

|                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | MRKVISMLLVVAMLTTIFAAMIPQTVSAATMTVEIGKVTAAVGSKVEIPITLKGVP SKGMANCDFVLGYDPNVLEVTEVVKPGSIIKDPDP SKSFDSAIYP<br>MRKVISMLLVVAMLTTIFAAMIPQTVSAATMTVEIGKVTAAVGSKVEIPITLKGVP SKGMANCDFVLGYDPNVLEVTEVVKPGSIIKDPDP SKSFDSAIYP<br>MRKVISMLLVVAMLTTIFAAMIPQTVSAATMTVEIGKVTAAVGSKVEIPITLKGVP SKGMANCDFVLGYDPNVLEVTEVVKPGSIIKDPDP SKSFDSAIYP<br>MRKVISMLLVVAMLTTIFAAMIPQTVSAATMTVEIGKVTAAVGSKVEIPITLKGVP SKGMANCDFVLGYDPNVLEVTEVVKPGSIIKDPDP SKSFDSAIYP<br>MRKVISMLLVVAMLTTIFAAMIPQTVSAATMTVEIGKVTAAVGSKVEIPITLKGVP SKGMANCDFVLGYDPNVLEVTEVVKPGSIIKDPDP SKSFDSAIYP<br>MRKVISMLLVVAMLTTIFAAMIPQTVSAATMTVEIGKVTAAVGSKVEIPITLKGVP SKGMANCDFVLGYDPNVLEVTEVVKPGSIIKDPDP SKSFDSAIYP                                                                                                                        | 100<br>100<br>100<br>100<br>100<br>100    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | DRKMIVFLFAEDSGRGTYAITQDGVFATIVATVKSAAAAPITLLEVGA FADNDLVEISTTFVAGGVNLGSSVP TTPQNPVPSDGVVVEIGKVTG SVGTTTVE<br>IPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKS FDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITTFDEVG<br>DRKMIVFLFAEDSGRGTYAITQDGVFATIVATVKSAAAAPITLLEVGA FADNDLVEISTTFVAGGVNLGSSVP TTPQNPVPSDGVVVEIGKVTG SVGTTTVE<br>DRKMIVFLFAEDSGRGTYAITQDGVFATIVATVKSAAAAPITLLEVGA FADNDLVEISTTFVAGGVNLGSSVP TTPQNPVPSDGVVVEIGKVTG SVGTTTVE<br>DRKMIVFLFAEDSGRGTYAITQDGVFATIVATVKSAAAAPITLLEVGA FADNDLVEISTTFVAGGVNLGSSVP TTPQNPVPSDGVVVEIGKVTG SVGTTTVE<br>DRKMIVFLFAEDSGRGTYAITQDGVFATIVATVKSAAAAPITLLEVGA FADNDLVEISTTFVAGGVNLGSSVP TTPQNPVPSDGVVVEIGKVTG SVGTTTVE<br>DRKMIVFLFAEDSGRGTYAITQDGVFATIVATVKSAAAAPITLLEVGA FADNDLVEISTTFVAGGVNLGSSVP TTPQNPVPSDGVVVEIGKVTG SVGTTTVE | 200<br>200<br>200<br>200<br>200<br>200    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | IPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKS FDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITTFDEVG<br>IPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKS FDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITTFDEVG<br>IPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKS FDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITTFDEVG<br>IPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKS FDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITTFDEVG<br>IPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKS FDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITTFDEVG<br>IPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKS FDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVF AKIRATVKSSAPGYITTFDEVG                                                                                                                        | 300<br>300<br>300<br>300<br>300<br>300    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | GFADNDLVEQKVSFIDGGVNVGNATPTKGATP TINTATPTKSATATPTRPSVP TINTPTNPANTPVSGNLKVEFYN SNPSD TTNSINPQFKVINTGSSAID<br>GFADNDLVEQKVSFIDGGVNVGNATPTKGATP TINTATPTKSATATPTRPSVP TINTPTNPANTPVSGNLKVEFYN SNPSD TTNSINPQFKVINTGSSAID<br>GFADNDLVEQKVSFIDGGVNVGNATPTKGATP TINTATPTKSATATPTRPSVP TINTPTNPANTPVSGNLKVEFYN SNPSD TTNSINPQFKVINTGSSAID<br>GFADNDLVEQKVSFIDGGVNVGNATPTKGATP TINTATPTKSATATPTRPSVP TINTPTNPANTPVSGNLKVEFYN SNPSD TTNSINPQFKVINTGSSAID<br>GFADNDLVEQKVSFIDGGVNVGNATPTKGATP TINTATPTKSATATPTRPSVP TINTPTNPANTPVSGNLKVEFYN SNPSD TTNSINPQFKVINTGSSAID<br>GFADNDLVEQKVSFvg*.....▲.....                                                                                                                                                                                         | 400<br>400<br>400<br>400<br>388<br>316    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | LSKLTlRYYTVDGQKQDTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSN DYSFKSAS<br>LSKLTlRYYTVDGQKQDTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSN DYSFKSAS<br>LSKLTlRYYTVDGQKQDTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSN DYSFKSAS<br>LSKLTlRYYTVDGQKQDTFWCDHAAIIGSNGSYNGITSNVKGT FVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAKNDWSNYTQSN DYSFKSAS<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                    | 500<br>500<br>500<br>500<br>388<br>316    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | <div>CipAup</div> QFVEWDQVTAYLNGVLVWGKEPGGSVVPSTQPV TTPPATTKPPATTKPPATTIPPSSDDPNAIKIKVDTVNAKPGDTVNI PVRFSGIPSKGIANCDFVYSY<br>QFVEWDQVTAYLNGVLVWGKEPGGSVVPSTQPV TTPPATTKP.....PATTIPPSSDDPNAIKIKVDTVNAKPGDTVNI PVRFSGIPSKGIANCDFVYSY<br>QFVEWDQVTAYLNGVLVWGKEPGGSVVPSTQPV TTPPATTKP.....PATTIPPSSDDPNAIKIKVDTVNAKPGDTVNI PVRFSGIPSKGIANCDFVYSY<br>QFVEWDQVTAYLNGVLVWGKEPGGSVVPSTQPV TTPPATTKP.....PATTIPPSSDDPNAIKIKVDTVNAKPGDTVNI PVRFSGIPSKGIANCDFVYSY<br>.....<br>.....                                                                                                                                                                                                                                                                                                             | 600<br>594<br>594<br>594<br>388<br>316    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | DPNVLEIIIEIKP GELIVDPNPKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGV<br>DPNVLEIIIEIKP GELIVDPNPKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGV<br>DPNVLEIIIEIKP GELIVDPNPKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQRTQFFDGGV<br>DPNVLEIIIEIKP GELyres*.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                           | 700<br>694<br>694<br>614<br>388<br>316    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | NVGDTTVPTTPTTPVTPTTDDSNAVRIKVDTVNAKPGDTVRI PVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGDIIVDPNPKS FDTAVYPDRKII V<br>NVGDTTVPTTPTTPVTPTTDDSNAVRIKVDTVNAKPGDTVRI PVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGDIIVDPNPKS FDTAVYPDRKII V<br>NVGDTTVPTTPTTPVTPTTDDSNAVRIKVDTVNAKPGDTVRI PVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGDIIVDPNPKS FDTAVYPDRKII V<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                              | 800<br>794<br>794<br>614<br>388<br>316    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | FLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTTEPATPTTPVTTP TTTTDDLDAVRIKVDTVNAK<br>FLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQFFD G.....<br>FLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQFFD G.....<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 900<br>855<br>855<br>614<br>388<br>316    |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | PGDTVRI PVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGDIIVDPNPKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKSGAPNGL<br>.....<br>.....<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 1000<br>855<br>855<br>614<br>388<br>316   |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | SVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTTEPATPTTPVTTP TTTTDDLDAVRIKVDTVNAKPGDTVRI PVRFSGIPSKGIANCDFVYSYDPNVLEI<br>.....<br>.....<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 1100<br>855<br>855<br>614<br>388<br>316   |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | IEIEPGDIIVDPNPKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTTE<br>.....<br>.....<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1200<br>855<br>855<br>614<br>388<br>316   |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | PATPTTPVTTP TTTTDDLDAVRIKVDTVNAKPGDTVRI PVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGELIVDPNPTKS FDTAVYPDRKMIVFLFAE<br>.....<br>.....<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1300<br>855<br>855<br>614<br>388<br>316   |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | DSGTGAYAITEDGVFATIVAKVKSGAPNGLSVIKFVEVGGFANNDLVEQKTQFFDGGVNVGDTTTEPATPTTPVTTP TTTTDDLDAVRIKVDTVNAKPGDTV<br>.....<br>.....<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1400<br>899<br>899<br>614<br>388<br>316   |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | RIPVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGDIIVDPNPKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKF<br>RIPVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGDIIVDPNPTKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKF<br>RIPVRFSGIPSKGIANCDFVYSYDPNVLEIIIEIEPGDIIVDPNPTKS FDTAVYPDRKIIVFLFAEDSGTGAYAITKDGVFATIVAKVKEGAPNGLSVIKF<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1500<br>999<br>999<br>614<br>388<br>316   |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | VEVGGFANNDLVEQKTQFFDGGVNVGDTTVPTTSP TTTTPEPTITPNKLTlKIGRAEGRPGDTVEIPVNLYGVPQKGIASGDFVVSYPNVLEIIIEIEPG<br>VEVGGFANNDLVEQKTQFFDGGVNVGDTTVPTTSP TTTTPEPTITPNKLTlKIGRAEGRPGDTVEIPVNLYGVPQKGIASGDFVVSYPNVLEIIIEIEPG<br>VEVGGFANNDLVEQKTQFFDGGVNVGDTTVPTTSP TTTTPEPTITPNKLTlKIGRAEGRPGDTVEIPVNLYGVPQKGIASGDFVVSYPNVLEIIIEIEPG<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1600<br>1099<br>1099<br>614<br>388<br>316 |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | ELIVDPNPTKS FDTAVYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKEGAPGFS AIEISEFGAFADNDLVEVETDLINGVLVTNKP VIEGYKV<br>ELIVDPNPTKS FDTAVYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKEGAPGFS AIEISEFGAFADNDLVEVETDLINGVLVTNKP VIEGYKV<br>ELIVDPNPTKS FDTAVYPDRKMIVFLFAEDSGTGAYAITEDGVFATIVAKVKEGAPGFS AIEISEFGAFADNDLVEVETDLINGVLVTNKP VIEGYKV<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1700<br>1199<br>1199<br>614<br>388<br>316 |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | <div>CipA down</div> SGYILPDFSF DATVAPLVKAGFKVEIVGT ELYAVTDANGYFEITGVPANASGYTLKISRATYLD RVIANVVVTGDTSVSTSQAPIMMWGDIVKDNSINL<br>SGYILPDFSF DATVAPLVKAGFKVEIVGT ELYAVTDANGYFEITGVPANASGYTLKISRATYLD RVIANVVVTGDTSVSTSQAPIMMWGDIVKDNSINL<br>SGYILPDFSF DATVAPLVKAGFKVEIVGT ELYAVTDANGYFEITGVP Acdakasqmi vplaqgartgpvvppiva*.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                      | 1800<br>1299<br>1274<br>614<br>388<br>316 |
| ATCC27405 CipA<br>DSM1313 (wT) CipA<br>CipA-ΔXDocII (CipA3740s)<br>CipA-Δ6CohI (CipA1827s)<br>CipA-ΔCBM-1 (CipA1158a)<br>CipA-ΔCBM-2 (CipA942a) | LDVAEIVRCFNATKGSANYVEELDINRNGAINM QDIMIVHKHFGATSSDYDA*<br>LDVAEIVRCFNATKGSANYVEELDINRNGAINM QDIMIVHKHFGATSSDYDA*<br>.....<br>.....<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 1852<br>1351<br>1274<br>614<br>388<br>316 |

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Additional file 4. Amino acid sequences of the CipA proteins of *C. thermocellum* strains.

The wild-type CipA sequences are from the genome sequence of *C. thermocellum* ATCC27405 (accession number YP\_001039466) and *C. thermocellum* DSM 1313 (accession number ADU73707), which may contain two more CohI modules according to our PCR analysis (Additional file 1). The horizontal arrows ahead of amino acids 509-517 and amino acids 1715-1723 indicate the binding sites of the PCR primers used to investigate the difference between *cipA* genes of ATCC27405 and DSM1313 (Additional file 1 and 2). Black triangles indicate the insertion sites of the targettrons, and the amino acids derived from intron sequences in frame with *cipA* are in lower case. The XDocII target peptide sequence at the C-terminus of CipA of WT is framed in a black box.