Genomic DNA extraction and PCR

Mark Dewitt & Julia Wong

Abstract

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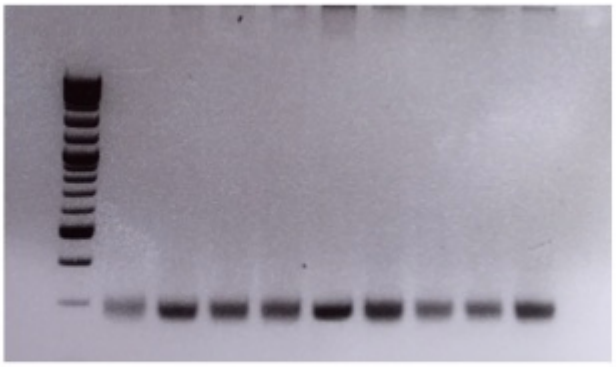
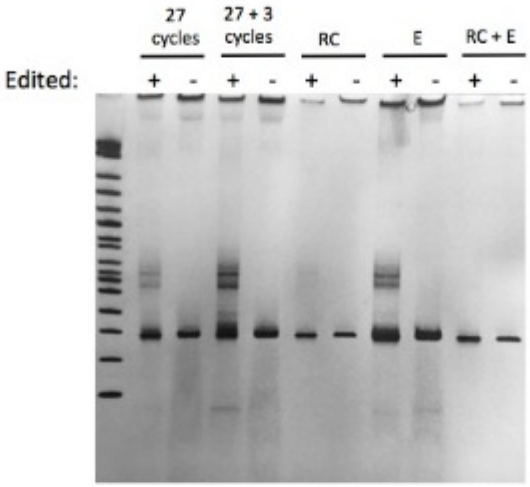
Guidelines

Example of two PCR'd gDNA extracts, one from a nucleofected cell and one from an unzapped control, saw heteroduplexing in the edited sample and tried to get rid of it by reconditioning and/or adding more polymerase.



2% agarose, HBB region PCR'd from K562 gDNA



RC = 10-fold dilution in master mix (same composition), additional 3-cycle PCR
E = add 2x amount of enzyme to original master mix
RC + E = 10-fold dilution in master mix with double amount of enzyme, additional 3-cycle PCR

Protocol

Extraction

Step 1.
Count cells

Extraction

Step 2.
Determine volume of cells and volume of QuickExtract DNA extraction solution needed to achieve concentration of 500K cells per 200 µL QE solution.

Extraction

Step 3.
Spin down appropriate volume of cells: 300xg, 10 minutes

DURATION
00:10:00

Extraction

Step 4.
Resuspend in appropriate volume of QE solution, transfer to 1.5mL tubes

Extraction

Step 5.
Pre-heat heating block to 65°C

Extraction

Step 6.
Vortex samples thoroughly for 15 seconds each.

DURATION
00:00:15

Extraction

Step 7.
Heat samples on block (65°C) for 6 minutes

DURATION
00:06:00

Extraction

Step 8.
Take samples off block, pre-heat block to 98°C (It's very important for block to reach this temperature! Not enough heat can lead to a failed extraction.)

Extraction

Step 9.
Vortex samples again, 15 seconds each.

DURATION
00:00:15

Extraction

Step 10.
Heat samples on block (98°C) for 2 minutes.

DURATION
00:02:00

Initial PCR

Step 11.
Mix for 1rxn

uL	component
29	H2O
10	5x Q5 Reaction Buffer (NEB)
1	10mM dNTPs
2.5	10uM Forward Primer
2.5	10uM Reverse Primer
0.5	Q5 DNA polymerase (NEB 2U/uL)
5	extract DNA

run PCR:
1.98C 30s
2.98C 10s
3.66C 30s
4. 72C 20s
5. #2-4, 27 cycles
6.72C 2min*

*This final extenstion time may not be necessary for our short amplicons, and might be contributing to unwanted re-annealing and heteroduplexing.

The products of this initial PCR often contain heteroduplexes that can interfere with downstream analysis. To get rid of heteroduplexes, we use a "reconditioning" step (see next.)

Reconditioning PCR

Step 12.
The products of this initial PCR often contain heteroduplexes that can interfere with downstream analysis. To get rid of heteroduplexes, we use a "reconditioning" step (see next.)

Reconditioning PCR

Step 13.
Reconditioning PCR
This is essentially a 10-fold dilution of the initial PCR products in more master mix.
Mix for 1rxn

uL	component
29	H2O
10	5x Q5 Reaction Buffer (NEB)
1	10mM dNTPs
2.5	10uM Forward Primer
2.5	10uM Reverse Primer
1.0*	Q5 DNA polymerase (NEB 2U/uL)
5	INITIAL PCR PRODUCT

*0.5uL is fine too, but we have seen that using double the concentration of polymerase in the reconditioning master mix helps to further eliminate heteroduplexing.

run PCR:
1.98C 30s
2.98C 10s
3.66C 30s
4. 72C 20s
5. #2-4, 27 cycles
no final extension

Step 14.
Gel: Add 1-2 µL 6X Purple Loading Dye to 5µL of reconditioned PCR pdt, run on either 2% agarose or 4-20% TBE acrylamide (to confirm successful elimination of heteroduplexes) at 180V for 40 minutes.

DURATION
00:40:00