There is no 703

# 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