Genomic DNA prep with Quick Extract (QE) buffer

Hannah.Long [1]

[1]University of Edinburgh

Mar 22, 2022

1

protocol .

Hannah.Long

This protocol describes a high throughput extraction method to isolate genomic DNA for screening clones (e.g. from 24 well plate).

There are two options: i) you can split clones onto two plates and extract gDNA from one of those replica plates, or ii) during the process of splitting cells, you can keep some material for genotyping. This protocol describes the second option.

This protocol is for hESCs, but could work for other cell-types too.

https://www.lucigen.com/QuickExtract-DNA-Extraction-Solution/

Hannah.Long 2022. Genomic DNA prep with Quick Extract (QE) buffer . **protocols.io** https://protocols.io/view/genomic-dna-prep-with-quick-extract-qe-buffer-b6kkrcuw

genomic DNA, gDNA, QuickExtract, QE, clones, screening

protocol ,

Mar 22, 2022

Mar 22, 2022

59756

QuickExtract (QE) buffer

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231**

ReLeSR™ 100 mL **Stemcell Technologies Catalog #5872**

mTeSR™1 500 mL Kit **Stemcell Technologies Catalog #85850**

QuickExtract DNA Extraction Solution **Lucigen Catalog #QE09050**

Grow cells to around 50% confluency.

1 Grow clones on a multi-well format, e.g. 24-well plate.

2 Prepare new **matrigel** coated plates for passaging clones and label PCR strip tubes corresponding to the position of the clones on the plates. E.g. 1-A1 would refer to plate 1, position A1 on the plate.

3 When clones are ready to be split, remove media and add **ReLeSR** for **00:00:30** . Aspirate **ReLeSR** and leave in incubator for **00:03:00 (up to 5 minutes)** until colonies appear cracked.

3m 30s

4 In the meantime, prepare new plate by removing **matrigel** and adding some warmed **mTeSR1** media.

5 Add appropriate amount of warmed mTeSR1 media for splitting, and tap plate gently. Using a P1000, gently scratch well to release remaining cells and put half media/cells in PCR tube and half in new plate.

6 Tap new plate to distribute cells and return to incubator. Monitor the following day and replace media (may see a lot of death).

7 Pulse spin strip tubes on bench-top mini-centrifuge. You may see pellet of cells. Remove media and replace with **50 µL** **QuickExtract buffer**. Tap to dislodge pellet. Could pipette up and down.

8 Place in thermocycler with the following settings:

65°C 8-10 min

98°C 10 min

9 DNA samples are now ready to be used for PCR genotyping. For example, first try 1uL sample in a 10uL reaction (e.g. GXL PrimeStar - or Q5 polymerase). If PCR fails, try a dilution 1:5 of DNA, then 1:25 dilution etc.