Extraction plan for APCL18_659-APCL18_732

2018-07-26

Organize samples into plate format:

1. Sample_id plate locations

	1	2	3	4	5	6	7	8	9	10
A	APCL18_659	APCL18_667	APCL18_674	APCL18_682	APCL18_690	APCL18_698	APCL18_706	APCL18_714	APCL18_721	APCL18_729
B	APCL18_660	APCL18_668	APCL18_675	APCL18_683	APCL18_691	APCL18_699	APCL18_707	APCL18_715	APCL18_722	APCL18_730
C	APCL18_661	APCL18_669	APCL18_676	APCL18_684	APCL18_692	APCL18_700	APCL18_708	APCL18_716	APCL18_723	APCL18_731
D	APCL18_662	XXXX	APCL18_677	APCL18_685	APCL18_693	APCL18_701	APCL18_709	APCL18_717	APCL18_724	APCL18_732
E	APCL18_663	APCL18_670	APCL18_678	APCL18_686	APCL18_694	APCL18_702	APCL18_710	XXXX	APCL18_725	NA
F	APCL18_664	APCL18_671	APCL18_679	APCL18_687	APCL18_695	APCL18_703	APCL18_711	APCL18_718	APCL18_726	NA
G	APCL18_665	APCL18_672	APCL18_680	APCL18_688	APCL18_696	APCL18_704	APCL18_712	APCL18_719	APCL18_727	NA
H	APCL18_666	APCL18_673	APCL18_681	APCL18_689	APCL18_697	APCL18_705	APCL18_713	APCL18_720	APCL18_728	NA

2. Extraction_id plate locations

	1	2	3	4	5	6	7	8	9	10
A	E5223	E5231	E5239	E5247	E5255	E5263	E5271	E5279	E5287	E5295
B	E5224	E5232	E5240	E5248	E5256	E5264	E5272	E5280	E5288	E5296
C	E5225	E5233	E5241	E5249	E5257	E5265	E5273	E5281	E5289	E5297
D	E5226	E5234	E5242	E5250	E5258	E5266	E5274	E5282	E5290	E5298
E	E5227	E5235	E5243	E5251	E5259	E5267	E5275	E5283	E5291	NA
F	E5228	E5236	E5244	E5252	E5260	E5268	E5276	E5284	E5292	NA
G	E5229	E5237	E5245	E5253	E5261	E5269	E5277	E5285	E5293	NA
H	E5230	E5238	E5246	E5254	E5262	E5270	E5278	E5286	E5294	NA

Write the newly assigned extraction_ids to the database

```
lab <- write_db("Laboratory")
```

```
## Loading required package: DBI
```

```
dbWriteTable(lab, "extraction", plate, row.names = F, overwrite = F, append = T)
```

```
## [1] TRUE
```

```
dbDisconnect(lab)
```

```
## [1] TRUE
```

```
rm(lab)
```

Prepare a solution of Lifton's Buffer

Lifton's Buffer				
ingredient	initial_conc	initial_vol_mL	final_conc	final_vol_mL
mM_EDTA	500	50.00	100	250
mM_Tris-HCl_pH7.5	1000	6.25	25	250
perc_SDS	20	12.50	1	250
pH2O	NA	181.25	NA	250

Prepare Lifton's buffer with proteinase K

Lifton's with Proteinase K				
ingredient	initial_conc	initial_vol_uL	final_conc	final_vol_uL
mM_DTT	1000	262.5	75.0	3500
mg/ml_ProteinaseK	20	735.0	4.2	3500
Liftons	NA	2502.5	NA	3500

Prepare hybridization solution

Hybridization Buffer				
ingredient	initial_conc	initial_vol	final_conc	final_vol
M_NaCl	4.45	2808.98876	2.5	5000
perc_PEG_8000	50.00	2000.00000	20.0	5000
mM_DTT	1000.00	125.00000	25.0	5000
pH2O	NA	66.01124	NA	5000

Prepare low TE solution

Low TE buffer				
ingredient	initial_conc	initial_vol	final_conc	final_vol
mM_Tris-HCl_pH7.5	1000	150	10.0	15000
mM_EDTA	500	3	0.1	15000
pH2O	NA	14847	NA	15000

Load samples for lysis

1. Add 80uL Lifton's buffer to each well of a 96 well plate.

2. Add fin clips measuring 2-25mmˆ2 to each well.

3. Add 40uL enzyme solution to each well, pipet up and down to mix.

4. Seal plate and incubate at 55C overnight.

Extract DNA from lysate

1. To a new plate add 45uL hybridization buffer to each well.
2. Add 15uL Agencourt Ampure beads.
3. Add 45uL of the lysate and pipet up and down to mix.
4. Incubate at room temperature for 5 minutes, then place on magnet and allow the wells to clear.
5. Remove supernatant.
6. Remove from the magnet and use 150uL freshly prepared 80% EtOH to resuspend the beads.
7. Wash with ethanol again
8. wash with ethanol a 3rd time.
9. Allow beads to air dry on the magnet
10. Add 100uL low TE to elute DNA from the beads