Apr 17, 2020

gDNA Extraction with QuickExtract

DOI
dx.doi.org/10.17504/protocols.io.7kyhkxw

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DOI: https://dx.doi.org/10.17504/protocols.io.7kyhkxw

Protocol Citation: Eric Danner 2020. gDNA Extraction with QuickExtract. **protocols.io https://dx.doi.org/10.17504/protocols.io.7kyhkxw**

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Protocol status: Working
We use this protocol and it's working

Created: September 23, 2019

Last Modified: April 17, 2020

Protocol Integer ID: 28024

Keywords: gdna extraction with quickextract, gdna extraction, hundreds of gdna extract, gdna extract, gdna, quick extract solution, quickextract, uncounted small amount of cell, extraction

Abstract

This uses the Quick Extract solution to rapidly obtain gDNA.

I found this protocol on-line written by Patrick Hsu but can no longer find the original link. The protocol is nice because it does not require an extra vortexing step. It has worked well for hundreds of gDNA extracts.

For quick work I often pellet an uncounted small amount of cells and use 30ul QE and it has always worked. This has worked for me with mouse/human T and B cells, iPSCs, HeLa, K562.

Guidelines

Store initial bottle -20. Do not subject the QuickExtract Solution to repeated freeze/thaw cycles. Aliquot out small amounts and store -20. Upon using, store at 4C. I have had in fridge for 6 months and it still seemed fine.

Materials

MATERIALS

Epicentre QuickExtract™ DNA Extraction Solution **Epicentre Catalog #**QE09050

Troubleshooting

Pellet Cells

1 **Count cells if you want gDNA concentration.**
The quick extract interferes with the nanodrop and so quantification of gDNA is easier by adding a known volume to a known about of cells.

2N human cell = 6 pg gDNA
→ 100ng is 16,000 cells. (32k alleles)

For 100ul of 50ng/ul: 8,000,000 cells re-suspend in 100ul QuickExtract (this will give you roughly the right concentration)

Normally for quick genotyping I just pellet some cells then resuspend in 20-50ul QE. I do PCR with 35x cycles (PrimeStar GXL is best polymerase). This has always worked and is very low energy required.

Count cells and then pellet the desired amount in 96 well plate (2000rpm 2 min).

2 Wash once with PBS (or just tap dry). Then re-pellet if you did a wash.
Resus in QE, pipette up and down to mix, transfer to PCR strip.

*Often I don't wash and this hasn't seemed to create an issue during PCR. *

3 To extract the gDNA from the cells:
Put on thermocyclcer
65C for 15 min
68C for 15 min
98C for 10 min
Hold at 4C

*I have left at 4C for months and things work fine. Also I have frozen and thawed and it works fine.