Planning SACR DNA Methylation Analysis

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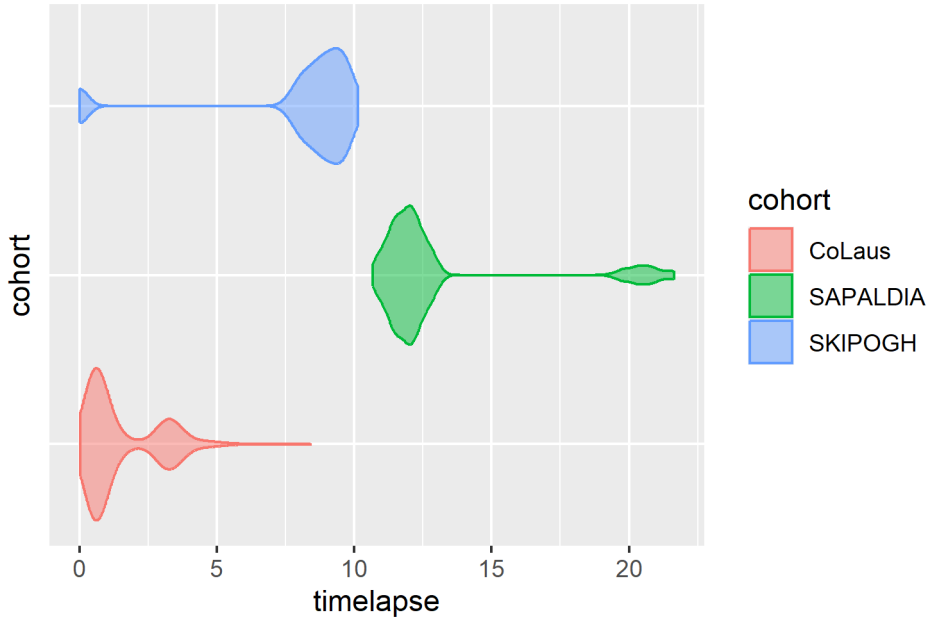
1. SACR samples for methylome analysis

Cohort	Sample Type 1	Type 1 Sample Size	Mean year(s) blood collection to MRI	Sample Type 2	Type 2 Sample Size	Mean year(s) blood collection to MRI	Age range at MRI
SAPALDIA N=403	Extracted DNA 2µg at 50ng/µl SAPALDIA 3	353	11.9	Extracted DNA SAPALDIA 2 2µg at various concentrations	50	20.5	50-89
(Psy)CoLaus N=346	EDTA Blood	346	1.5	-	-		60-93
SKIPOGH N=142	SKIPOGH2 Buffy Coat	128*	9.0	SKIPOGH3 (SACR) Buffy Coat	14	0.0	27-93*

* MRI not done yet for 16 samples

Time lapse in years since blood collection to MRI differs by cohort, in the range of 10-21 years for SAPALDIA, 0-8 years for CoLaus, and 0-10 years for SKIPOGH.



2. Cohort-specific datasets on participants & samples for methylome analysis

The following cohort specific datasets are provided by cohorts.

Variable	Description
sample_ID	ID by which the submitted samples are labeled
sacr_ID	SACR specific participant ID
cohort	SAPALDIA / SKIPOGH / CoLaus
survey	e.g. SAPALDIA2, SAPALDIA3, etc

study_center	Study Centre ID (does not apply for CoLaus)
sex	Female / Male
age_blood	Age at the time point of blood collection [years]
date_blood	Date at the time point of blood collection [dd/mm/yyyy]
date_mri	Date at the time point of MRI [dd/mm/yyyy]
sample_type	Extracted DNA / EDTA blood / Buffy coat
storage_temp	storage temperature of the blood sample (e.g. -80 degrees)
bench_time	Duration between blood collection and final storage [hours]
fasting	Overnight fasting yes/no at the time point of blood collection
fasting_time	Duration between last eaten and blood collection in case no overnight fasting [hours]
Thawing_cycle	0 (never thawed), 1 (thawed once), …

These datasets are used to design the randomization of the DNA samples to minimize batch effects, and will be used to characterize biospecimens for future publications.

These datasets will be uploaded to the SACR project space along with the methylome data, once the methylome analysis is done.

3. Shipment of DNA or blood samples

Each cohort is shipping their biospecimens separately to analysis lab in the Netherlands, following the instruction described below.

Instructions for biospecimen shipment

Timeline of shipment and analysis: All samples must arrive at the laboratory in July 2023. Analysis will only start once samples from all cohorts are at the GenomeScan laboratory, as we need to randomize the biospecimens from all cohorts to avoid batch effects.

Cost and billing of shipment: Due to accounting reasons (billing date **before June 30** and shipment in July), we chose sending the sample using the bplogistics.

- The samples of each cohort can be shipped separately and separate bills per shipment will be established by bplogistics.
- Bplogistics needs to know the number of aliquots, the volume of the primary tubes, the dimension of the secondary containers (boxes).
- Bplogistics provides dry ice and shipping containers.
- Each cohort provides a proforma-invoice and an indication if the samples are UN3373 or human exempt specimen, as to allow the bplogistic partner in SKIPOL Amsterdam NL to check the papers for smooth transfer through customs.
- Each cohort needs to inform pblogistic in advance to prepare shipment on the beginning of a week (on a Monday or Tuesday). There are no state holidays until the end of the July in NL.
- **It is each cohort's responsibility to inform the laboratory of the shipment date and assure that the samples are received and kept frozen until analysis.**

- Bplogistics picks up samples and brings them to the airport in Switzerland and bplogistic partner in SKIPOL Amsterdam NL assures transfer through customs, maintenance of cold chain and delivers samples at Genome Scan in Leiden, NL.
- The shipping address and contact details are listed below.

  Shipping address:
  GenomeScan
  Plesmanlaan 1d, 4th floor
  2333 BZ LEIDEN
  The Netherlands

  Contact persons at GenomeScan:
  NameFahim Behrouz, f.behrouz@genomescan.nl
  Evita Karelioti, E.Karelioti@genomescan.nl
  Company Telephone: +31 (0)71 568 1050

- The contact details of bglogistics:
  Urs Suter
  General Manager
  B.P.L. AG
  Binningerstrasse 96
  4123 Allschwil / Schweiz
  Direkt: +41 61 485 15 61
  Mobile: +41 79 939 83 38
  Mail: urs.suter@bplogistics.chv

  For contact person in French of bglogistics:
  Claudine Felber
  Phone: 061 485 15 63
  Mail: claudine.felber@bplogistics.ch

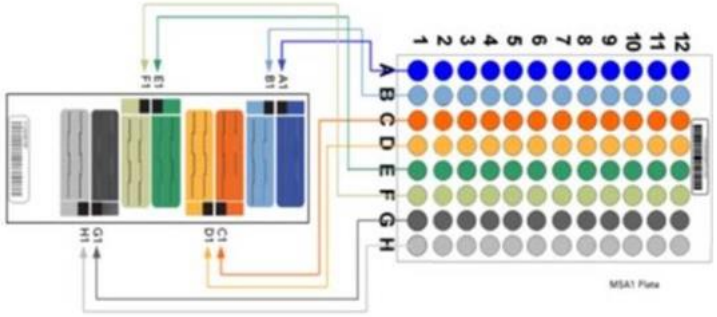
4. Description of analysis pipeline at Genomescan

DNA will be extracted in the Netherlands from EDTA blood or buffy coat for cohorts that do not provide extracted DNA.

Extracted DNA will be subjected to:

a) Bisulfate treatment in 96-well plates, each containing 1 positive control DNA (Genomic DNA - Human Adult Normal Tissue: Brain, from a single donor, ordered by GenomeScan)
b) Hybridization on the Illumina EPIC v2.0 BeadChips in batches of 8 samples (A01 to H01; A02 to H02; and so on)

During the hybridization, samples are transferred column by column from 96-well plates to 8-position chips.



Samples from each column of the 96-well plate will be applied onto the same chip (e.g. 1st column (8 samples from A01 to H01) are transferred to chip1, 2nd column (8 samples from A02 to H02) are transferred to chip2, and so on). The sequence of the 8 samples in each column are fixed (e.g. A01 to the first position of the chip1, B01 to the second position, and so on).

Each 96-well plate contains two positive control samples (Genomic DNA from a single donor), i.e. one at a fixed position and another at a random position. The positive control samples are hybridized to the chip along with other samples.

5. Distribution DNA samples for bisulfite treatment and hybridization

Given the number of samples under 1 (891 samples), 10 plates are required for bisulfate treatment (9 full plates and 47 wells filled in the last plate). 911 samples (including 20 control samples) will be hybridized to 114 chips (113 full chips and 7 positions filled in the last chip). To fill up the last chip, a random CoLaus sample is duplicated. A total of 912 samples are distributed across 10 plates and 114 chips.

We first distributed control samples to fulfill the following conditions:

- 2 control samples per plate
- 1 control per plate at a fixed position A01
- 1 control per plate at a random position
- Each position of the chip receives at least one control sample
- Control samples are as widely distributed as possible across columns on plates, i.e. each control sample takes a different column on plates

Using omixerRand from R package “Omixer” (Sinke et al 2021), randomization was run multiple times to search for a layout such that:

- No evidence of correlation between technical covariates (plate, chip, position on the chip) and selected biological variables
- The layout with the minimal sum of correlation is chosen

For the technical variables, we considered:

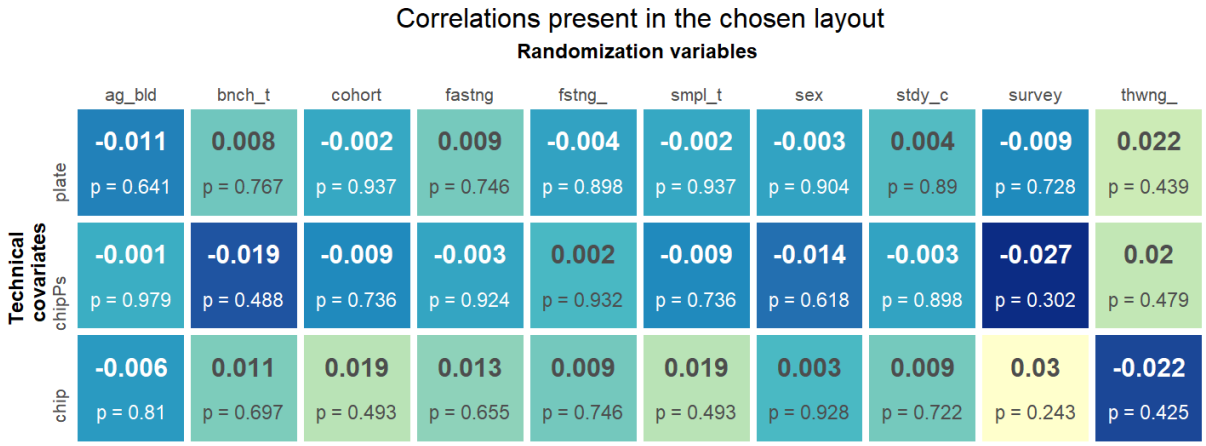
- Plate: which plate the sample is located
- Chip: which chip the sample is located
- Position on chip: which well on the chip the sample is located

For the biological variables, we considered the following variables as defined under 2:

- cohort
- survey
- study_center
- sex
- age_blood
- sample_type
- thawing_cycle
- bench_time
- fasting
- fasting_time

There are varying number of missing values for these variables. Missing values are omitted by omixerRand.

At the end of the procedure, a layout is produced with no evidence of correlation.



The layout also showed no correlation for the time lapse since blood collection to MRI. Correlation of the time lapse since blood collection to MRI with each of the three technical variables is as follows:

- plate: -0.004 (p = 0.862)
- chip: 0.010 (p = 0.674)
- chip position: -0.017 (p = 0.485)

6. Matched randomization

As a result of the procedure under 5, the final sample order was produced, where the samples from each cohort are distributed over chips and plates as follows:

N chips containing … control samples		total N chips	total N samples
0	1		
94	20	114	20

N chips containing … SAP2 samples				total N chips	total N samples
0	1	2	3		
74	31	8	1	114	50

N chips containing … SAP3 samples							total N chips	total N samples
0	1	2	3	4	5	6		
2	13	22	33	27	13	4	114	353

N chips containing … SKIPOGH2 samples					total N chips	total N samples
0	1	2	3	4		
28	53	26	5	2	114	128

N chips containing … SKIPOGH3 samples			total N chips	total N samples
0	1	2		
101	12	1	114	14

N chips containing … CoLaus samples							total N chips	total N samples
0	1	2	3	4	5	6		
3	12	25	34	23	11	6	114	347

N 96-well plates containing … control samples	total N plates	total N samples
2		
10	10	20

N 96-well plates containing … SAP2 samples							total N plates	total N samples
2	3	4	5	6	7	8		
1	1	1	4	1	1	1	10	50

N 96-well plates containing … SAP3 samples								total N plates	total N samples
17	28	30	35	37	38	43	51		
1	1	1	1	3	1	1	1	10	353

N 96-well plates containing … SKIPOGH2 samples								total N plates	total N samples
8	9	11	12	13	17	18	19		
2	1	1	1	2	1	1	1	10	128

N 96-well plates containing … SKIPOGH3 samples			total N plates	total N samples
0	1	2		
2	2	6	10	14

N 96-well plates containing … CoLaus samples								total N plates	total N samples
17	28	35	36	37	39	40	42		
1	1	1	2	2	1	1	1	10	347

The sample distribution including controls within the plates as a result of the procedure under 5 are illustrated in the Appendix B.

Reference

Sinke, L., et al. (2021) Omixer: multivariate and reproducible sample randomization to proactively counter batch effects in omics studies. Bioinformatics

Appendix A. randomization_scheme_using_omixer.R

```
setwd('c:/work/projects/SACR/methylome/randomization_scheme/')


library(Omixer)


set.seed(20230814)



# read in data
d.sap <- readRDS('../biospecimen_information/SAPALDIA/SAPALDIA_final.rds')
d.skip <- readRDS('../biospecimen_information/SKIPOGH/SKIPOGH_final.rds')
d.cola <- readRDS('../biospecimen_information/CoLaus/CoLaus_final.rds')

vars <- c("sample_id", "sacr_id", "cohort", "survey", "study_center", "sex",
          "age_blood", "date_blood", "date_mri", "sample_type", "storage_temp",
          "bench_time", "fasting", "fasting_time", "thawing_cycle")
#all(vars %in% colnames(d.sap))
#all(vars %in% colnames(d.skip))
#all(vars %in% colnames(d.cola))
d <- rbind(d.sap[,vars], d.skip[,vars], d.cola[,vars])



#######
# determine the number of plates and chips needed

n.sap <- sum(d$cohort=='SAPALDIA')
n.skip <- sum(d$cohort=='SKIPOGH')
n.cola <- sum(d$cohort=='CoLaus')

n.sample <- n.sap + n.skip + n.cola
n.plate <- ceiling(n.sample/95) # 94 samples per plate (2 wells reserved for control)
n.control <- n.plate*2 # 1 control per plate at a fixed position + 1 control per plate at a
random position
n.chip <- ceiling((n.sample+n.control)/8)

cat(n.plate-1, 'full plates and', (n.sample+n.control)%%96, 'wells filled in the last
plate\n')
# 9 full plates and 48 wells filled in the last plate
cat(n.chip-1, 'full chips and', (n.sample+n.control)%%8, 'positions filled in the last
chip\n')
# 114 full chips and 0 positions filled in the last chip



#######
# masking the non-sample wells
```

```
# - wells that remain unused in the last plate

a.mask <- array(0, dim=c(8, 12, n.plate))

# masking the empty wells in the last plate
a.mask[,,n.plate] <- matrix(c(rep(0, (n.sample+n.control)%%96), rep(1, 96-
(n.sample+n.control)%%96)), nrow=8, byrow=F)

# masking the wells for control samples at a fixed position
# note: 1 control per plate at a fixed position, i.e. the first position (H12)
a.mask[1,1,] <- 1

# masking the wells for control samples at a random position
# note: 1 control per plate at a random position, but to cover all 8 positions on the chip
#       and to be as widely distributed as possible across columns on plates;
#       the random procedure is repeated until all the 8 positions are covered by at least
one control sample
k <- 0
repeat{
  k <- k+1
  pos.cont <- data.frame(row=rep(NA, n.plate), col=rep(NA, n.plate))
  for(i in 1:n.plate){
    idx <- which(a.mask[,,i]==0, arr.ind=T) # unmasked wells
    pos.cont[i,] <- idx[sample(i:nrow(idx), 1),]
  }
  if(all(1:8 %in% pos.cont$row) & length(unique(pos.cont$col))==n.plate){
    print(sprintf('success at the %d-th iteration', k))
    break
  }
}
for(i in 1:n.plate){
  a.mask[pos.cont$row[i], pos.cont$col[i], i] <- 1
}

# concatenate to a vector
v.mask <- NULL
for(i in 1:n.plate){
  for(j in 1:12){
    v.mask <- c(v.mask, a.mask[,j,i])
  }
}

# generate the scheme by running omixerRand{Omixer}
# note: omixerRand runs the randomization many times (defined by iterNum) and selects a
layout, so that
#       - no evidence of correlation between technical covariates and selected biological
variables (defined by randVars)
#       - the layout with the minimal sum of correlation is chosen
# note: omixerRand uses Kendall's rank based correlation test
```

```r
# note: NA handling is defined by the global setting (getOption("na.action")), i.e. by
default na.omit
randVars <- c('cohort', 'survey', 'study_center', 'sex', 'age_blood', 'sample_type',
'thawing_cycle',
             'bench_time', 'fasting', 'fasting_time')
scheme <- omixerRand(d, sampleId='sample_id',
                     iterNum=100, wells=96, div="col", positional=T,
                     plateNum=n.plate, randVars=randVars, mask=v.mask)

saveRDS(scheme, 'omixer_chosen_layout.rds')


# clean layout to share
final <- scheme[1:(n.sample+n.control), c('plate', 'well', 'row', 'column', 'chip',
'chipPos', 'sampleId')]
idx <- which(is.na(final$sampleId))
final$sampleId[idx] <- 'control'
final$chip <- (final$plate-1)*12 + final$chip


# manual replacement of a wrong sample
# note: CoLaus sent B00RXI instead of B00RWD by mistake; B00RWD will be replaced by a
random CoLaus sample so that
#       the random sample will have duplicate measurements and all 114 chips are filled up
final$sampleId[which(final$sampleId=='B00RWD')] <- sample(d.cola$sample_id, 1)

head(final)
write.csv(final, 'SACR_final_layout.csv', row.names=F)


# visualize the scheme per plate
omixerSheet(scheme, group='cohort')
for(i in 1:n.plate){
  idx <- (i-1)*96 + (1:96)
  this.plate <- matrix(scheme$sampleId[idx], nrow=8, byrow=F)
  this.plate[pos.cont$row[i], pos.cont$col[i]] <- 'control'
  colnames(this.plate) <- 1:12
  rownames(this.plate) <- c('A','B','C','D','E','F','G','H')
  sink(sprintf('plate%02d.txt', i))
  print(this.plate)
  sink()
}
```

Appendix B. Plate composition as a result of the randomization scheme

SAPALDIA
SKIPOGH
CoLaus
Control

	Plate01											
	1	2	3	4	5	6	7	8	9	10	11	12
A	control	308925	302289	B00SCN	B00T95	205001	320681	B00RLP	B00OLP	206220	308079	control
B	320934	320253	4051521188	304292	B00T44	B00PWU	B00Q7H	B00OLH	320723	B00PV2	307326	B00TJY
C	309640	B00T8I	B00RYI	300047	320953	B00TMZ	301100	302695	302320	302501	320243	B00RXL
D	320252	B00S8M	320571	304678	300017	320903	B00S6I	B00S7L	B00Q3T	B00Q4W	320309	301559
E	301298	B00SJ2	4051521451	309456	B00T0W	320703	308631	300566	320682	B00N9X	320892	208007
F	303906	302485	307162	305719	305226	300647	B00Q66	B00Q9H	300730	300429	B00QPG	320522
G	B00TDJ	B00PKU	305064	207850	303801	B00TJ4	300436	B00O0L	B00Q9Z	200543	B00T2B	B00S9Y
H	B00Q88	305130	320364	320546	B00SYM	200392	B00PIF	B00T76	209353	320618	203540	320441

	Plate02											
	1	2	3	4	5	6	7	8	9	10	11	12
A	control	308805	B00QW8	308130	B00S74	302479	B00P7E	B00T8G	301755	306964	320292	B00S7W
B	301496	B00TYM	B00PL4	320991	307881	B00MZ3	302873	B00OOO	204567	308480	304083	B00PYF
C	306167	308943	B00PZB	305628	307729	300448	320932	320656	B00Q1Q	320924	B00OTB	200591
D	200138	B00TIM	320504	B00T35	B00OQB	320508	B00OAZ	307355	control	B00T0O	320500	B00QIR
E	308625	300739	4051427468	304496	B00Q2X	B00Q5M	B00OOI	B00OE2	B00RG3	308658	B00ODS	304150
F	305358	330097	B00P35	B00RL7	320011	B00N7A	B00SHM	306330	306511	306217	205645	B00RHC
G	302687	B00QPT	B00RRX	B00NBD	305174	308342	B00Q3L	304035	306160	B00NGA	B00QMV	301001
H	4020043155	B00SXX	B00SVX	305945	320421	302863	305612	320155	206961	B00OO1	302264	B00RNG

	Plate03											
	1	2	3	4	5	6	7	8	9	10	11	12
A	control	306927	304508	305584	303227	B00P85	301871	304618	308510	302119	205905	303810
B	B00RK9	B00NA2	304609	B00QAQ	307498	320978	B00Q15	308046	320531	307911	control	203279
C	305354	B00Q0B	320482	308681	205627	304498	B00O6Q	305409	B00RJC	304597	305365	4051520877
D	304820	B00T1O	308502	B00OOJ	304417	B00TOA	320755	B00TC1	306010	B00PUX	205510	305419
E	B00QYP	304229	303807	308308	320711	301341	B00MWD	320563	300052	308315	308370	303496
F	B00T42	305298	B00S4K	304001	204084	302020	305431	308186	B00T1M	320091	4051420022	B00Q0P
G	B00TZR	B00NB7	B00SB0	308424	300843	309230	303253	320371	305442	B00Q8P	308332	B00Q60
H	306514	303999	308953	301337	B00SKW	B00OHJ	308970	B00OPT	309406	305171	B00Q7N	307221

	Plate04											
	1	2	3	4	5	6	7	8	9	10	11	12
A	control	B00QAM	307334	B00OAT	201345	303554	320994	307633	B00TJ0	304728	B00RI6	307978
B	B00Q0V	320674	307217	306510	B00QC6	B00T19	B00TEC	4051427483	B00MZA	B00SE8	B00QS7	B00T7P
C	320474	B00QPV	B00SBK	302561	B00TJT	307392	302210	308768	B00T87	204072	B00QEK	302421
D	203724	B00Q1J	320384	B00NEZ	303266	320872	B00RIV	320733	B00NG0	309299	301310	B00SGW
E	308134	320261	B00OQ0	B00S8I	320525	301666	320974	B00RAE	B00S7X	B00OZN	B00PQI	B00S7N
F	B00QC5	B00HWA	B00Q3G	control	320184	306667	320512	320853	309057	B00P3O	305565	309095
G	204715	B00O2V	301409	301551	304126	301054	320114	B00PSQ	B00TIS	B00S6G	320673	320212
H	B00RGM	320423	B00OLT	B00SFU	B00T84	308675	301558	300796	330627	B00RWD	320821	320582

	Plate05											
	1	2	3	4	5	6	7	8	9	10	11	12
A	control	306883	308473	300914	303170	B00RJJ	B00RFW	304453	B00Q52	300828	320732	320877
B	B00P78	206309	B00P2R	303930	B00OH1	300453	303184	B00P42	B00P86	309290	B00NFD	303934
C	302855	305559	320923	320805	304468	B00UTE	B00SBC	308759	203187	B00OFQ	B00RK8	302230
D	303335	301815	308955	306809	B00PYE	B00Q5T	302303	320661	320305	302657	207831	300530
E	307693	B00Q43	B00OZ1	B00S9J	302514	B00SCC	control	301929	300725	304760	B00RJX	303418
F	300258	303008	306621	B00SJB	308976	205829	B00PXR	B00RFT	300265	306936	B00QND	308498
G	B00PMG	B00P7J	B00UZ8	B00PIK	308132	B00Q9A	B00TED	320153	B00N38	303203	306523	B00TJO
H	301697	B00TOQ	B00SE0	B00N0P	320925	B00PHJ	309061	300798	304376	320144	203245	B00RTA

Plate06

	1	2	3	4	5	6	7	8	9	10	11	12
A	control	B00Q0J	4051419987	B00TGW	306730	320841	B00Q67	B00PYT	B00S92	200115	B00RL3	304694
B	304218	307052	303278	306839	B00OC1	307259	307510	B00SJL	205974	307381	309431	B00TLR
C	B00PAX	206656	203629	B00QN1	B00SGE	B00TN7	305882	309588	B00T8A	320422	B00MYZ	307135
D	B00OVH	304234	B00QQI	306381	B00Q9W	303506	306564	B00Q50	B00S6E	200496	320602	309075
E	B00Q6J	B00Q6N	302008	B00RMQ	320051	B00SBA	B00O6G	B00TOD	301234	320554	B00S9X	B00QVQ
F	320262	301467	302967	320222	302775	320115	301410	320983	303276	300670	B00PZH	B00RLR
G	308848	B00TYJ	320683	300904	302482	306533	308853	B00OAJ	306265	303945	B00SZ8	4051419455
H	320844	320992	300178	300452	control	204196	B00T1D	B00P15	B00RGT	301567	320702	301126

Plate07

	1	2	3	4	5	6	7	8	9	10	11	12
A	control	307258	B00MSQ	B00PR0	304983	320213	300219	305505	B00PHR	207041	301127	302974
B	B00PUZ	303541	B00P32	306233	320751	B00P9G	320052	B00QFX	B00TKD	B00QEY	306414	302589
C	320382	309125	203457	305933	302872	B00PWC	320692	301950	B00Q57	B00S6C	309223	300581
D	301074	B00SZ3	320442	304573	B00Q4K	B00N1S	208465	303740	306695	307420	B00ORL	B00V09
E	303617	207180	B00SOZ	207699	B00RJ3	305883	320506	B00RGL	B00RIO	320655	B00PYO	308199
F	B00OC8	B00TCR	303267	320142	B00PZE	305511	B00OYM	305327	303816	B00PYY	B00PIB	B00T3K
G	307628	320526	B00QQV	B00SZF	B00TLV	302895	320513	301028	B00T34	304874	B00O7U	307093
H	304782	B00RJV	control	B00RN3	B00PQB	303786	303983	B00P21	301842	320811	320610	B00RL1

Plate08

	1	2	3	4	5	6	7	8	9	10	11	12
A	control	320935	300879	206862	B00QAJ	306628	304945	209342	303977	206694	B00RH3	B00RTK
B	B00POH	B00PVJ	B00S87	320721	B00SCB	320187	320653	300372	B00OUF	B00QWJ	B00U5K	301897
C	B00R2D	B00Q18	320373	B00SBJ	301729	B00OT1	302061	305139	4051521135	control	304828	305242
D	B00Q04	306493	305845	306938	302261	B00PSV	320752	302702	301393	208520	B00N1J	303249
E	B00SFS	B00RL5	308956	320803	B00QFY	B00Q92	B00O1Q	300434	B00PWK	206534	B00T5R	207854
F	306561	306005	302257	303228	303130	320372	202898	320354	301354	B00Q38	B00TG3	B00QN5
G	307453	307514	306200	305199	B00PXH	302677	320071	304965	B00SYJ	306100	300982	B00SL6
H	B00Q5O	B00T2Q	B00PXK	B00RFU	307292	B00QCD	B00TH4	307979	303888	B00RUF	308247	320081

Plate09

	1	2	3	4	5	6	7	8	9	10	11	12
A	control	320742	302950	305069	307901	302632	306643	B00O30	B00OKA	320612	208393	300196
B	B00MWQ	B00PQ3	320634	320092	308891	B00O16	302634	B00NXM	B00T32	302177	4051427501	B00T06
C	B00OZO	B00SGD	B00RGI	306041	320662	320614	B00TH3	B00N03	304619	B00T4Y	B00PZ6	320902
D	308241	320851	320312	B00SG6	309046	B00T7T	B00T8S	320912	306636	300212	B00N4S	B00RTZ
E	320311	307884	B00OKS	300522	B00RG0	B00RHF	304459	B00Q90	B00PTM	B00MWH	B00SD5	304991
F	301776	301553	300031	301363	309638	300276	320388	301647	303071	B00QUG	307280	320013
G	320882	307752	306392	308017	204421	B00RKH	B00T6Q	control	320509	307243	B00RX0	B00OLX
H	320352	302165	307236	200082	320201	4051520873	300246	301144	B00RUY	306285	B00TIH	B00S6O

Plate10

	1	2	3	4	5	6	7	8	9	10	11	12
A	control	B00RJN	302683	320304	304808	303179						
B	302587	B00SAY	B00SD4	B00Q86	B00Q8E	320873						
C	4051521582	B00Q27	4051427509	B00OUX	B00RIH	320973						
D	B00NGR	B00N91	305329	305615	303321	320874						
E	301069	320754	307138	B00SE6	320587	B00N1V						
F	301870	B00PVT	320542	B00TLP	305005	307459						
G	306747	305924	320753	B00N39	208414	B00T3C						
H	206537	control	301286	B00OY0	304013	302302						