

University of Groningen

Intramolecular Activation Mechanism of the Dictyostelium LRRK2 Homolog Roco Protein GbpC

Egmond, Wouter N. van; Kortholt, Arjan; Plak, Katarzyna; Bosgraaf, Leonard; Bosgraaf, Sylvia; Keizer-Gunnink, Ineke; Haastert, Peter J.M. van

Published in:
The Journal of Biological Chemistry

DOI:
[10.1074/jbc.M804265200](https://doi.org/10.1074/jbc.M804265200)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Egmond, W. N. V., Kortholt, A., Plak, K., Bosgraaf, L., Bosgraaf, S., Keizer-Gunnink, I., & Haastert, P. J. M. V. (2008). Intramolecular Activation Mechanism of the Dictyostelium LRRK2 Homolog Roco Protein GbpC. *The Journal of Biological Chemistry*, 283(44). <https://doi.org/10.1074/jbc.M804265200>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Supplementary Information

Primer	Sequence (5'-3')	Restriction sites	Amino acid coverage
Fw1	GGGATCC AAAAAAAT <u>G</u> TCCAAAAACAAAAACCAAT	<i>Bam</i> HI	1-524
Rv1	<u>GTTATCTAGATGGTACCTTCTCTTTGAGATAAGGTTGAG</u>	<i>Kpn</i> I, <i>Xba</i> I	
Fw2	GGGTACCAAGTAGTTTCTTTACACTC	<i>Kpn</i> I	522-868
Rv2	GGATATCTACCATTGAAATATCTG	<i>Eco</i> RV	
Fw3	GGGATCC AAAAAT <u>G</u> GATATCCAATACCAAGATTTAATTTAAG	<i>Bam</i> HI, <i>Eco</i> RV	867-1160
Rv3	<u>GTTATCTAGAACCTAGGATTTGACAATACTTGTA</u> AAAAG	<i>Avr</i> II, <i>Xba</i> I	
Fw4	GCCTAGGTCAAATTTAAAAGAAATTTGC	<i>Avr</i> II	1159-1411
Rv4	GTGGTACCGCATTTTTACCCGCTGTATCTGAT	<i>Kpn</i> I	
Fw5	GAGATCT AAAAAT <u>G</u> GCGGTACCACCACATTGGAGAAT	<i>Bgl</i> II, <i>Kpn</i> I	1408-1615
Rv5	<u>GTTATCTAGAAAGTCGCGAGATTTAAAATACTCTTGATAC</u>	<i>Nru</i> I, <i>Xba</i> I	
Fw6	GAGATCT AAAAAT <u>G</u> GCTCGCGACTGGTATGAGAGATCCATT	<i>Bgl</i> II, <i>Nru</i> I	1613-2008
Rv6	<u>GTTATCTAGAACTCGAGGTCAAACCACCACCATTCTC</u>	<i>Xho</i> I, <i>Xba</i> I	
Fw7	GAGATCT AAAAAT <u>G</u> ACCTCGAGTAATTTCTTTGGTAATGGTTC	<i>Bgl</i> II, <i>Xho</i> I	2005-2334
Rv7	<u>GTTATCTAGAAAGTCGACGTTTGAGTACGACCTAAATAAG</u>	<i>Sal</i> I, <i>Xba</i> I	
Fw8	GAGATCT AAAAAT <u>G</u> ACGTCGACTTCACCATTGAATGAAGG	<i>Bgl</i> II, <i>Sal</i> I	2331-2631
Rv8A	GTCTAGATTA AGCATAAAGTTGTGATTC	<i>Xba</i> I	
Rv8B	GTCTAGA AGCATAAAGTTGTGATTCTCT	<i>Xba</i> I	

SI Table I: Cloning of *gbc* cDNA in *Dictyostelium* expression vectors.

gbc cDNA was amplified with PCR from cDNA in eight parts of approximately equal size. Part 8 was amplified in two versions that differ in the presence of a Stop codon, to discriminate for expression of GbpC, and a GbpC-GFP fusion construct. Sequences in red represent restriction sites for direct cloning of separate parts in MB74-expression plasmids, sequences in bold represent restriction sites for fusion of parts, and Start/Stop codons are underlined.