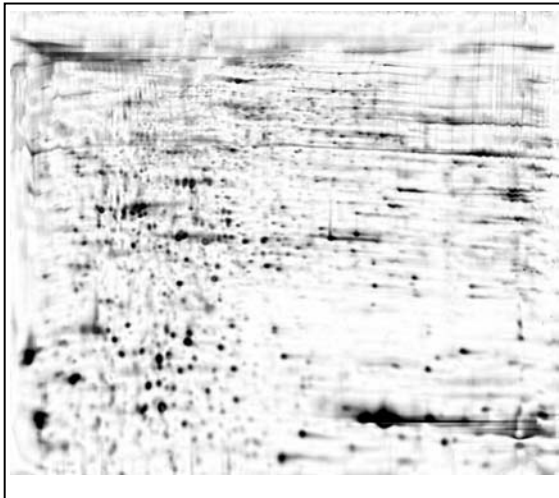
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2



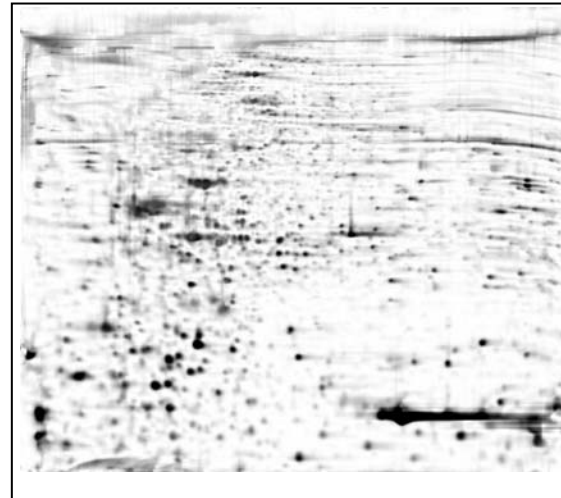
Valid spot count:984

3



Valid spot count:944

4



Valid spot count:834

Human Glomerulus Proteome Database

Example 1

Number	Protein	Observed		Theoretical		Sequence coverage	Access number	Gene name	DNA Reference Access No
		pI	Mw (kDa)	pI	Mw (kDa)				
QHKG1165	Cell division control protein CDC46	4.81	16.4	8.8	26.8	27	pir: S43199		
QHKG1166	Gamma actin	4.82	18.2	5.3	41.9	25	pir: JC5818		
QHKG1167	Unknown protein*	4.82	17.5	5.3	41.9	14	gb: AAH12854		
QHKG1168	Myosin VIIA	4.82	19.3	9.1	256.6	13	ref: NP_000251	MYO7A	NM_00260
QHKG1169	Beta galactosidase soluble lectin	4.84	14.9	5.3	14.9	52	ref: NP_002296	LGALS1	NM_002305
QHKG1170	Beta galactosidase soluble lectin	4.86	14.6	5.3	14.9	49	ref: NP_002296	LGALS1	NM_002305
QHKG1174	Unknown protein*	4.93	18.4	5.7	41.5	20	gb: AAH04223		BC004223
QHKG1177	sudD homolog	5.03	17.5	5.5	59.6	13	ref: NP_003822	SUDO	NM_003831

Human Glomerulus Proteome Database

Example 2

Number	Protein	Observed		Theoretical		Sequence coverage	Access number	Gene name	DNA Reference Access No
		pI	Mw (kDa)	pI	Mw (kDa)				
QHKG1179	Immunoglobulin epsilon chain	5.08	13.8	9.1	47.7	14	gb: AAB59395	IgH	J00221
QHKG1181	KIAA0472 protein	5.14	19.2	7.1	26.2	24	ref: XP_050147	KIAA0472	XM_050147
QHKG1182	Unknown protein	5.14	19.5	5.2	18.0	38	gb: AAH09544		BC009544
QHKG110e	Protein disulfide-isomerase	5.73	15.3	6.0	57.2	38	pir: JC5704		D83485
QHKG111d	Endocytosis protein HSTNB*	6.36	13.2	6.7	100.6	14	gb: AAK76362		AF380833
QHKG1250	Hypothetical protein XP_012952	4.78	22.7	8.6	39.6	26	ref: XP_012952	LOC57761	XM_012952
QHKG1251	Suppression of tumorigenecity protein	4.79	25.0	9.7	127.7	16	emb: CAC35387	ST5	AJ400879
QHKG1253	Hypothetical protein FLJ22313	4.82	28.6	5.9	42.8	20	ref: XP_015485		

Human Glomerulus Proteome Database

Example 3

Number	Protein	Observed		Theoretical		Sequence coverage	Access number	Gene name	DNA Reference Access No
		pI	Mw (kDa)	pI	Mw (kDa)				
QHKG1275	Keratin 1	5.30	23.5	8.3	66.2	32	ref: XP_049904	LOC144511	XM_049902
QHKG1278	Gamma actin (frangment)	5.34	29.8	5.6	26.2	23	gb: AAA51580		
QHKG1282	Thiol specific antioxidant	5.44	25.4	5.2	18.5	24	emb: CAA57764	TSA	X82321
QHKG1291	Vimentin	5.80	29.7	5.1	53.7	28	ref: NP_003371	VIM	NM_003380
QHKG120r	Hypothetical protein XP_030811*	6.31	29.1	7.9	26.5	30	ref: XP_030811		XM_030811
QHKG120w	Hypothetical protein XP_030392*	6.40	29.5	7.2	214.4	11	ref: XP_030392	RNAH	XM_030392
QHKG121h	orf ^x *	6.59	25.7	9.3	40.2	34	emb: CAB03630		Z81330
QHKG1348	Zinc finger protein SALL1	4.73	31.7	6.6	141.9	12	emb: CAB41399	SALL1	Y18264

Human Glomerulus Proteome Database

Example 4

Number	Protein	Observed		Theoretical		Sequence coverage	Access number	Gene name	DNA Reference Access No
		pI	Mw (kDa)	pI	Mw (kDa)				
QHKG1359	Gamma actin	5.07	30.4	5.3	41.9	45	pir: JC5818		
QHKG1360	Unknown protein*	5.10	37.5	5.2	74.2	24	gb: AAH15620		BC015620
QHKG1361	Ro alternative protein 60e2*	5.15	30.4	9.6	23.6	39	gb: AAB81552		U44388
QHKG1425	KIAA0336 gene product	4.79	46.2	5.1	185.6	18	ref: XP_017779	KIAA0336	XM_017779
QHKG1426	ATP synthase beta chain	4.84	49.3	5.3	56.5	34	sp: P06576	ATP5B	NM_001686
QHKG1427	Discoidin receptor tyrosine kinase isoform b	4.91	46.2	6.0	98.0	12	ref: NP_001945	DDR1	NM_001945
QHKG1429	Vimentin	4.99	46.4	5.1	53.7	34	ref: NP_003380		NM_003380
QHKG1423	Gamma Actin	5.16	47.0	5.3	42.1	27	ref: NP_001614	ACYG1	NM_001604