Glucosylceramidase Beta (*GBA*) Genotyping

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ABSTRACT

This protocol details the steps for GBA genotyping. This protocol has been adapted from the PRoBaND Clinical Consortium (incorporating methods described by Neuman et al., 2009 and Stone et al., 2000) and has been used for all publications for PRoBaND / Tracking Parkinson's describing clinical data and outcomes with respect to GBA status

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KEYWORDS

Amplification, GBA gene, Genotyping, Intronic Regions, Exons, Parkinson's disease, PCR, Sequencing, ASAPCRN

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GUIDELINES

- To avoid amplification of the pseudogene, design primer sequences to DNA regions exclusively found within the *GBA* gene.
- As an internal control, calculate the size of the PCR products resulting from amplification of the *GBA* pseudogene.
- Refer to Table 2 (Steps) for the PCR conditions for amplification of the *GBA* gene.

MATERIALS TEXT

1. Fast start PCR master mix (Roche) - https://lifescience.roche.com/en_gb/products/faststart-essential-dna-probes-master.html
2. Primers
3. Deionised water
4. Genomic DNA template (50 ng/µl)

SAFETY WARNINGS

Ethidium bromide is hazardous chemical. It is a risk to the environment, toxic by inhalation and highly toxic as a mutagen. Refer to the product safety data sheet and institutional health & safety policies and procedures and risk assessments.

BEFORE STARTING

Check that you have the correct forward and reverse primers and required materials, supplies and equipment.

Primer Sequences

1

Use the following primer sequences:

	Forward primer	Reverse primer
Fragment 1 (exons 1-5)	5'-CCTAAAGTTGTCACCCATAC-3'	5'-AGCAGACCTACCCTACAGTTT-3'
Fragment 2 (exons 5-7)	5'-GACCTCAAATGATATACCTG-3'	5'-AGTTTGGGAGCCAGTCATTT-3'
Fragment 3 (exons 8-11)	5'-TGTGTGCAAGGTCCAGGATCAG-3'	5'-ACCACCTAGAGGGGAAAGTG-3'

Table 1: Forward and reverse primers used by Stone DL, Tayebi N, Orvisky E, Stubblefield B, Madike V, Sidransky E. Glucocerebrosidase gene mutations in patients with type 2 Gaucher disease. Hum Mutat. 2000;15(2):181-8. doi: 10.1002/(SICI)1098-1004(200002)15:2<181::AID-HUMU7>3.0.CO;2-S. PMID: 10649495.

Amplification of the *GBA* gene

2

Three different PCR reactions are performed. Refer to Table 2 for a detailed description of the PCR conditions used for each fragment.

2.1 In order to avoid amplification of the pseudogene, primer sequences are

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designed to bind to DNA regions exclusively found within the *GBA* gene. As an internal control, calculate the size of the PCR products resulting from amplification of the pseudogene for these three fragments. Confirm they are an alternative size to those amplified from *GBA*.

2.2 Amplify three distinct fragments spanning all exonic and most intronic sequences of *GBA*.

2.3 PCR conditions

	Initial PCR Conditions	Optimized PCR Conditions
Fragment 1[a]	94°C-15min 94°C-0:45min 60°C-0:45min 37 cycles 72°C-3min 72°C-7min 4°C-hold	94°C-15min 94°C-0:45min 58°C-0:45min 37 cycles 72°C-3min 72°C-7min 4°C-hold
Fragment 2[b,d]	94°C-15min 94°C-0:45min 62-60°C-0:45min 20 cycles 72°C-3min 94°C-0:45min 60°C-0:45min 18 cycles 72°C-3min 72°C-7min 4°C-hold	94°C-15min 94°C-0:45min 61°C-0:45min 37 cycles 72°C-3min 72°C-7min 4°C-hold
Fragment 3[c,d]	94°C-15min 94°C-0:45min 62-60°C-0:45min 20 cycles 72°C-3min 94°C-0:45min 60°C-0:45min 18 cycles 72°C-3min 72°C-7min 4°C-hold	94°C-15min 94°C-0:45min 62-60°C-0:45min 20 cycles 72°C-3min 94°C-0:45min 60°C-0:45min 18 cycles 72°C-3min 72°C-7min 4°C-hold

[a]Fragment 1-5 spans the region from exon 1 to exon 5
[b]Fragment 5-7 spans the region from exon 5 to exon 7
[c]Fragment 8-11 spans the region from exon 8 to exon 11
[d]For fragment 8-11 and the initial PCR for fragment 5-7 a touch down PCR was performed, decreasing the annealing temperature by 0.1°C in each cycle for 20 cycles.

Table 2: PCR conditions for amplification of the *GBA* gene. Run all PCR products on a 1% agarose gel with ethidium bromide to confirm successful amplification of the *GBA* gene and to rule out accidental amplification of the *GBA* pseudogene (*GBAP*).

PCR

3

Prepare the following reagents for the PCR in a total reaction volume of **15 µL** :

1. **7.5 µL** fast start PCR master mix (Roche)
2. **1 µL** of 10 µM Forward primer

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3. **4.5 µL** Deionised water
4. **1 µL** Genomic DNA template (50 ng/µl)

4

Run all PCR products on a 1% agarose gel with ethidium bromide and size check to rule out amplification of the *GBA* pseudogene *(GBAP)*.

Ethidium bromide is a hazardous chemical. It is a risk to the environment, toxic by inhalation and highly toxic as a mutagen. Refer to the product safety data sheet, institutional health & safety policies, procedures and risk assessments.

5 If the initial primers failed or showed an incomplete read then and alternative set of primers can be used:
Table 3.

Exon	Forward Primer	Reverse Primer
1	5'-ctgtgtcatgtgacgctcct-3'	5'-cagtgccaggattccagaag-3'
2	5'-cctgcccaggagagtagttg-3'	5'-ctctgtgctacctccccact-3'
3	5'-caaggggtgaggaattttga-3'	5'-tatcagtacccagcgggaaa-3'
4	5'-ttcccgctgggtactgatac-3'	5'-gacagaatgggcagagtgaga-3'
5	5'-caggagcccaagttccc-3'	5'-tgtctgtacaagcagacctaccc-3'
6	5'-gctgaaccggatgcactg-3'	5'-gctaaatgggaggccagtc-3'
7	5'-acacccagctggtctggtc-3'	5'-tggatgctggatttgaaggt-3'
8	5'-agttccagaagcctgtgtgc-3'	5'-cttctgtcagtctttggtgaaa-3'
9	5'-cccacatgtgacccttacct-3'	5'-tgtaggagatgataggcctggt-3'
10 + 11	5'-gggtccgtgggtgggt-3'	5'-tgctgtgccctctttagtca-3'

[1]The primers shown here were only used if sequencing with the initial primers (Stone *et al.*, 2000) failed or showed an incomplete read.

Table 3: Alternative set of *GBA* sequencing primers reported by Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, Li A, Holton J, Guerreiro R, Paudel R, Segarane B, Singleton A, Lees A, Hardy J, Houlden H, Revesz T, Wood NW. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. Brain. 2009 Jul;132(Pt 7):1783-94. doi: 10.1093/brain/awp044. Epub 2009 Mar 13. PMID: 19286695; PMCID: PMC2702833.

6 Perform cycle sequencing for each exon and the flanking intronic sequences using the Dye Terminator Sequencing Kit (Applied Biosystems) and run on an ABI 3700xl genetic analyzer (Applied Biosystems).

7 Sequence all amplicons both in the forward and the reverse direction.

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8

Analyse sequence chromatograms using Sequencher software (Genecodes).

When this sequencing was carried out NM_001005749 was used.

This RefSeq entry was permanently suppressed because currently there is insufficient support for the transcript and the protein.

We would now recommend used of variant nomenclature based on the Human Genome Variation Society guidelines (den Dunnen et al., 2016) using GenBank reference sequence NM_000157.4 - describing a 536 amino acid form for glucocerbrosidase.

NB The traditional numbering for GBA missense mutations omits the first 39 amino acids of the current accepted transcript.

9

Analyse all exons and the flanking intronic regions when clean, complete sequence reads are obtained.

Key references

Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, Li A, Holton J, Guerreiro R, Paudel R, Segarane B, Singleton A, Lees A, Hardy J, Houlden H, Revesz T, Wood NW. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. Brain. 2009 Jul;132(Pt 7):1783-94. doi: 10.1093/brain/awp044. Epub 2009 Mar 13. PMID: 19286695; PMCID: PMC2702833.

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