**RNA and DNA Extraction from CSF (Batch 03)**

15 CSF samples were selected for sequencing in batch 03 according to the criteria: volume, final diagnosis and cell count. 3 water controls and 1 one extracted control were also included. The table below entails the selected sample IDs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Batch SI** | **StudyID** | **AdmDate** | **TLC** | **RemVolume** | **Hprv4Ct** | **Remark** |
| 1 | 12204004541 |  | 60 | 830 | 20.57 | MRI Case |
| 2 | 12203026991 |  | 40 | 850 | 13.84 |  |
| 3 | 12203024741 |  | 20 | 2000 | 13.29 |  |
| 4 | 12201034831 | 17-Jan-22 | 350 | 500 | 17.5 |  |
| 5 | 12105004921 | 18-Apr-21 | 10 | 2000 | 19.62 |  |
| 6 | 12103015842 | 10-Mar-21 | 180 | 350 | 18.11 |  |
| 7 | 12101025462 | 23-Jan-21 | 20 | 800 | 17.58 |  |
| 8 | 12010030701 | 22-Oct-20 | 150 | 570 | 20.42 |  |
| 9 | 12010015311 | 24-Sep-20 | 60 | 800 | 19.28 |  |
| 10 | 12003023362 | 22-Mar-20 | 10 | 600 | 20.43 |  |
| 11 | 12003016171 | 23-Feb-20 | 300 | 2000 | 14.23 |  |
| 12 | 12002019032 | 16-Feb-20 | 10 | 750 | 18.52 |  |
| 13 | 12002002564 | 4-Feb-20 | 80 | 1200 | 14.62 |  |
| 14 | 12001029023 | 28-Jan-20 | 30 | 500 | 17.25 |  |
| 15 | 12001027713 | 27-Jan-20 | 20 | 400 | 19.72 |  |

**Date: 10.04.2022**

**Name of experiment: DNA/RNA extraction from CSF samples**

1. CSF sample was aliquoted in a 1.5ml Eppendorf tube. For samples having 400-600ul volume (1,2,3,5,7,9,11,13), 500ul was aliquoted from each sample. Aliquots were centrifuged at 14000rpm for 10 min to separate the serum and pellet down any cell debris.
2. Without touching the pellet, 200ul supernatant was added in 200ul of 2x RNA shield and mixed by pipetting.

* **Quick DNA/RNA Miniprep Plus kit was used for extraction.**

1. Master mix of 10ul proteinase K buffer and 5ul proteinase K was prepared. For 400ul sample volume, 30ul of master was added in each sample.
2. Incubation at 55C for 30min was followed.
3. Samples were centrifuged for 2 min at 16000g.
4. 400ul supernatant was added in 400ul DNA/RNA lysis buffer and mixed by pipetting.
5. 800ul was transferred in IC-XM (yellow) collection tube and centrifuged for 1 minute at 16000g
   * Due to mishandling of the centrifuge, the lids of samples 1,3,4,10, and 11 broke. These samples were excluded from the further procedure and stored in 200ul of 2x RNA shield at -80C for them to be extracted later. We continued with sample number- 2,5,6,7,8,10,12,13,14,15,16,17,18 and EC.
6. The flow through in the collection tube contains RNA and the column contains DNA.
7. The flow through was transferred to a new 1.5ml tube.
8. 800ul 100% ethanol was added to the flow through and mixed by pipetting.
9. 1600ul was transferred to the IC column (green) and centrifuged for 1min at 16000g.flow through was discarded.
10. Step 11 was repeated until all the sample passed through the column. The column contains RNA.
11. To RNA columns (green) only:
    * 800ul DNA/RNA wash buffer was added and centrifuged for 1 min at 16000g
    * Prepared master mix of 5ul DNase+35ul DNA digestion buffer. 80ul master mix was added in each RNA column and incubated for 15min at room temperature.
12. THE NEXT STEPS ARE FOR ALL COLUMNS (DNA AND DNase TREATED RNA)
13. 400ul DNA/RNA prep buffer was added and centrifuged for 1min at 16000g. Flow through was discarded.
14. 700ul DNA/RNA wash buffer was added and centrifuged for 1min at 16000g. Flow though was discarded.
15. 400ul DNA/RNA wash buffer was added and centrifuged for 2min at 16000g.
16. Columns were transferred to labelled (DNA or RNA) Eppendorf tubes.
17. 30ul nuclease free water was added straight to each of the columns and centrifuged at 16000g for 1min.
18. Flow through was put back into column and centrifuged for 1min at 16000g (double elution).

***Extracted DNA and RNA are kept at -80C.***