Genomic DNA extraction and PCR V.1

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Works for me

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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.

**Extraction**

1. Count cells

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

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

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

5. Pre-heat heating block to 65°C

6. Vortex samples thoroughly for 15 seconds each.
   \[00:00:15\]

7. Heat samples on block (65°C) for 6 minutes
   \[00:06:00\]

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.)

9. Vortex samples again, 15 seconds each.
   \[00:00:15\]

10. Heat samples on block (98°C) for 2 minutes.
    \[00:02:00\]

**Initial PCR**

11. Mix for 1rxn

<table>
<thead>
<tr>
<th>µL</th>
<th>component</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>H2O</td>
</tr>
<tr>
<td>10</td>
<td>5x Q5 Reaction Buffer (NEB)</td>
</tr>
<tr>
<td>1</td>
<td>10mM dNTPs</td>
</tr>
<tr>
<td>2.5</td>
<td>10uM Forward Primer</td>
</tr>
<tr>
<td>2.5</td>
<td>10uM Reverse Primer</td>
</tr>
<tr>
<td>0.5</td>
<td>Q5 DNA polymerase (NEB 2U/µL)</td>
</tr>
<tr>
<td>5</td>
<td>extract DNA</td>
</tr>
</tbody>
</table>

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

*This final extension 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

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.)

This is essentially a 10-fold dilution of the initial PCR products in more master mix.

Mix for 1rxn

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<td>10uM Reverse Primer</td>
</tr>
<tr>
<td>1.0*</td>
<td>Q5 DNA polymerase (NEB 2U/uL)</td>
</tr>
<tr>
<td>5</td>
<td>INITIAL PCR PRODUCT</td>
</tr>
</tbody>
</table>

*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

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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.