

# **CPAP3 proteins in the mineralized cuticle of a decapod crustacean**

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## Supplementary Tables

**Table S1.** Names and sequences of the different primers used in this research

| Primer           | Sequence 5'- 3'              |
|------------------|------------------------------|
| T7P              | TAATACGACTCACTATAGGG         |
| dsCqCPAP3A-F     | TGAGGGCTCTTCCCTACAGA         |
| dsCqCPAP3A-R     | AGATTAGTCCGCCAGCCTTT         |
| dsCqCPAP3A-F+T7  | T7P-TGAGGGCTCTTCCCTACAGA     |
| dsCqCPAP3A-R+T7  | T7P-AGATTAGTCCGCCAGCCTTT     |
| dsCqCPAP3E-F     | ACCCAGAACTTCACCTGTCC         |
| dsCqCPAP3E-R     | CCTCCTCCTCTGTGGTCTGT         |
| dsCqCPAP3E-F+T7  | T7P-ACCCAGAACTTCACCTGTCC     |
| dsCqCPAP3E-R+T7  | T7P-CCTCCTCCTCTGTGGTCTGT     |
| dsCqCPAP3B1-F    | TGCTGATCCAACAGACTGTCA        |
| dsCqCPAP3B1-R    | GTCGCTTTGGAACCGTGT           |
| dsCqCPAP3B1-F+T7 | T7P- TGCTGATCCAACAGACTGTCA   |
| dsCqCPAP3B1-R+T7 | T7P- GTCGCTTTGGAACCGTGT      |
| dsCqCPAP3B2-F    | TGAAGCTCTTAGTCTGCCTACTG      |
| dsCqCPAP3B2-R    | GCAGGAGAACCCAAGGTAGG         |
| dsCqCPAP3B2-F+T7 | T7P- TGAAGCTCTTAGTCTGCCTACTG |
| dsCqCPAP3B2-R+T7 | T7P- GCAGGAGAACCCAAGGTAGG    |

**Table S2.** Accession numbers of newly found CPAP3 proteins in different crustaceans.

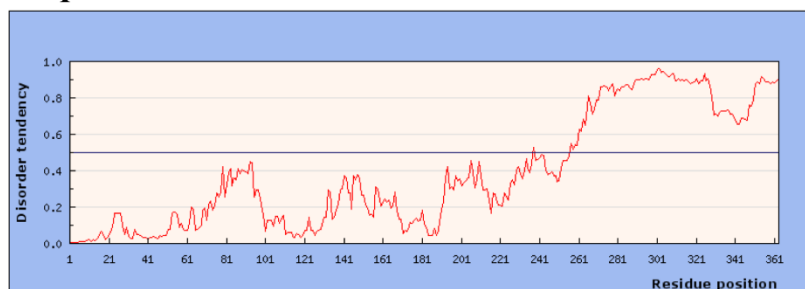
| CPAP3 name       | Accession number |
|------------------|------------------|
| <i>MrCPAP3B</i>  | MF407550         |
| <i>MrCPAP3E1</i> | MF407551         |
| <i>MrCPAP3E2</i> | MF407552         |
| <i>LvCPAP3A1</i> | MF415537         |
| <i>LvCPAP3A2</i> | MF415538         |
| <i>LvCPAP3B1</i> | MF415536         |
| <i>LvCPAP3B2</i> | MF415535         |
| <i>LvCPAP3E</i>  | MF415539         |
| <i>LvCPAP3I1</i> | MF415540         |
| <i>LvCPAP3I2</i> | MF415541         |
| <i>LvCPAP3J</i>  | MF415542         |
| <i>LvCPAP3A1</i> | MF415537         |
| <i>FcCPAP3A</i>  | MF415532         |
| <i>FcCPAP3B1</i> | MF415531         |
| <i>FcCPAP3B2</i> | MF415530         |
| <i>FcCPAP3I</i>  | MF415533         |
| <i>FcCPAP3J</i>  | MF415534         |
| <i>EcCPAP3A1</i> | MF415523         |
| <i>EcCPAP3A2</i> | MF415524         |
| <i>EcCPAP3C</i>  | MF415525         |
| <i>EcCPAP3D</i>  | MF415528         |
| <i>EcCPAP3E1</i> | MF415526         |

|                  |          |
|------------------|----------|
| <i>EcCPAP3E2</i> | MF415527 |
| <i>EcCPAP3J</i>  | MF415529 |

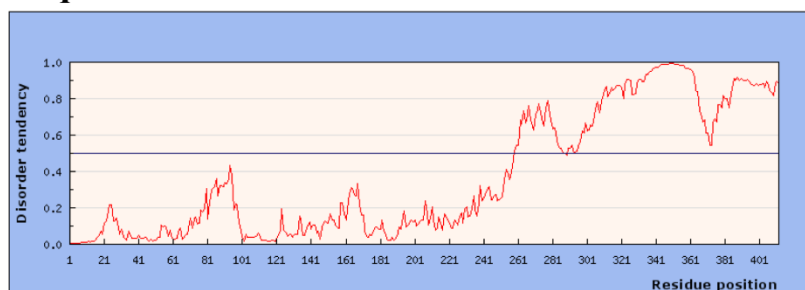
## Supplementary Figures

Abehsera et al Figure S1

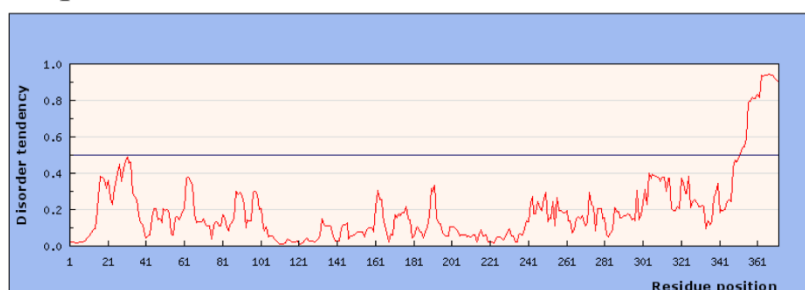
### CqCPAP3B1



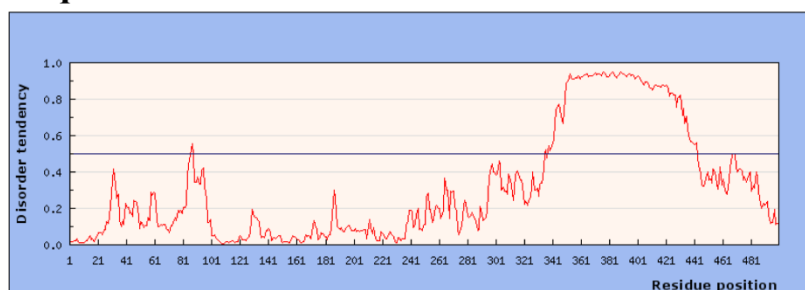
### CqCPAP3B2



### CqCPAP3J1

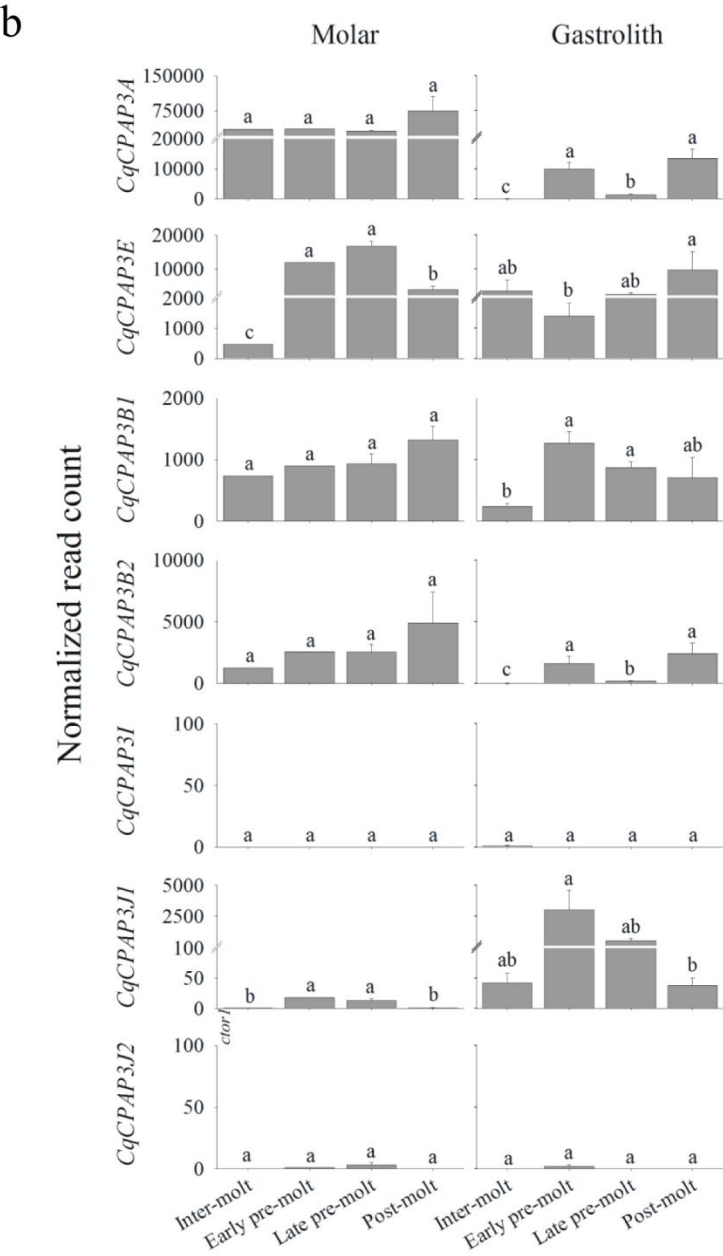
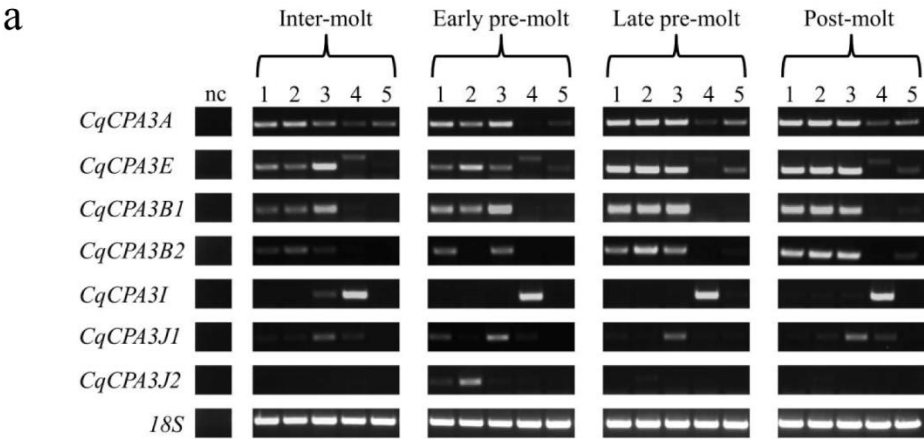


### CqCPAP3J2

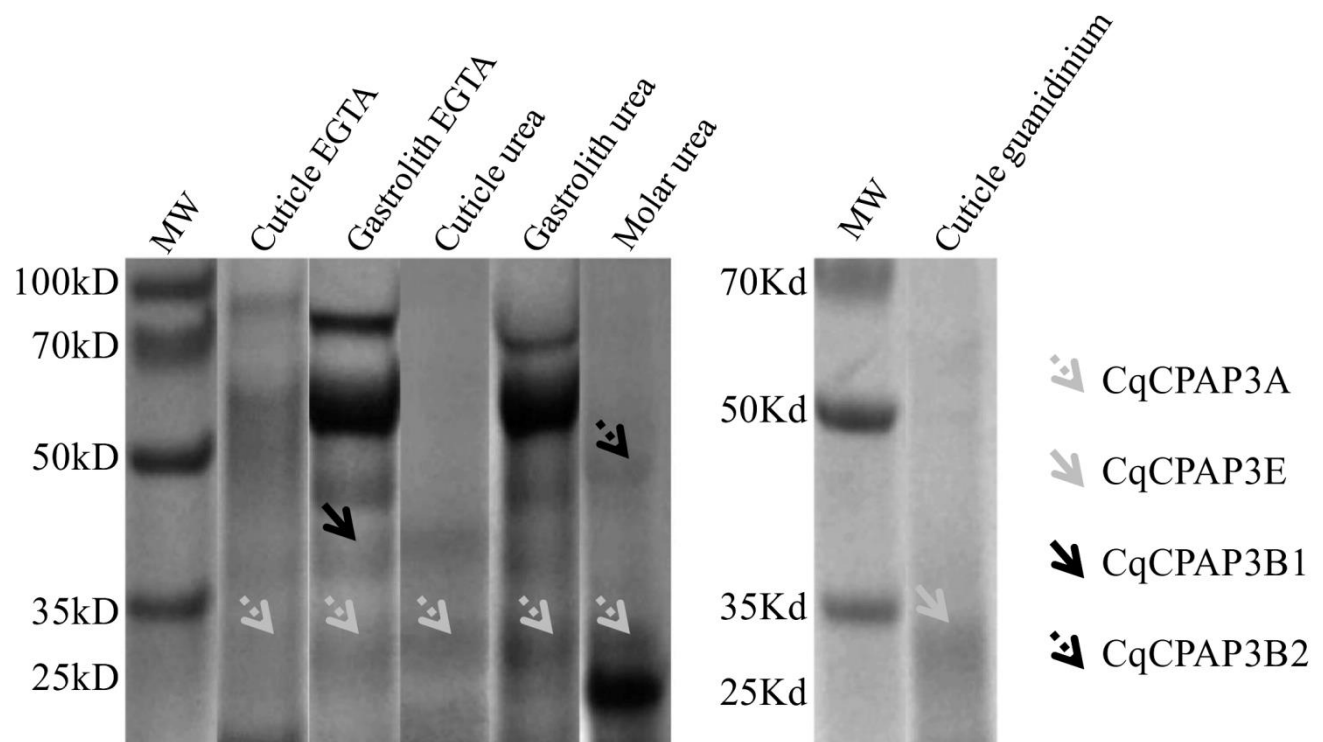


**Figure S1.** Prediction of intrinsically disordered regions by the server IUPRED in different CPAP3 from *C. quadricarinatus*.

Abehsera et al Figure S2

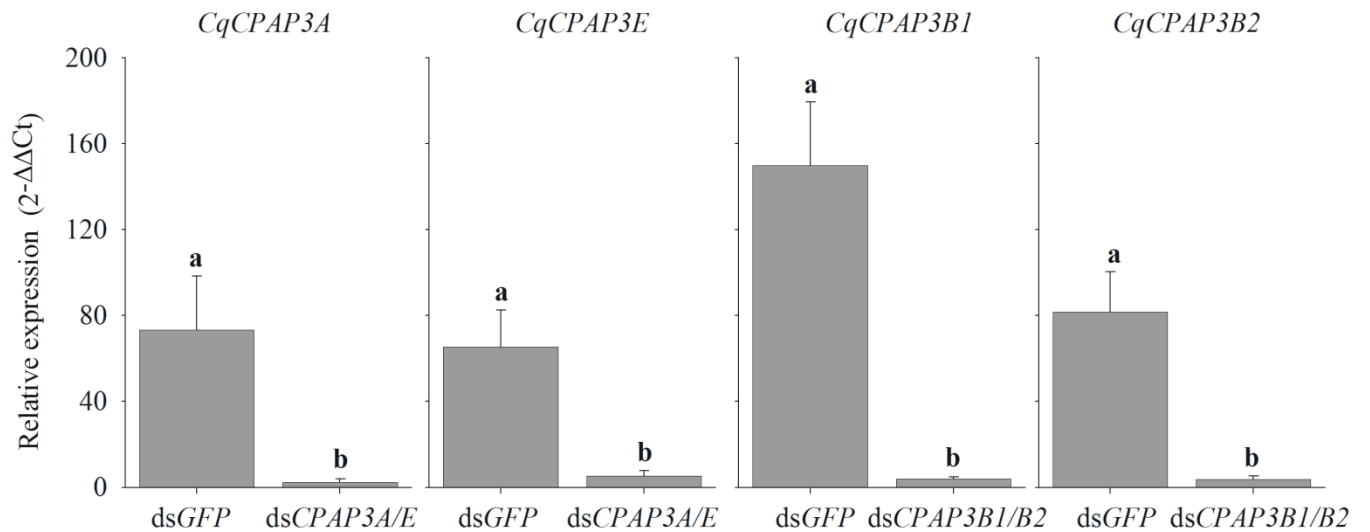


**Figure S2.** CqCPAP3A, E, B1 and B2 are the most active CPAP3 proteins in the cuticular-forming epithelia of *Cherax quadricarinatus*. a) *In vitro* spatial-temporal expression of the seven *CqCPAP3* transcripts found in *Cherax quadricarinatus*. For each transcript, spatial-temporal expression is shown in five tissues sampled in animals from four molt stages. NC- negative control, 1- molar-forming epithelium, 2- carapace cuticle-forming epithelium, 3- gastrolith-forming epithelium, 4- hepatopancreas, and 5- abdominal muscle. b) *In silico* spatial-temporal expression based on data from our molt-related transcriptomic library showing normalized read count of the seven *CqCPAP3* transcripts found in the molt-related transcriptomic library of *Cherax quadricarinatus* from the molar-forming epithelium (left) and gastrolith-forming epithelium (right). Letters represent statistical groups that are significantly different (p-value <0.05); error bars represent standard error.



**Figure S3.** CqCPAP3 proteins are found in the mature cuticle of *Cherax quadricarinatus*. Coomassie staining of the SDS/PAGE-generated protein profile of the extraction of proteins from different cuticular structures and soluble-fractions in which CqCPAP3 proteins were found. Arrows point at the location of different CqCPAP3 proteins.

Abehsera et al Figure S4



**Figure S4.** Relative expression levels following dsRNA-injections. Relative expression levels of *CqCPAP3A*, *E*, *B1* and *B2* (right to left) transcript in different dsRNA-injected groups, dsGFP-injected (n = 5) as control and either ds*CqCPAP3A/E* -2 (n=5) or ds*CqCPAP2B1/B2* (n = 5). Different letters represent statistical groups that are significantly different (p-value <0.05); error bars represent standard error.

## CPAP3 protein sequences used in the phylogeny study

### >CqCPAP3-A

QFQCPPDDGFYVDPRQCDKYYDCYRGQTTEKLCPDGLIFDTTQGPRVEQCNYPFIVECEGSSSLQTPQPS  
GIECPRQNGYFEHENAKNCESYYECTNGLAVLRKCATGLVFDEFTGVCQWSHTGVRSGCGFVPEVLADG  
FSCPNNTQLHTNGQELDHARYVNPNDCRFFYVCEGDGIHPREVGC PKGTVFNDVNLLCDAPENVPGCEN  
YYP AEDVRS AKAGGLI

### >CqCPAP3-E

WGQSCPPEDVHYFSDSQCDKYVECRDGVATEQLCPDGLLFNDKIKNGRYP CDYPSEVDCGSRGRTQP  
PQPTEFCQH QWGYFGSGNRAECGYFYNCVNGVAHLVNCPAGLAFSSSTYRCEWADESSDCDAEAYLGF  
SCPNSVDTNQLLLSGYPQFRSPRDCRQFFICVNTSPRLQFCQLGLVFNEETYTCDEPQNVRCENYYTPPEE  
LALYREQRERQRIAE EKRAELDTLRQQLAARRKQQLQ

### >CqCPAP3-B1

LCDGQELASECPAPNGYFADARQCDRYE CVDNVM SVRLCPDGM AFNDFNPAVEKCDLLSQVDCTGR  
PDLQPARPTEHCDRQYGNFAPKETGNCQSAYRCVDGVGT LISCEGLAFSLETGICDWPDQSGRKDCDK  
QKTQNFCTPKVNFDAVSHPRYADPHDCQYFYVCINGDIPRRNGCAFGQVFNTNTTTCDSPKEVPECAD  
YYTEYFDEYFRTLEKDPGKMSADIVAAAVAAGYDVPKFRDRVRINPGAATPLRRGTTSPPVVPRTTEEPLR  
PLAGKPGESRDGKPGDRPQRRRPGGIRRKRTLTTTTTTTTTTPAPDDY EYYYDYPEGSEATAAAPGPSRT  
TLPI

### >CqCPAP3-B2

VEAQGVVPECPAPDGGFFADAEQCDRYE CVDNVLTEKMCADGLAFVDLNPRIEKCDYISSVDCTGRPKL  
QPAQPTDVCPRQNGYFQHPDETVCNKFYFCNAGTGNLVTCP EGLVYGLDSHNCVWPDVAARQGCSSD  
VIAEFDCPKVTDDIAVSHPRYADPTDCQYFYVCISGVNPRRNGCAFGQVFNSKLAACDAPQEVPECADYY  
TAYFEDYFNTLTATDGRVSVDILAAALASGYDVPSIQERFHVDPKLLQTQPRPSRARGVTAAPAAAAPAA  
VAPAAATVRKAGKLPARRQEELVSQDGDVSPPSLTAGAPRNTVPKRRFRPQPPTTTTTPPPPPPPPQGG  
PEDYTDYTDYTDGAVYDYGAEVPLPPPTTTTAAPVQPTS IKRRIPLRRPRPAN

### >CqCPAP3-I

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DTNYCKDLCS PRAIQCEPGTMLPSPTDCQLYKICLDNGFLDVSCPPEYPNYNFETSDCTNDDTVCYNLC  
DPCEVYCVAEGKIPDPKNCNGYLYCHPPEVAYFLCPEGEAFNSDLLCEPFAGNCTNTC

### >CqCPAP3-J1

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PNQPSDCSCDAPNQFKEDSYNEARFLFCEATGSDPQIFQCPDDHVF DSTMNQCIHYNGLPECTSIGVFA  
NSRNC SQFYNCIPTIDGWVQKPFSCNNETHKHLMYNEAAGSCEDPCTWGN GNFKCEE EGRFADPLNC  
QRYEYECVKDTSEASDFRQALHECPEGYEW DQSARNSYGHCVLEGNLNP CVP AVANKCSVDRSQCPSTT  
EEPDDTTDQPTTRQHE

>CqCPAP3-J2

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SKTGARQQPDDLCSDIVNGFVCS DCKTLVLCLEGEAYTEPCDAGDFCDVREEDFGGGVCYPNWDTVCS  
SDEQCFSRDVYDDQKFVYCWPDV D KADVYRCPDGMRF SKEQQKCVSTIGNITCAQNGVFANPDDCTEY  
YDCVYTDIGWKTRIHKCS DGLMLNENTGNCE DPCTWPTGDFSCTEEGRYPDPLDCGRYYVCIAEGEGFR  
QLQRQCPELYYWDPSEKTGQGICVKATISTCSPISASRCQVPEEWCVSYPSDQDTEVDQDAEADRTTDA  
DQTTDEDQTTDEDQTTDADQTTDADQTTDEDQTTNEDQTTNADHSTNADHSTNADHSTDTDQSADA  
DHSTKVDTHALPSGCELPLL GAGRDCLFITGEATQATDFARECEIAGGEAVSLTDSHIQAIQDYIDPVLLKN  
VGWVVTGPF SITRKFLWPDGTRVNM RQVMGTTWDHDSFCLYITSNIKFPFSLGYCNHFKRVLCKVRVK

>MrCPAP3-B

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PEEAGRTNCASEKVFNFCTPKVGM DTA VTHPRYADPQDCQYFYVCINGETPRRNGCSFGQVFDTKSVTC  
AEPKDVPDCADYYIEYFENYFETLEAGARPSGDILTAARASGYEIP EFRERVIRPGDAVPTRRGTTTASPR  
GERPEGRPLRRRRRPGGNGGLRRRRKRPTTTTTTTTTTTPPEYYDDYYYYDETYDNVDNKDDSSY

>MrCPAP3-E1

AQ SCKDDEILYYPDSQECDKYTQCKFGVVTEEYCPPGLLFNDKVTNGRYPCDYPIDVDCGSR SRTQPAVSS  
ENCPNAWGYGTGDRANCYFFNCVDGQAF PFTCPEGLAFSSATYRCEWPDESPDCDSSAFLGFTCPE  
QQDPTQLLLYG HARLRSRDCRQFFICVGSPRLNHCELGQVFNEAANSCDEPEN VAGCENYYPREELAA  
IRDRKEKQRIAAAKRQEDFEQLRQQLVRRN

>MrCPAP3-D

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PKCANYFKK

>FcCPAP3-A

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>FcCPAP3-B1

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>FcCPAP3-B2

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LPILSRN

>FcCPAP3-I

GLGFCHGKLCIPDCSEMREGDKVPDPTNCFRYYYCSDPGETGELIHSSEPLDCPEGYYFNAELHILECEPIV  
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>FcCPAP3-J

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VEDYGISRDDHNFHDCSLPQDWCPNSGIGPTVRGIPRTFRPEYEGDNIQTASASQDLNVIWVGSKSKN

>EcCPAP3-A1

LVTGLPQEIQFVSNTNSRGKDFICQEDYGVYEDEYQCDKYWVCEDGIAKAQLCDDGLVDFIFKANAGHV  
DPCESPYVVDGQRLQLPATFTSEVCPKNGIFADPDSTNCIRYYTCIKGVATATDCTTGLHFDETTGN  
AWPSTANRSNCSELQNNCIGDFCCPGTPVVTADGVTLPHPTYANVEDCQKFWVCLSGVTPQEISSCALG  
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>EcCPAP3-A2

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>EcCPAP3-C

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EVFQE

>EcCPAP3-D2

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>EcCPAP3-D1

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CANYFK

>EcCPAP3-E

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>EcCPAP3-J

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CIASQNGWQKPFSCSDGLMYNEIKGQCEDPCSWDTGKFTCQREGRFSDPLSCDKYECVLLSDGSFRQ  
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>LvCPAP3-A1

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TEACPRRNGIFAHPESSVCHLFYTCVDGAAVQTECAAGLVFDENTGTCTWPESAGRQGCFFKAGRLPDG  
FECPTVEVKDVRGLTEIHPKYPHEDCAKFYVCLNRIEPRLLTCDFKVFNVKESFCDDPENVPDCEDYYGDV  
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>LvCPAP3-A2

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DGFSCPNETQVHTNGQALDHTRHLKPSDCRFFYICNEGKYPREVGCPQGTVFNDATLNCDDPRNVPGC  
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>LvCPAP3-B1

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NKFTCPEPLPNDGGVVNPNPLYADPEDCQHfYVCINNVEPRRNGCPLGYVFNDDTKQCDDPANVPECK  
DFYGEVEEK

>LvCPAP3-B2

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GRSGCASTNVLNFTCPTSGFDIQRDAHPRFPDPEDCQYfYVCINGKDPRRNGCAFGQVfNSVTKACDSP  
KEVPECADYYSQYfDEYfQTLSSGGRVSADIAAAIAAGYDVPDLKDRVRVATGGSGSGATRRRGQTPAP  
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YDYAYEDYPADALYDEPAPAPATAAPPPPPAPAATPNRGRVVNRRPLVRPRN

>LvCPAP3-E

AAAQNCPEEGVAYFADRDFCDRYSQCRDGVYTEEQCPDGLLFNDLVTNGRYPCDYPSEVDCGSRKRQ  
PAQPTEFCAHQWGYFGSGDRAQCGYfYNCVDGGAHQFACPDGLAFSSATYRCEWPDESPDCDSAAFL  
GFTCPAEVDLRQVQLFGHSRLRSPRDCRQFFICVGASPRLNyCDLGLVFNEAISSCDEPENVQGCQNYYP  
PEELAAIRDRKEKARLAQLKREEFQQLRQQLAQRRQN

>LvCPAP3-I1

VGLGFCHGKLCFPDCAEMREGDKVPDPTNCFRYYYCSDPEGTGDLIHSSEPLDCPEGYYfNAELHILECEPI  
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NLCDPCDLYCTHEGKVPDPVDCTMYYYCDPPLLShFECPNDETfNPDTLVCEQSLTGnCTnLC

>LvCPAP3-I2

VSPSVQVCAPDCTGVDPGTKVRDPTDCTRYVVCVDATGSGVLVPSIDPVECPDGQYfNDQHTSPRCDPI  
NSAPNGFCSQLCNPCEPHCTHAGEVTPDPLDCSTYYVCLQDDHfMSVGCPAETPyFDfMAGECQGDST  
LCfHNCDVCEPHCTQQNervPDPTDChrfYLCTPPTMSSfLCPHNEIfNRETGVCEEgATCIEECGGGVT  
DGI

>LvCPAP3-J

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TACQQPRRQARALFEDNAfSYMCSEgKDGFFCADCKTLINCVDRHAYKLTCVNGDMCAHKESfGGGIC  
YPKEPAECTCQEPNSfKEDLYDNrkFFyCNDTSSDPVfYECDEGSafDAEKsQCKNKNGLPEChRSGVfA  
DINNCTQYYACISTRNGWVQKAFSCNQSNLMYNDLTGACEDPCTWQVGsFACTAEGRYPDPKDCQRYI  
ECVADEGGLKQVRRSCPEDYSWDPTARGGVGHCVKASTNTHCTPATQNKCIIPDGTCASAATAATP  
APEPTSPSTLVVSAELNVATTTTEAAPTTPPKAPVARRRRRLNRRLLN

>AmCPAP3-C

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GGFTCPAAGEVSGASGSFSRHAHPDDCRKYICLEGIAREYGCPIGTVFKIGDADGSGACEDPEDVPGCE  
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>AmCPAP3-D

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>AmCPAP3-B

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PT

>TcCPAP3-B

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CDLPFNIDCTSRPKLQEPQPSQHCPRKHGYFAHEEPHICDKFYCYVDGKYNMITCPNGLVYNDKAGICSW  
PDEAKKKGCSSEEVFQFECPKVNETVAATHPRYADPDDCQYFYVCINGDTPRRSGCKLGQVFDDVGKKC  
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>TcCPAP3-D1

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VVGFKCPTKVPSNSPAAKFWPYPRFAVPGDCHRLITCVNGFPRLISCGEGKAFDQHSLTCEEPELVPHCA  
NHIRK

>ApCPAP3-C

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ARSQLEPAIGGPHCPRLYGIFPDDVKCDTFWNCWGGEASRYQCSPGLAYDRESRVCMWADQVPECKID  
EVANGFNCPAAGELLASVGSFSRHAHPDDCRKYICMEGTAREYGCPIGTVFKIGDSGSGSCESPEDVP  
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>ApCPAP3-B

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>TcCPAP3-E

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DGVENVTGCVGGAQGGANFRNYRI

>NvCPAP3-A1

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PPQPTKKCPRKNGFFAHPDPAVCNIFYNCIDGEAIEITCTTGLHFDEYSGTCVWPDSAGRKGCGVVGKTL  
SDGFECPKDAGVDSRGLAVDHPKFAHPEDCQKFYVCLNGVTPREQGSDGTVYNEVQQRCDAPENV  
GCEDWYKDDDKKP

>NvCPAP3-D1

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CNYNWAVHCGHRKADLTPLSSPGCEYQFGIYPDSDESCSTTIKAYGEPHQAHC DAGLAYDDKSHTCVW  
PDQLIPYCNPEAVVGFKCPAKPPTGAAARFWPFPRFPVPGDCGRLITCVEGHPRLITCGEDKLF DSETLSCL  
DKDELPHCA

>NvCPAP3-E

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FYKSPNDCQHYYICVDNRRLQNCGAGHAFNELINACDAAENVTGCEPPQTFNEVQQVRLDQPQHQN  
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NGF

>NvCPAP3-B

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>Daphnia magna CPAP3-E

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>Daphnia magna CPAP3-D2

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>TcCPAP3-A2

DPVQCDLYYVCSKGEYEEKLCPDGLVFDARDPNHERCDIPANVDCDERTELQEPHPSPGCPRANGYYRH  
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>TcCPAP3-A1

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>ApCPAP3-A1

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>ApCPAP3-D1

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CNPADIIGFSCPEKADPHSVSAKFEPYPRYALPGDSHRLITCVHGHPRLISCGEDSVVDESSLTCVEKGPHY  
RKRK

>DmObstructorA

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HPDPAVCNIFYNCIEGDALETKCTVGLHFDEYSGTCVWPDTAKREGCNPEQRTSETGFVCPKDQPKTDD  
RGQVVTHPKYPHPTDCQKFYVCLNGEDPRDLGCQLGEVYNDATEMCDAPENVPGCEDWYKDVEDDKK  
D

>DmObstructorB

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KFYFCVDGQFNMITCPAGLVFNPKTGICGWPDQVGVGTGCKSEDFDFECPKVNESIAVTHPRYADPNDC  
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>DmObstructorJ

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>DmObstructorF

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>Daphnia magna CPAP3-A

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>Daphnia magna CPAP3-B

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>DpCPAP3-B1

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>DpCPAP3-A1

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>DpCPAP3-B2

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KDVVAFDCPARVLEADPVGPQFIDPLYADPTDCQFYFVCIGGKEPRRNGCTTGLVFNDLTRCDRPRNPV  
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>DpCPAP3-A2

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VCPHIGSGANPSASLRFGDHDLAHTPSCRHFYMCLLTGMPRLGGCTYGLVFNPNVSGRCDQPQNVRCG  
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>DpCPAP3-A3

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TCPHGSNVQSDEVIPSRNSITKFNNKKQKGDCRHFFMCLKSGRPRLGGCPLGTIYNPATFFCDKPENVE  
W

>DmObstructor-H

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HAMEMHCDNELYFNSLTGQCDYDPKVQCAFEDPRSHKCLPHMTEFFPHPDNCNYFYCYIKGFLTQQC  
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>DmObstructor-G

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>DmObstructor-I

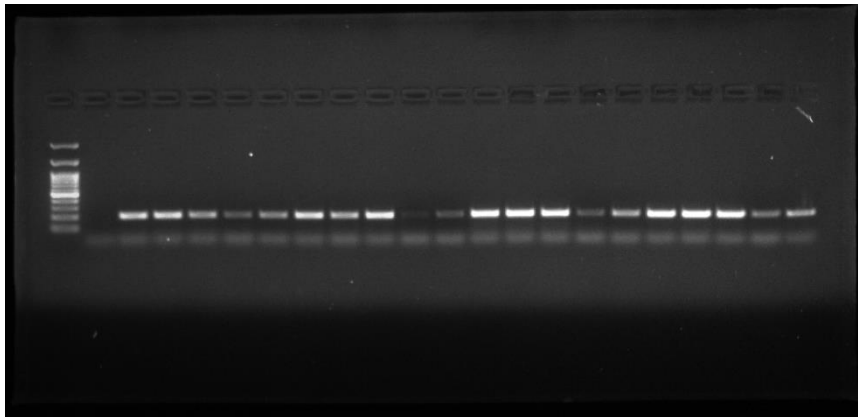
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## **Gel blots pictures**

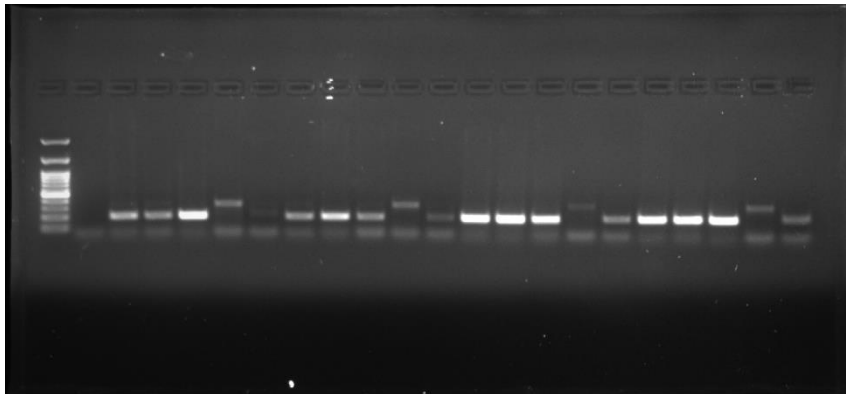
Columns key of RT-PCR gel blots from left to right

1. Nc
2. molar-forming epithelium - Intermolt
3. carapace cuticle-forming epithelium - Intermolt
4. gastrolith-forming epithelium - Intermolt
5. hepatopancreas - Intermolt
6. abdominal muscle – Intermolt
7. molar-forming epithelium – Early premolt
8. carapace cuticle-forming epithelium - Early premolt
9. gastrolith-forming epithelium - Early premolt
10. hepatopancreas - Early premolt
11. abdominal muscle – Early premolt
12. molar-forming epithelium - Late premolt
13. carapace cuticle-forming epithelium - Late premolt
14. gastrolith-forming epithelium - Late premolt
15. hepatopancreas - Late premolt
16. abdominal muscle – Late premolt
17. molar-forming epithelium - Postmolt
18. carapace cuticle-forming epithelium - Postmolt
19. gastrolith-forming epithelium - Postmolt
20. hepatopancreas - Postmolt
21. abdominal muscle – Postmolt

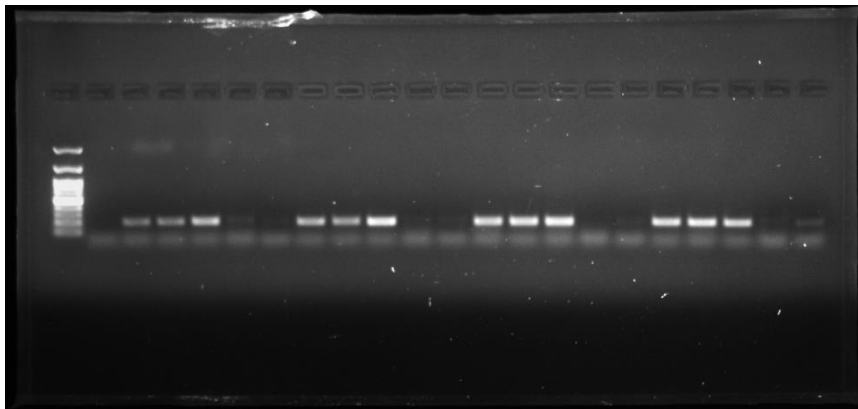
*CqCPAP3A* transcript blot



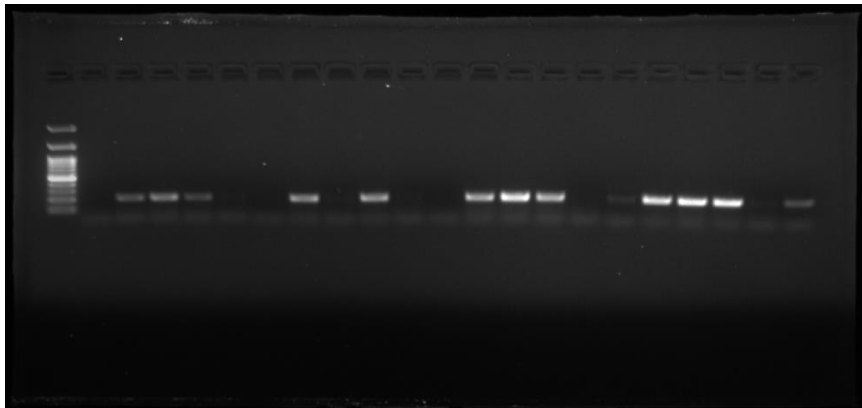
*CqCPAP3E* transcript blot



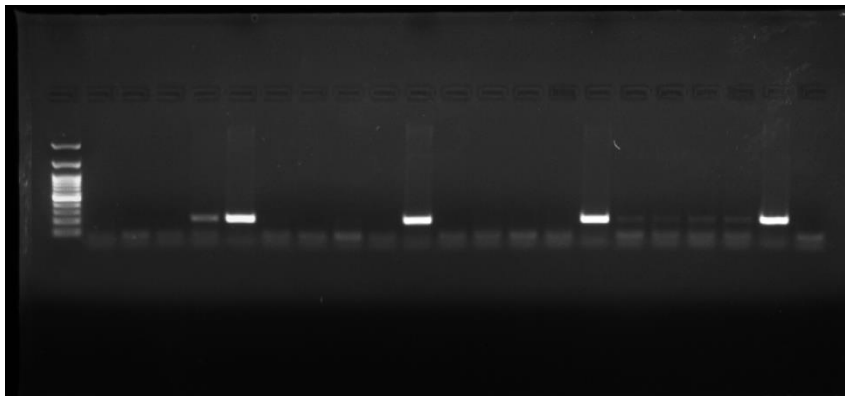
*CqCPAP3B1* transcript blot



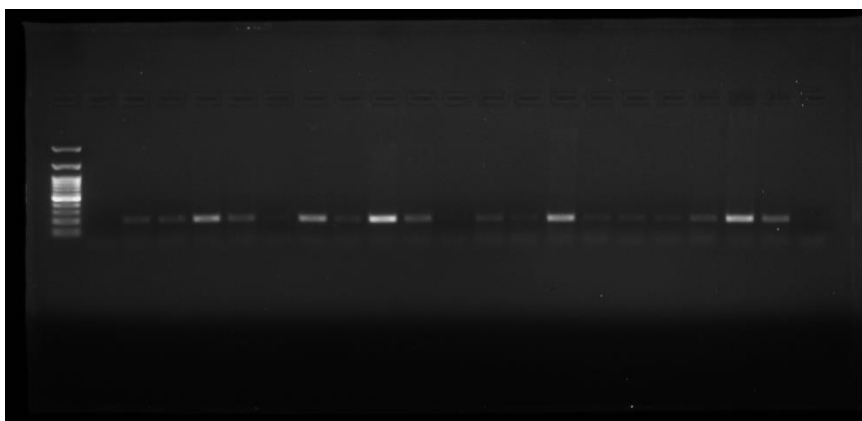
*CqCPAP3B2* transcript blot



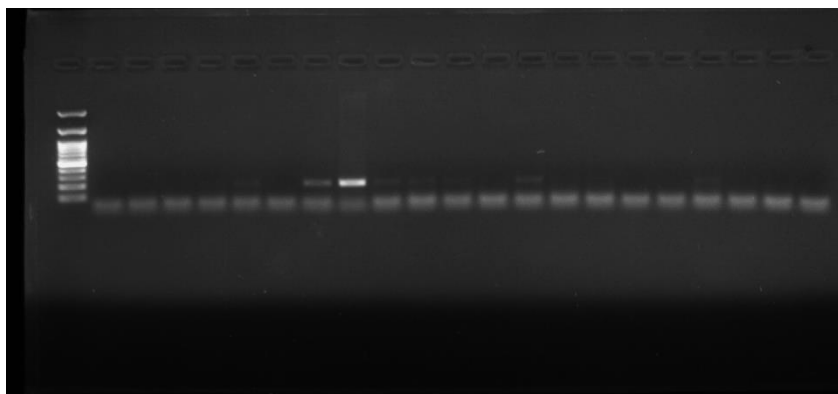
*CqCPAP3I* transcript blot



*CqCPAP3J1* transcript blot



*CqCPAP3J2* transcript blot

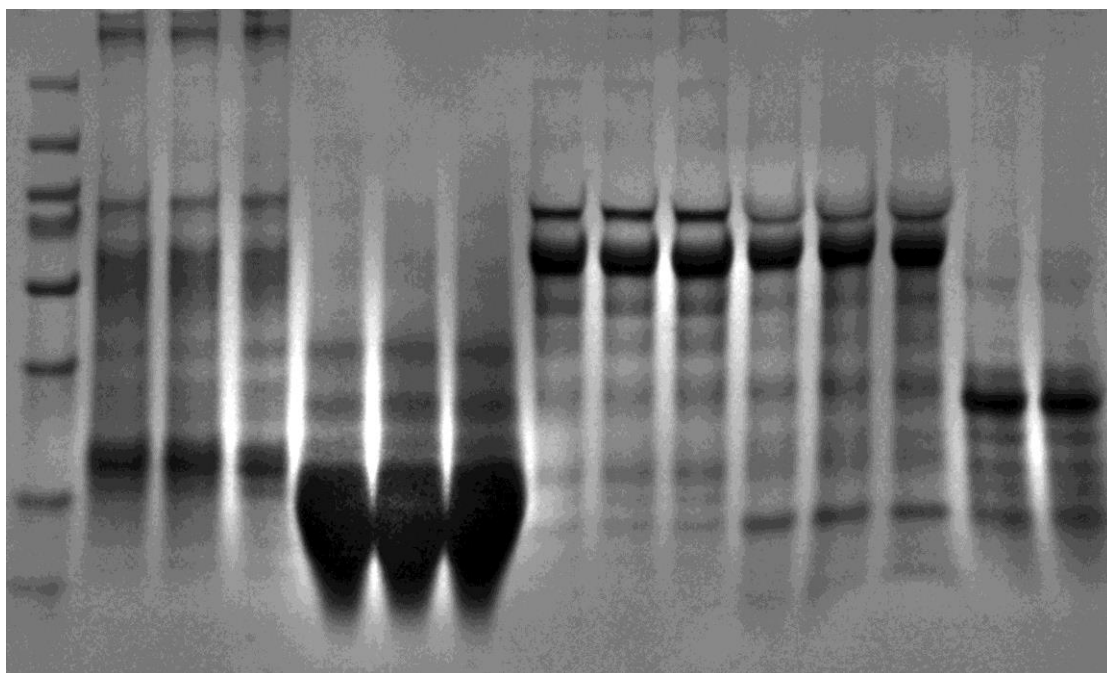


## **Protein Blot**

First extraction

Columns key of gel blot of first extraction from left to right

1. Cuticle EGTA
2. Cuticle EGTA
3. Cuticle EGTA
4. Cuticle Urea
5. Cuticle Urea
6. Cuticle Urea
7. Gastrolith EGTA
8. Gastrolith EGTA
9. Gastrolith EGTA
10. Gastrolith Urea
11. Gastrolith Urea
12. Gastrolith Urea
13. Molar Urea
14. Molar Urea



## Second extraction

Columns key of gel blot of second extraction from left to right

1. Cuticle guanidinium
2. Molar Urea

