**Additional Information on “Quantitative proteome analysis of Merkel cell carcinoma cell lines using SILAC”**

**HPLC Separation**

The nano HPLC Separation was performed using a nanoRSLC UltiMate 3000 HPLC system by Thermo Fisher.

Mobile phases applied for sample loading, desalting, and separation were:

* 0.1% aqueous TFA solution cooled to 3°C and delivered to the trap column in the column oven at 30µl/min
* Mobile phases for peptide separation on the nano separation column were:

 A: 95% Acetonitrile, 5% Water, 0.1% Formic acid

 B: 50% Acetonitrile, 30% Methanol, 10% 2,2,2-Trifluoroethanol, 10% Water, 0.1% Formic acid

* Autosampler loading solvent was 0.1% aqueous TFA
* Injector and trap column wash were performed with 100% 2,2,2-Trifluoroethanol

A cleaning run was performed between each sample in order to prevent carryover. The Chromelon v. 6.8 parameters are shown below as a text file. It can be copied and pasted in any Chromeleon v. 6.8, or earlier. For newer Chromeleon versions or different LC control systems this has to be adapted accordingly.

|  |  |  |  |
| --- | --- | --- | --- |
| Nano Separation Gradient |  |  |  |
|  |  |  |  |
| **Retention time (min)** | **Flow (µl/min)** | **%A** | **%B** |
| 0 | 0.30 | 95.00 | 5.00 |
| 10 | 0.30 | 95.00 | 5.00 |
| 100 | 0.30 | 90.00 | 10.00 |
| 200 | 0.30 | 75.00 | 25.00 |
| 275 | 0.30 | 60.00 | 40.00 |
| 285 | 0.30 | 40.00 | 60.00 |
| 290 | 0.30 | 10.00 | 90.00 |
| 295 | 0.30 | 10.00 | 90.00 |
| 296 | 0.30 | 95.00 | 5.00 |
| 300 | 0.30 | 95.00 | 5.00 |
|  |  |  |  |
|  |  |  |  |
| Column Valve Switching Times |  |  |  |
|  |  |  |  |
| **Retention time (min)** | **Valve A** | **Valve B** |  |
| 0 | 1\_2 | 1\_2 |  |
| 10 | 1\_2 | 10\_1 |  |
| 286 | 1\_2 | 1\_2 |  |
|  |  |  |  |

Mascot Search parameters used in ProteinScape are shown below:



**HPLC Program**

Sampler.TempCtrl = On

 Sampler.Temperature.Nominal = 5.0 [°C]

 Sampler.Temperature.LowerLimit = 4.0 [°C]

 Sampler.Temperature.UpperLimit = 45.0 [°C]

 Sampler.ReadyTempDelta = None

 ColumnOven.TempCtrl = On

 ColumnOven.Temperature.Nominal = 60.0 [°C]

 ColumnOven.Temperature.LowerLimit = 20.0 [°C]

 ColumnOven.Temperature.UpperLimit = 75.0 [°C]

 EquilibrationTime = 0.5 [min]

 ColumnOven.ReadyTempDelta = 1.0 [°C]

 LoadingPump.Pressure.LowerLimit = 0 [bar]

 LoadingPump.Pressure.UpperLimit = 500 [bar]

 LoadingPump.MaximumFlowRampDown = 10 [µl/min²]

 LoadingPump.MaximumFlowRampUp = 10 [µl/min²]

 LoadingPump.%A.Equate = "%A"

 LoadingPump.%B.Equate = "%B"

 %C.Equate = "%C"

 NC\_Pump.Pressure.LowerLimit = 0 [bar]

 NC\_Pump.Pressure.UpperLimit = 800 [bar]

 NC\_Pump.MaximumFlowRampDown = 0.500 [µl/min²]

 NC\_Pump.MaximumFlowRampUp = 0.500 [µl/min²]

 NC\_Pump.%A.Equate = "%A"

 NC\_Pump.%B.Equate = "%B"

 DrawSpeed = 1000 [nl/s]

 DrawDelay = 5000 [ms]

 DispSpeed = 2000 [nl/s]

 DispenseDelay = 2000 [ms]

 WasteSpeed = 4000 [nl/s]

 WashSpeed = 4000 [nl/s]

 LoopWashFactor = 2.000

 SampleHeight = 3.000 [mm]

 PunctureDepth = 7.000 [mm]

 WashVolume = 100.000 [µl]

 RinseBetweenReinjections = No

 LowDispersionMode = Off

 InjectMode = UserProg

 LoadingPump\_Pressure.Step = 0.01 [s]

 LoadingPump\_Pressure.Average = On

 NC\_Pump\_Pressure.Step = 0.01 [s]

 NC\_Pump\_Pressure.Average = On

 NC\_Pump\_Press\_RightBlk.Step = 0.01 [s]

 NC\_Pump\_Press\_RightBlk.Average = On

 NC\_Pump\_Press\_LeftBlk.Step = 0.01 [s]

 NC\_Pump\_Press\_LeftBlk.Average = On

 ColumnOven\_Temp.Step = 0.01 [s]

 ColumnOven\_Temp.Average = Off

 UV.Data\_Collection\_Rate = 2.0 [Hz]

 TimeConstant = 1.20 [s]

 UV\_VIS\_1.Wavelength = 214 [nm]

 ValveRight = 1\_2

 ReagentAVial= R1

 ReagentBVial= R2

 Start\_MS.State Off

 ValveLeft = 1\_2

 PrepVial= B5

 UdpInjectValve Position=Inject

 UdpSyringeValve Position=Needle

 UdpDraw From=ReagentAVial, Volume=15.000, SyringeSpeed=1000, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpDraw From=ReagentAVial, Volume=0.000, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpInjectValve Position=Load

 UdpDraw From=SampleVial, Volume=Volume, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpDraw From=SampleVial, Volume=0.000, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpDraw From=ReagentAVial, Volume=15.000, SyringeSpeed=1000, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpDraw From=ReagentAVial, Volume=0.000, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpInjectValve Position=Inject

 UdpInjectMarker

 UdpMixNeedleWash Volume=100.000

 UdpMixWait Duration=800

 UdpDraw From=ReagentBVial, Volume=25.000, SyringeSpeed=4000, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpDispense To=Drain, Volume=10.000, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpInjectValve Position=Load

 UdpDraw From=ReagentBVial, Volume=25.000, SyringeSpeed=4000, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpDraw From=ReagentBVial, Volume=0.000, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpInjectValve Position=Inject

 UdpMixWait Duration=300

 UdpInjectValve Position=Load

 UdpDraw From=ReagentAVial, Volume=25.000, SyringeSpeed=4000, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpDraw From=ReagentAVial, Volume=0.000, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpInjectValve Position=Inject

 UdpMixNeedleWash Volume=300.000

 0.000 Autozero

 NC\_Pump.Flow = 0.300 [µl/min]

 NC\_Pump.%B = 5.0 [%]

 LoadingPump.Flow = 20.000 [µl/min]

 LoadingPump.%B = 0.0 [%]

 %C = 0.0 [%]

 Wait UV.Ready and LoadingPump.Ready and NC\_Pump.Ready and ColumnOven.Ready and Sampler.Ready and PumpModule.Ready

 Inject

 LoadingPump\_Pressure.AcqOn

 NC\_Pump\_Pressure.AcqOn

 NC\_Pump\_Press\_RightBlk.AcqOn

 NC\_Pump\_Press\_LeftBlk.AcqOn

 ColumnOven\_Temp.AcqOn

 UV\_VIS\_1.AcqOn

 NC\_Pump.Flow = 0.300 [µl/min]

 LoadingPump.Flow = 20.000 [µl/min]

 LoadingPump.%B = 0.0 [%]

 %C = 0.0 [%]

 NC\_Pump.%B = 5.0 [%]

10.000 NC\_Pump.%B = 5.0 [%]

 ValveLeft = 1\_2

 ValveRight = 10\_1

 NC\_Pump.Flow = 0.300 [µl/min]

15.000 Start\_MS.State On

15.100 Start\_MS.State Off

30.000 LoadingPump.Flow = 20.000 [µl/min]

 LoadingPump.%B = 0.0 [%]

 %C = 0.0 [%]

32.000 LoadingPump.Flow = 2.000 [µl/min]

 LoadingPump.%B = 0.0 [%]

 %C = 0.0 [%]

100.000 NC\_Pump.%B = 10.0 [%]

 NC\_Pump.Flow = 0.300 [µl/min]

200.000 NC\_Pump.%B = 25.0 [%]

 NC\_Pump.Flow = 0.300 [µl/min]

275.000 NC\_Pump.%B = 40.0 [%]

 NC\_Pump.Flow = 0.300 [µl/min]

280.000 LoadingPump.Flow = 2.000 [µl/min]

 LoadingPump.%B = 0.0 [%]

 %C = 0.0 [%]

285.000 NC\_Pump.%B = 60.0 [%]

 NC\_Pump.Flow = 0.300 [µl/min]

286.000 ValveLeft = 1\_2

 LoadingPump.Flow = 20.000 [µl/min]

 LoadingPump.%B = 0.0 [%]

 %C = 0.0 [%]

 ValveRight = 1\_2

290.000 NC\_Pump.%B = 90.0 [%]

 NC\_Pump.Flow = 0.300 [µl/min]

295.000 NC\_Pump.%B = 90.0 [%]

 NC\_Pump.Flow = 0.300 [µl/min]

296.000 NC\_Pump.%B = 5.0 [%]

 NC\_Pump.Flow = 0.300 [µl/min]

300.000 NC\_Pump.Flow = 0.300 [µl/min]

 NC\_Pump.%B = 5.0 [%]

 LoadingPump\_Pressure.AcqOff

 NC\_Pump\_Pressure.AcqOff

 NC\_Pump\_Press\_RightBlk.AcqOff

 NC\_Pump\_Press\_LeftBlk.AcqOff

 ColumnOven\_Temp.AcqOff

 UV\_VIS\_1.AcqOff

 End

**HPLC Program used for column cleaning**

Sampler.TempCtrl = On

 Sampler.Temperature.Nominal = 15.0 [°C]

 Sampler.Temperature.LowerLimit = 4.0 [°C]

 Sampler.Temperature.UpperLimit = 45.0 [°C]

 Sampler.ReadyTempDelta = None

 ColumnOven.TempCtrl = On

 ColumnOven.Temperature.Nominal = 60.0 [°C]

 ColumnOven.Temperature.LowerLimit = 20.0 [°C]

 ColumnOven.Temperature.UpperLimit = 75.0 [°C]

 EquilibrationTime = 0.5 [min]

 ColumnOven.ReadyTempDelta = 1.0 [°C]

 LoadingPump.Pressure.LowerLimit = 0 [bar]

 LoadingPump.Pressure.UpperLimit = 500 [bar]

 LoadingPump.MaximumFlowRampDown = 10 [µl/min²]

 LoadingPump.MaximumFlowRampUp = 10 [µl/min²]

 LoadingPump.%A.Equate = "%A"

 LoadingPump.%B.Equate = "%B"

 %C.Equate = "%C"

 NC\_Pump.Pressure.LowerLimit = 0 [bar]

 NC\_Pump.Pressure.UpperLimit = 800 [bar]

 NC\_Pump.MaximumFlowRampDown = 0.300 [µl/min²]

 NC\_Pump.MaximumFlowRampUp = 0.300 [µl/min²]

 NC\_Pump.%A.Equate = "%A"

 NC\_Pump.%B.Equate = "%B"

 DrawSpeed = 1000 [nl/s]

 DrawDelay = 5000 [ms]

 DispSpeed = 20000 [nl/s]

 DispenseDelay = 2000 [ms]

 WasteSpeed = 40000 [nl/s]

 WashSpeed = 40000 [nl/s]

 LoopWashFactor = 2.000

 SampleHeight = 3.000 [mm]

 PunctureDepth = 7.000 [mm]

 WashVolume = 300.000 [µl]

 RinseBetweenReinjections = No

 LowDispersionMode = Off

 InjectMode = UserProg

 LoadingPump\_Pressure.Step = 0.01 [s]

 LoadingPump\_Pressure.Average = On

 NC\_Pump\_Pressure.Step = 0.01 [s]

 NC\_Pump\_Pressure.Average = On

 NC\_Pump\_Press\_RightBlk.Step = 0.01 [s]

 NC\_Pump\_Press\_RightBlk.Average = On

 NC\_Pump\_Press\_LeftBlk.Step = 0.01 [s]

 NC\_Pump\_Press\_LeftBlk.Average = On

 ColumnOven\_Temp.Step = 0.01 [s]

 ColumnOven\_Temp.Average = Off

 UV.Data\_Collection\_Rate = 2.0 [Hz]

 TimeConstant = 1.20 [s]

 UV\_VIS\_1.Wavelength = 214 [nm]

 LoadingPump.Flow = 5.000 [µl/min]

 LoadingPump.%B = 0.0 [%]

 %C = 0.0 [%]

 LoadingPump.Curve = 5

 ReagentAVial= R1

 ReagentBVial= R2

 Start\_MS.State Off

 ValveLeft = 10\_1

 PrepVial= B5

 UdpInjectValve Position=Inject

 UdpSyringeValve Position=Needle

 UdpDraw From=ReagentBVial, Volume=16.000, SyringeSpeed=1000, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpDraw From=ReagentBVial, Volume=0.000, SyringeSpeed=GlobalSpeed, SampleHeight=4.000

 UdpInjectValve Position=Load

 UdpDraw From=ReagentBVial, Volume=20.000, SyringeSpeed=1000, SampleHeight=4.000

 UdpMixWait Duration=2

 UdpInjectValve Position=Inject

 UdpInjectMarker

 UdpMixNeedleWash Volume=300.000

 0.000 Autozero

 NC\_Pump.Flow = 0.300 [µl/min]

 NC\_Pump.%B = 99.0 [%]

 Wait UV.Ready and LoadingPump.Ready and NC\_Pump.Ready and ColumnOven.Ready and Sampler.Ready and PumpModule.Ready

 Inject

 LoadingPump\_Pressure.AcqOn

 NC\_Pump\_Pressure.AcqOn

 NC\_Pump\_Press\_RightBlk.AcqOn

 NC\_Pump\_Press\_LeftBlk.AcqOn

 ColumnOven\_Temp.AcqOn

 UV\_VIS\_1.AcqOn

 NC\_Pump.Flow = 0.300 [µl/min]

 NC\_Pump.%B = 99.0 [%]

 8.000 ValveLeft = 10\_1

13.000 Start\_MS.State On

13.100 Start\_MS.State Off

40.000 NC\_Pump.Flow = 0.300 [µl/min]

 NC\_Pump.%B = 99.0 [%]

41.000 NC\_Pump.Flow = 0.300 [µl/min]

 NC\_Pump.%B = 3.0 [%]

45.000 ValveLeft = 1\_2

60.000 NC\_Pump.Flow = 0.300 [µl/min]

 UV\_VIS\_1.AcqOff

 LoadingPump\_Pressure.AcqOff

 NC\_Pump\_Pressure.AcqOff

 NC\_Pump\_Press\_RightBlk.AcqOff

 NC\_Pump\_Press\_LeftBlk.AcqOff

 ColumnOven\_Temp.AcqOff

 NC\_Pump.%B = 3.0 [%]

 End