The Fleischman Lab

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Title: CALR^{DEL} and CALR^{INS} Screening

Introduction

Calreticulin (CALR) mutations are common in patients with essential thrombocythemia (ET) and myelofibrosis (MF). The majority of patients with CALR mutations have either a 52-base pair deletion (CALR^{DEL}) or a 5-base pair insertion (CALR^{INS}) in exon 9. This protocol detects CALR^{DEL} and CALR^{INS} mutations, as well as other less common CALR deletion mutations (e.g. the 61-base pair deletion found in the Marimo cell line). The CALRgenoF1 + CALRgenoR primer set amplifies the muated region of the gene, while the CALRgenoF2 primer is specific to the 5-base pair insertion and, along with CALRgenoR, generates a smaller PCR product. CALR mutations may then be identified via PCR product size discrimination.

This protocol is optimized for genomic DNA purified from ACK pellets or cell lines using a commercial kit or our *Isolation of Genomic DNA from Peripheral Blood* protocol. DNA isolated from colonies grown in methylcellulose may also be used; for best results, pick colonies into 100µl of nuclease-free water and incubate in the thermal cycler at 97°C for 15 minutes with heated lid to lyse cells. Store plates at -20°C.

For more information, see Jeong, et al. (2016). Screening PCR versus Sanger sequencing: detection of CALR mutations in patients with thrombocytosis. *Annals of Laboratory Medicine, 36(4),* 291-299.

Materials

1. Nuclease-free water
2. Dream*Taq* green DNA polymerase and 10x PCR buffer
3. dNTPs (10mM)
4. Primers (10µM):
   - CALRgenoF1: 5'-GCAGCAGAGAAACAAATGAAGG-3'
   - CALRgenoF2: 5'-GCAGAGGACAATTGTCGGA-3'
   - CALRgenoR: 5'-AGAGTGGAGGAGGGGAACAA-3'
5. 96-well plates and seals or PCR tube strips
6. 1.5ml microcentrifuge tube or reagent reservoir
7. Thermal cycler
8. 2% DNA gel and low-range DNA ladder (25-700bp)

Protocol

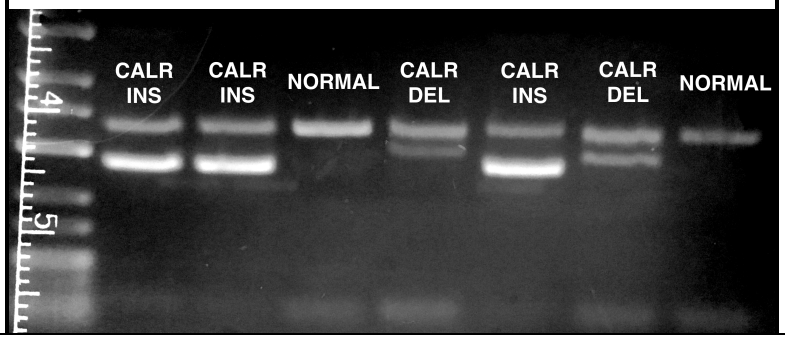
A. PCR

Notes

1. Allow DNA samples to thaw on ice. Prepare master mix as follows:

Reagent	1 well
10x PCR buffer	2 µl
dNTP mix (10 mM)	0.4 µl
CALRgenoF1 primer (10 µM)	0.5 µl
CALRgenoF2 primer (10 µM)	0.5 µl
CALRgenoR primer (10 µM)	1 µl
Nuclease-free H_2O	14.44 µl
DreamTaq DNA polymerase	0.16 µl
DNA (~10ng/µl)	1 µl

Be sure to make enough master mix for at least 1 additional well. For a full 96-well plate, make enough master mix for 110 wells and mix in a reagent reservoir.

<table>
<tr><td>2.</td><td>Transfer 19µl master mix to each well or tube. Add 1µl DNA per well. Seal plate well and briefly centrifuge in plate spinner.</td><td>Always balance plate spinner. For tube strips, spin down using the tube strip adapter for the minicentrifuge.</td></tr>
<tr><td>3.</td><td>Place plate into thermal cycler and run on the following program:
Lid temp: 105°C
<table><tr><td>1x</td><td>94°C</td><td>5 min</td></tr><tr><td>39x</td><td>94°C
64°C
72°C</td><td>30 sec
30 sec
30 sec</td></tr><tr><td>1x</td><td>72°C</td><td>7 min</td></tr><tr><td></td><td>4°C</td><td>∞</td></tr></table></td><td>For tube strips, be sure to use the green tube support insert.</td></tr>
<tr><td>4.</td><td>Prepare 2% agarose DNA gel(s):
Prepare 2 large gels for each 96-well plate. Each large gel uses 2 combs and is about 200-250ml. Use 12-25µl EtBr for each gel.
For fewer samples, prepare a small gel using the 8-well combs. Each small gel is about 50ml and uses 5µl EtBr.</td><td>Allow the gel to cool somewhat before adding EtBr! Add EtBr directly to the gel tray and use a comb to mix thoroughly.</td></tr>
<tr><td>1.</td><td>Run PCR and digested products on a 2% agarose gel alongside 8µl of low-range DNA ladder. Load 15-20µl PCR product and/or 25-30µl digested product.
 
</td><td>CALR^{WT} = 357 bp
CALR^{INS} = 272 bp
CALR^{DEL} = 302 bp (varies based on patient's specific mutation; this estimate reflects the 52-bp deletion, which is most common)</td></tr>
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