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|---------------|---------------------------------------------------------------|
| <b>AmePNG</b> | -----MRIFTIVLSLVLTVWFAGAVASAAPIVEGDTDNPVTAAPGVTRPDSPHC        |
| <b>RcPNG</b>  | -----MSLAGPTLAQAQALTVGSRNVATADPLVPRPPTPC                      |
| <b>DjPNG</b>  | MVPGGFVR--HLAVCVLAATLVG-----GFSVRAADPVVGSTNVATADPTVPRPPGQPC   |
| <b>TrPNG</b>  | -MPRILCRPLSLQRCVLAASLTFAVTAQAQVTVDTKPVVGSSNPATAEPPVTRPTSAPC   |
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| <b>AmePNG</b> | SVTLADGFRSNAADGSPQFYEGTLAPPKACPGPWAKVVMQTVTV--SGRQYDRIGDLRIG  |
| <b>RcPNG</b>  | TVTLFSGQQFADFNNKPF----DYTPAAGCAGPWEKVVLAVDFSITGTRQYDRTARIWLG  |
| <b>DjPNG</b>  | VVTLFTNVAFDDFGTRPY----SYAPPTSCSGSWSKVLEADFSVTAGRQYDRTATLWLG   |
| <b>TrPNG</b>  | TVTLFDNQVFKDYSAPKL----TYAPPTGCRGHWAKVVLTVDFTVTAGRQYDRTASLYLG  |
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| <b>AmePNG</b> | GTEVWWGTTEEPSGEGHRPITYHFDKDLTPYSALLRTPQPFHGIE---NYNSAIYTG     |
| <b>RcPNG</b>  | NSVIYFGTTQEPSIPGGGTTSHWVERDLTDYSALLAQASGYAELGNFVGTNGVVDGI     |
| <b>DjPNG</b>  | GVNLYFGTTQEPSKT--VSPAWHVERDLTDYAALLRNAGQGQAIIGNLVN---STYTG    |
| <b>TrPNG</b>  | NANIFYGTTAEPRAA--LSPSWHVERDVTDLALLKTSQSGQAIYVNVVD---STYGTI    |
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| <b>AmePNG</b> | YAQTVTLTYQADRKHPAAVTPDRVAGFGHADATP-----AAPTVHFSQAQGLPRNITRA   |
| <b>RcPNG</b>  | ITGTATLYFYSGDGNDG-KRPDQVLSLNQN--GGIASLAQPSDQLSAAFSALPKNIERA   |
| <b>DjPNG</b>  | IHGSAARLLFPASAGARAAEAPDAVYPLGSDPVGSTVSLNTSSDKLSRT-LTLPTNVERA  |
| <b>TrPNG</b>  | IYGTAALLFPADFRNPPAVVPDVVIVPVSQT--NSPYELDTTSSQLTTS-VVAPRNTVKA  |
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| <b>AmePNG</b> | YLEVTLEGHACDFQWFFDDVPDAVAAKYPAAGLCGKGSYREANFAIDGTSAGSAFTFPHIY |
| <b>RcPNG</b>  | YLDVFTQSQSADFFWFFDLDDIAPLFQ---DTGSTAFKEALVTIDGQAGISPIYPWIY    |
| <b>DjPNG</b>  | YLDVFTQSQGGDEFWYTCVPDAYAAQVS---ECGGGNFREAEISIDGQAGVAPVYPWIY   |
| <b>TrPNG</b>  | YLDVIAQSQSSDEFWYLCAPNDVAAKLQ---SCPSTAFRETEITIDGKPAVAPVYPWIY   |
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| <b>AmePNG</b> | SGGIVPQLWRPVVAIDTFSLHAETYDVTFFAGRLVDGGTHDLSFSFPDIGGEFTVVPDLL  |
| <b>RcPNG</b>  | TGGADPILWRPIPGVQTLSEFYPRLDLTPFVGLGDGSAHTVAISVYNAANYFATAANLL   |
| <b>DjPNG</b>  | TGGVDPYLRPTPGVQTLNFMPIYRVDLTPFAGQLSDGNPHEVAVQVAGANGYFSAATAALL |
| <b>TrPNG</b>  | TGGIDPYLWEPIVGVQTLNFKPYRVDLTPFAGVLSDGAPHTVGVSVYNATSYFLATANLL  |
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| <b>AmePNG</b> | LYTDKNATQTSALTQHDVAAAPARQ---ETVKD---ISGGVNVTVTAKRHDVTAGYVDT   |
| <b>RcPNG</b>  | LYLDAGASTVSGSVTTNTLS-ATPVNVNVENVTSAPDGSASGSVSVTSNRSYITITGTANT |
| <b>DjPNG</b>  | VYRDAHAKQVTGKITRNTLAGQAPTPTIGNTLVTASNGDIAGDITKLDHRHFVIEGYAMT  |
| <b>TrPNG</b>  | LYTDPFRAQTGGGLLENTLS-LAPTPLVVEKLTTDASGTTTGTVTVDARSFTTVKGVNS   |
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| <b>AmePNG</b> | SAGRVYTRVERTRDYRNSDDVTGGGFTQHVQADAGQOTS SVSTV-----DGR-VRSA    |
| <b>RcPNG</b>  | SHGAVTTQIDSKIAFSSVQQLATSNT---VYQQDTVQRTDIDTLTRTQNGKGKGGTQT    |
| <b>DjPNG</b>  | SHGRITSSVTQNTGFDDTQFTINSS---TYRQLTSQRTWTD SKSESRLGNF----PVAE  |
| <b>TrPNG</b>  | ALGRVETTVEQKVNFNSAQT FNVGTN---DIQNAVQT-STVSSKTTSQLG-F----LKET |
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| <b>AmePNG</b> | DRHTWSYPLTTDATANITDDQNLRISGSADMT-----KILGDLT-GDGRWTRPV-RAS    |
| <b>RcPNG</b>  | LHEVRSYPLTFDYDYITAADGSATLHSFADQEFQQRVDAGVSGNAIKTATRDNHVVTVT   |
| <b>DjPNG</b>  | RQQHVSYPFTLDYNQQLASDGSTSAASTVHQGYRQDELHLFGLLAYGANVDNTVDSQDT   |
| <b>TrPNG</b>  | TDKQISFPLTLNYAYTVNNDGTSAQVVTSDQKDVETETK SINGLPIFGSSRNEVHTTDT  |
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| <b>AmePNG</b> | REWLASSGVLARTNGVNTEADGRSKTLFTGTDDRGRPYFHYVASEHGLITEN---RELPG  |
| <b>RcPNG</b>  | RNY-DASGALVT-----TPATASQTYTYADPFGACYSRKVEAQDRLLTAVTDGAGCPG    |
| <b>DjPNG</b>  | LVF-DASGSLTS-----HSGQQSAQTFAFHDTRGSCYRAALTQAGAVASYNAGQGPCD    |
| <b>TrPNG</b>  | LNYSAGALVS-----ATPTSASATYKAEDTLGKCFSRQVTSTARAVTNVTDGAGCPV     |
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| <b>AmePNG</b> | GR-----                                                       |
| <b>RcPNG</b>  | GHNQLSWHDQFAKFVSNAGATVKLLP--                                  |
| <b>DjPNG</b>  | GTNRVSWFAHPDGSP-----SSSLLPGH                                  |
| <b>TrPNG</b>  | KR-----                                                       |

**Supplementary Figure S1:** Sequence alignment showing the moderate homology between AmePNG and other PNGase H<sup>+</sup> enzymes. Pairwise sequence identities are approximately 31.3% between AmePNG and RcPNG, 31.1% between AmePNG and DjPNG, and 31.4% between AmePNG and TrPNG. In contrast, RcPNG, DjPNG, and TrPNG share higher sequence identities ranging from 45% to 55%. Catalytically relevant residues are highlighted in green.