Main guidelines to successfully apply the R based algorithm for pattern reconstruction procedure of chlorinated paraffins.

Background: In majority of cases there are 2 types of reference standards available for quantification of chlorinated paraffins. Namely, single-chain standards (e.g. Chloroparaffin C11 65.25% Cl) or standards which contain either C10-C13 (SCCPs), C14-C17 (MCCPs) or C18-C21 (LCCPs) altogether in one mix (e.g. Chloroparaffin C10-C13 55,5% Cl). This algorithm can be used for both types. However, different calibration sheets must be used. In case of single-chain standards please use sheets, which end with "Single chains". In case of group standards, please use sheets, which end with “Multiple chains”.

This quantification approach can be applied for both – GC-MS and LC-MS analysis to process results for SCCPs, MCCPs or LCCPs. It is also possible to substitute all area values with ratios (Target/ISTD) in case internal standard approach has been used. General workflow for pattern reconstruction and quantification of CPs is as follows:

1.) Measurement of calibration solutions.
2.) Fill acquired calibration data in corresponding Excel sheets.
3.) Sample measurement.
4.) Fill acquired sample data in corresponding Excel sheet.
5.) Pattern reconstruction and quantification in R.

Contact ingus.perkons[at]bior.lv if you experience any serious problems with the R. code or provided .xlsx documents. This is a part of supplementry material for article (link). Please refer to the article for further information.

Note: Authors do not take any responsibility for the accuracy of the quantification/pattern reconstruction data obtained with this approach. Additionally, this algorithm should be used for research/educational purposes only.

If using multiple chain standards – measure a calibration curve for each standard mixture. In order to accurately enter calibration results – please use Excel document - **"CP calibration - multiple chains.xlsx".**

If using single chain standards – I suggest to create several specific mixtures of standard solutions, where one measurement covers four different $C_{n\text{-}n+3}$ standards. Although, single-chain CP standards can be measured separately, there is no need to analyze them one by one, as standards with different carbon chain lengths do not overlap, thus 4 different single-chain standards can be measured in one single run. Nevertheless, it possible to measure mixtures which contain e.g. only two chain length standards. In such case – leave the unnecessary homologue rows blank. In order to accurately enter calibration results – please use Excel document - **"CP calibration - single chains.xlsx".**

Each Excel document which is used to store calibration data, contains 3 sheets - "SCCPs","MCCPs", and "LCCPs". Unfortunately, this R algorithm can only simultaneously process data for either SCCPs, MCCPs or LCCPs, but not all three (or two) groups together.

Each sheet contains several important columns:

Column A - "Standard mixture" – Contains arbitrary name for each calibration mixture. Must be different for each $C_{n\text{-}n+3}$ CP group. I personally prefer to name them “Type-A/B/C/D/E” (e.g. SCCP-A, MCCP-C, etc.).

Column B - "Standard used" – Contains name of reference standard used for the particular standard mixture in calibration curve.

Column C - "Chain length" – Chain length of each CP homologue

Column D - "Type" – Chlorinated paraffin type: SCCP, MCCP or LCCP

Column E - "Homologue" – Molecular formula of CP homologues.

Columns F,G,H,I,J,K,L,M - "Calibration levels" – Calibration levels used to construct calibration curve. By default, there are a maximum of 8 calibration levels, however, you don’t need to fill all of them. Concentration levels (conc. values) can be changed as you want and number of levels is up to you. However, it’s important that the last calibration level is in column “M”. You can delete unnecessary/unused calibration levels starting from column “F”.

Columns N,O,P,Q,R,S,T,U - "Areas" – From column “N” to “U” you can store acquired peak areas (or ratios in case of internal standard applications) for each homologue. If no homologue is detected, just leave those cells blank (it will be greyed out).

Columns V and W - contains R^2 (square of the Pearson product moment correlation coefficient) and response factor (RF) for each homologue (slope of the linear regression line through data points, b = 0). Both columns are calculated automatically from the given calibration data.

Columns Z,AA,AB,AC,AD,AE - contain all the necessary data, which must be copied (using Paste->Values) to “input_ref.xls” document, which will be later used for pattern reconstruction procedure in R.

Running ".R" scripts

To run the code you'll need to install R-Stats from one of the CRAN mirrors (https://cran.r-project.org/mirrors.html) and, preferably, RStudio - an integrated development environment (IDE), which can be downloaded from https://www.rstudio.com/products/rstudio/#Desktop. For this code three R packages are necessary ("readxl", "ggplot2", and "dplyr"). When running the code for the first time they will be installed automatically. An in-depth guide for running the algorithm is provided below. Additional information regarding R and RStudio can be found online in developer homepages - https://cran.r-project.org and https://www.rstudio.com, respectively.

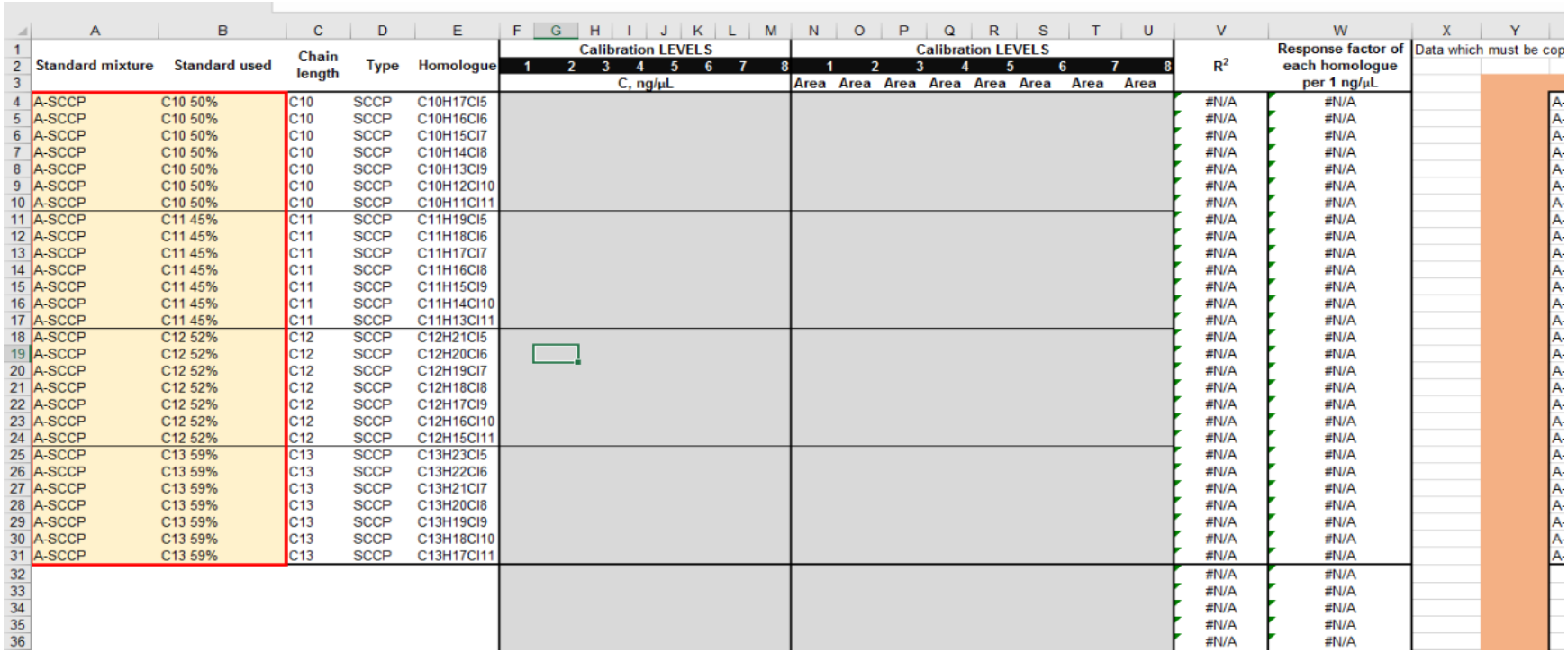
Note: There are two ".R" files uploaded in repository. In case of single chain CP standards use "ParaffinCrawler-Chain.R", while for multiple chain standards use "ParaffinCrawler-Group.R"

A guide on how to perform the procedure is illustrated below (example of single chain SCCP standards)

1.) Measure reference mixtures at different calibration levels
An example composition

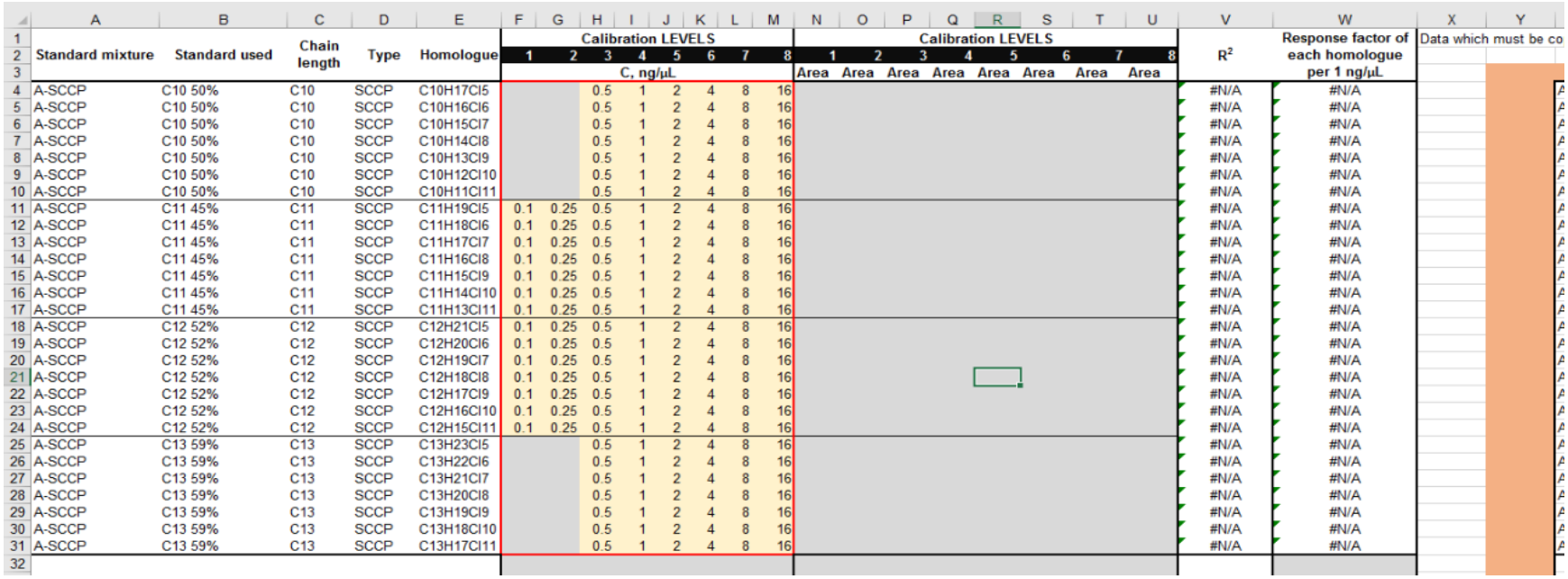
Standard	SCCP-A	SCCP-B	SCCP-C	SCCP-D	SCCP-E
C10 50%	x				
C11 45%	x				
C12 52%	x				
C13 59%	x				
C10 55%		x			
C11 50%		x			
C12 55%		x			
C13 65%		x			
C10 60%			x		
C11 55%			x		
C12 60%			x		
C13 69%			x		
C10 65%				x	
C11 60%				x	
C12 65%				x	
C13 75%				x	
C11 69%					x
C12 69%					x

2.) Fill standard mixture "SCCP-A" information data in "CP calibration - single chains.xlsx" document columns A-B.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1						Calibration LEVELS								Calibration LEVELS											
2	Standard mixture	Standard used	Chain length	Type	Homologue	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	R^2	Response factor of each homologue per 1 ng/µL	Data which must be cop	
3						C, ng/µL								Area	Area	Area	Area	Area	Area	Area	Area				
4	A-SCCP	C10 50%	C10	SCCP	C10H17Cl5																	#N/A	#N/A		
5	A-SCCP	C10 50%	C10	SCCP	C10H16Cl6																	#N/A	#N/A		
6	A-SCCP	C10 50%	C10	SCCP	C10H15Cl7																	#N/A	#N/A		
7	A-SCCP	C10 50%	C10	SCCP	C10H14Cl8																	#N/A	#N/A		
8	A-SCCP	C10 50%	C10	SCCP	C10H13Cl9																	#N/A	#N/A		
9	A-SCCP	C10 50%	C10	SCCP	C10H12Cl10																	#N/A	#N/A		
10	A-SCCP	C10 50%	C10	SCCP	C10H11Cl11																	#N/A	#N/A		
11	A-SCCP	C11 45%	C11	SCCP	C11H19Cl5																	#N/A	#N/A		
12	A-SCCP	C11 45%	C11	SCCP	C11H18Cl6																	#N/A	#N/A		
13	A-SCCP	C11 45%	C11	SCCP	C11H17Cl7																	#N/A	#N/A		
14	A-SCCP	C11 45%	C11	SCCP	C11H16Cl8																	#N/A	#N/A		
15	A-SCCP	C11 45%	C11	SCCP	C11H15Cl9																	#N/A	#N/A		
16	A-SCCP	C11 45%	C11	SCCP	C11H14Cl10																	#N/A	#N/A		
17	A-SCCP	C11 45%	C11	SCCP	C11H13Cl11																	#N/A	#N/A		
18	A-SCCP	C12 52%	C12	SCCP	C12H21Cl5																	#N/A	#N/A		
19	A-SCCP	C12 52%	C12	SCCP	C12H20Cl6																	#N/A	#N/A		
20	A-SCCP	C12 52%	C12	SCCP	C12H19Cl7																	#N/A	#N/A		
21	A-SCCP	C12 52%	C12	SCCP	C12H18Cl8																	#N/A	#N/A		
22	A-SCCP	C12 52%	C12	SCCP	C12H17Cl9																	#N/A	#N/A		
23	A-SCCP	C12 52%	C12	SCCP	C12H16Cl10																	#N/A	#N/A		
24	A-SCCP	C12 52%	C12	SCCP	C12H15Cl11																	#N/A	#N/A		
25	A-SCCP	C13 59%	C13	SCCP	C13H23Cl5																	#N/A	#N/A		
26	A-SCCP	C13 59%	C13	SCCP	C13H22Cl6																	#N/A	#N/A		
27	A-SCCP	C13 59%	C13	SCCP	C13H21Cl7																	#N/A	#N/A		
28	A-SCCP	C13 59%	C13	SCCP	C13H20Cl8																	#N/A	#N/A		
29	A-SCCP	C13 59%	C13	SCCP	C13H19Cl9																	#N/A	#N/A		
30	A-SCCP	C13 59%	C13	SCCP	C13H18Cl10																	#N/A	#N/A		
31	A-SCCP	C13 59%	C13	SCCP	C13H17Cl11																	#N/A	#N/A		
32																						#N/A	#N/A		
33																						#N/A	#N/A		
34																						#N/A	#N/A		
35																						#N/A	#N/A		
36																						#N/A	#N/A		

3.) Fill standard mixture "SCCP-A" calibration levels in "CP calibration - single chains.xlsx" document columns F-M.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1						Calibration LEVELS								Calibration LEVELS											
2	Standard mixture	Standard used	Chain length	Type	Homologue	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	R^2	Response factor of each homologue per 1 ng/µL	Data which must be co	
3						C, ng/µL								Area	Area	Area	Area	Area	Area	Area	Area				
4	A-SCCP	C10 50%	C10	SCCP	C10H17Cl5			0.5	1	2	4	8	16									#N/A	#N/A		
5	A-SCCP	C10 50%	C10	SCCP	C10H16Cl6			0.5	1	2	4	8	16									#N/A	#N/A		
6	A-SCCP	C10 50%	C10	SCCP	C10H15Cl7			0.5	1	2	4	8	16									#N/A	#N/A		
7	A-SCCP	C10 50%	C10	SCCP	C10H14Cl8			0.5	1	2	4	8	16									#N/A	#N/A		
8	A-SCCP	C10 50%	C10	SCCP	C10H13Cl9			0.5	1	2	4	8	16									#N/A	#N/A		
9	A-SCCP	C10 50%	C10	SCCP	C10H12Cl10			0.5	1	2	4	8	16									#N/A	#N/A		
10	A-SCCP	C10 50%	C10	SCCP	C10H11Cl11			0.5	1	2	4	8	16									#N/A	#N/A		
11	A-SCCP	C11 45%	C11	SCCP	C11H19Cl5	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
12	A-SCCP	C11 45%	C11	SCCP	C11H18Cl6	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
13	A-SCCP	C11 45%	C11	SCCP	C11H17Cl7	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
14	A-SCCP	C11 45%	C11	SCCP	C11H16Cl8	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
15	A-SCCP	C11 45%	C11	SCCP	C11H15Cl9	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
16	A-SCCP	C11 45%	C11	SCCP	C11H14Cl10	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
17	A-SCCP	C11 45%	C11	SCCP	C11H13Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
18	A-SCCP	C12 52%	C12	SCCP	C12H21Cl5	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
19	A-SCCP	C12 52%	C12	SCCP	C12H20Cl6	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
20	A-SCCP	C12 52%	C12	SCCP	C12H19Cl7	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
21	A-SCCP	C12 52%	C12	SCCP	C12H18Cl8	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
22	A-SCCP	C12 52%	C12	SCCP	C12H17Cl9	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
23	A-SCCP	C12 52%	C12	SCCP	C12H16Cl10	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
24	A-SCCP	C12 52%	C12	SCCP	C12H15Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
25	A-SCCP	C13 59%	C13	SCCP	C13H23Cl5			0.5	1	2	4	8	16									#N/A	#N/A		
26	A-SCCP	C13 59%	C13	SCCP	C13H22Cl6			0.5	1	2	4	8	16									#N/A	#N/A		
27	A-SCCP	C13 59%	C13	SCCP	C13H21Cl7			0.5	1	2	4	8	16									#N/A	#N/A		
28	A-SCCP	C13 59%	C13	SCCP	C13H20Cl8			0.5	1	2	4	8	16									#N/A	#N/A		
29	A-SCCP	C13 59%	C13	SCCP	C13H19Cl9			0.5	1	2	4	8	16									#N/A	#N/A		
30	A-SCCP	C13 59%	C13	SCCP	C13H18Cl10			0.5	1	2	4	8	16									#N/A	#N/A		
31	A-SCCP	C13 59%	C13	SCCP	C13H17Cl11			0.5	1	2	4	8	16									#N/A	#N/A		
32																									

4.) Fill acquired areas for standard mixture "SCCP-A" calibration levels in "CP calibration - single chains.xlsx" document columns N-U.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1						Calibration LEVELS								Calibration LEVELS											
2	Standard mixture	Standard used	Chain length	Type	Homologue	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	R^2	Response factor of each homologue per 1 ng/µL	Data which must be c	
3						C, ng/µL								Area	Area	Area	Area	Area	Area	Area	Area				
4	A-SCCP	C10 50%	C10	SCCP	C10H17Cl5	0.13	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
5	A-SCCP	C10 50%	C10	SCCP	C10H16Cl6	0.1	0.25	0.5	1	2	4	8	16				25000	49000	94000	201000		0.998447235	25157		
6	A-SCCP	C10 50%	C10	SCCP	C10H15Cl7	0.1	0.25	0.5	1	2	4	8	16			12500	24000	50000	110000	200000	400000	0.999205151	24953		
7	A-SCCP	C10 50%	C10	SCCP	C10H14Cl8	0.1	0.25	0.5	1	2	4	8	16	1000	2500	5000	10000	20000	40000	80000	160000	0.999997653	10005		
8	A-SCCP	C10 50%	C10	SCCP	C10H13Cl9	0.1	0.25	0.5	1	2	4	8	16	500	1250	2500	5000	10000	20000	40000	80000	0.999997653	5002		
9	A-SCCP	C10 50%	C10	SCCP	C10H12Cl10	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
10	A-SCCP	C10 50%	C10	SCCP	C10H11Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
11	A-SCCP	C11 45%	C11	SCCP	C11H19Cl5	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
12	A-SCCP	C11 45%	C11	SCCP	C11H18Cl6	0.1	0.25	0.5	1	2	4	8	16	3000	7500	15000	30000	60000	120000	240000	480000	0.999997653	30014		
13	A-SCCP	C11 45%	C11	SCCP	C11H17Cl7	0.1	0.25	0.5	1	2	4	8	16	4000	10000	20000	40000	80000	160000	320000	640000	0.999997653	40018		
14	A-SCCP	C11 45%	C11	SCCP	C11H16Cl8	0.1	0.25	0.5	1	2	4	8	16	2100	5250	10500	21000	42000	84000	148000	336000	0.996516383	20635		
15	A-SCCP	C11 45%	C11	SCCP	C11H15Cl9	0.1	0.25	0.5	1	2	4	8	16	700	1750	3500	7000	14000	28000	56000	112000	0.999997653	7003		
16	A-SCCP	C11 45%	C11	SCCP	C11H14Cl10	0.1	0.25	0.5	1	2	4	8	16	300	750	1500	3000	6000	12000	24000	48000	0.999997653	3001		
17	A-SCCP	C11 45%	C11	SCCP	C11H13Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
18	A-SCCP	C12 52%	C12	SCCP	C12H21Cl5	0.1	0.25	0.5	1	2	4	8	16	500	1250	2500	5000	10000	20000	40000	80000	0.999997653	5002		
19	A-SCCP	C12 52%	C12	SCCP	C12H20Cl6	0.1	0.25	0.5	1	2	4	8	16	4500	11250	22500	45000	90000	180000	360000	720000	0.999997653	45020		
20	A-SCCP	C12 52%	C12	SCCP	C12H19Cl7	0.1	0.25	0.5	1	2	4	8	16	3700	9250	18500	37000	74000	148000	296000	592000	0.999997653	37017		
21	A-SCCP	C12 52%	C12	SCCP	C12H18Cl8	0.1	0.25	0.5	1	2	4	8	16	2000	5000	10000	20000	40000	80000	160000	320000	0.999997653	20009		
22	A-SCCP	C12 52%	C12	SCCP	C12H17Cl9	0.1	0.25	0.5	1	2	4	8	16			2500	5000	10000	20000	40000	80000	1	5000		
23	A-SCCP	C12 52%	C12	SCCP	C12H16Cl10	0.1	0.25	0.5	1	2	4	8	16				5000	10000	20000	40000	80000	1	5000		
24	A-SCCP	C12 52%	C12	SCCP	C12H15Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
25	A-SCCP	C13 59%	C13	SCCP	C13H23Cl5			0.5	1	2	4	8	16				7000	14000	28000	56000	112000	1	7000		
26	A-SCCP	C13 59%	C13	SCCP	C13H22Cl6			0.5	1	2	4	8	16			15000	30000	60000	120000	240000	480000	1	30000		
27	A-SCCP	C13 59%	C13	SCCP	C13H21Cl7			0.5	1	2	4	8	16	4500	11250	22500	45000	90000	180000	360000	720000	1	45000		
28	A-SCCP	C13 59%	C13	SCCP	C13H20Cl8			0.5	1	2	4	8	16	2700	6750	13500	27000	54000	108000	216000	432000	1	27000		
29	A-SCCP	C13 59%	C13	SCCP	C13H19Cl9			0.5	1	2	4	8	16	1200	3000	6000	12000	24000	48000	96000	192000	1	12000		
30	A-SCCP	C13 59%	C13	SCCP	C13H18Cl10			0.5	1	2	4	8	16	200	500	1000	2000	4000	8000	16000	32000	1	2000		
31	A-SCCP	C13 59%	C13	SCCP	C13H17Cl11			0.5	1	2	4	8	16									#N/A	#N/A		
32																						#N/A	#N/A		
33																						#N/A	#N/A		

5.) Repeat steps 2.-4. for all remaining standard mixtures.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
22	A-SCCP	C12 52%	C12	SCCP	C12H17Cl9	0.1	0.25	0.5	1	2	4	8	16			2500	5000	10000	20000	40000	80000	1	5000			A-SCCP	C12 52%	C12	SCCP
23	A-SCCP	C12 52%	C12	SCCP	C12H16Cl10	0.1	0.25	0.5	1	2	4	8	16				5000	10000	20000	40000	80000	1	5000			A-SCCP	C12 52%	C12	SCCP
24	A-SCCP	C12 52%	C12	SCCP	C12H15Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A			A-SCCP	C12 52%	C12	SCCP
25	A-SCCP	C13 59%	C13	SCCP	C13H23Cl5			0.5	1	2	4	8	16				7000	14000	28000	56000	112000	1	7000			A-SCCP	C13 59%	C13	SCCP
26	A-SCCP	C13 59%	C13	SCCP	C13H22Cl6			0.5	1	2	4	8	16			15000	30000	60000	120000	240000	480000	1	30000			A-SCCP	C13 59%	C13	SCCP
27	A-SCCP	C13 59%	C13	SCCP	C13H21Cl7			0.5	1	2	4	8	16	4500	11250	22500	45000	90000	180000	360000	720000	1	45000			A-SCCP	C13 59%	C13	SCCP
28	A-SCCP	C13 59%	C13	SCCP	C13H20Cl8			0.5	1	2	4	8	16	2700	6750	13500	27000	54000	108000	216000	432000	1	27000			A-SCCP	C13 59%	C13	SCCP
29	A-SCCP	C13 59%	C13	SCCP	C13H19Cl9			0.5	1	2	4	8	16	1200	3000	6000	12000	24000	48000	96000	192000	1	12000			A-SCCP	C13 59%	C13	SCCP
30	A-SCCP	C13 59%	C13	SCCP	C13H18Cl10			0.5	1	2	4	8	16	200	500	1000	2000	4000	8000	16000	32000	1	2000			A-SCCP	C13 59%	C13	SCCP
31	A-SCCP	C13 59%	C13	SCCP	C13H17Cl11			0.5	1	2	4	8	16									#N/A	#N/A			A-SCCP	C13 59%	C13	SCCP
32	B-SCCP	C10 55%	C10	SCCP	C10H17Cl5		0.25	0.5	1	2	4	8	16									#N/A	#N/A			B-SCCP	C10 55%	C10	SCCP
33	B-SCCP	C10 55%	C10	SCCP	C10H16Cl6		0.25	0.5	1	2	4	8	16				4000	8000	16000	32000		1	4000			B-SCCP	C10 55%	C10	SCCP
34	B-SCCP	C10 55%	C10	SCCP	C10H15Cl7	0.1	0.25	0.5	1	2	4	8	16	2000	4000	8000	16000	32000	64000	128000	256000	1	16000			B-SCCP	C10 55%	C10	SCCP
35	B-SCCP	C10 55%	C10	SCCP	C10H14Cl8	0.1	0.25	0.5	1	2	4	8	16	4000	8000	16000	32000	64000	128000	256000	512000	1	32000			B-SCCP	C10 55%	C10	SCCP
36	B-SCCP	C10 55%	C10	SCCP	C10H13Cl9	0.1	0.25	0.5	1	2	4	8	16		1000	2000	4000	8000	16000	32000	64000	1	4000			B-SCCP	C10 55%	C10	SCCP
37	B-SCCP	C10 55%	C10	SCCP	C10H12Cl10	0.1	0.25	0.5	1	2	4	8	16				1600	3200	6400	12800	25600	1	1600			B-SCCP	C10 55%	C10	SCCP
38	B-SCCP	C10 55%	C10	SCCP	C10H11Cl11		0.25	0.5	1	2	4	8	16									#N/A	#N/A			B-SCCP	C10 55%	C10	SCCP
39	B-SCCP	C11 50%	C11	SCCP	C11H19Cl5	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A			B-SCCP	C11 50%	C11	SCCP
40	B-SCCP	C11 50%	C11	SCCP	C11H18Cl6	0.1	0.25	0.5	1	2	4	8	16				8000	16000	32000	64000	128000	1	8000			B-SCCP	C11 50%	C11	SCCP
41	B-SCCP	C11 50%	C11	SCCP	C11H17Cl7	0.1	0.25	0.5	1	2	4	8	16			18000	36000	72000	144000	288000	576000	1	36000			B-SCCP	C11 50%	C11	SCCP
42	B-SCCP	C11 50%	C11	SCCP	C11H16Cl8	0.1	0.25	0.5	1	2	4	8	16		7000	14000	28000	56000	112000	224000	448000	1	28000			B-SCCP	C11 50%	C11	SCCP
43	B-SCCP	C11 50%	C11	SCCP	C11H15Cl9	0.1	0.25	0.5	1	2	4	8	16	2100	4200	8400	16800	33600	67200	134400	268800	1	16800			B-SCCP	C11 50%	C11	SCCP
44	B-SCCP	C11 50%	C11	SCCP	C11H14Cl10	0.1	0.25	0.5	1	2	4	8	16		960	1920	3840	7680	15360	30720	61440	1	3840			B-SCCP	C11 50%	C11	SCCP
45	B-SCCP	C11 50%	C11	SCCP	C11H13Cl11				1	2	4	8	16									#N/A	#N/A			B-SCCP	C11 50%	C11	SCCP
46	B-SCCP	C12 55%	C12	SCCP	C12H21Cl5	0.1	0.25	0.5	1	2	4	8	16				4000	8000	16000	32000	64000	1	4000			B-SCCP	C12 55%	C12	SCCP
47	B-SCCP	C12 55%	C12	SCCP	C12H20Cl6	0.1	0.25	0.5	1	2	4	8	16		9000	18000	36000	72000	144000	288000	576000	1	36000			B-SCCP	C12 55%	C12	SCCP
48	B-SCCP	C12 55%	C12	SCCP	C12H19Cl7	0.1	0.25	0.5	1	2	4	8	16	3700	7400	14800	29600	59200	118400	236800	473600	1	29600			B-SCCP	C12 55%	C12	SCCP
49	B-SCCP	C12 55%	C12	SCCP	C12H18Cl8	0.1	0.25	0.5	1	2	4	8	16	2000	4000	8000	16000	32000	64000	128000	256000	1	16000			B-SCCP	C12 55%	C12	SCCP
50	B-SCCP	C12 55%	C12	SCCP	C12H17Cl9	0.1	0.25	0.5	1	2	4	8	16	1500	3000	6000	12000	24000	48000	96000	192000	1	12000			B-SCCP	C12 55%	C12	SCCP
51	B-SCCP	C12 55%	C12	SCCP	C12H16Cl10	0.1	0.25	0.5	1	2	4	8	16				4000	8000	16000	32000	64000	1	4000			B-SCCP	C12 55%	C12	SCCP
52	B-SCCP	C12 55%	C12	SCCP	C12H15Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A			B-SCCP	C12 55%	C12	SCCP
53	B-SCCP	C13 65%	C13	SCCP	C13H23Cl5		0.25	0.5	1	2	4	8	16									#N/A	#N/A			B-SCCP	C13 65%	C13	SCCP
54	B-SCCP	C13 65%	C13	SCCP	C13H22Cl6	0.1	0.25	0.5	1	2	4	8	16			4800	9600	19200	38400	76800	153600	1	9600			B-SCCP	C13 65%	C13	SCCP
55	B-SCCP	C13 65%	C13	SCCP	C13H21Cl7	0.1	0.25	0.5	1	2	4	8	16	6000	12000	24000	48000	96000	192000	384000	768000	1	48000			B-SCCP	C13 65%	C13	SCCP
56	B-SCCP	C13 65%	C13	SCCP	C13H20Cl8	0.1	0.25	0.5	1	2	4	8	16	3500	7000	14000	28000	56000	112000	224000	448000	1	28000			B-SCCP	C13 65%	C13	SCCP
57	B-SCCP	C13 65%	C13	SCCP	C13H19Cl9	0.1	0.25	0.5	1	2	4	8	16	2000	4000	8000	16000	32000	64000	128000	256000	1	16000			B-SCCP	C13 65%	C13	SCCP
58	B-SCCP	C13 65%	C13	SCCP	C13H18Cl10	0.1	0.25	0.5	1	2	4	8	16			2800	5600	11200	22400	44800	89600	1	5600			B-SCCP	C13 65%	C13	SCCP
59	B-SCCP	C13 65%	C13	SCCP	C13H17Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A			B-SCCP	C13 65%	C13	SCCP
60	C-SCCP	C10 60%	C10	SCCP	C10H17Cl5	0.13	0.25	0.5	1	2	4	8	16									#N/A	#N/A			C-SCCP	C10 60%	C10	SCCP
61	C-SCCP	C10 60%	C10	SCCP	C10H16Cl6	0.1	0.25	0.5	1	2	4	8	16				1600	3200	6400	12800	25600	1	1600			C-SCCP	C10 60%	C10	SCCP
62	C-SCCP	C10 60%	C10	SCCP	C10H15Cl7	0.1	0.25	0.5	1	2	4	8	16	4000	8000	16000	32000	64000	128000	256000	512000	1	32000			C-SCCP	C10 60%	C10	SCCP
63	C-SCCP	C10 60%	C10	SCCP	C10H14Cl8	0.1	0.25	0.5	1	2	4	8	16	7000	14000	28000	56000	1E+05	224000	448000	896000	1	56000			C-SCCP	C10 60%	C10	SCCP
64	C-SCCP	C10 60%	C10	SCCP	C10H13Cl9	0.1	0.25	0.5	1	2	4	8	16	3500	7000	14000	28000	56000	112000	224000	448000	1	28000			C-SCCP	C10 60%	C10	SCCP
65	C-SCCP	C10 60%	C10	SCCP	C10H12Cl10	0.1	0.25	0.5	1	2	4	8	16		2000	4000	8000	16000	32000	64000	128000	1	8000			C-SCCP	C10 60%	C10	SCCP
66	C-SCCP	C10 60%	C10	SCCP	C10H11Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A			C-SCCP	C10 60%	C10	SCCP
67	C-SCCP	C11 55%	C11	SCCP	C11H19Cl5	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A			C-SCCP	C11 55%	C11	SCCP
68	C-SCCP	C11 55%	C11	SCCP	C11H18Cl6	0.1	0.25	0.5	1	2	4	8	16				1600	3200	6400	12800	25600	1	1600			C-SCCP	C11 55%	C11	SCCP

6.) Check if there are no unexpected errors in your sheet and copy the calculated information from column Z-AE to an Excel document "input_ref.xlsx" starting from 2nd row (use Paste->Paste special->Values). Do not clear the first row of "input_ref.xlsx" sheet!

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1						Calibration LEVELS								Calibration LEVELS											
2	Standard mixture	Standard used	Chain length	Type	Homologue	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	R^2	Response factor of each homologue per 1 ng/µL		Data which must be
3						C, ng/µL								Area	Area	Area	Area	Area	Area	Area	Area				
4	A-SCCP	C10 50%	C10	SCCP	C10H17Cl5	0.13	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
5	A-SCCP	C10 50%	C10	SCCP	C10H16Cl6	0.1	0.25	0.5	1	2	4	8	16				25000	49000	94000	201000		0.998447235	25157		
6	A-SCCP	C10 50%	C10	SCCP	C10H15Cl7	0.1	0.25	0.5	1	2	4	8	16			12500	24000	50000	110000	200000	400000	0.999205151	24953		
7	A-SCCP	C10 50%	C10	SCCP	C10H14Cl8	0.1	0.25	0.5	1	2	4	8	16	1000	2500	5000	10000	20000	40000	80000	160000	0.999997653	10005		
8	A-SCCP	C10 50%	C10	SCCP	C10H13Cl9	0.1	0.25	0.5	1	2	4	8	16	500	1250	2500	5000	10000	20000	40000	80000	0.999997653	5002		
9	A-SCCP	C10 50%	C10	SCCP	C10H12Cl10	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
10	A-SCCP	C10 50%	C10	SCCP	C10H11Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
11	A-SCCP	C11 45%	C11	SCCP	C11H19Cl5	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
12	A-SCCP	C11 45%	C11	SCCP	C11H18Cl6	0.1	0.25	0.5	1	2	4	8	16	3000	7500	15000	30000	60000	120000	240000	480000	0.999997653	30014		
13	A-SCCP	C11 45%	C11	SCCP	C11H17Cl7	0.1	0.25	0.5	1	2	4	8	16	4000	10000	20000	40000	80000	160000	320000	640000	0.999997653	40018		
14	A-SCCP	C11 45%	C11	SCCP	C11H16Cl8	0.1	0.25	0.5	1	2	4	8	16	2100	5250	10500	21000	42000	84000	148000	336000	0.996516383	20635		
15	A-SCCP	C11 45%	C11	SCCP	C11H15Cl9	0.1	0.25	0.5	1	2	4	8	16	700	1750	3500	7000	14000	28000	56000	112000	0.999997653	7003		
16	A-SCCP	C11 45%	C11	SCCP	C11H14Cl10	0.1	0.25	0.5	1	2	4	8	16	300	750	1500	3000	6000	12000	24000	48000	0.999997653	3001		
17	A-SCCP	C11 45%	C11	SCCP	C11H13Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
18	A-SCCP	C12 52%	C12	SCCP	C12H21Cl5	0.1	0.25	0.5	1	2	4	8	16	500	1250	2500	5000	10000	20000	40000	80000	0.999997653	5002		
19	A-SCCP	C12 52%	C12	SCCP	C12H20Cl6	0.1	0.25	0.5	1	2	4	8	16	4500	11250	22500	45000	90000	180000	360000	720000	0.999997653	45020		
20	A-SCCP	C12 52%	C12	SCCP	C12H19Cl7	0.1	0.25	0.5	1	2	4	8	16	3700	9250	18500	37000	74000	148000	296000	592000	0.999997653	37017		
21	A-SCCP	C12 52%	C12	SCCP	C12H18Cl8	0.1	0.25	0.5	1	2	4	8	16	2000	5000	10000	20000	40000	80000	160000	320000	0.999997653	20009		
22	A-SCCP	C12 52%	C12	SCCP	C12H17Cl9	0.1	0.25	0.5	1	2	4	8	16			2500	5000	10000	20000	40000	80000	1	5000		
23	A-SCCP	C12 52%	C12	SCCP	C12H16Cl10	0.1	0.25	0.5	1	2	4	8	16				5000	10000	20000	40000	80000	1	5000		
24	A-SCCP	C12 52%	C12	SCCP	C12H15Cl11	0.1	0.25	0.5	1	2	4	8	16									#N/A	#N/A		
25	A-SCCP	C13 59%	C13	SCCP	C13H23Cl5			0.5	1	2	4	8	16				7000	14000	28000	56000	112000	1	7000		
26	A-SCCP	C13 59%	C13	SCCP	C13H22Cl6			0.5	1	2	4	8	16			15000	30000	60000	120000	240000	480000	1	30000		
27	A-SCCP	C13 59%	C13	SCCP	C13H21Cl7			0.5	1	2	4	8	16	4500	11250	22500	45000	90000	180000	360000	720000	1	45000		
28	A-SCCP	C13 59%	C13	SCCP	C13H20Cl8			0.5	1	2	4	8	16	2700	6750	13500	27000	54000	108000	216000	432000	1	27000		
29	A-SCCP	C13 59%	C13	SCCP	C13H19Cl9			0.5	1	2	4	8	16	1200	3000	6000	12000	24000	48000	96000	192000	1	12000		
30	A-SCCP	C13 59%	C13	SCCP	C13H18Cl10			0.5	1	2	4	8	16	200	500	1000	2000	4000	8000	16000	32000	1	2000		
31	A-SCCP	C13 59%	C13	SCCP	C13H17Cl11			0.5	1	2	4	8	16									#N/A	#N/A		

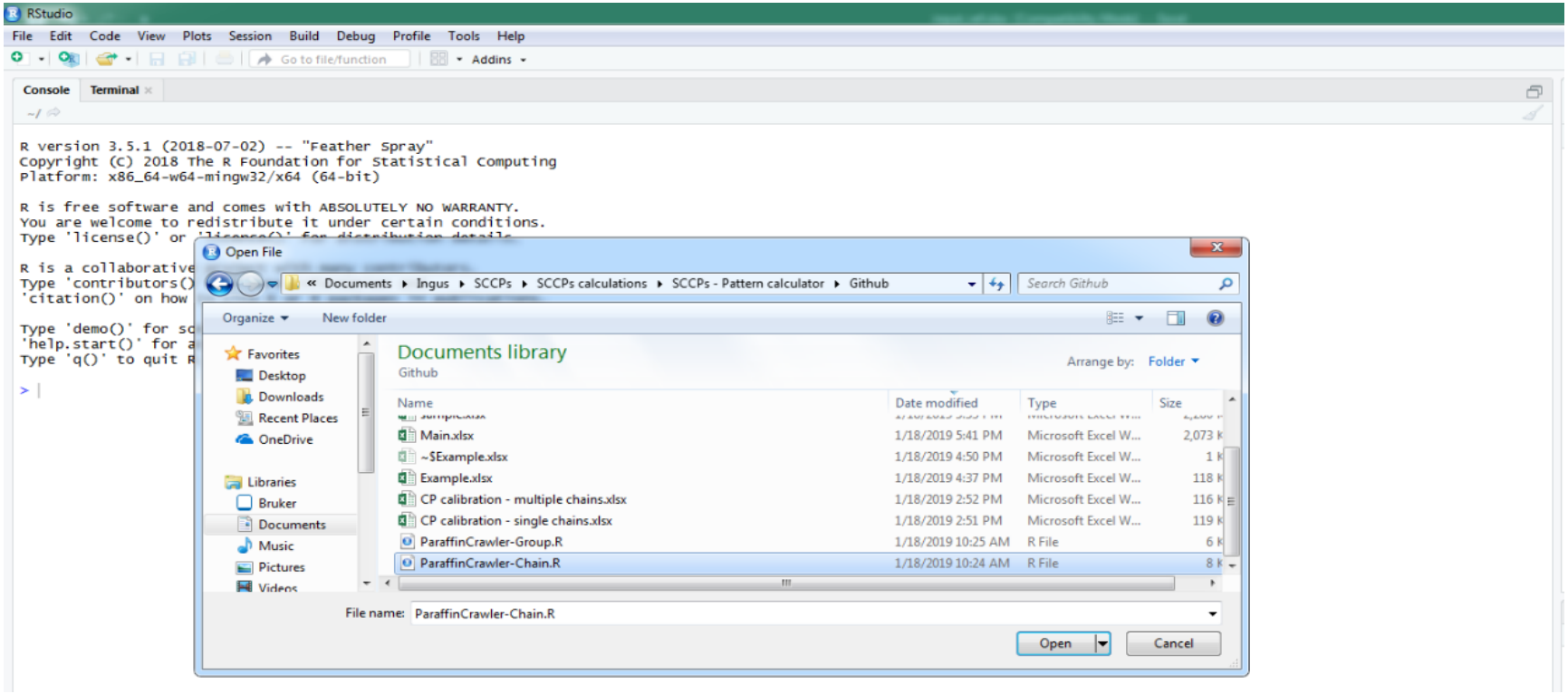
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	STD_code	Reference	Chain_leng	Type	Homologue	Response_factor															
2	A-SCCP	C10 50%	C10	SCCP	C10H17Cl5																
3	A-SCCP	C10 50%	C10	SCCP	C10H16Cl6	25157															
4	A-SCCP	C10 50%	C10	SCCP	C10H15Cl7	24953															
5	A-SCCP	C10 50%	C10	SCCP	C10H14Cl8	10005															
6	A-SCCP	C10 50%	C10	SCCP	C10H13Cl9	5002															
7	A-SCCP	C10 50%	C10	SCCP	C10H12Cl10																
8	A-SCCP	C10 50%	C10	SCCP	C10H11Cl11																
9	A-SCCP	C11 45%	C11	SCCP	C11H19Cl5																
10	A-SCCP	C11 45%	C11	SCCP	C11H18Cl6	30014															
11	A-SCCP	C11 45%	C11	SCCP	C11H17Cl7	40018															
12	A-SCCP	C11 45%	C11	SCCP	C11H16Cl8	20635															
13	A-SCCP	C11 45%	C11	SCCP	C11H15Cl9	7003															
14	A-SCCP	C11 45%	C11	SCCP	C11H14Cl10	3001															
15	A-SCCP	C11 45%	C11	SCCP	C11H13Cl11																
16	A-SCCP	C12 52%	C12	SCCP	C12H21Cl5	5002															
17	A-SCCP	C12 52%	C12	SCCP	C12H20Cl6	45020															
18	A-SCCP	C12 52%	C12	SCCP	C12H19Cl7	37017															
19	A-SCCP	C12 52%	C12	SCCP	C12H18Cl8	20009															
20	A-SCCP	C12 52%	C12	SCCP	C12H17Cl9	5000															
21	A-SCCP	C12 52%	C12	SCCP	C12H16Cl10	5000															
22	A-SCCP	C12 52%	C12	SCCP	C12H15Cl11																
23	A-SCCP	C13 59%	C13	SCCP	C13H23Cl5	7000															
24	A-SCCP	C13 59%	C13	SCCP	C13H22Cl6	30000															
25	A-SCCP	C13 59%	C13	SCCP	C13H21Cl7	45020															
26	A-SCCP	C13 59%	C13	SCCP	C13H20Cl8	27012															

7.) Save “input_ref.xlsx” file in desired directory. Open “sample.xlsx” file to enter results for sample measurement.

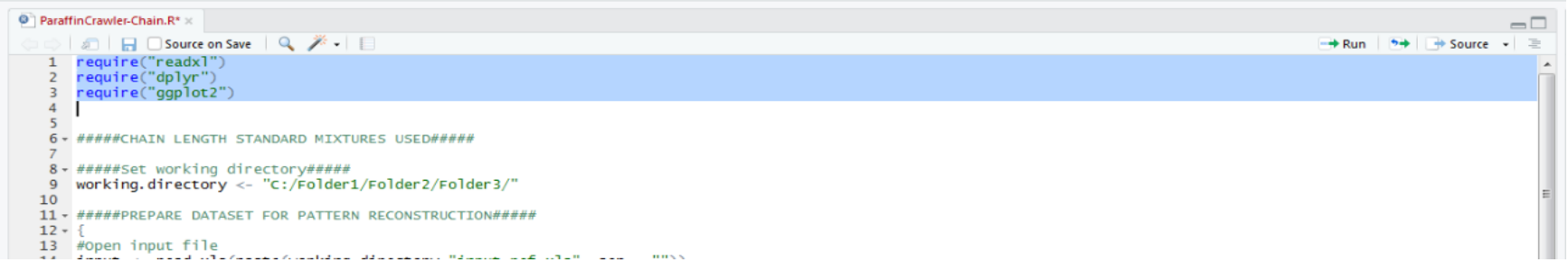
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Chain_length	Type	Homologue	Area																	
2	C10	SCCP	C10H17Cl5	0																	
3	C10	SCCP	C10H16Cl6	21600																	
4	C10	SCCP	C10H15Cl7	112000																	
5	C10	SCCP	C10H14Cl8	236800																	
6	C10	SCCP	C10H13Cl9	151200																	
7	C10	SCCP	C10H12Cl10	80000																	
8	C10	SCCP	C10H11Cl11	16000																	
9	C11	SCCP	C11H19Cl5	0																	
10	C11	SCCP	C11H18Cl6	40000																	
11	C11	SCCP	C11H17Cl7	204000																	
12	C11	SCCP	C11H16Cl8	208800																	
13	C11	SCCP	C11H15Cl9	226400																	
14	C11	SCCP	C11H14Cl10	123200																	
15	C11	SCCP	C11H13Cl11	19200																	
16	C12	SCCP	C12H21Cl5	20000																	
17	C12	SCCP	C12H20Cl6	180000																	
18	C12	SCCP	C12H19Cl7	172000																	
19	C12	SCCP	C12H18Cl8	158400																	
20	C12	SCCP	C12H17Cl9	218400																	
21	C12	SCCP	C12H16Cl10	155200																	
22	C12	SCCP	C12H15Cl11	68800																	
23	C13	SCCP	C13H23Cl5	0																	
24	C13	SCCP	C13H22Cl6	48000																	
25	C13	SCCP	C13H21Cl7	273600																	
26	C13	SCCP	C13H20Cl8	212000																	
27	C13	SCCP	C13H19Cl9	224000																	
28	C13	SCCP	C13H18Cl10	180000																	
29	C13	SCCP	C13H17Cl11	48000																	
30																					

8.) If using MCCPs or LCCPs instead of SCCPs, replace columns A, B, and C with corresponding information (Chain_length, Type and Homologue) from calibration sheets.
9.) Save “sample.xlsx” file in desired directory.

10.) Go to RStudio software and open “ParaffinCrawler-Chain.R”. Open “ParaffinCrawler-Group.R” if using multiple chain length standard mixtures.

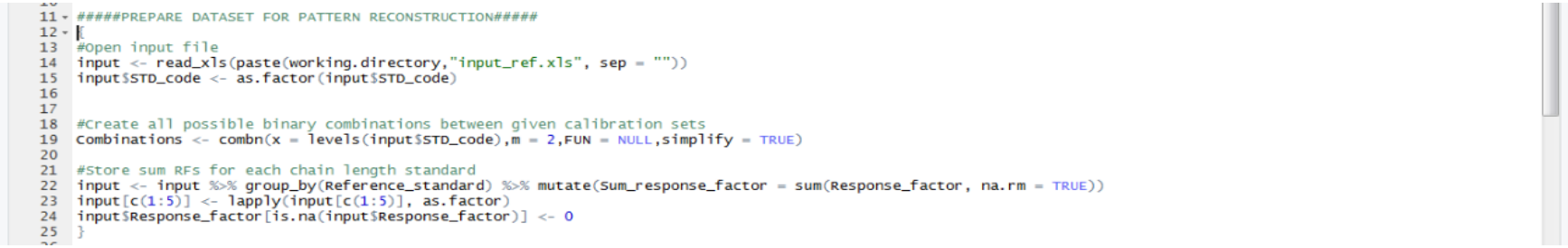


11.) Select first three lines and execute code with "Ctrl+Enter" to load required libraries.



12.) In line 9, edit the **working.directory** to the location of "input_ref.xlsx" and "sample.xlsx". Folders must be separated with "/", not "\"!

13.) Click in line 12 on the left side before curly bracket **"{".** Press "Ctrl+Enter" to run the first part of the code.



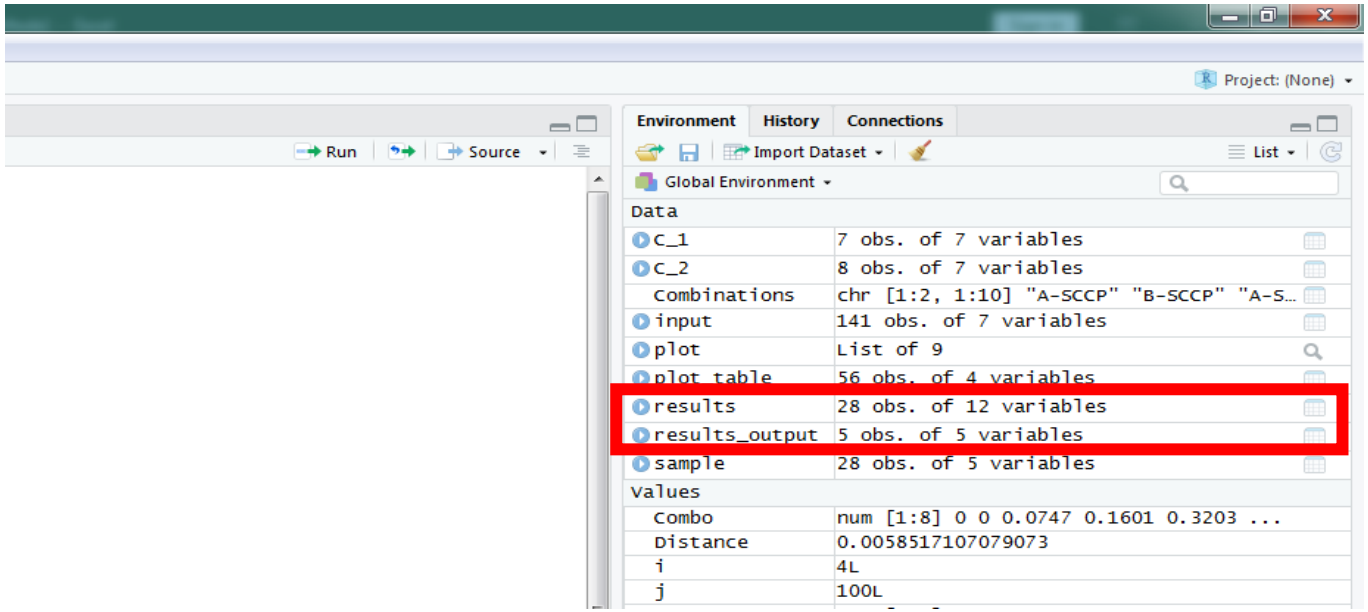
14.) You do not have to repeat steps 10. - 13. for each sample. Once they have been run, there is no need to do that for each sample unless new Rstudio session (or restart) has occurred.

15.) Enter sample name in line 34.

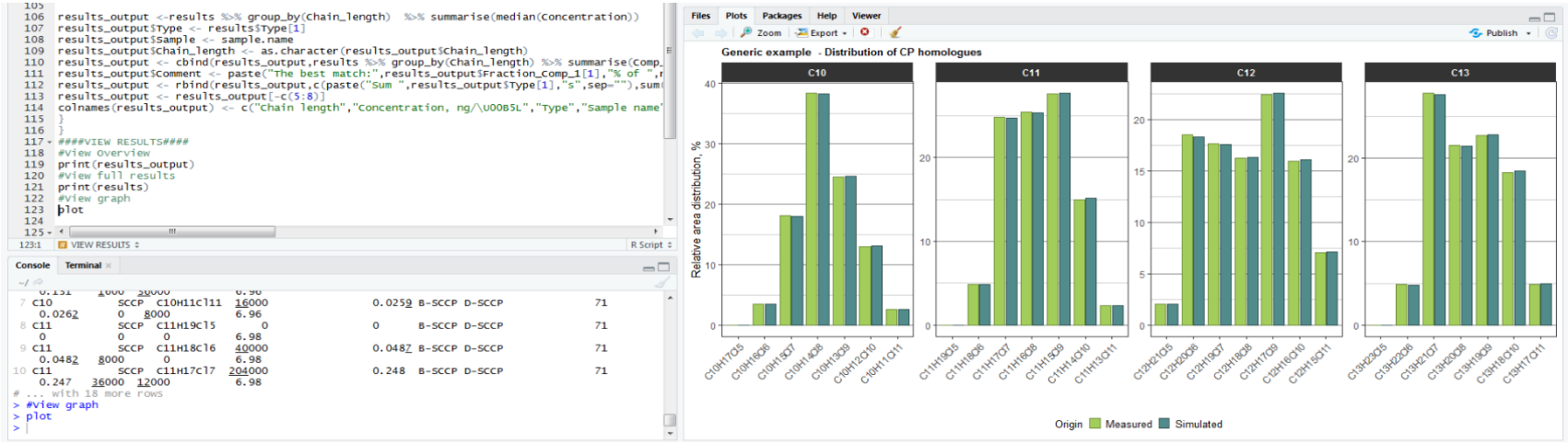
16.) Click in line 32 on the left side before curly bracket "**{**". Press "Ctrl+Enter" to run the second part of the code.

```
31 ####Open sample data####
32 {
33 sample <- read_xls(paste(working.directory,"sample.xls", sep = ""))
34 ####Set sample name####
35 sample.name <- "Generic example"
36 ####RUN PATTERN RECONSTRACTION FOR SELECTED (LOADED) SAMPLE####
37 {
38 sample$Chain_length <- as.factor(sample$Chain_length)
39 sample$Area <- as.numeric(sample$Area)
```

17.) Step 16 executed pattern reconstruction procedure and quantification. Now you can see and save the results.
18.) To see results summary and full data table open data frames on the right side panel. Click "play" button on "results_output" to see the summary, click "results" to see the full data.frame.



19.) To visualize the calculated patterns click in line 123 and execute it by pressing "Ctrl+Enter". The plots will be visualized in right panel ("Plots").



20.) To save all files go to line 127, edit save.directory and execute lines 130, 132, and 134 by clicking to each line and pressing "Ctrl+Enter" to save summary of results (.txt separated by TAB), full results (.txt separated by TAB) and relative distribution plot (.tiff), respectively. All these files will be named by sample.name, which was defined in step 15 and can be located in save.directory.

```
125 ####SAVE RESULTS####
126 #Set save directory
127 save.directory <- "C:/folder1/folder2/folder3/"
128
129 #Save Overview
130 write.table(results_output, paste(save.directory,sample.name,"-overview", ".txt", sep = ""), s
131 #Save full results
132 write.table(results, paste(save.directory,sample.name,"-full_results", ".txt", sep = ""), sep=
133 #Save distribution plot
134 ggsave(filename = paste(sample.name,"-CP_distribution_plot",".tiff", sep =""),device = "tiff",
135
```

21.) Repeat the procedure from step 15 to 20 for each sample. Remember to replace data in "sample.xlsx" file when running a different sample.

Acknowledgments

The backbone for this approach was largely inspired/adapted from two publications:

Schinkel, L., Lehner, S., Knobloch, M., Lienemann, P., Bogdal, C., McNeill, K., & Heeb, N. V. (2018). Transformation of chlorinated paraffins to olefins during metal work and thermal exposure – Deconvolution of mass spectra and kinetics. Chemosphere, 194, 803–811. https://doi.org/10.1016/j.chemosphere.2017.11.168

Bogdal, C., Alsberg, T., Diefenbacher, P. S., Macleod, M., & Berger, U. (2015). Fast quantification of chlorinated paraffins in environmental samples by direct injection high-resolution mass spectrometry with pattern deconvolution. Analytical Chemistry, 87(5), 2852–2860. https://doi.org/10.1021/ac504444d

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