

BRUSH MPQFDKDGVPVLLT--HAQQSDEFMDSNCRRLSYRTRSAS-ISIPMVPIEPYEGGTHLVGHTGPLRSVRKPPSGQMSGPLYATTAGA  
LJCNGC.IVA3 .A.....E.M.S...R...F..LE.F.V..F.S.....M.....SM...DTKPN.....LK.T.FV.....P..T  
LJCNGC.IVA4 .ASSENNEH.T...A--EE.SYG.PLHTRFQ..ASN.Q...C.....S..S..KE.S..KQRS.....T  
LJCNGC.IVA5 .AN.KN.E..M.A..KAR.RPG.GPF..KFQ..VT..Q...T..V.LSSM.Q...D.SVIR.....Q.K.NA.LM.....K..P..T

\* CD

BRUSH GNHFQHSIAVPGKKAVERGKTQQLSTFDGTDENLWNNYDRKNEHLLRSGQLGMCNDPYCTTCPTYIKASQKG--NPKVSTVFDKSFHN  
LJCNGC.IVA3 .L.L.....A.E.TA.SN.DDF.....S...H.....N.....F.P...R.....A.....P...  
LJCNGC.IVA4 .LL.Q..CLT.N.PE.SN.GSI.....YSDK...N..K...R.V.I...CF..YA.NS...HSQLVY..A..IS...  
LJCNGC.IVA5 A.LL.QH.IGI.NR.E.R..ENFAA.RS.GP.Y.D..H.....FN...QR..K..P..I..H...

TM1 TM2

BRUSH SLYGEAKGFGRLKFSFCSSCVPGVMPNPHAKVVQWKNFLAMFCLMAIFVDPLFFFLFYVRKDSNCIVIDLTMTKILLLLRSINDVVYL  
LJCNGC.IVA3 F..D.....L.L.....H.....QI.GI.F.V.....I..K...K..A.NW...TIVS.....F  
LJCNGC.IVA4 A..D.....A.NHN..Y..Y.....T.FI...V..I...V.....L..QR.F...A.NWRL..A.VIV.....FI.F  
LJCNGC.IVA5 V..D.....A..YL...YF.....T..I.....I.....L..QQ.YK...NWA..IG.V...F...

TM3 TM4

BRUSH LNILLQFRLAYVSPSTVVGAGDLVDNPKKIALNYVKGYFFDFLVVSPPLPQIMILFVLPTSLGSPGPNSTKNVLHVAVLLQYVPRL  
LJCNGC.IVA3 F.....H.....L.....L.....L.....N...S.A...L.RA...V..I...  
LJCNGC.IVA4 F...V.....R.....H.....H.L.....I..F.....L.L...E.M..R.A.YA..L.RAVI.V..I...  
LJCNGC.IVA5 ..F..K.....H..R..RH.LR...LI...I..F.....T..NT..W.A.HA..L.RA.I.V..I.K.

TM5

BRUSH FRFLPLLIGQSPTGFI FESA WNFVINLLIFMLSGHVVGSCWYLFGLQRVNQCLQNCHSSIKH--GCTELIDCDSRM-----GQM  
LJCNGC.IVA3 ..V.....A..I.....S.....RDA..R.NISNMI..M.....PK.....ANL  
LJCNGC.IVA4 ..A..I.....Y..AS.....FR.A...SVP...LRF...GIDHDRNYLQ.PK  
LJCNGC.IVA5 ..A.....R.A.S.....RDA..K.NIT...MKF...GRGRGLN...NT

F TM6

BRUSH SAMWRNNTNATACLNSTS-GSPYGIYDRAVALTTETKVVKYVFALFWGFQQISTLAGNQNPSPYFEWEVLFMTAIIIGLGLLLFALLI  
LJCNGC.IVA3 .RL.SI.E.....D.A..NA.....K.....R..N..Y.....E..V.....S.....  
LJCNGC.IVA4 .EQ.N...D.I..WG.P.T...D...GN..P..K.DM.T...S.....LT..N.V.....T...S.....  
LJCNGC.IVA5 .NQ.K...D.IN...PP.D.G.....EL..P..I..N..N..YS.....I..V...V.....S.M.....I...

GNIQNFLQALGRRRLEMQLRGRDVEQWMSHRRLEPLRRKVRQAERYSWAATRGVNEEMLENLPEDLQTDIRRHLEKFAKKVRI FAL

BRUSH GNINQNFLQALGRRRLEMQLRGRDVEQWMSHRRLEPLRRKVRQAERYSWAATRGVNEEMLENLPEDLQTDIRRHLEKFAKKVRI FAL  
LJCNGC.IVA3 .....G.....Q.....R..F.....RY...D...R.....T.....MV.....R.....V...L.S.  
LJCNGC.IVA4 .....K.....K.....Q.....I.....VD..R.....N.....VM.....R.....R.V.EI...S.  
LJCNGC.IVA5

CNBD

BRUSH MDEPILDAIRERLKQKTYIKGSRI LSRGSLVEKMFVVRGKLESIGEDGIGVPLSEGDACGEELLTWYLENSSVSKDGKKVRLPGQRL  
LJCNGC.IVA3 .....C.....T...Q...V.NK.G.....A.....TR...V.....H.....T...IK...G.  
LJCNGC.IVA4 .....VC...R.....K...Q...I.....TRM.....M.....H.....S..R.....  
LJCNGC.IVA5

BRUSH LSNRTVRCCLTNVEAFSLHAADLEEVTFLFTRFLRSPQVQGALRYESPWRS LAANRIQVAWRYRKRLGRARANISQSDQTPKS  
LJCNGC.IVA3 Q.....K.....G...R.....I.....D.....S...NTSQGL.....  
LJCNGC.IVA4 ..S..IK.....R...R...L...H..R.....T.....QN..HSSL.G.....  
LJCNGC.IVA5 V.....K.....S.....I.....Q...S.VNSSVTTKH.....

**Figure 1-figure supplement 3. Protein sequence comparison of Group IVA CNGCs from *Lotus japonicus*.** Protein sequence alignment of BRUSH (CNGC.IVA1), CNGC.IVA3, CNGC.IVA4, and CNGC.IVA5. Residues identical to BRUSH are shown as dots and gaps are shown as dashes. Shown is the Group IVA CNGC conserved domain (CD, green), *brush* mutation (red asterisk), predicted transmembrane domains (TM, light blue), putative selectivity filter (F, orange) in the pore region, and the cyclic nucleotide-binding domain (CNBD, purple). CNGC.IVA3 (81%), CNGC.IVA4 (65%), and CNGC.IVA5 (72%) show high sequence identity to BRUSH.