

# LOWESS normalization tutorial

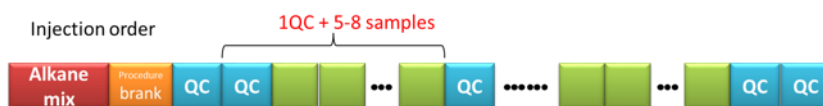
## Objective

The normalization part of MS-DIAL and MRMPROBS programs was separated. The powerful mathematics especially for cohort studies is essential to normalize the drifts of MS-signal intensities. The basic concept of this method is to use the intensities over an analytical run of 'quality control (QC)' sample which is a mixture of all biological samples.

Briefly, measurement data of QC samples are smoothed by the LOWESS of the single-degree least-squares. And then, the coefficient values between QC samples are interpolated by the cubic spline. Lastly, the entire datasets is aligned to the spline result.

## Experimental design

The QC samples should be periodically (5-8 samples each) analyzed throughout an analytical run as shown the below figure.



## How to use

1. Text (tab-delimited) data table should be prepared. The first, second, and third columns should contain the sample name, QC or Sample by TRUE or FALSE, and injection order, respectively. Then, from the fourth column, each cell should include the intensities of each metabolite.

Name	Type	Order	Acylcarnitini	Acylcarnitiri	Acylcarnititri	Acylcarnititri	Acylcarnititri	Acylcarnititri	Acylcarnititri	Acylcarnititri	Acylcarnititri	Cholic acid	Cholic acid
01_Serum mix	TRUE	1	3399	1835	5111	14119	4948	9822	2974	25013	2206	2840	924
02_Serum Human_01	FALSE	2	6924	4141	1099	9243	3191	6109	3719	37371	293	0	1466
02_Serum Human_02	FALSE	3	10025	3140	1014	8529	2746	6707	3348	39360	497	841	669
02_Serum Human_03	FALSE	4	8469	2073	1048	9474	2599	6301	4543	45926	337	481	944
02_Serum Human_04	FALSE	5	9104	3053	1064	10496	3129	6766	4290	62995	400	533	1110
02_Serum Human_05	FALSE	6	6159	3037	1240	9317	3684	7065	3226	72066	386	824	650
03_Serum mix	TRUE	7	2984	1688	2452	16144	6100	10429	2568	34260	1533	2941	1733
04_Serum Human_06	FALSE	8	7054	3165	1610	9015	3761	8201	5835	106827	604	237	1133
04_Serum Human_07	FALSE	9	8695	2938	1161	9154	2647	5159	3957	125700	204	585	1102
04_Serum Human_08	FALSE	10	5931	2431	1261	9026	2501	5467	3945	107253	281	395	1245
04_Serum Human_09	FALSE	11	7370	3418	1493	7932	3692	7565	4329	147894	408	503	1634
04_Serum Human_10	FALSE	12	6829	2984	1054	9224	4125	7690	4973	170592	57	889	1182
05_Serum mix	TRUE	13	3215	2601	3164	17444	3554	8384	3539	47212	2062	3430	1262
06_Serum Mouse_01	FALSE	14	408	853	4033	12900	3498	9891	1099	63676	2747	1232	1328
06_Serum Mouse_02	FALSE	15	0	2053	3593	14436	3640	8828	640	61961	1351	704	1484
06_Serum Mouse_03	FALSE	16	293	761	4302	16044	3457	8953	2600	62195	1973	1326	1248
06_Serum Mouse_04	FALSE	17	512	866	3289	15655	3492	8572	1129	90589	1631	705	1164
06_Serum Mouse_05	FALSE	18	293	928	3751	15052	3116	6807	1708	64360	1475	773	564
07_Serum mix	TRUE	19	2640	2760	3755	19587	4693	8339	2872	53826	800	1791	1386
08_Serum Mouse_06	FALSE	20	306	1104	5248	14045	2920	10341	2001	65475	1404	966	1475
08_Serum Mouse_07	FALSE	21	204	967	5128	16622	4064	9791	801	70818	1773	1373	1093
08_Serum Mouse_08	FALSE	22	102	2069	4680	14335	4332	9953	2162	78095	2355	768	1142
08_Serum Mouse_09	FALSE	23	136	791	4421	15125	4066	8166	1442	84259	1890	748	511
08_Serum Mouse_10	FALSE	24	0	906	4270	14311	4770	10126	1474	79941	1840	640	737
09_Serum mix	TRUE	25	2820	1439	4564	16352	5008	10196	2624	81079	2012	1712	1906
10_Serum Rat_01	FALSE	26	751	1744	5469	17243	5752	10312	1916	21803	3556	4518	1186
10_Serum Rat_02	FALSE	27	1120	1845	4892	21842	8119	9168	3959	23142	6139	7121	1705
10_Serum Rat_03	FALSE	28	502	1800	5502	22845	6966	9012	3225	23417	3369	6169	1000
10_Serum Rat_04	FALSE	29	508	2231	5598	22329	6009	9694	3194	29070	5908	4368	1182
10_Serum Rat_05	FALSE	30	685	3223	4888	20873	7927	7356	2140	26974	3735	5000	2062
11_Serum mix	TRUE	31	2499	2582	4668	14925	5855	8243	2807	82011	1951	2278	844

2. Open 'LowessNormalizationSample.exe'
3. Click 'Browse' and select your data matrix.
4. Click 'Load'. You will find your QC number and the minimum span number for LOWESS smoothing if you can correctly load the data matrix.
5. If you click 'Span opt.', you can see the optimal 'Span' value which is coming from 'seven-fold cross validation' in the QC samples.
6. Click 'Export', then you will find the normalized result in the same directly of your data.

