Proteins/peptides purification by a three-well OFFGEL electrophoresis with immobilized ultra narrow pH gradient gels

---- Supporting Information ----

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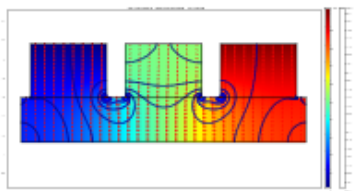E-mail: hubert.girault@epfl.ch

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Fax number: +41-21-693 3667

SI-1: Finite element simulations

The model, mesh size, boundary conditions and domain equations used are summarized in the generated COMSOL report file below:

COMSOL Model Report

1. Table of Contents

- Title - COMSOL Model Report
- Table of Contents
- Model Properties
- Geometry
- Geom1
- Solver Settings
- Postprocessing
- Variables

2. Model Properties

Property	Value
Model name	
Author	
Company	
Department	
Reference	
URL	
Saved date	Mar 11, 2013 4:55:23 PM
Creation date	Mar 11, 2013 3:10:22 PM
COMSOL version	COMSOL 3.5.0.603

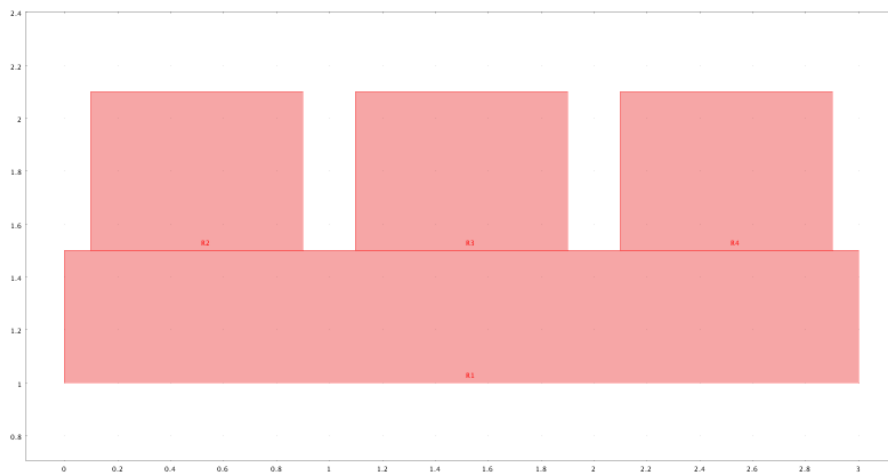File name: /home/dmitry/Desktop/3 well E field.mph

Application modes and modules used in this model:
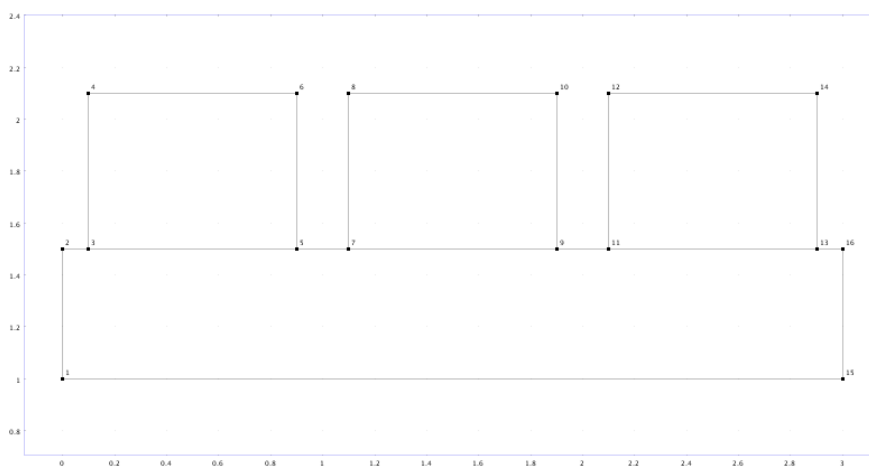
- Geom1 (2D)
  - Electrostatics (AC/DC Module)

3. Geometry

Number of geometries: 1

3.1. Geom1

3.1.1. Point mode

3.1.2. Boundary mode
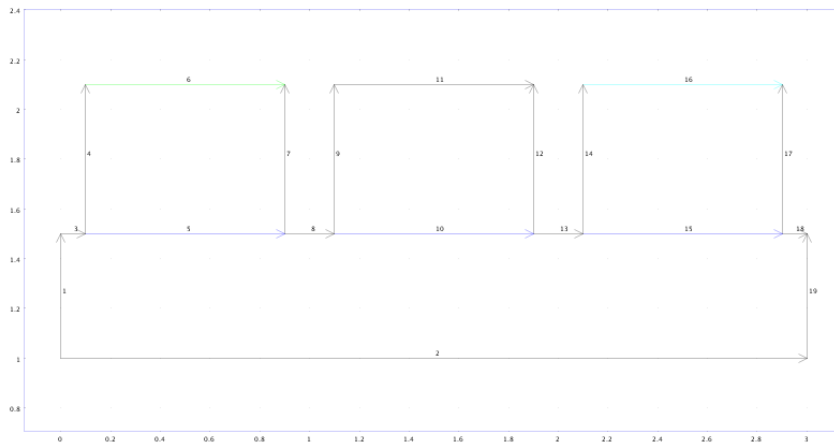
3.1.3. Subdomain mode
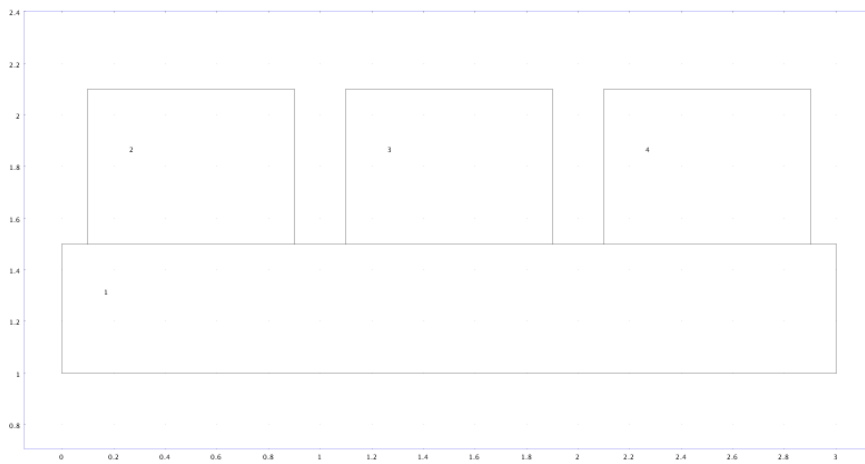
4. Geom1

Space dimensions: 2D

Independent variables: x, y, z

4.1. Mesh

4.1.1. Mesh Statistics

Number of degrees of freedom	31186
Number of mesh points	7946
Number of elements	15295
Triangular	15295
Quadrilateral	0
Number of boundary elements	796
Number of vertex elements	16
Minimum element quality	0.815
Element area ratio	0
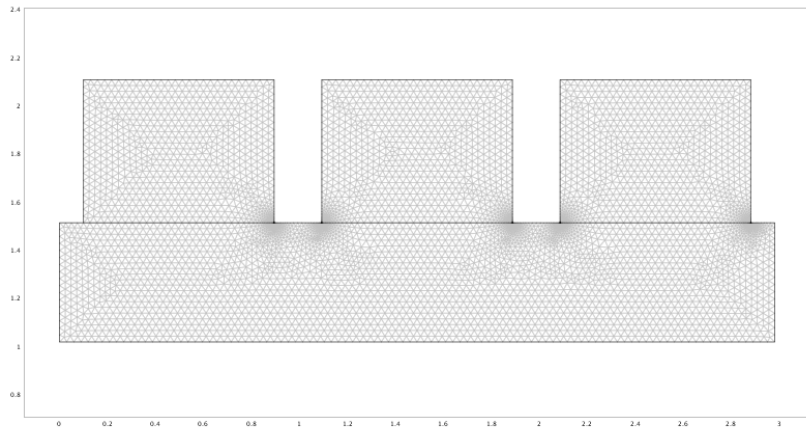
4.2. Application Mode: Electrostatics (emes)

Application mode type: Electrostatics (AC/DC Module)

Application mode name: emes

4.2.1. Scalar Variables

Name	Variable	Value	Unit	Description
epsilon0	epsilon0_emes	8.854187817e-12	F/m	Permittivity of vacuum

4.2.2. Application Mode Properties

Property	Value
Default element type	Lagrange - Quadratic
Input property	Forced voltage
Frame	Frame (ref)
Weak constraints	Off
Constraint type	Ideal

4.2.3. Variables

Dependent variables: V

Shape functions: shlag(2,'V')
Interior boundaries not active

4.2.4. Boundary Settings

Boundary		1-4, 7-9, 11-14, 17-19	6	16
Type		Zero charge/Symmetry	Electric potential	Electric potential
Electric potential (V0)	V	0	0	**1**

4.2.5. Subdomain Settings

Subdomain		1-4
Relative permittivity (epsilonr)	1	**{78,0;0,78}**

5. Solver Settings

Solve using a script: off

Auto select solver	On
Solver	Stationary
Solution form	Automatic
Symmetric	auto
Adaptive mesh refinement	Off
Optimization/Sensitivity	Off
Plot while solving	Off

5.1. Direct (UMFPACK)

Solver type: Linear system solver

Parameter	Value
Pivot threshold	0.1
Memory allocation factor	0.7

5.2. Stationary

Parameter	Value
Linearity	Automatic
Relative tolerance	1.0E-6
Maximum number of iterations	25
Manual tuning of damping parameters	Off
Highly nonlinear problem	Off
Initial damping factor	1.0
Minimum damping factor	1.0E-4
Restriction for step size update	10.0

6. Postprocessing

Surface: Electric potential [V]   Contour: Electric field, norm [V/m]   Arrow: Electric field
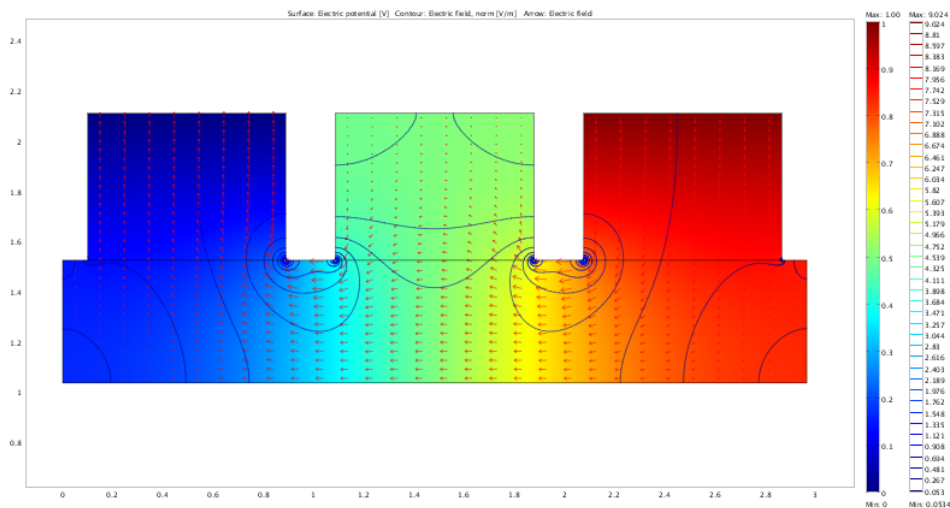
7. Variables

7.1. Boundary

Name	Description	Unit	Expression
unTEx_emes	Maxwell surface stress tensor (x)	Pa	-0.5 * (up(Dx_emes) * up(Ex_emes)+up(Dy_emes) * up(Ey_emes)) * dnx+(dnx * up(Dx_emes)+dny * up(Dy_emes)) * up(Ex_emes)
unTEy_emes	Maxwell surface stress tensor (y)	Pa	-0.5 * (up(Dx_emes) * up(Ex_emes)+up(Dy_emes) * up(Ey_emes)) * dny+(dnx * up(Dx_emes)+dny * up(Dy_emes)) * up(Ey_emes)
dnTEx_emes	Maxwell surface stress tensor (x)	Pa	-0.5 * (down(Dx_emes) * down(Ex_emes)+down(Dy_emes) * down(Ey_emes)) * unx+(unx * down(Dx_emes)+uny * down(Dy_emes)) * down(Ex_emes)
dnTEy_emes	Maxwell surface stress tensor (y)	Pa	-0.5 * (down(Dx_emes) * down(Ex_emes)+down(Dy_emes) * down(Ey_emes)) * uny+(unx * down(Dx_emes)+uny * down(Dy_emes)) * down(Ey_emes)
unTx_emes	Exterior Maxwell stress tensor (u), x component	Pa	unTEx_emes+unTMx_emes
unTMx_emes	Exterior magnetic Maxwell stress tensor (u), x component	Pa	0
unTy_emes	Exterior Maxwell stress tensor (u), y component	Pa	unTEy_emes+unTMy_emes
unTMy_emes	Exterior magnetic Maxwell stress tensor (u), y component	Pa	0
dnTx_emes	Exterior Maxwell stress tensor (d), x component	Pa	dnTEx_emes+dnTMx_emes
dnTMx_emes	Exterior magnetic Maxwell stress tensor (d), x component	Pa	0
dnTy_emes	Exterior Maxwell stress tensor (d), y component	Pa	dnTEy_emes+dnTMy_emes
dnTMy_emes	Exterior magnetic Maxwell stress tensor (d), y component	Pa	0
dVolbnd_emes	Volume integration contribution	m	d_emes
nD_emes	Surface charge density	C/m^2	unx * (down(Dx_emes)-up(Dx_emes))+uny * (down(Dy_emes)-up(Dy_emes))

7.2. Subdomain

Name	Description	Unit	Expression
dr_guess_emes	Width in radial direction default guess	m	0
R0_guess_emes	Inner radius default guess	m	0
Sx_emes	Infinite element x coordinate	m	x
S0x_guess_emes	Inner x coordinate default guess	m	0
Sdx_guess_emes	Width in x direction default guess	m	0
Sy_emes	Infinite element y coordinate	m	y
S0y_guess_emes	Inner y coordinate default guess	m	0
Sdy_guess_emes	Width in y direction default guess	m	0
dVol_emes	Volume integration contribution	m	detJ_emes * d_emes
Dx_emes	Electric displacement, x component	C/m^2	epsilonxx_emes * Ex_emes+epsilonxy_emes * Ey_emes
Dy_emes	Electric displacement, y component	C/m^2	epsilonyx_emes * Ex_emes+epsilonyy_emes * Ey_emes
epsilon_emes	Permittivity	F/m	epsilon0_emes * epsilonr_emes
epsilonxx_emes	Permittivity, xx component	F/m	epsilon0_emes * epsilonrxx_emes
epsilonxy_emes	Permittivity, xy component	F/m	epsilon0_emes * epsilonrxy_emes
epsilonyx_emes	Permittivity, yx component	F/m	epsilon0_emes * epsilonryx_emes
normD_emes	Electric displacement, norm	C/m^2	sqrt(abs(Dx_emes)^2+abs(Dy_emes)^2)
normDr_emes	Remanent displacement, norm	C/m^2	sqrt(abs(Drx_emes)^2+abs(Dry_emes)^2)
normP_emes	Electric polarization, norm	C/m^2	sqrt(abs(Px_emes)^2+abs(Py_emes)^2)
We_emes	Electric energy density	J/m^3	0.5 * ((Dx_emes+Drx_emes) * Ex_emes+ (Dy_emes+Dry_emes) * Ey_emes)
dW_emes	Integrand for total energy	N/m	dVol_emes * We_emes
Ex_emes	Electric field, x component	V/m	-Vx
Ey_emes	Electric field, y component	V/m	-Vy

SI-2: Purification of *E. coli* extract on UNPG gels
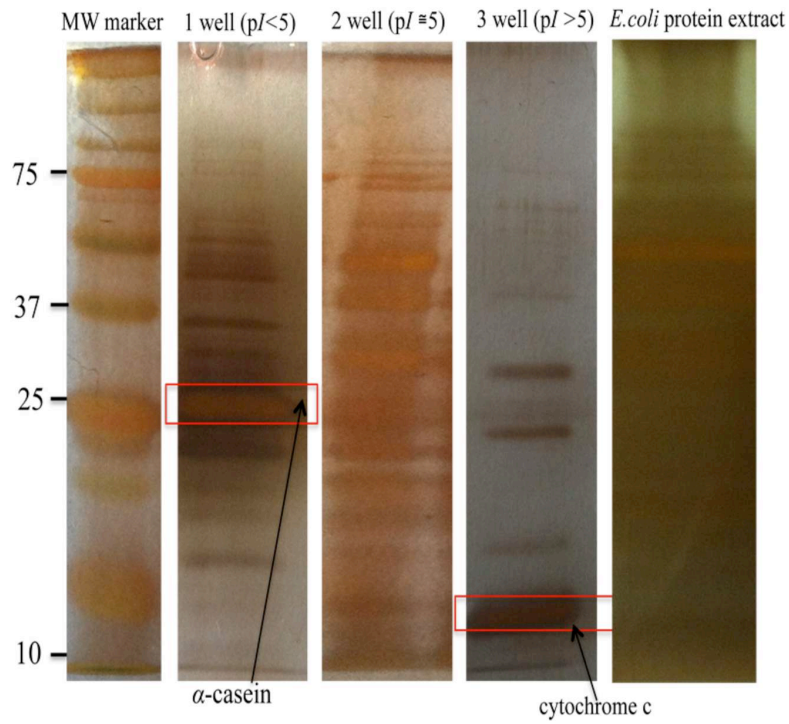
Figure 1: a) Silver stained SDS PAGE of *E. coli* protein extract spiked with α-casein and cytochrome C after fractionation on a UNPG gel with a pH gradient = 5.0-5.27. Each SDS-PAGE matches the protein fractions collected from a respective well after OFFGEL separation. Pre-stained Molecular weight protein markers appear on left side of the gel and are as follows from top to bottom: 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa.

SI-3: Purification of digested *E.coli* extract digest followed by LC-MS/MS analysis

E.coli extract digest was firstly fractionated in a gel with pH gradient 6.0-6.27, after the experiment was finished the collected fractions from each of three wells were lyophilised by vacuum centrifugation. Afterwards, the fractions were diluted in 30μL of water and analysed by LC-MS/MS. MS/MS detection was operating in a positive ion mode with 400-2000 *m/z* scanning range and collision induced dissociation. LC-MS/MS data processing was performed with the Trans-Proteomic Pipeline (TPP), which is a set of integrated tools for MS/MS proteomics, developed at the Seattle Proteome Center of the Institute for Systems Biology in USA (http://tools.proteomecenter.org/wiki/index.php?title=Software:TPP). The peptide assignment was performed with X! Tandem as search engine and UniProtKB as database. Results validation was performed with the PeptideProphet of TPP.

Result obtained from the <u>fraction No.1</u> (anodic side).

probability	peptide	protein	calc_neutral_pep_m	pI
1	K.C[160.03]DM[147.04]VDDEELLELVEM[147.04]EVR.E	sp|P0CE47	2254.9647	3.62
0.9993	K.AFQELNAIDVL.-	sp|P0AB71	1231.6447	3.67
1	K.ADEQILDIGDASAQELAEILK.N	sp|P0A799	2241.1377	3.71
0.8432	K.SGM[147.04]NPFLLDSEDVFIDLLTDSGTGAVTQSM[14	sp|P0A853	3911.7577	3.71
0.9997	K.DQIIEAVAAM[147.04]SVM[147.04]DVVELISAM[147	sp|P0A7K2	2768.3167	3.77
1	K.VEVETPEENTGDVIGDLSR.R	sp|P0A6M8	2057.9757	3.77
1	K.VALQDAGLSVSDIDDVILVGGQTR.M	sp|P0A6Y8	2440.2807	3.77
0.9997	R.TTDVTGTIELPEGVEM[147.04]VM[147.04]PGDNIK.N	sp|P0CE47	2577.2187	3.83
0.9993	R.TTDVTGTIELPEGVEM[147.04]VM[147.04]PGDNIK.N	sp|P0CE47	2577.2187	3.83
0.9999	K.VGDTVIEFDLPLLEEK.A	sp|P69783	1815.9507	3.83
1	R.VDDLAVDLVER.F	sp|P09373	1242.6457	3.84
1	K.DDSFFDVYTEC[160.03]R.T	sp|P0A853	1552.6137	3.84
1	R.ELLSQYDFPGDDTPIVR.G	sp|P0CE47	1963.9527	3.84
0.9999	K.EGQNLDFVGGAE.-	sp|P0ADZ0	1234.5467	3.91
1	R.SGETEDATIADLAVGTAAGQIK.T	sp|P0A6P9	2117.0487	3.92
0.9937	R.EM[147.04]LIADGIDPNELLNSLAAVK.S	sp|P0ACF8	2241.1567	3.92
1	K.AAGAELVGM[147.04]EDLADQIK.K	sp|P0A7L0	1745.8507	3.92
1	R.AFDQIDNAPEEK.A	sp|P0CE47	1375.6257	3.92
1	K.TLAEGQNVEFEIQDGQK.G	sp|P0A9Y6	1904.9117	4
0.9999	K.GM[147.04]NTAVGDEGGYAPNLGSNAEALAVIAEAVK	sp|P0A6P9	3004.4447	4
0.9999	K.VLYEM[147.04]DGVPEELAR.E	sp|P0ADY7	1635.7817	4
0.9966	K.ALEGDAEWEAK.I	sp|P0CE47	1217.5567	4
0.9945	K.ILELAGFLDSYIPEPER.A	sp|P0CE47	1961.0147	4
1	R.DIADAVTAAGVEVAK.S	sp|P0A7R1	1428.7457	4.03
1	K.ANDAAGDGTTTATVLAQAIITEGLK.A	sp|P0A6F5	2401.2337	4.03
0.9999	K.GGDTVTLNETDLTQIPK.V	sp|P0AET2	1800.9107	4.03
0.9992	R.AGDNAPM[147.04]AYIELVDR.S	sp|P0AG44	1649.7717	4.03
1	K.LINDAYDSEYFATK.V	sp|P0A6F3	1648.7617	4.03
0.9911	K.DGVIADFFVTEK.M	sp|P0A9X4	1339.6657	4.03
1	K.DKPEDAVLDVQGIATVTPAIVQAC[160.03]TQDK.Q	sp|P0AES9	2981.5017	4.04
1	K.DKPEDAVLDVQGIATVTPAIVQAC[160.03]TQDK.Q	sp|P0AES9	2981.5017	4.04
0.9993	K.DAM[147.04]VPM[147.04]GGLLC[160.03]M[147.04	sp|P0A853	3004.2417	4.04
1	K.ALEEAGAEVEVK.-	sp|P0A7K2	1243.6297	4.09
1	K.YLGGEELTEAEIK.G	sp|P0A6M8	1450.7187	4.09
0.9999	K.LKDGEDPGYTLYDLSER.L	sp|P69908	1969.9267	4.11
0.8707	K.LKDGEDPGYTLYDLSER.L	sp|P69908	1969.9267	4.11
0.9992	R.EQIIFPEIDYDKVDR.V	sp|P62399	1878.9367	4.11
0.9913	R.MDAAQLTEEGYYSVFGK.S	sp|P36683	1907.8607	4.14
1	R.LPGLYYIETDSTGER.T	sp|P37647	1712.8257	4.14
1	K.AAAFEGELIPASQIDR.L	sp|P0A7J3	1686.8577	4.14
0.2873	R.NVEEGGSLTIIATALIDTGSK.M	sp|P0AG30	2088.0947	4.14
0.9848	K.IQTIDEIQSTETLIVLQNPIM[147.04]R.T	sp|P0ACI6	2570.3627	4.14
1	R.NIGEILELAGC[160.03]DR.L	sp|P0A870	1458.7137	4.14
1	K.YDEAPSNVAQAVIEAR.G	sp|P0A6M8	1731.8427	4.14
1	K.GATVELADGVEGYLR.A	sp|P0AG67	1548.7787	4.14
1	K.EGEATLAPSLDLVGKI.-	sp|P0AE08	1611.8717	4.14
1	R.ATFVVDPQGIIQAIEVTAEGIGR.D	sp|P0AE08	2383.2747	4.14
0.3578	K.GNFDLEGLER.G	sp|P0A853	1148.5457	4.14
1	K.DIALGEEFVNK.-	sp|P61889	1233.6237	4.14
0.2801	R.TIAMSLPEFEQSLFMAAQPDNLLLATAPR.Y	sp|P31463	3174.6097	4.14
1	R.ATFVVDPQGIIQAIEVTAEGIGR.D	sp|P0AE08	2383.2747	4.14

	Peptide	Protein	Mass	RT
1	K.VAEAIAASFGSFADFK.A	sp|P0AGD3	1629.8037	4.37
0.9989	K.TIATENAPAAIGPYVQGVDLGNM[147.04]IITSGQIPV	sp|P0AF93	3564.8497	4.37
0.999	K.QYDINEAIALLK.E	sp|P0A7L0	1389.7507	4.37
1	K.ALAINLVDPAAAGTVIEK.A	sp|P0AEE5	1764.9987	4.37
1	R.GWQVPAFTLGGEATDIVVM[147.04]R.I	sp|P69908	2162.0827	4.37
1	K.DLVESAPAALK.E	sp|P0A7K2	1112.6077	4.37
1	R.AQYLIDQLLAEAR.K	sp|P0AFG8	1502.8097	4.37
1	K.LVTDELVIALVK.E	sp|P69441	1311.8017	4.37
1	K.GLTDAAQQVVAAVEGK.-	sp|P0A836	1555.8207	4.37
0.9997	R.GLSVLM[147.04]LEAQDLAC[160.03]ATSSASSK.L	sp|P13035	2254.0817	4.37
0.9956	R.VTITIAADSIETAVK.S	sp|P0A850	1530.8507	4.37
0.9989	K.IVDLLTER.A	sp|P0A7D4	957.5497	4.37
1	K.AILAAAGIAEDVK.I	sp|P0A7S9	1240.7027	4.37
0.9925	R.YWDVELR.E	sp|P69908	979.4767	4.37
0.9991	K.TQLIDVIAEK.A	sp|P0ACF0	1128.6387	4.37
0.5307	K.ISDIPEFVR.G	sp|P0AC38	1074.5707	4.37
1	K.EVVFASIDTGTPAK.F	sp|Q46938	1433.7397	4.37
0.5328	K.TLQQLNASIAVEGLDAK.K	sp|P33362	1769.9527	4.37
1	K.LVADSITSQLER.R	sp|P0A7V3	1330.7097	4.37
1	R.IINEPTAAALAYGLDK.G	sp|P0A6Y8	1658.8877	4.37
0.9645	K.QLGEDPWVAIAK.R	sp|P0AG67	1325.6977	4.37
1	K.EGDAVQLVGFGTFK.V	sp|P0ACF0	1466.7407	4.37
0.9795	K.GQLKEFLDANLA.-	sp|P0AA25	1317.6927	4.37
1	R.DLALIEINPLVITK.Q	sp|P0A836	1550.9287	4.37
1	K.VQNASYQVAAYLADEIAK.L	sp|P69908	1952.9847	4.37
0.9984	K.VQNASYQVAAYLADEIAK.L	sp|P69908	1952.9847	4.37
0.9184	K.LGDIEYR.E	sp|P68066	864.4337	4.37
1	K.VVADIAGVPAQINIAEVR.K	sp|P0A7V3	1834.0317	4.37
0.972	R.TSTFLDVYIER.D	sp|P09373	1342.6767	4.37
0.6786	K.VYAAIEAGDK.A	sp|P0A7U7	1035.5237	4.37
0.9894	K.ELANVQDLTVR.G	sp|P02925	1256.6727	4.37
0.9998	K.VAIANVLKEEGFIEDFKVEGDTKPELELTLK.Y	sp|P0A7W7	3473.8547	4.38
0.9999	R.EAEGSHIM[147.04]LGAQNVDLNLSGAFTGETSAAM	sp|P0A858	3293.5547	4.4
0.9995	R.EAEYKDWTIEQITR.E	sp|P0A853	1780.8627	4.41
0.9989	K.AALESTLAAITESLKEGDAVQLVGFGTFK.V	sp|P0ACF0	2965.5647	4.41
0.9996	K.EYDHIKDVNDLPELLK.A	sp|P0A7A9	1939.9887	4.43
1	K.IALESVLLGDKE.-	sp|P0ABP8	1285.7127	4.43
1	R.GVVVAIDKDVVLVDAGLKSESAIPAEQFK.N	sp|P0AG67	2996.6437	4.44
1	K.AFTSEEFTHFLEELTK.Q	sp|P0A6P9	1927.9207	4.48
1	K.VGEEVEIVGIKETQK.S	sp|P0CE47	1656.8937	4.49
0.4239	R.VGFFNPIASEKEEGTRLDLDR.I	sp|P0A7T3	2392.2027	4.51
0.9999	R.VLENAEGDRTTPSIIAYTQDGETLVGQPAKR.Q	sp|P0A6Y8	3328.6897	4.51
0.9936	K.RFPLHEM[147.04]RDDVAFQIINDELYLDGNAR.Q	sp|P69908	3262.5827	4.52
1	K.EAYELVAPILTK.I	sp|P00350	1345.7497	4.53
0.9999	K.GNTGENLLALLEGR.L	sp|P0A7V8	1455.7677	4.53
0.9996	K.STAESIVYSALETLAQR.S	sp|P02359	1837.9417	4.53
0.9999	K.ISYISTGGGAFLEFVEGK.V	sp|P0A799	1873.9457	4.53
1	K.TQLPSGSELSLYDIAPVTPGVAVDLSHIPTAVK.I	sp|P61889	3374.7977	4.53
1	K.STAESIVYSALETLAQR.S	sp|P02359	1837.9417	4.53
1	R.GIEEVGPNNVPYIVATITSNSAGGQPVSLANLK.A	sp|P0A853	3308.7257	4.53
1	R.GIEEVGPNNVPYIVATITSNSAGGQPVSLANLK.A	sp|P0A853	3308.7257	4.53
1	K.AALESTLAAITESLK.E	sp|P0ACF0	1516.8347	4.53

1	K.FESEVYILSK.D	sp|P0CE47	1213.6227	4.53
0.9994	R.EM[147.04]LPVLEAVAK.A	sp|P0A6F5	1214.6577	4.53
0.9997	K.AALELAEQR.E	sp|P0A7V8	999.5347	4.53
1	R.LTGLEGEQLGIVSLR.E	sp|P0A707	1583.8887	4.53
0.9716	K.IEIEAIAVR.R	sp|P0AF93	1012.5917	4.53
0.992	K.TVPM[147.04]FNEALAELNK.I	sp|P0A7V0	1591.7917	4.53
1	K.AM[147.04]VEVFLER.G	sp|P0A825	1108.5587	4.53
0.9993	K.QAIVAEVSEVAK.G	sp|P0A7J3|	1242.6817	4.53
0.9995	K.NIEFFEAR.R	sp|P0A7R1	1024.4977	4.53
1	R.VPEGIGETAIVQIR.N	sp|P27248	1480.8247	4.53
0.3364	R.AAVEEGVVAGGGVALIR.V	sp|P0A6F5	1566.8727	4.53
1	R.VATEFSETAPATLK.S	sp|P0A799	1463.7507	4.53
0.9999	R.LATLPTYEEAIAR.L	sp|P0A7J3|	1446.7717	4.53
0.7658	K.LEVVVNER.R	sp|P0ACF8	956.5287	4.53
0.9984	K.GLNVMQNLLTAHPDVQAVFAQNDEM[147.04]ALGAL	sp|P02925	3351.6707	4.54
1	R.HASDDEPFSALAFK.I	sp|P0A6M8	1533.7097	4.54
1	K.ITFIDGDEGILLHR.G	sp|P0ABH7	1597.8467	4.54
0.9999	K.VFVADEYTM[147.04]VYSHIDR.I	sp|Q46938	1959.9037	4.54
0.3464	R.E[111.03]AATHAADAADSAR.A	sp|P76072	1337.5957	4.54
1	K.GLNVM[147.04]QNLLTAHPDVQAVFAQNDEM[147.0	sp|P02925	3367.6657	4.54
0.9525	K.AKIELSSAQQTDVNLPYITADATGPK.H	sp|P0A6Y8	2730.4077	4.56
0.9945	R.AIDKPFLLPIEDVFSISGR.G	sp|P0CE47	2116.1567	4.56
0.9925	R.ILTGDKVTVELTPYDLSK.G	sp|P69222	1991.0827	4.56
0.9999	R.AIDKPFLLPIEDVFSISGR.G	sp|P0CE47	2116.1567	4.56
0.9988	K.LTKPVELIATLDDSAK.S	sp|P35340	1712.9557	4.56
0.9178	K.QDVPSFRPGDTVEVK.V	sp|P0A7K6	1672.8417	4.56
0.9997	K.LQTLGLTQGTVVTISAEGEDEQKAVEHLVK.L	sp|P0AA04	3192.6877	4.57
0.9936	K.LGVLGFEVDHER.N	sp|P0A6A3	1369.6987	4.65
0.998	R.ELGVINIGGAGTITVDGQC[160.03]YEIGHR.D	sp|Q46938	2628.2967	4.65
0.8833	K.DLEHPIEVPVGK.A	sp|P0ABB4	1331.7087	4.65
1	R.SGKSELEAFEVALENVRPTVEVK.S	sp|P02359	2530.3277	4.65
0.9754	R.LGANPVPLQLAIGAEEHFTGVVDLVK.M	sp|P0A6M8	2686.4697	4.65
1	R.ILENGEVKPLDVK.V	sp|P0A6F9	1452.8187	4.68
0.9962	K.TLDTQGLRNEFLVEK.V	sp|Q46938	1761.9257	4.68
1	K.TQLIDVIAEKAELSK.T	sp|P0ACF0	1656.9297	4.68
0.9987	K.DGISYTFSIVPNALGKDDEVRK.T	sp|P09373	2423.2337	4.68
0.9963	K.STLTPVVISNM[147.04]DEIKELIK.L	sp|P69783	2145.1607	4.68
0.9956	K.LGPYEFIC[160.03]TGRPDEGIPAVC[160.03]FK.L	sp|P69908	2525.2087	4.68
0.999	K.WKEGEATLAPSLDLVGKI.-	sp|P0AE08	1926.0467	4.68
0.9795	R.AKLESLVEDLVNR.S	sp|P0A6Y8	1484.8197	4.68
0.9978	R.NHETGELLATFELK.T	sp|P36683	1600.8097	4.75
0.9998	R.GNPTVEAEVHLEGGFVGM[147.04]AAAPSGASTGSR.E	sp|P0A6P9	2871.3457	4.75
1	R.ELPELTAEFIKR.F	sp|P0A850	1444.7927	4.79
0.675	K.E[111.03]VLIFGGQEVETKR.F	sp|P60752	1585.8467	4.79
1	R.EVPVEVKPEVR.V	sp|P68066	1279.7137	4.79
1	K.HEDM[147.04]YTAINELINKLER.Q	sp|P0AD49	2104.0257	4.83
0.9999	R.GYLSPYFINKPETGAVELESPFILLADKK.I	sp|P0A6F5	3238.7167	4.87
0.619	K.Q[111.03]LPC[160.03]PAELLR.L	sp|P0A853	1178.6117	5.99
0.5839	E.LSKTQAKAALESTLAAITESLK.E	sp|P0ACF0	2273.2847	8.5
0.4233	R.GQSSAALALGM[147.04]THWQSM[147.04]K.L	sp|P0AER5	1934.8977	8.76
0.5485	R.VHLPPLRERR.E	sp|P0AFB8	1271.7577	11.7

LC-MS/MS result obtained from the <u>fraction No.2</u> (middle well, where the sample was loaded) after OFFGEL fractionation of an *E.coli* protein digest.

probability	peptide	protein	calc_neutral_pep_mass	pI
0.1577	K.Q[111.03]SSAALESYLN.R	sp|P37325|YBCH_ECOI	1164.5297	4
0.1626	K.IGTGATQA.S	sp|P31678|OTSB_ECOI	717.3657	5.52
0.1659	K.NANETKSGAG.Q	sp|P08957|T1MK_ECO	947.4307	6
0.1717	K.VTKIFV.D	sp|P61175|RL22_ECOL	705.4427	8.72
0.1965	K.ILAFAHI.M	sp|P0AAD6|SDAC_ECO	783.4647	6.74
0.207	K.VTKIFV.D	sp|P61175|RL22_ECOL	705.4427	8.72
0.2104	K.GDIGAFD.S	sp|P76104|YDCP_ECOI	693.2967	3.89
0.2105	R.VSSPM[147.04]VA.A	sp|P30176|YBIA_ECOL	705.3367	5.49
0.3067	K.NIIPQFRIP.A	sp|Q46811|YGFK_ECO	1096.6397	9.75
0.3439	K.Q[111.03]NLPGAEEGD.G	sp|P36929|RSMB_ECO	1011.4147	3.88
0.3489	Q.Q[111.03]DDM[147.04]TK.N	sp|P0A705|IF2_ECOLI,:	735.2747	4.21
0.3823	A.GPFGKSDR.A	sp|P76090|YNBA_ECO	862.4297	8.75
0.5704	K.FGM[147.04]AGGS.I	sp|P37645|YHJG_ECOL	641.2477	5.52
0.5828	K.GMIATGEG.T	sp|P10906|UGPE_ECO	734.3267	4
0.7303	-.MKVGFIGLGIM[147.04].G	sp|P0ABQ2|GARR_ECC	1180.6347	8.5
0.7998	K.LLTWGFRFFE.T	sp|P0AEB2|DACA_ECO	1314.6757	6.22
0.8364	R.NLTNRHIQLIA.I	sp|P0AAE0|CYCA_ECO	1291.7357	9.76
0.8387	R.GVRQLLELDP.G	sp|P31802|NARP_ECO	1138.6347	4.37
0.8653	R.HSGMESR.Q	sp|P60869|YBJL_ECOLI	802.3387	6.75
0.8842	R.Q[111.03]GKASVND.A	sp|P38035|RTCR_ECOI	800.3667	6.09
0.9809	R.LPNNFWVPR.C	sp|P0A8P1|LFTR_ECOL	1141.6037	9.75

LC-MS/MS result obtained from the <u>fraction No.3</u> (cathodic side) after OFFGEL fractionation of an *E.coli* protein digest

probability	peptide	protein	calc_neutral_pep_mass	pI
0.7346	K.NMITGAAQMDGAILVVAATD	sp|P0CE47|EFTU1_ECOLI	2728.3347	4.21
0.9976	R.TLTLSGMLAEAIR.R	sp|P0A717|KPRS_ECOLI	1374.7537	5.66
0.891	Y.TAINELINKLER.Q	sp|P0AD49|RAIA_ECOLI	1412.7987	5.81
0.9924	W.TLLTELLKEIPAK.-	sp|P15288|PEPD_ECOLI	1467.8917	5.81
0.9981	Y.VVPAFTGLGAPYWDPYAR.G	sp|P0A6F3|GLPK_ECOLI	1978.9937	5.81
0.9998	K.IQGIGAGFIPANLDLK.L	sp|P0ABK5|CYSK_ECOLI	1625.9137	5.84
0.9999	K.IATDPFVGNLTFFR.V	sp|P0A6M8|EFG_ECOLI	1596.8297	5.84
0.9004	R.GVNVLADAVK.V	sp|P0A6F5|CH60_ECOLI	984.5607	5.84
0.9953	K.GGTRIPISGIAGDQQAALF.G	sp|P0A6F3|GLPK_ECOLI	1870.9897	5.84
0.9882	K.AADIVLQAAIAAGAPK.D	sp|P0A9Q7|ADHE_ECOLI	1478.8457	5.88
0.9986	K.AGIALNDNFVK.L	sp|P0A9B2|G3P1_ECOLI	1160.6187	5.88
0.9997	K.AGPLAGYPVVDMGIR.L	sp|P0A6M8|EFG_ECOLI	1514.7917	5.88
0.999	K.KYDIPVVMDSAR.F	sp|P0A853|TNAA_ECOLI	1392.7067	5.96
1	K.LLDNAAADLAAISGQKPLITK.A	sp|P62399|RL5_ECOLI	2122.1997	5.96
0.9226	L.IMDKIYDVLR.A	sp|P0C8J8|GATZ_ECOLI	1264.6847	5.96
0.9976	R.KIDGIPALLDR.A	sp|P37769|KDUD_ECOLI	1209.7077	5.96
0.6715	F.LVDLVDKNLTGK.E	sp|P0A825|GLYA_ECOLI	1313.7557	5.96
0.8699	Y.VVVAALGELAK.R	sp|P0AFG8|ODP1_ECOLI	1068.6547	5.97
0.9999	K.VILAGEVTTPVTVR.G	sp|P02413|RL15_ECOLI	1453.8507	5.97
0.9697	K.VLPELNGKLTGMAF.R	sp|P0A9B2|G3P1_ECOLI	1488.8007	5.97
1	R.VVVGLLLGEVIR.T	sp|P0A850|TIG_ECOLI	1265.8067	5.97
0.995	K.VIVEGINLVK.K	sp|P60624|RL24_ECOLI	1082.6697	5.97
1	R.VFMQPASEGTGIIAGGAMR.A	sp|P0A7W1|RS5_ECOLI	1891.9287	5.97
0.9999	R.GSVNPEC[160.03]TLAQLGAA	sp|P0A9G6|ACEA_ECOLI	1614.8037	5.99
0.9934	K.QLPC[160.03]PAELLR.L	sp|P0A853|TNAA_ECOLI	1195.6387	5.99
0.9997	R.FGGESVLAGSIIVR.Q	sp|P0A7L8|RL27_ECOLI	1403.7777	6
0.9998	R.GAEQIYIPVLIK.K	sp|P0A853|TNAA_ECOLI	1342.7857	6
0.9998	R.GAEQIYIPVLIK.K	sp|P0A853|TNAA_ECOLI	1342.7857	6
0.9998	R.LQAFEGVVIAIR.N	sp|P0A7K6|RL19_ECOLI	1314.7657	6
0.986	R.FAENAYFIK.Q	sp|P0A853|TNAA_ECOLI	1101.5497	6
0.9991	K.FNQIGSLTETLAAIK.M	sp|P0A6P9|ENO_ECOLI	1604.8777	6
0.9993	K.GGFTVELNGIR.A	sp|P0AG67|RS1_ECOLI	1161.6137	6
0.9963	R.GAEQIYIPVLIK.K	sp|P0A853|TNAA_ECOLI	1342.7857	6
0.9898	Y.AAIDGLIDKLAR.Q	sp|P0AFX0|HPF_ECOLI	1254.7297	6
0.8134	R.LQAFEGVVIAIR.N	sp|P0A7K6|RL19_ECOLI	1314.7657	6
0.9928	K.GLTFTYEPK.V	sp|P0A853|TNAA_ECOLI	1054.5337	6
0.9906	K.LFNELGPR.F	sp|P0AG44|RL17_ECOLI	944.5077	6
0.9666	K.GLTFTYEPK.V	sp|P0A853|TNAA_ECOLI	1054.5335	6
0.9991	K.LAEVLAAANAR.A	sp|P0A7R1|RL9_ECOLI	1097.6197	6
0.9999	R.ALLNSMVIGVTEGFTK.K	sp|P0AG55|RL6_ECOLI	1678.8967	6.05
0.9994	K.ALGANLVLTEGAK.G	sp|P0ABK5|CYSK_ECOLI	1255.7137	6.05
0.9945	R.AGENVGVLLR.G	sp|P0CE47|EFTU1_ECOLI	1026.5817	6.05
0.9721	R.AGENVGVLLR.G	sp|P0CE47|EFTU1_ECOLI	1026.5817	6.05
0.9797	W.ATQSSTLVEVLAK.A	sp|P0A6F3|GLPK_ECOLI	1345.7457	6.05
0.9965	K.AANIIGIQIEFAK.V	sp|P02413|RL15_ECOLI	1386.7867	6.05
0.9932	R.KLEDLLAAKL.-	sp|P0A867|TALA_ECOLI	1112.6807	6.07
0.9987	K.ITLNMGVGEAIADKK.L	sp|P62399|RL5_ECOLI	1558.8387	6.07
0.8173	Y.KFNGWELDINSR.S	sp|P0A9Q1|ARCA_ECOLI	1477.7317	6.07
0.8123	R.KAVEFQDILK.M	sp|P0AC38|ASPA_ECOLI	1189.6707	6.07
0.9995	R.LRGWQVPAFTLGGEATDIVVN	sp|P69908|DCEA_ECOLI	2415.2737	6.07
1	R.KVVADIAGVPAQINIAEVR.K	sp|P0A7V3|RS3_ECOLI	1962.1257	6.07

0.9998	R.LKGNTGENLLALLEGR.L	sp|P0A7V8|RS4_ECOLI	1696.9467	6.14
0.9799	R.KISWMEIYTGEK.S	sp|P08200|IDH_ECOLI	1483.7377	6.14
0.944	F.THAGTENLLALVSAM[14:	sp|P0C8J6|GATY_ECOLI	1741.9037	6.41
0.9979	F.THAGTENLLALVSAMAK.(	sp|P0C8J6|GATY_ECOLI	1725.9087	6.41
0.9865	R.MVQLFFPDPWHK.A	sp|P0A8I5|TRMB_ECOLI	1543.7647	6.5
0.8306	-.MENFKHLPEPFR.I	sp|P0A853|TNAA_ECOLI	1543.7607	6.51
0.9995	K.VLDLIAHISK.-	sp|P0A9B2|G3P1_ECOLI	1107.6647	6.71
0.8149	A.VEPHAVATLSC[160.03]I	sp|P39361|SGCR_ECOLI	1423.7497	6.71
0.999	R.VFQTHSPVVDSISVK.R	sp|P0A7K6|RL19_ECOLI	1641.8727	6.71
0.7811	K.WMYHYC[160.03].C	sp|P36979|RLMN_ECOLI	958.3467	6.73
0.8847	R.DGHPLFAGFVK.A	sp|P0A7E5|PYRG_ECOLI	1186.6137	6.74
0.9113	R.GGVSLRPGDGVIHSW.L	sp|P36683|ACON2_ECOL	1535.7847	6.74
0.9437	R.HVAILGDLQGPK.I	sp|P21599|KPYK2_ECOLI	1246.7037	6.74
0.9983	K.GPFIDLHLLK.K	sp|P0A7U3|RS19_ECOLI	1151.6707	6.74
0.8063	R.RVAALEGDVLGSYQHGAI	sp|P0A6P1|EFTS_ECOLI	1897.9757	6.75
0.6622	K.AIQLHPLVC[160.03]AAY	sp|P0A8T7|RPOC_ECOLI	1354.7067	6.77
0.9394	R.ALPLPVSVPSHC[160.03].	sp|P0AAI9|FABD_ECOLI	1459.7857	6.78
0.9999	R.AVLEVAGVHNVLAK.A	sp|P0A7W1|RS5_ECOLI	1418.8247	6.79
0.9997	R.AVLEVAGVHNVLAK.A	sp|P0A7W1|RS5_ECOLI	1418.8247	6.79
0.9899	R.KVLNIFPSIDTGVC[160.0:	sp|P0A862|TPX_ECOLI	2046.0927	8.22
0.9917	R.SNTGLVIDPYFSGTKVK.V	sp|P0A6F3|GLPK_ECOLI	1824.9617	8.22
0.9983	K.TGKYDAVIALGTVIR.G	sp|P61714|RISB_ECOLI	1575.8987	8.25
0.9923	Y.SNKVLDLIAHISK.-	sp|P0A9B2|G3P1_ECOLI	1436.8347	8.33
0.9998	K.TTLTAAITTVLAK.T	sp|P0CE47|EFTU1_ECOLI	1302.7757	8.41
0.9737	K.TPPAAVLLK.K	sp|P0A7J7|RL11_ECOLI	908.5697	8.41
0.7166	L.TAAITTVLAK.T	sp|P0CE47|EFTU1_ECOLI	987.5967	8.41
0.9997	K.SAGGIVLTGSAAAK.S	sp|P0A6F9|CH10_ECOLI	1201.6667	8.47
0.9675	Y.SHIDRIIVGGIMPITK.T	sp|Q46938|KDUI_ECOLI	1748.9967	8.49
0.8914	-.MITGIQITK.A	sp|P68066|GRCA_ECOLI	1003.5737	8.5
0.9996	R.GAEQIYIPVLIKK.R	sp|P0A853|TNAA_ECOLI	1470.8807	8.5
0.945	-.MKVAVLGAAGGIGQAL.A	sp|P61889|MDH_ECOLI	1454.8277	8.5
0.8538	K.KLQLVGVGY.R	sp|P0AG55|RL6_ECOLI	975.5757	8.59
0.9899	K.KLADSGLNIIAAK.G	sp|P0A836|SUCC_ECOLI	1312.7717	8.59
0.914	K.GKPFAPLLEK.N	sp|P60422|RL2_ECOLI	1098.6437	8.59
0.9974	K.FVNILMVDGKK.S	sp|P02359|RS7_ECOLI	1262.7057	8.59
0.9998	R.GVNVLADAVKVTLGPK.G	sp|P0A6F5|CH60_ECOLI	1579.9297	8.59
0.9989	K.KGPFIDLHLLK.K	sp|P0A7U3|RS19_ECOLI	1279.7657	8.6
0.6623	R.AANKFPAIIY.G	sp|P68919|RL25_ECOLI	1106.6127	8.63
0.7071	K.VKAPVIVQF.S	sp|P0AB71|ALF_ECOLI	999.6117	8.72
0.9671	W.VGQGATISDRVAALIK.E	sp|P0A7T3|RS16_ECOLI	1597.9147	8.72
0.9996	K.IAAANVPAFVSGK.A	sp|P0ACF0|DBHA_ECOLI	1243.6927	8.75
0.9743	Y.GVAIADGPLKGLAAR.A	sp|P0A862|TPX_ECOLI	1407.8197	8.75
0.9907	K.KLNSAVFPGGQGGPL.M	sp|P0A825|GLYA_ECOLI	1440.7727	8.75
0.9916	Y.IVATITSNSAGGQPVSLAN	sp|P0A853|TNAA_ECOLI	2040.1217	8.75
0.9987	Y.IVATITSNSAGGQPVSLAN	sp|P0A853|TNAA_ECOLI	2040.1217	8.75
0.9997	K.IAAANVPAFVSGK.A	sp|P0ACF0|DBHA_ECOLI	1243.6927	8.75
0.9997	R.IIVGGIMPITK.T	sp|Q46938|KDUI_ECOLI	1140.6937	8.75
0.9092	R.IILLGAPGAGKGTQAQF.I	sp|P69441|KAD_ECOLI	1640.9247	8.75
0.9359	R.DVILFPAMRPVK.-	sp|P0A8N3|SYK1_ECOLI	1384.7897	8.75
0.9992	K.AYGSTNPINVVR.A	sp|P0A7W1|RS5_ECOLI	1289.6728	8.79
0.6968	K.EITIAAAKVPSFR.A	sp|P0ACF4|DBHB_ECOLI	1401.7977	8.85
0.9064	R.SQWLGGWR.E	sp|P0AFW2|RMF_ECOLI	988.4877	9.47

0.9987	R.AFLPGSLVDVRPVR.D	sp|P0AG67|RS1_EC	1524.8777	9.64
0.9345	Q.KKAEAAAAALK.K	sp|P19934|TOLA_E	1070.6447	9.7
0.9347	Q.KKAEAAAAALK.K	sp|P19934|TOLA_E	1070.6447	9.7
0.9899	K.VQLLGSGSILR.H	sp|P0AFG8|ODP1_	1141.6817	9.72
0.9752	R.VVGQLGQVLGPR.G	sp|P0A7L0|RL1_EC	1221.7197	9.72
0.9996	R.VVGQLGQVLGPR.G	sp|P0A7L0|RL1_EC	1221.7197	9.72
0.8168	R.RAVQLNSLSGF.C	sp|P0A7D4|PURA_	1190.6407	9.75
0.9684	K.ITVVPILR.A	sp|P0A8F0|UPP_EC	909.6007	9.75
0.687	Y.KPGNVVLTPTILR.D	sp|P0AB71|ALF_EC	1406.8607	11
0.7176	T.PVTRGVR.V	sp|P75779|YBIX_EC	783.4717	12