Manuscript #:	SREP-16-15686
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Title:	Identification of candidate genes for congenital heart defects on proximal chromosome 8p

Supplemental Table S1. **gene list of 18.5-Mb region**

gene	OMIM number	expression status in embryonic hearts (MGI database)	cardiovascular phenotypes in knockout mouse (MGI database)	Congential heart disease/phenotype (HGMD database, etc.)
PSD3	614440	—	—	—
INTS10	611353	—	—	—
LPL	609708	E14.5: heart atrium, heart ventricle	abnormal cardiac cell glucose uptake, abnormal cardiovascular system physiology, cardiac fibrosis, cardiac interstitial fibrosis, increased left ventricle diastolic pressure, increased left ventricle systolic pressure	—
SLC18A1	193002	—	—	—
ATP6V1B2	606939	—	—	—
LZTS1	606551	—	—	—
GFRA2	601956	—	—	—
DOK2	604997	—	—	—
XPO7	606140	E14.0, E18.0: heart	—	—
NPM2	608073	—	—	—
FGF17	603725	E7.5, E8.0: heart; E12.5: arch of aorta, innominate artery,dorsal aorta; E15.5: arterial system; E16.5: pulmonary artery, descending thoracic aorta	—	—
EPB49	125305	—	—	—
HR	602302	—	—	—
REEP4	609349	—	—	—
LGI3	608302	—	—	—
SFTPC	178620	—	abnormal lung vasculature morphology	—
BMP1	112264	E7.5:cardiogenic plate; E8.5:heart; E11.0:atrioventricular canal, endocardial cushion; E12.5: atrioventricular endocardial cushion, aortico-pulmonary spiral septum	—	—
PHYHIP	608511	—	—	—

MIR320A	614112	—	—	—
PIWIL2	610312	—	—	—
SLC39A14	608736	—	—	—
PPP3CC	114107	—	—	—
SORBS3	610795	E8.5, E9.5: heart; E10.5: atrioventricular canal, outflow tract; E13.5: heart; E14.5: heart ventricle	—	—
PDLIM2	609722	—	—	—
KIAA1967	607359	—	—	—
BIN3	606396	—	—	—
EGR3	602419	—	—	—
PEBP4	612473	—	—	—
RHOBTB2	607352	E13.5: heart	—	—
TNFRSF10B	603612	—	—	—
TNFRSF10C	603613	—	—	—
TNFRSF10D	603614	—	—	—
TNFRSF10A	603611	—	—	—
CHMP7	611130	—	—	—
LOXL2	606663	—	abnormal liver vasculature morphology , abnormal interventricular septum morphology	—
ENTPD4	607577	E14.5: heart atrium, cardiovascular system	—	—
SLC25A37	610387	—	—	—
NKX3-1	602041	E9.5: heart, dorsal aorta; E11.5:vertebral artery, dorsal aorta, common dorsal aorta; E13.5: common carotid artery; E15.5:aorta	—	—
NKX2-6	611770	E8.0, E8.5: heart; E8.5: sinus venosus; E9.0: heart; E10.0: outflow tract	Nkx2-5-/Nkx2-5-, Nkx2-6-/Nkx2-6+: absent atrioventricular cushions,embryonic lethality during organogenesis；Nkx2-5-/Nkx2-5-, Nkx2-6-/Nkx2-6-: absent atrioventricular cushions, decreased cell proliferation, embryonic growth retardation, embryonic lethality during organogenesis, complete penetrance, increased apoptosis,	common arterial trunk, TFO, DORV, and VSD

			pericardial effusion, pharynx hypoplasia	
STC1	601185	E8.5: heart; E11.5: atrium cardiac muscle,ventricle cardiac muscle; E13.5: ventricle cardiac muscle; E15.5: heart	—	—
ADAM28	606188	—	—	—
ADAMDEC1	606393	—	—	—
ADAM7	607310	—	—	—
NEFM	162250	—	—	—
NEFL	162280	—	—	—
GNRH1	152760	—	—	—
EBF2	609934	—	—	—
PPP2R2A	604941	—	—	—
BNIP3L	605368	—	abnormal heart left ventricle morphology (late after pressure overload, mice exhibit reduced left ventricular end-diastolic diameter, left ventricular dilation, and ventricular remodeling), cardiac fibrosis, increased cardiac muscle contractility, decreased cardiomyocyte apoptosis, altered response of heart to induced stress	—
PNMA2	603970	—	—	—
DPYSL2	602463	—	—	—
ADRA1A	104221	—	decreased vasoconstriction, decreased heart rate variability, hypotension	—
PTK2B	601212	—	abnormal physiological neovascularization, abnormal vascular endothelial cell migration, decreased vasodilation	—
CHRNA2	118502	—	—	—
EPHX2	132811	E14.5: heart atrium, heart ventricle, cardiovascular system	decreased systemic arterial systolic blood pressure, abnormal retinal vasculature morphology, abnormal vascular branching morphogenesis	—
CLU	185430	E14.5: heart atrium, heart ventricle, cardiovascular system,	abnormal glomerular capillary morphology, abnormal heart	—

		blood vessel	morphology, abnormal myocardium layer morphology, abnormal myocardial fiber morphology, decreased myocardial fiber number, abnormal heart ventricle morphology, decreased cardiac muscle contractility, heart inflammation, myocarditis	
SCARA3	602728	—	—	—
ESCO2	609353	E14.5: heart atrium, heart ventricle, cardiovascular system	—	—
PBK	611210	E14.5: heart atrium, heart ventricle, cardiovascular system	—	—
SCARA5	611306	E12.5: heart	—	—
ELP3	612722	—	—	—
PNOC	601459	—	—	—
ZNF395	609494	—	—	—
FBXO16	608519	—	—	—
FZD3	606143	—	—	—
EXTL3	605744	E14.5: heart atrium, heart ventricle, cardiovascular system, heart valve	—	—
INTS9	611352	—	—	—
KIF13B	607350	—	—	—
DUSP4	602747	—	—	—
TMEM66	614768	—	—	—
LEPROTL1	607338	—	abnormal heart left ventricle morphology, thick ventricular wall, increased heart rate	—
MBOAT4	611940	—	—	—
DCTN6	612963	—	—	—
RBPMS	601558	E9.5: heart atrium, outflow tract, primitive ventricle, sinus venosus; E10.5: heart atrium, primitive ventricle	—	—
GTF2E2	189964	—	—	—
GSR	138300	—	abnormal retinal vasculature morphology	—
UBXN8	602155	—	—	—
PPP2CB	176916	—	—	—
TEX15	605795	—	—	—
WRN	604611	—	aortic valve stenosis, cardiac interstitial fibrosis, increased	—

			left ventricle systolic pressure, decreased systemic arterial diastolic blood pressure, heart inflammation	
NRG1	142445	E8.5: primitive ventricle endocardial tube; E9.5: common atrial chamber endocardial lining, endocardial cushion, primitive ventricle endocardial lining; E11.5: endocardial cushion; E12.0: endocardial lining; E12.5: endocardial cushion; E13.5: endocardial cushion; E15.0: interventricular septum endocardial lining, left atrium auricular region endocardial lining, left atrium endocardial lining, left ventricle endocardial lining, right atrium auricular region endocardial lining, right atrium endocardial lining, right ventricle endocardial lining, endocardial tissue; E18.0: interventricular septum endocardial lining, left atrium auricular region endocardial lining, left atrium endocardial lining, left ventricle endocardial lining, right atrium auricular region endocardial lining, right atrium endocardial lining, right ventricle endocardial lining, endocardial tissue	cardiovascular system phenotype, abnormal heart morphology, abnormal atrioventricular cushion morphology, failure of atrioventricular cushion closure,abnormal myocardium layer morphology, decreased heart weight, enlarged heart,abnormal heart ventricle morphology, absent trabeculae carneae, trabecula carnea hypoplasia, thin ventricular wall,dilated heart ventricle, enlarged pericardium, abnormal cardiovascular system, decreased cardiac output physiology, poor circulation, decreased cardiac muscle contractility, decreased ventricle muscle contractility, creased left ventricle systolic pressure, decreased heart rate, irregular heartbeat	—
TTI2	614426	—	—	—

Supplemental Table S2. **The data quality of exome sequencing**

Samples Name	Total reads	Good reads	Total base	Q20(%)[1]	Q30(%)[1]	Mapped Reads	Mapped Ratio(%)
ASD patient	84,357,684	84,357,684	12,653,652,600	96.48	91.70	84,275,124	99.9
Proband	81,857,278	81,857,278	12,278,591,700	96.35	91.43	81,776,072	99.9

Samples Name	Unique Mapped Reads	Unique Mapped Ratio(%)	Reads on target Region (%)	Mean depth	Coverage ≥1X(%)	Coverage ≥10X(%)	Coverage ≥20X(%)
ASD patient	73,803,937	87.49	74.58	113.97	99.33	98.36	96.99
Proband	72,029,667	87.99	74.50	111.65	99.34	98.36	96.94

1. Q20 means bases of Q>=20 / all bases of sequencing, Q30 means bases of Q>=30 / all bases of sequencing

Supplemental Table S3. The sequencing quality of other CHD genes in the ASD patient

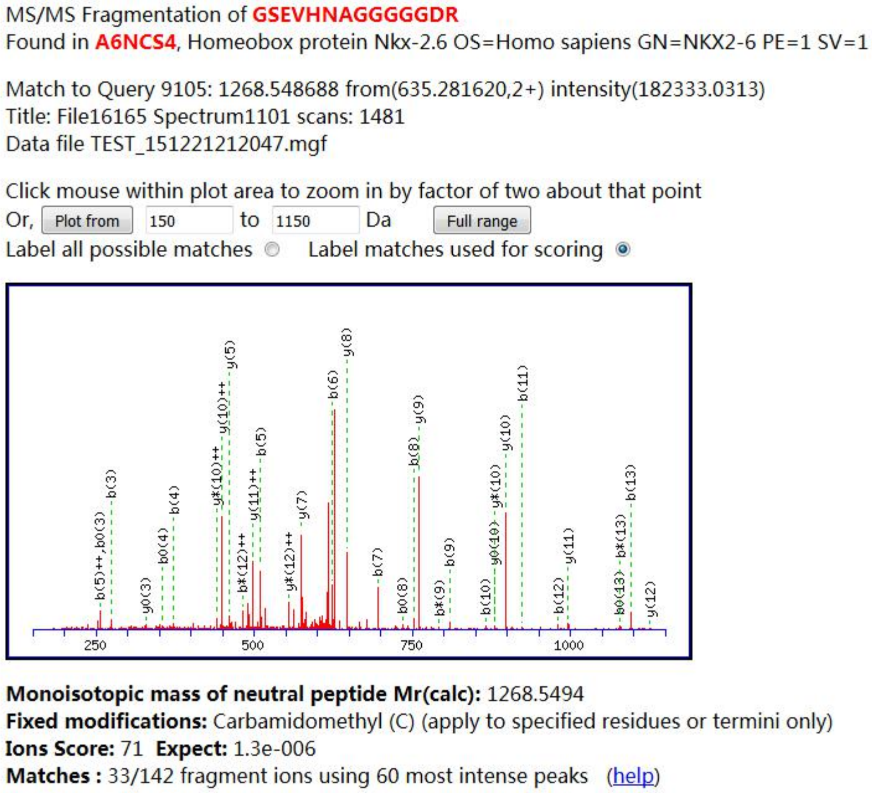
Target gene	Sequencing data amount	Target base	Mean depth	Coverage ≥ 1X(%)	Coverage ≥10X(%)	Coverage ≥20X(%)
GATA4	177353	2254	78.68	100	99.78	90.42
NKX2-5	183402	1606	114.2	100	94.65	93.65
TBX5	305702	2652	115.27	100	100	100
JAG1	810728	7000	115.82	100	100	99.66
NOTCH1	1398368	10650	131.3	100	99.38	96.3
NOTCH2	1241114	11136	111.45	100	100	99.69
TBX1	259406	2291	113.23	100	99.87	94.85
GATA6	196661	2149	91.51	100	90.55	83.76
CITED2	177824	1007	176.59	100	100	100
ZFPM2 (FOG2)	611859	5278	115.93	100	100	100
ISL1	221116	1631	135.57	100	100	100
MEF2C	314712	2501	125.83	100	100	99.24
NR2F2	323789	1875	172.69	100	100	100
PITX2	316664	2123	149.16	100	100	100
SRF	383763	2713	141.45	100	100	99.48

The sequencing quality of other CHD genes in the proband

Target gene	Sequencing data amount	Target base	Mean depth	Coverage ≥ 1X(%)	Coverage ≥10X(%)	Coverage ≥20X(%)
GATA4	175042	2254	77.66	100	98.67	84.65
NKX2-5	216595	1606	134.87	100	99.38	95.45
TBX5	312055	2652	117.67	100	100	100
JAG1	823861	7000	117.69	100	100	99.46
NOTCH1	1463326	10650	137.4	100	99.59	96.15
NOTCH2	1204863	11136	108.2	100	100	99.87
TBX1	270063	2291	117.88	100	100	98.34
GATA6	216836	2149	100.9	100	100	83.25
CITED2	189900	1007	188.58	100	100	100
ZFPM2 (FOG2)	594682	5278	112.67	100	100	100
ISL1	233953	1631	143.44	100	100	99.45
MEF2C	312051	2501	124.77	100	100	100
NR2F2	382517	1875	204.01	100	100	100
PITX2	327443	2123	154.24	100	100	99.29
SRF	376498	2713	138.78	100	99.19	96.39

Supplemental Figure S1. The mass spectrogram of c.1A>T mutant NKX2-6 protein

(a)



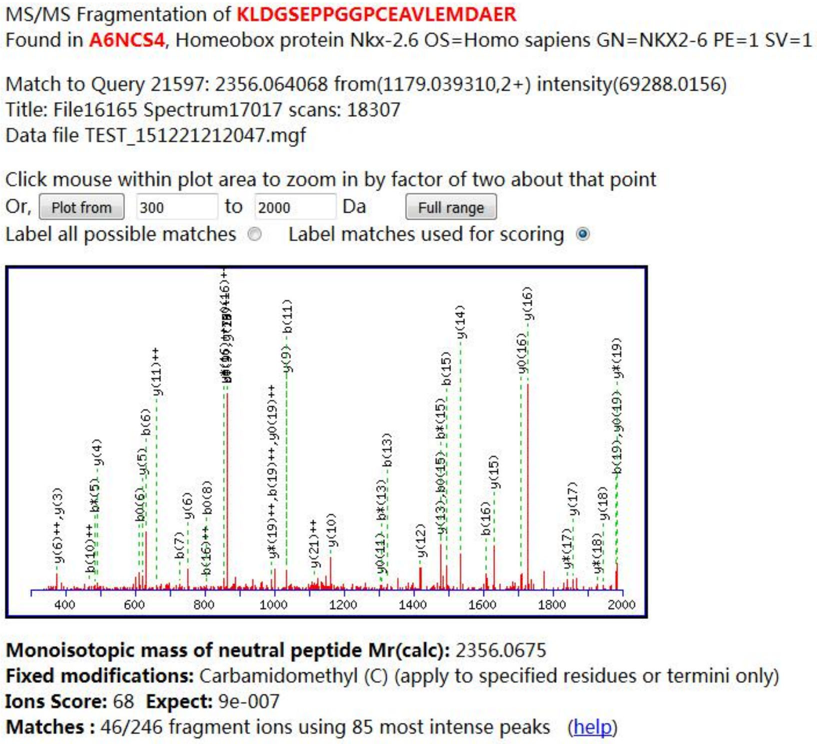
MS/MS Fragmentation of **GSEVHNAGGGGGDR**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 9105: 1268.548688 from(635.281620,2+) intensity(182333.0313)
Title: File16165 Spectrum1101 scans: 1481
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 150 to 1150 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 1268.5494
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 71 **Expect:** 1.3e-006
Matches : 33/142 fragment ions using 60 most intense peaks (help)

(b)



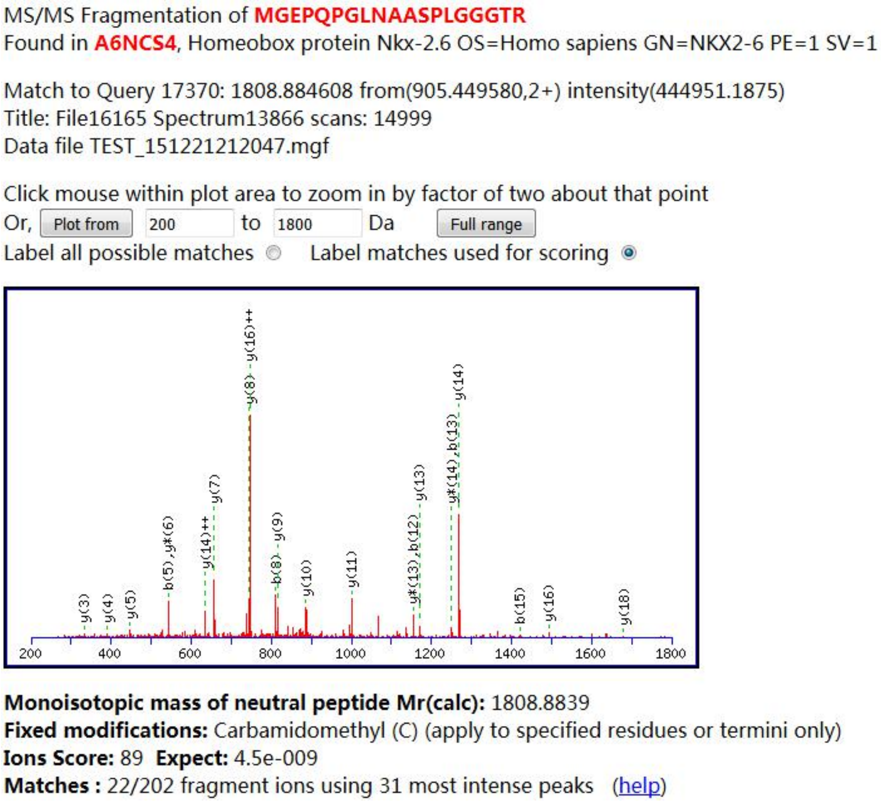
MS/MS Fragmentation of **KLDGSEPPGGPCEAVLEMDAER**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 21597: 2356.064068 from(1179.039310,2+) intensity(69288.0156)
Title: File16165 Spectrum17017 scans: 18307
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 300 to 2000 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 2356.0675
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 68 **Expect:** 9e-007
Matches : 46/246 fragment ions using 85 most intense peaks (help)

(c)



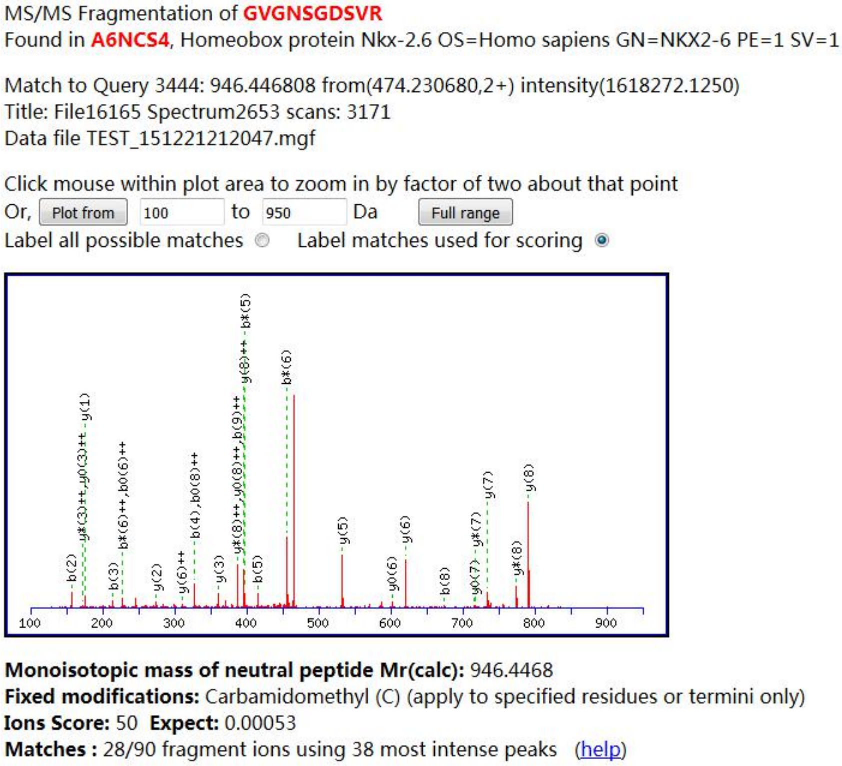
MS/MS Fragmentation of **MGEPQPGLNAASPLGGGTR**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 17370: 1808.884608 from(905.449580,2+) intensity(444951.1875)
Title: File16165 Spectrum13866 scans: 14999
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 200 to 1800 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 1808.8839
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 89 **Expect:** 4.5e-009
Matches : 22/202 fragment ions using 31 most intense peaks (help)

(d)



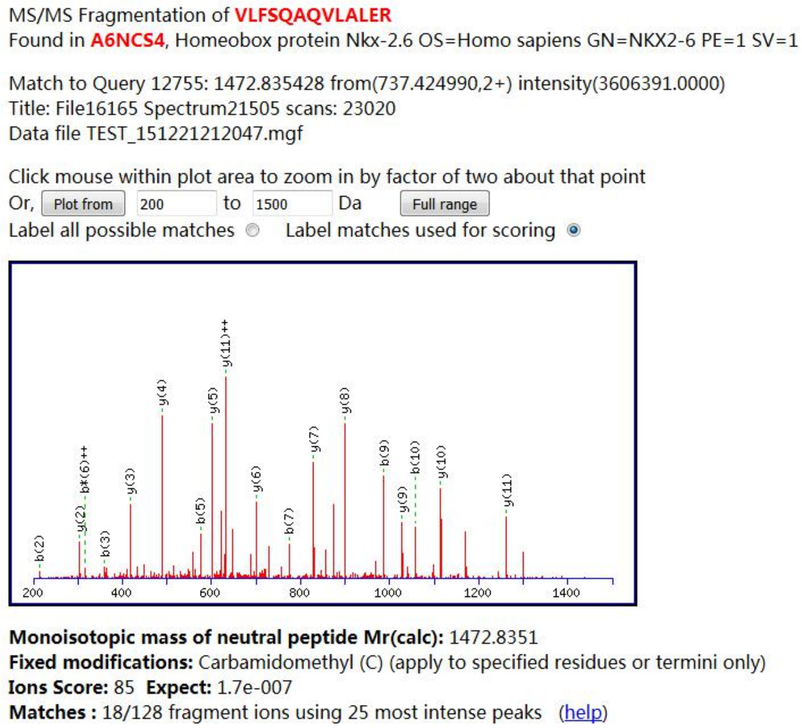
MS/MS Fragmentation of **GVGNSGDSVR**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 3444: 946.446808 from(474.230680,2+) intensity(1618272.1250)
Title: File16165 Spectrum2653 scans: 3171
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 to 950 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 946.4468
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 50 **Expect:** 0.00053
Matches : 28/90 fragment ions using 38 most intense peaks (help)

(e)



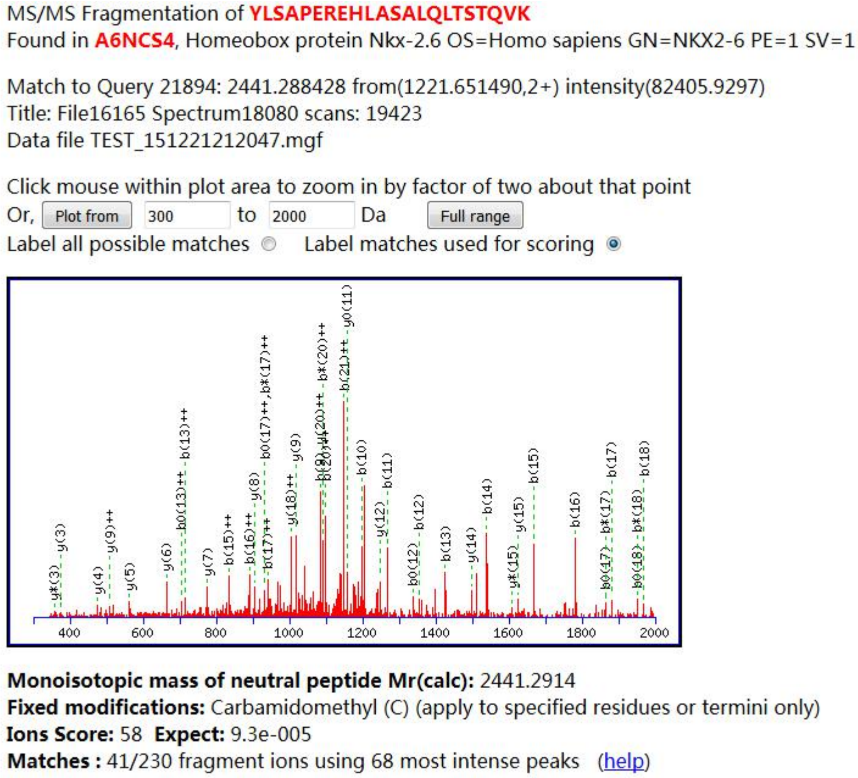
MS/MS Fragmentation of **VLFSQAQVLALER**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 12755: 1472.835428 from(737.424990,2+) intensity(3606391.0000)
Title: File16165 Spectrum21505 scans: 23020
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 200 to 1500 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 1472.8351
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 85 **Expect:** 1.7e-007
Matches : 18/128 fragment ions using 25 most intense peaks (help)

(f)



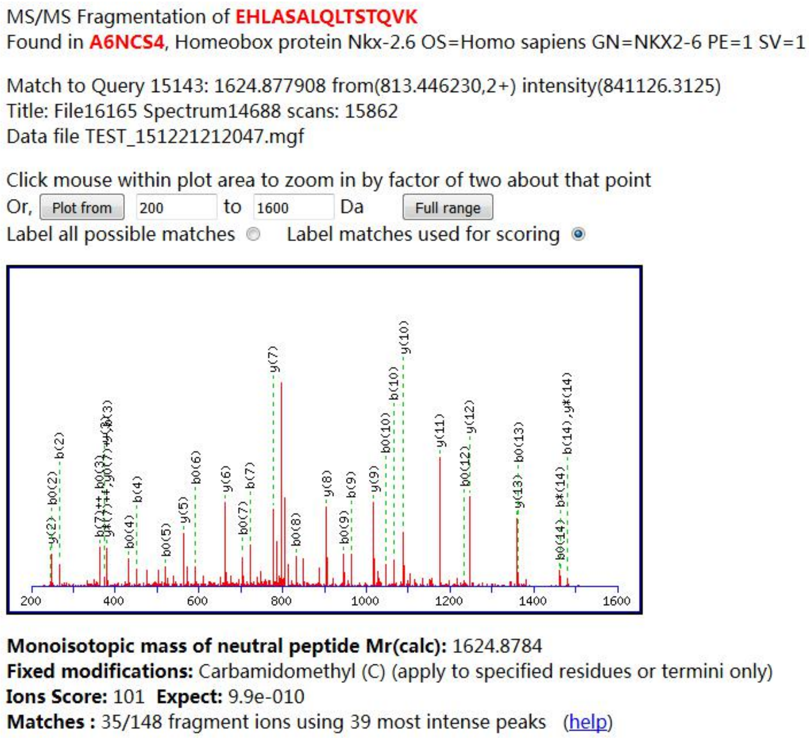
MS/MS Fragmentation of **YLSAPEREHLASALQLTSTQVK**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 21894: 2441.288428 from(1221.651490,2+) intensity(82405.9297)
Title: File16165 Spectrum18080 scans: 19423
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 300 to 2000 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 2441.2914
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 58 **Expect:** 9.3e-005
Matches : 41/230 fragment ions using 68 most intense peaks (help)

(g)



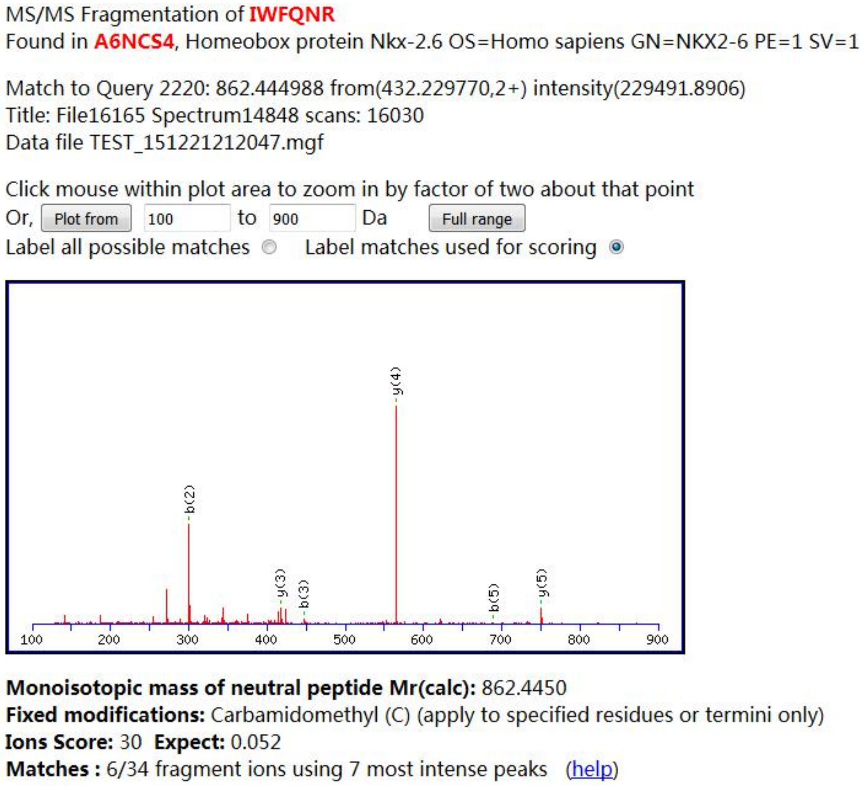
MS/MS Fragmentation of **EHLASALQLTSTQVK**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 15143: 1624.877908 from(813.446230,2+) intensity(841126.3125)
Title: File16165 Spectrum14688 scans: 15862
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 200 to 1600 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 1624.8784
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 101 **Expect:** 9.9e-010
Matches : 35/148 fragment ions using 39 most intense peaks (help)

(h)



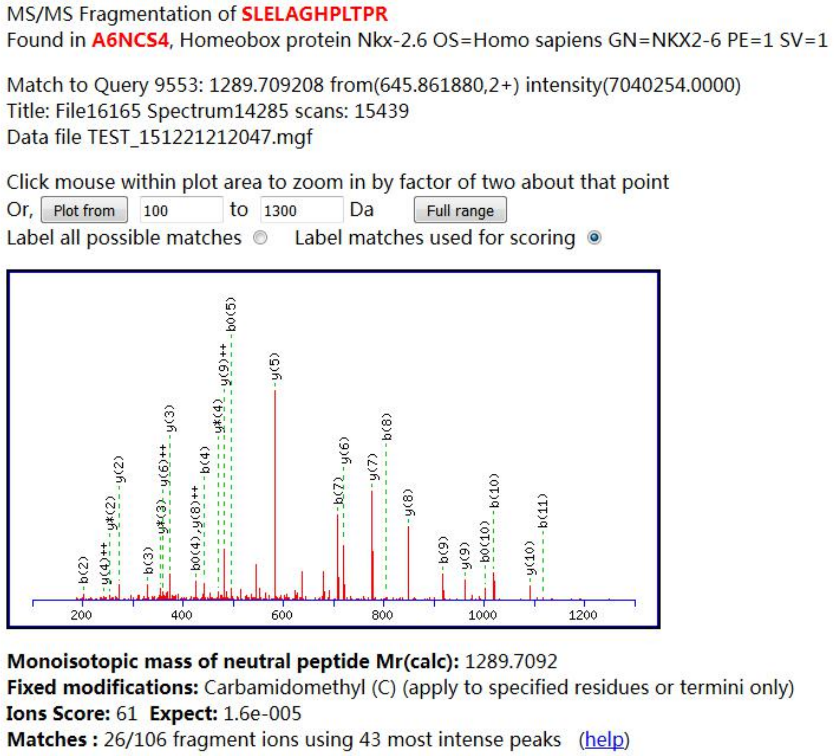
MS/MS Fragmentation of **IWFQNR**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 2220: 862.444988 from(432.229770,2+) intensity(229491.8906)
Title: File16165 Spectrum14848 scans: 16030
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 to 900 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 862.4450
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 30 **Expect:** 0.052
Matches : 6/34 fragment ions using 7 most intense peaks (help)

(i)



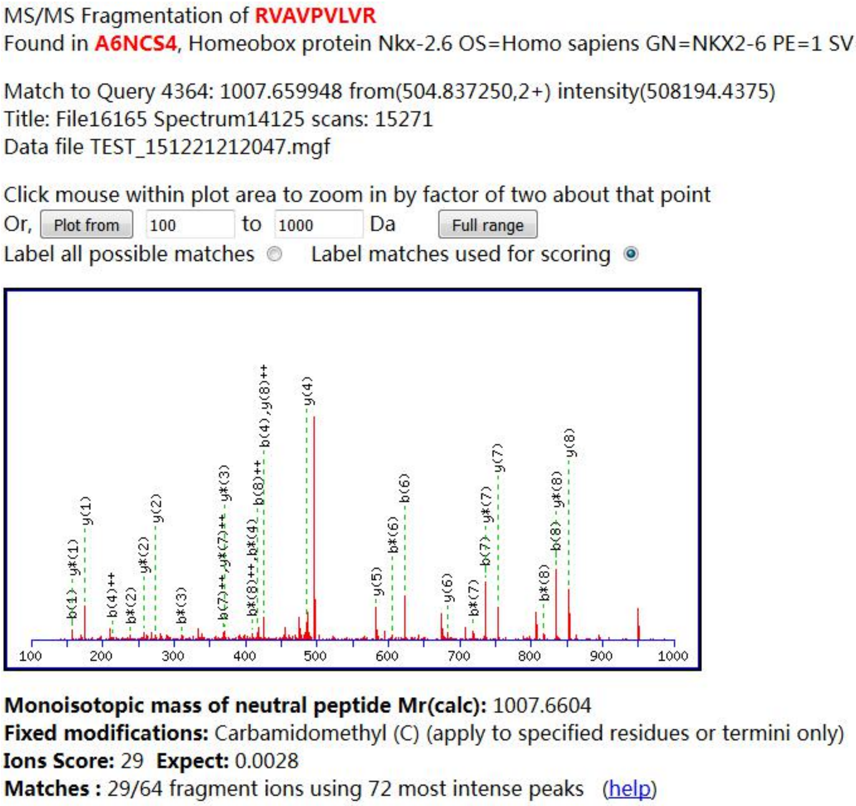
MS/MS Fragmentation of **SLELAGHPLTPR**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 9553: 1289.709208 from(645.861880,2+) intensity(7040254.0000)
Title: File16165 Spectrum14285 scans: 15439
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 to 1300 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 1289.7092
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 61 **Expect:** 1.6e-005
Matches : 26/106 fragment ions using 43 most intense peaks (help)

(j)



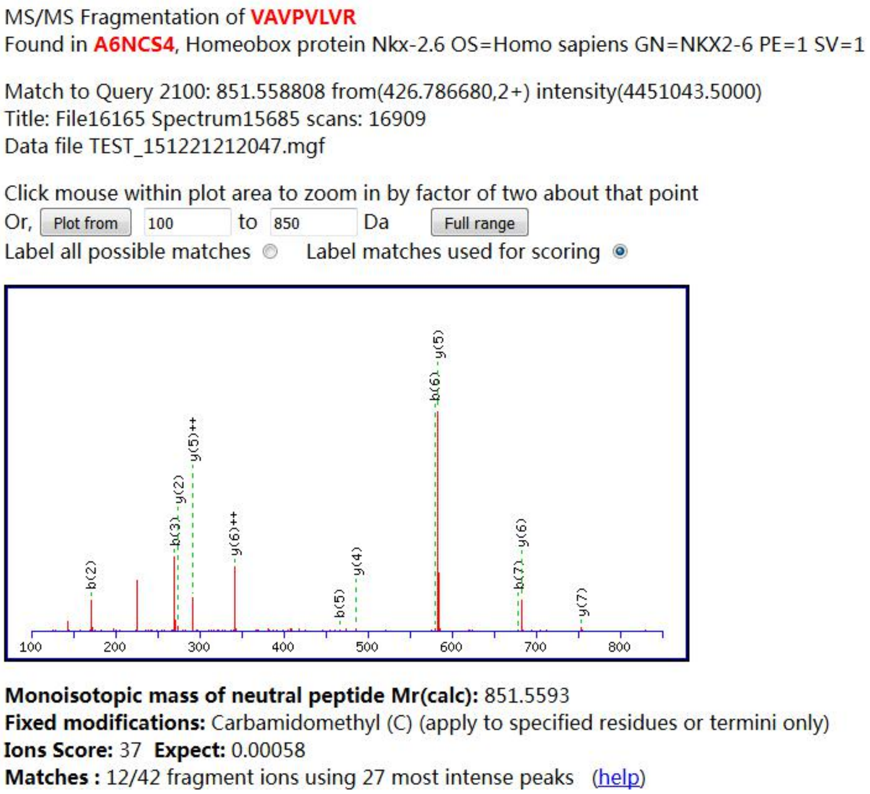
MS/MS Fragmentation of **RVAVPVLVR**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 4364: 1007.659948 from(504.837250,2+) intensity(508194.4375)
Title: File16165 Spectrum14125 scans: 15271
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 to 1000 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 1007.6604
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 29 **Expect:** 0.0028
Matches : 29/64 fragment ions using 72 most intense peaks (help)

(k)



MS/MS Fragmentation of **VAVPVLVR**
Found in **A6NCS4**, Homeobox protein Nkx-2.6 OS=Homo sapiens GN=NKX2-6 PE=1 SV=1

Match to Query 2100: 851.558808 from(426.786680,2+) intensity(4451043.5000)
Title: File16165 Spectrum15685 scans: 16909
Data file TEST_151221212047.mgf

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 to 850 Da Full range
Label all possible matches ○ Label matches used for scoring ●

Monoisotopic mass of neutral peptide Mr(calc): 851.5593
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 37 **Expect:** 0.00058
Matches : 12/42 fragment ions using 27 most intense peaks (help)