

# PKA Subunits in the Human Pathogen *Paracoccidioides*: An *in Silico* Approach

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**Research Article** 

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### Abstract

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Paracoccidioides brasiliensis and P. lutzii is the causative agent of paracoccidioidomycosis, a primary systemic mycosis with different clinical manifestations. Infection occurs when hypha-fragments or spores are inhaled by the host, an event that induces *Paracoccidioides* cells to switch to the yeast form. The cAMP-PKA signaling pathway is important in controlling the morphological transition, as well as several development processes in fungal pathogens. PKA holoenzyme is an inactive tetramer composed of two regulatory and two catalytic subunits. When cAMP binds to the regulatory subunits, the catalytic subunits are released and become active. These active subunits subsequently phosphorylate protein kinases, transcription factors, and other substrates. In this study, we show an *in silico* characterization of the PKA subunits from three strains of *Paracoccidioides*. The genome of each strain encodes a single regulatory, PbPKA-R, and two catalytic subunits, PbPKA-C1 and PbPKA-C2. The subunits are highly conserved among the strains analyzed. The computer-based prediction suggests that PKA can be found in more than one subcellular location: PKA-R and PKA-C2 are mainly localized in the nucleus, whereas PKA-C1 seems to act predominantly in the cytosol. Each PKA-R subunit presents two tandem copies of the cyclic nucleotide monophosphate-binding (CNMP) domain at the C-terminus and each PKA-C has a protein kinase and an AGC-kinase C-terminal domain. Alignment of human and Paracoccidioides PKA subunits reveals a huge discrepancy in the N-terminal sequences of these two organisms. This sequence divergence between human and Paracoccidioides subunits combined with the pivotal role that PKA plays in many cellular processes, makes this protein an interesting target for antifungal drugs.

Keywords: PKA; Paracoccidioides; in silico

#### Introduction

Protein kinases are key regulatory enzymes that play crucial roles in cellular biochemistry and physiology. These enzymes act as mediators in several signal transduction pathways by reverse phosphorylating specific amino acid residues [1]. Protein kinase A (PKA), a serine/threonine kinase that mediates cAMP signaling in eukaryotic cells, belongs to the AGC group of kinases. The holoenzyme is an inactive tetramer composed of two regulatory (PKA-R) and two catalytic (PKA-C) subunits. In response to external signals that increase intracellular cAMP levels, this second messenger binds to the PKA-R subunits triggering conformational changes that release the PKA-C subunits. The free PKA-C became active and subsequently phosphorylate protein kinases. transcription factors, and other substrates to control various physiological processes, such as cell growth and metabolism, DNA replication, cell division and actin cvtoskeleton rearrangements [2,3].

Although evolutionary conserved, the PKA subunits can vary in number and isoforms among different species. Several variants of the PKA-C and PKA-R subunits have been identified in human cells. Four PKA-R subunits designated RIa, RIB, RIIa, and RIIB are transcribed from separate genes [4]. Five different PKA-C subunit genes have been identified in human: PRKACA, PRKACB, PRKACG, PRKX, and PRKY. Three of these genes, PRKACA, PRKACB, and PRKX, have been demonstrated to be transcribed and translated into functional protein kinases, termed PKA C $\alpha$ , PKA C $\beta$ , and PRKX, respectively [5]. In the nonpathogenic yeast Saccharomyces cerevisiae PKA consists of a PKA-R subunit encoded by a single gene, BCY1, and three PKA-C subunits encoded by the TPK1, TPK2, and TPK3 genes. These Tpk isomers are functionally redundant for cell viability, but they regulate different processes [6].

The cAMP-PKA signaling pathway mediates the microbial response to changes in the environment. In yeast, PKA is activated in response to nutrients and subsequently regulates metabolism and growth [7]. This pathway is also associated with pathogenesis in several fungal species, regulating morphological changes and virulence of several plant and animal fungi [8-12]. In the human pathogenic fungus of the *Paracoccidioides* genus, PKA controls morphological changes that are imperative to the establishment of infection [13,14].

The *Paracoccidioides* genus comprises two closely related species: *P. brasiliensis* and *P. lutzii.* Both can infect

humans, but they can vary in virulence and induce different immune responses by the host [15,16]. These thermally dimorphic fungi are the etiological agents of paracoccidiodomycosis, a mycosis that affects millions of people in Latin America [17]. Infection occurs when the host inhales the hypha-fragments or spores released from mycelium, which differentiates to the yeast form in the host lungs. This morphological switch is essential for the pathogenicity of these fungi since strains that are unable differentiate is often a virulent [18]. The cAMP-PKA pathway is important in controlling the morphological transition from mycelium to yeast [13,14]. Here we describe an in silico analysis of the PKA subunits encoded genome of two reference strains of P. in the brasiliensis (Pb03, Pb18) and one strain of P. lutzii (Pb01).

### Methodology

# Identification of the PKA subunits in the genome of *Paracoccidioides* and protein sequence analysis

*Paracoccidioides* PKA subunits were searched in the protein database available on the Broad Institute website [19,20], whose data have been relocated to the fungal ftpsite

(http://archive.broadinstitute.org/ftp/pub/annotation/f ungi/paracoccidioides/genomes/). The corresponding amino acid sequences were analyzed in silico as follows: similarity searches were performed using BLASTp [21]; the theoretical molecular weight and isoelectric point of each subunit were determined using theCompute pI/Mw tool, available at http://web.expasy.org/compute\_pi/; the multiple alignment sequence analysiswere performed the using t-coffee program [22] at the http://www.ebi.ac.uk/Tools/msa/tcoffee/, under default settings; the protein domain identification were done using Prosite, a large database of protein domains, families and functional sites [23] and the cellular localization of the protein subunits was predicted using the PsortII program [24], recommended for yeast sequences.

#### **Protein Sequence accession number**

The uniprot accession numbers for the *human* proteins used in the multiple sequence alignment are: KAP0\_HUMAN cAMP-dependent protein kinase type Ialpha regulatory subunit (P10644), KAP1\_HUMAN cAMPdependent protein kinase type I-beta regulatory subunit (P31321), KAP2\_HUMAN cAMP-dependent protein kinase type II-alpha regulatory subunit (P13861), KAP3\_HUMAN cAMP-dependent protein kinase type II-beta regulatory subunit (P31323), KAPCA\_HUMAN cAMP-dependent protein kinase catalytic subunit alpha (P17612), KAPCB\_HUMAN cAMP-dependent protein kinase catalytic subunit beta (P22694), KAPCG\_HUMAN cAMP-dependent protein kinase catalytic subunit gamma (P22612), PRKX\_HUMAN cAMP-dependent protein kinase catalytic subunit PRKX (P51817) and PRKY\_HUMAN Putative serine/threonine-protein kinase PRKY (O43930).

### **Results and Discussion**

# Overview of PKA subunits encoded in the *Paracoccidioides* genome

We identified and analyzed the PKA subunits encoded in the genomes of three phylogenetic species from *Paracoccidioides*. Each genome encodes a single PKA-R and two PKA-C subunits.

The Paracoccidioides PKA-R subunits are approximately 47.0 kDa, slightly acidic proteins, with pI ranging from 5.5 to 5.7 (table 1). Only the Pb01 strain present a PKA-R subunit annotated in the genome (locus number PAAG 07887). However, BLASTp analyses revealed hypothetical proteins in Pb03 (PABG\_07470) and Pb18 (PADG\_08191) strains that share over 97% amino acid identity with Pb01PKA-R subunit (figure 1). In addition to the strong conservation observed between these proteins in the three Paracoccidioides strains (figure 1A), they share the same predicted subcellular localization (table 2) and the same domain structures (figure 2), as described below. So, we believe that these computer-based analyses strongly indicate that Pb03 PABG\_07470 and Pb18 PADG\_08191 are, indeed, cAMP-dependent protein kinase regulatory subunits.

Each *Paracoccidioides* PKA-C strain presents two PKA-C subunits, which we called PKA-C1 and PKA-C2, according to the BLASTp best hit with either Tpk1 or Tpk2 from the model organism *S. cerevisiae*, respectively. The PKA-C1 subunits predicted mass are about 48kDa, with pI ranging from 5.5 to 6.0. The PKA-C2 are larger proteins, with 64.5 to 67.5 kDa, basic proteins, with pI between 8.1 to 8.8 (table 1). All PKA-C2 subunits exhibit large N-terminal regions that are not present at the PKA-C1 (S1 figure).

PKA-C1 subunits are highly conserved in the three strains. Pb01PKA-C1 shares 93% and 94% identity with Pb18 and Pb03 subunits, respectively; while the Pb03 and Pb18 subunits are 99% identical. PKA-C2 subunits are also highly conserved in the three strains analyzed, sharing over 91% amino acid identity (figure 1B). The respective guide tree (figure 1C) places PKA-C subunits from Pb03 and Pb18 closer to each other than to Pb01. This evolutionary evidence is also described for other molecular markers that place Pb01 as a separate Paracoccidioides species [15,16]. The extensive conservation of PKA-C1 and *PKA-C2 protein sequences* among representatives of these phylogenetic species three maior within the Paracoccidioides genus suggests that PKA is likely highly conserved in all Paracoccidioides strains.

We observe that PKA-C1 are clearly distinct from PKA-C2 subunits, sharing only 29% to 31% amino acid identity. This low identity between PKA-C1 and PKA-C2 subunits is due mainly to the N-terminus present only in the PKA-C2 sequences.

Name	Species (strain)	*Gene name	*Description	Type of subunit	Number of aminoacids	Molecula r weight (kDa)	Isoeletri cpoint
Pb01PKA-R	<i>P. lutzii</i> (Pb01)	PAAG_0 7887	cAMP-dependent protein kinase regulatory subunit	regulatory	445	47.49	5.75
Pb03PKA-R	P. brasiliensis (Pb03)	PABG_0 7470	Hypotheticalprotein	regulatory	440	47.07	5.55
Pb18PKA-R	P. brasiliensis (Pb18)	PADG_0 8191	Hypotheticalprotein	regulatory	440	47.04	5.64
Pb01PKA-C1	<i>P. lutzii</i> (Pb01)	PAAG_0 4050	Serine/threonine-protein kinase PRKX	catalyticty pe 1	417	47.77	5.56
Pb03PKA-C1	P. brasiliensis (Pb03)	PABG_0 3878	AGC/PKA proteinkinase	catalyticty pe 1	425	48.72	5.99
Pb18PKA-C1	P. brasiliensis (Pb18)	PADG_0 7326	AGC/PKA proteinkinase	catalyticty pe 1	426	48.87	5.99
РЬ01РКА-С2	<i>P. lutzii</i> (Pb01)	PAAG_0 0108	cAMP-dependent protein kinase catalytic subunit pkaC	catalyticty pe 2	585	65.08	8.15

Pb031PKA-C2	P. brasiliensis (Pb03)	PABG_0 5134	AGC/PKA proteinkinase	catalyticty pe 2	580	64.45	8.41
Pb018PKA-C2	P. brasiliensis (Pb18)	PADG_0 7154	AGC/PKA proteinkinase	catalyticty pe 2	606	67.48	8.74
*htt	p://www.broad	institute.org	g/annotation/genome/paraco	ccidioides_br	asiliensis/MultiH	Iome.html	

Table 1: Survey of the PKA subunits encoded in the genomes of *Paracoccidioides* species and strains.



Figure 1: Relationship between the PKA subunits from the *Paracoccidioides* species and strains. A and B-Percentage of identity between PKA-R and PKA-C, respectively. C- Cladogram representing the distance between the PKA-C sequences.

Name	Predicted Localization	Name	Predicted Localization	Name	Predicted Localization
	78.3 %: nuclear		60.9 %: cytoplasmic		60.9 %: nuclear
Pb01PKA-R	13.0 %: cytoplasmic	Pb01PKA-	26.1 %: nuclear		13.0 %: cytoplasmic
	4.3 %: cytoskeletal	C1	8.7 %: mitochondrial	Pb01PKA- C2	13.0 %: cytoskeletal
	4.3 %: mitochondrial		4.3 %: vacuolar		8.7 %: Golgi
	69.6 %: nuclear		56.5 %: cytoplasmic		4.3 %: plasma membrane
Pb03PKA-R	17.4 %: mitochondrial	Pb03PKA-	26.1 %: nuclear	Pb031PKA- C2	73.9 %: nuclear
	8.7 %: cytoskeletal	C1	13.0 %: mitochondrial		13.0 %: cytoplasmic
	4.3 %: cytoplasmic		4.3 %: vacuolar		8.7 %: cytoskeletal
Pb18PKA-R	69.6 %: nuclear	Pb18PKA- C1	47.8 %: cytoplasmic		4.3 %: vesiclesofsecretory system

	30.4 %: nuclear		69.6 %: nuclear
17.4 %: mitochondrial	13.0 %: mitochondrial	Pb018PKA-	13.0 %: mitochondrial
8.7 %: cytoplasmic	4.3 %: vacuolar	C2	8.7 %: cytoplasmic
4.3 %: cytoskeletal	4.3 %: cytoskeletal		4.3 %: vesiclesofsecretory system

Table 2: Predicted cellular localization of the PKA subunits from *Paracoccidioides* according to the PSORT II program.



PRKACB PRKACG PRKX	MGNAATAKKEFIAKAK
PRKACG PRKX	HORALL HER HER
PRKX	MGNAPAKKDEFLAKAR
The second state of the se	MEAPGLAQAASR
PRKY	MEAPGPAQAASREVTEDAAESNSRSRSR
Pb01PKA-C1	MAAETIQGQETDVPAESTSSTSYRPETDVPAEST
Pb01PKA-C2	MRGLGNLLKKKKKRTKDSRISRDLDLSASPVAESTSSSPGDNNNNIIINISSPLSQTTAATATAPATVTEASTTNTLLQHPAFTSPARDSSCKDPASSPG
Pb03PKA-C1	MAAETIQGQEIDVPAESTEIDVPAEST
Pb03PKA-C2	MRGLGNLLKKKKKRTKDSRISRDLDLSASPVAESTSSSPGDNNNNII-NNSSPLFLTTAATATAPATVTAAPTTNTLLQHPAFTSPARDSSCKDPASSPG
PD18PKA-C1	MAAET1QGQ
PD18PKA-CZ	MKGLGNLLKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK
PRKACA	FLKFLK
PRKACB	FLKFLK
PRKACG	FLY
PRKX	
PRKY	
PhO1PKA-C2	
Pb03PKA-C1	BD IDTVNKK
Pb03PKA-C2	TNKI SSSSNPPASVELTA I PTSTKPEGVASSPPTGSTTAGA I TKI DSTHSAPMNPLOSPAPPSSNI AGD PSHEKOHOOOOOOPOOOSHHSONVASI KNI I
Pb18PKA-C1	KQQNHHHHHHQHPQKKSNGEK-PNSLDLSL
Pb18PKA-C2	TNKISSSSNPPASVFLTAIPTSTKPEGVASSPPTGSTTAGAITKIDSTHSAPMNPLHSPGPPSSNIAGDPSHFKQHQQQQQQPQQQSHHSQNVASIKNII
PRKACA	DD
PRKACB	
PRKACG	AONTASSDQFERLRTLGMGSFGRVMLVRHQE-
PRKX	-TPDGAPALCPSPTPDGAPALCPSP
PRKY	-AADWAPALCPSPAADWAPALCPSP
Pb01PKA-C1	DLSLGLDLACPASRETPQDILENRERIRQRLPAERLSIDDFDLLETVGTGTFARVWLARWAKRKEG
Pb01PKA-C2	$\tt NPPLNDEVAASSQRVGDGLHAAQQ-MHSQHSQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQ$
Pb03PKA-C1	DLSLGLDLSCPASQPETPQDILENRERIRQRLPAERLNIDDFDLLETVGTGTFARVWLARSVKRKEG
Pb03PKA-C2	$\label{eq:constraint} NPPLNDEVAASPQPVGDGLHAAQQRMHSQHPQQQQQQQ-QQQQQQQQQQQQVQQSVPSVSRQTKGKYSLDDFTLQTTGSFGRVHLVQSRH$
Pb18PKA-C1	DISLGLDLACPAS
Pb18PKA-C2	NPPLNDEVAASPQPVGDGLHAAQQRMHSQHPQQQQHQQQQQQQQQQQQQQQQQQQQQQQQQQ
PRKACA	${\tt TGNHYAMKILDKQKVVKLKQIEHTLNEKRILQAVN-FPFLVKLEFSFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLD-L$
PRKACB	${\tt TEQYYAMKILDKQKVVKLKQIEHTLNEKRILQAVN-FPFLVRLEYAFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLD-L$
PRKACG	TGGHYAMKILNKQKVVKMKQVEHILNEKRILQAID-FPFLVKLQFSFKDNSYLYLVMEYVPGGEMFSRLQRVGRFSEPHACFYAAQVVLAVQYLHSLD-L
PRKX	AKHFFALKVMSIPDVIRLKQEQHVHNEKSVLKEVS-HPFLIRLFWTWHDERFLYMLMEYVPGGELFSYLRNRGRFSSTTGLFYSAEIICAIEYLHSKE-I
PRKY	AKHFFALKVMSIPDVIRKQEQHVHNEKSVLKEVS-HPFLIRLFWTWHEERFLYMLMEYVPGGELFSYLRNRGHFSSTTGLFYSAEIICAIEYLHSKE-I
Pb01PKA-C1	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTF5DDQNLVMLLDYCPGGEVFTFLRRARCFSERTSQFYAAEIVLILEFLHEVHGV
PD01PKA-C2	NORY TAIN URKAQUVINIKQUEHTINDEKIMIQKVE-HPFLITLIGITSTEDUNALTINUMDFUEGGELFSLIKKSQRFPNPVARFTAREVTLALETLIHDH-I ODGIVATIVTI URVAQUVINIKQUEHTINDEKIMIQKVE-HPFLITLIGITSTEODINALTINUMDFUEGGELFSLIKKSQRFPNPVARFTAREVTLALETUHTUHTI
PD03PKA-C1	UDDY TATUTINADY TALKYYEN YAEVATI LAAVSGHFT ITSIISISISISISISTEMU TAUMAN EVERGET FY TAKAANA SEATSY TAAEVA TATUTI HIL T
Pb18PKA-C1	NOVE IN INVESTIGATING VEHITING INVESTIGATING INVESTIGATION OF A DESCRIPTION OF A DESCRIPTIO
TDTOTION OT	ODGVYAIKIIHKADVIKIKOVEHVRNEVRTIAAVSGHPEIISIISTESDOONIYMIIDYCPGGEVETEIRRAHRESERTSOFYAAEIVIIIEEIHEVHGV
Pb18PKA-C2	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDYCPGGEVFTFLRRAHRFSERTSQFYAAEIVLILEFLHEVHGV NORFYAIKVLKKAOVVKMKOVEHTNDERRMLORVK-HPFLITLWGTFODVKNLYMVMDFVEGGELFSLLRKSORFPNPVAKFYAAEVTLALEYLHDHH-I
Pb18PKA-C2	QDGVYAIKILHKADVIKLKQVEHHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDYCPGGEVFTFLRAHRFSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: **: **: * * :. : **::*:: **:::: **::::: **:::::: **::::::
Pb18PKA-C2	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTFLRAHRFSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: .*:: *: *: *: *: : : : *:::: **::: : **:::: **:::: **:::: *:::: *:::: *::::: *::::::
Pb18PKA-C2 PRKACA	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDYCPGGEVFTFLRAHRFSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: .*::**: *: *: *: *: : : : : : : :
Pb18PKA-C2 PRKACA PRKACB PRKACG	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: **: **: * :* :* :: **:::: **:::: **:::: **:::: IYRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDHQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDHQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX	QDGVYAIKILHKADVIKLKQVEHVVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDVCPGGEVFTPLRAAHRSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: **: **: **: * :: **:::: **:::: **::::: **::::: **::::: IYRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IHRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IHRDLKPENLLIDQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGRVRFPSH IHRDLKPENLLIDQGHIKUTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGRVRFPSH
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY	QDGVYAIKILHKADVIKLKQVEHVVREVRTLAAVSGHPFIISLISTFSDDQNLYMLLDYCPGGEVFTFLRAHRFSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: *:: **: **: **: **: *:: **:::: **:::: **:::: **:::: *:::: *:::: *::::: *::::::
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY Pb01PKA-C1	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDONLYMLLDYCPGGEVFTELRAAHRFSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNDVAKFYAAEVTLALEVLHDHH-I :*:*:: *:: *:: *:: *:: *::: *:::: **:::: **:::: **::::: **::::: IYRDLKPENLLIDQGYIQVTDFGFAKVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDQGYIQVTDFGFAKVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IHRDLKPENLLIDQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAGFPPFYAD-QPIQIYEKIVSGKVRFPSK VYRDLKPENLLIDQGYLQVTDFGFAKRVKGR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFFDD-NPFGIYQKILAGKIDFPRH VYRDLKPENILLDRDGHIKLTDFGFAKRVKDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFFDD-NPFGIYQKILAGKIYFPRH AYRDLKPENILLDRDGHIKLTDFGFAKRVKDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFFDD-NPFGIYQKILAGKIYFPRH
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY Pb01PKA-C1 Pb01PKA-C2	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: **: **: **: * :: **:: **::
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY Pb01PKA-C1 Pb01PKA-C2 Pb03PKA-C1	QDGVYAIKILHKADVIKLKQVEHVVREVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVVAKFYAAEVTLALEYLHDHH-I :*:*:: *::**: *::*::*:::::**:::::**:::::**:::::**:::::
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2	QDGVYAIKILHKADVIKLKQVEHVVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDVCPGGEVFTPLRAAHRSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: *::**: *: **: **: **: **: *: *::::**:::::**:::::**:::::**:::::**:::::
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY PRKY Pb01PKA-C1 Pb01PKA-C2 Pb03PKA-C1 Pb03PKA-C2 Pb18PKA-C1	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEVULILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: **:: **:: *:: *::: **::: *:::: **::::: **::::: **::::: **::::: IYRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFPAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFPAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFPAD-QPIQIYEKIVSGKVRFPSH IHRDLKPENLLIDQGYLQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAVGPPFPAD-QPIQIYEKIVSGKVRFPSH IHRDLKPENLLIDQGHIKLTDFGFAKRVKGR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFTDD-QPIQIYEKIVSGKVRFPSH VYRDLKPENILLDRDGHIKLTDFGFAKRVKDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFTDD-NPFGIYQKILAGKIYPPRH VYRDLKPENILLDRGHIKLTDFGFAKRVKDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFTDD-NPFGIYQKILAGKIYPPRH VYRDLKPENILLDRGHIKLTDFGFAKRVLOR-TWTLCGTPEYLAPEVISKGYNMSVDWWSLGILIFEMLGGFPFWDGSPMKIYENIIKCRVKYPSY VYRDLKPENILLDRGHLKLTDFGFAKUNGFTYTLCGTPEYLAPEVISKGYNMSVDWWSLGILIFEMLCGFTPFWDGSPMKIYENIIKCRVKYPSY YYRDLKPENLLLDRYGHLKITDFGFAKCWDI-TWTLCGTPDYLAPEVVSSKGYNMSVDWWSLGILIFEMLCGFTPFWDGGSPMKIYENIIKCRVKYPSY YYRDLKPENLLLDRYGHLKITDFGFAKQUWSRETYTLCGTPDYLAPEVVSKGYNMSVDWWSLGILIFEMLCGFTPFWDGGSPMKIYENIKCRVKYPSY YYRDLKPENILLDAGHLKLVDFGFAKQUWSRETYTLCGTPDYLAPEVVSKGYNMSVDWWSLGULIYEFIVGQPFFWDQ-NPMCIYEQIVQGRLRFPVN YYRDLKPENILLDAGHLKLVDFGFAKQUWSRETYTLCGTPEYLAPEVIHNKGHGLAVDWWALGVLIYEFIVGQPFFWDQ-NPMCIYEQIVQGRLRFPVN YYRDLKPENILLDAGHLKLVDFGFAKQUSSRETYTLCGTPEYLAPEVIHSKGYNMSVDWWSLGULIYEFIVGQPFFWDQ-NPMCIYEQIVQGRLRFPVN YYRDLKPENILLDACHLKVDFGFAKQUSSRETYTLCGTPEYLAPEVIHNKGHGLAVDWWALGVLIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKXC PRKY Pb01PKA-C1 Pb01PKA-C1 Pb03PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDONLYMLDYCPGGEVFTELRAAHRFSERTSQFYAAEVTLALEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVAKFYAAEVTLALEVHADHH-I :*:*:: *:: **: **: * : * : *:: **:: *:: **::: **:::: **:::: **:::: **:::: **:::: **::::: **::::: IYRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDQQYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDLKPENLLIDQQGYLQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IHRDLKPENLLIDQGYLQVTDFGFAKRVKGR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGVLIYEMAVGFPPFYAD-QPIQIYEKIVSGKVRFPSH IHRDLKPENILLDROGHIKLTDFGFAKKLVDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGULIYEMAVGFPPFYDD-NPFGIYQKILAGKLYPPRH YYRDLKPENILLDROGHIKLTDFGFAKKLVDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFFDD-NPFGIYQKILAGKLYPPRH AYRDLKPENILLDRYGHLKITDFGFAKCKVDI-TWTLCGTPEYLAPEVINSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN YYRDLKPENILLDAEGHLKLVDFGFAKQLWSRETYTLCGTPEYLAPEVIHNSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN IYRDLKPENILLDAEGHLKLVDFGFAKQLWSRETYTLCGTPEYLAPEVIHNSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN IYRDLKPENILLDAEGHLKLVDFGFAKQLWSRETYTLCGTPEYLAPEVIHNSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN IYRDLKPENILLDAEGHLKLVDFGFAKQLWSRETYTLCGTPEYLAPEVIHNSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN IYRDLKPENILLDAEGHLKLVDFGFAKQLWSI-TWTLCGTPEYLAPEVIHNSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN IYRDLKPENILLDRYGHLKITDFGFAKEVKDI-TWTLCGTPEYLAPEVIHNSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN IYRDLKPENILLDRYGHLKITDFGFAKEVKDI-TWTLCGTPEYLAPEVIHNSGHGLAVDWWALGULIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN IYRDLKPENILLDRYGHLKITDFGFAKEVKDI-TWTLCGTPEYLAPEVYSKSYMNSVDWWSLGILIFEMLCGFTPFWDGGSPMKIYENIIKCRVKYPSY
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PB01PKA-C1 Pb01PKA-C1 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEVULILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYHHDHH-I :*:*: *: *: **: **: *: *: *: *: *: *: *:
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACA	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEVTLALEVLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVAKFYAAEVTLALEVHADHH-I :*:*: *: *: **: * *: * *: **: *: **: *:
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKXCG PRKX PRKY Pb01PKA-C1 Pb01PKA-C2 Pb03PKA-C1 Pb18PKA-C1 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PB18PKA-C2	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDONLYMLDYCPGGEVFTPLRAAHRSERTSQFYAAEVTLALEYLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: **:: *:: **:: *:::: **::::: **::::: **::::::
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX Pb01PKA-C1 Pb01PKA-C1 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACB PRKACB PRKACG	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQVKLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEVULILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*: *: *: **: **: *: *: *: *: *: *: *:
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTLRAAHRSERTSQFYAAEVTLALEVLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEVHHDH-I :*:*:: *:: *:: *:: *::: *:::: **::::: **::::: **::::::
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTFLRAAHRSERTSQFYAAEVTLALEYHDHFI NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVAKFYAAEVTLALEYHHDHH-I :*:*:: *:: *:: *:: *:: *:::: *::::: **::::::
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Ph18PKA-C2 PRKACB PRKACB PRKX PRKX PRKX PRKX PRKX PD01PKA-C1 PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCACCB PRCA	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEVTLALEVHLAEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNVAKFYAAEVTLALEVHADHH-I :*:*: *: *: **: **: *: *: *: *: *: *: *:
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX Pb01PKA-C1 Pb03PKA-C1 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACB PRKACB PRKACB PRKXCB PRKXCB PRKXCB PRKX PRKY Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C2 Pb03PKA-C1 Pb01PKA-C2 Ph03PKA-C1 Pb01PKA-C2 PKACB PRKX PRKY Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C2 PKACB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKACCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKKCCB PKCCCB PKCCB PKCCB PKCCCB PKCCCB PKCCCB PKCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCCB PKCCB PKCCCB PKCCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTPLRAAHRSERTSQFYAAEVTLLEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYHHHH-I :*:*: *: *: **: **: *: *: *: *: *: *: *:
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACB PRKACB PRKACG PRKX PRKY Pb01PKA-C1 Pb01PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb03PKA-C1	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDYCPGGEVFTELRAAHRSERTSQFYAAEVTLALEVILDEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFIITLSUGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNDVAKFYAAEVTLALEVIHDH-I :*:*: *: *: **: *: *: *: *: *: *: *: *:
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACA PRKACB PRKACG PRKACG PRKX PRKY Pb01PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PKACA PKACA PKKACA PKKACA PKKACA PKKACA PKKACB PKKACA PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKKACB PKACCB PKACCB PKACC2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18PKA-C2 Ph18	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDYCPGGEVFTFLRAAHRSERTSQFYAAEVTLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTVNDERRMLQRVK-HPFLISLISTFSDDQNLYMLDYCPGGEVFTFLRAAHRSERTSQFYAAEVTLALEYHDHH-I :*:*:: *:: *:: *:: *::::: **:::::: **::::::
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX Pb01PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACB PRKACB PRKACB PRKACB PRKX PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb03PKA-C2 Pb18PKA-C1	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDVCPGGEVFTFLRAAHRFSERTSQFYAAEVTLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHVRDERRMLQRVK-HPFLITLWGTFQDVKNLYMVDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*: *: *: **: **: *: *: *: *: *: *: **: *:
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACB PRKACB PRKACG PRKX PRKY Pb01PKA-C1 Pb01PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PD19FKA-C1 Pb18PKA-C1 PD19FKA-C1 PD19FKA-C1 PD19FKA-C1 PD19FKA-C1 PD19FKA-C1 PD19FKA-C1 PD19FKA-C1 PD19FKA-C2 PD19FKA-C2 PD19FKA-C1 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FKA-C2 PD19FK	QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLDYCPGGEVFTFLRAAHRFSERTSQFYAAEVTLALEYLHDHH-I NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*: *: *: **: *: *: *: *: *: *: *: **: *:
Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACB PRKACB PRKACB PRKACB PRKACC PRKACC PRKY Pb01PKA-C1 Pb03PKA-C1 Pb03PKA-C1 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PKX PKX PKX PKX PKX PKX PKX PKX	QDGYYAIKILHKADVIKIKQVEHVENEVAFILAAVSGHPFIISLISTFSDDQNLYMLDYCPGGEVFTFLERAMRFSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPAKFYAAEVTLALEYLHDHH-I :*:*: *: *: *: *: * : * : * : * : * : *
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACB PRKXCB PRKXCB PRKXCB PRKXCCB PRKXC1 Pb01PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PKACB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKXCB PKCCB PKCCB PKCCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB PKCB	QDGYYAIKILHKADVIKLKQVEHVENEVARLAAVSGHPFIISLISTFSDDQRLYMLDYCPGGEVETFIARAMRFSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HFIITIWGTFQDVKNLYMVMDEVGGELFSLEKSQRFPNVAKTYAAEVTLALEYLHDHH-I :*:*: *: *: *: *: *: *: *: *: *: *: *: *
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PB01PKA-C1 Pb01PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACB PRKACB PRKACB PRKXCB PRKXCB PRKXCB PRKXCB PRKAC1 Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PB18PKA-C2 PB18PKA-C2 PB18PKA-C2 PB18PKA-C2 PB18PKA-C2 PB18PKA-C2 PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKX PRKACB PRKACB PRKACB PRKACB PRKX PRKX PRKX PRKX PRKACB PRKACCB PRKACCB PRKX PRKX PRKX PRKX PRKX PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKX PRKX PRKX PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB	QDGYYAIKILHKADVIKLKQUEHYRKEVRTLAAVSGHPFIISLISTFSDDQNLYMLDYCPGGEVFTLRAAHRSEKTSQFYAAEIVLILEFLHEVHGV NQRFYAIKULKKADVYKMKQUEHTNDERKNLQRVK-HPFIISLISTFSDDQNLYMLDYCMUEGELFSLLRAKSQRFPNPVAKFYAAEVTLALEYLHDHH-I :*:*:: *:: *:: *:: *:: *:: *:: *:: *::: **::: *::: **::: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *:: *::::::
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACB PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKY	<pre>QDGYYAIKILHKADVIKLKQVEHVENEVRILAAVSGHEPTISLISTTSDDQNLYHLDVCFGGEVFTELRAARFSERTSGFYAAEVILLEFIHEVHGV NQRFYAIKVLKKAQVVEMKQVEHTNEERRALGVK-HEFLITUKGFTQDVKNLYHVMDFVEGGEVFTELRAARFSERTSGFYAAEVILLEFIHEVHGV NQRFYAIKVLKKAQVVEMKQVEHTNEERRALGVK-HEFLITUKGFTQDVKNLYHVMDFVEGGEVFTELRAARFSERTSGFYAAEVILLEFIHEVHGV I***:: **: **: **: * :: .**: **: * :: .**:: **:: **: **</pre>
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACA PRKACB PRKXC PRKY Pb01PKA-C2 Pb03PKA-C1 Pb03PKA-C2 PD03PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 PB18PKA-C2 Pb03PKA-C2 PB18PKA-C2 Pb18PKA-C2 PB18PKA-C2 PB18PKA-C2 PRKACA PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACCB PRKY PD19KA-C2 PD19KA-C2 PD03PKA-C2 PRKACB PRKY PRKY PD01PKA-C2 PRKACB PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKACB PRKX PRKY PRKY PRKX PRKY PRKY PRKY PRKACB PRKACB PRKX PRKY PRKY PRKY PRCY PRKACB PRKX PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY PRKY	QDGYYAIKILHKADVIKLKQVEHVENEVRIJAAVSGHPFIISLISTFSDDQALYHLDYCEGEVFFILRAARFSERTSGFYAAEVILLEFILEVHGV NQRFYAIKVLKKAQVVENKGVEHTADERRMLGVK-HPFIISLISTFSDDQALYHLDYCEGEVFFILRAARFSERTSGFYAAEVILLEFILEVHGV NQRFYAIKVLKKAQVVENKGVEHTADERRMLGVK-HPFILIKUGTPOVINLYMVDFVEGEGEVFFILRAARFSERTSGFYAAEVILLEFILEVHGV IYRDIKPENLLIDQGYIQVTDFGFAKRVKGR-TWTLCGTPEVIAPEIILSKGYNKAVDWAALGVLIYEMAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDIKPENLLIDQGYIQVTDFGFAKRVKGR-TWTLCGTPEVIAPEIILSKGYNKAVDWAALGVLIYEMAGYPPFFAD-QPIQIYEKIVSGKVRFPSH IYRDIKPENLLIDRQGYLQVTDFGFAKRVKGR-TWTLCGTPEVIAPEIILSKGYNKAVDWAALGVLIYEMAVGFPPFAD-QPIQIYEKIVSGKVRFPSH VYRDIKPENILIDRQGHIKLTDFGFAKRVKGR-TWTLCGTPEVIAPEVIQSKGHGRAVDWAALGVLIYEMAVGFPPFAD-QPIQIYEKIVSGKVRFPSH NYRDIKPENILIDROHIKLTDFGFAKRVKGR-TWTLCGTPEVIAPEVIQSKGHGRAVDWAALGVLIYEFIVGGPPFFDD-NPFGIYQKILAGKLIPFRH AYRDIKPENILIDROHIKLTDFGFAKKVDR-TWTLCGTPEVIAPEVIQSKGHGRAVDWAALGVLIYEFIVGGPPFDD-NPFGIYQKILAGKLIPFRH AYRDIKPENILIDDAGHIKLTDFGFAKKVDN-TWTLCGTPEVIAPEVINSGHGAVDWAALGVLIYEFIVGGPPFDD-NPFGIYQKILAGKLIPFRH AYRDIKPENILLDAGHLKINDFGFAKGVNGI-TWTLCGTPEVIAPEVINNSGHGLAVDWAALGVLIYEFIVGGPPFNDG-NPMCIYEQIVQGRLRFPVN YYRDIKPENILLDAGHLKINDFGFAKGVNGI-TWTLCGTPEVIAPEVINNSGHGLAVDWAALGVLIYEFIVGQPPFNDGSSPMKIYENIIKCRVKYPSY YXRDIKPENILLDRYGHLKITDFGFAKGVNGI-TWTLCGTPEVIAPEVYINKGHGLAVDWAALGVLIYEFIVGGPPFNDGSSPMKIYENIIKCRVKYPSY :******::::::::::::::::::::::::::::::
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKXC PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 PRKACB PRKACB PRKACB PRKACB PRKACCB PRKACC PRKACC PB03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C2 PRKACB PRKACB PRKACB PRKACB PRKACB PRKACB PRKACCB PRKACB PRKACB PRKACCB PRKACCB PRKACB PRKACB PRKACCB PRKACCB PRKACCB PRKACB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKACCB PRKY PRKY Pb01PKA-C1	QDGYYAIKILHKADVIKLKQUEHVREVKTLAAVSGHEFIISLISTFSDQMLYMLLDYCGGEVFTFLRAHRSEKTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVINKKQUEHVREVKTLAAVSGHEFIISLSTSDDQMLYMLDYCGGEVFTFLRAHRSEKTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVINKKQUEHVREVKH.HFFIISLIMYMDFYEGGELFSLRKSQFYPRPVAAFYAAEVTLALEFLHOHH-I :*::: *:: *:: *:: *:: *:: *:: *:: *:: *
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY Pb01PKA-C2 Pb18PKA-C1 Pb01PKA-C2 Pb18PKA-C1 Pb18PKA-C2 Pb18PKA-C1 Pb18PKA-C2 PB18PKA-C1 Pb18PKA-C2 PRKACA PRKACB PRKACB PRKACA PRKACA PRKACA PRKACA PRKACA PRKY PB01PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACA PRKACA PRKACA PRKY PKY Pb01PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA PRKACA P	<pre>QDGYYAIKILHKADVIKLKQUEHVREVKTLAAVSGHEFIISLSTF5DDQNLYMLLDYCGGEVFTFLRAHRFSERTSQFYAAEIVLILEFLHEVN NQRFYAIKVLKKAQUHTDERRMLQRVK-HPFLITUGTFODVIKLIYMVDFVEGGELF5LLRSQRFPNPVAKTARAEUTLALEFLHDHH-I :*::: *:: *:: *:: *:: *:: *:: *:: *:: *</pre>
Pb18PKA-C2 PRKACA PRKACB PRKACB PRKX PRKY Pb01PKA-C1 Pb03PKA-C2 Pb03PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 PRKACA PRKACB PRKACG PRKX PRKY Pb01PKA-C2 Pb18PKA-C1 Pb18PKA-C1 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Pb18PKA-C2 Ph18PKA-C2 PKACA PRKXCB PRKXCB PRKX PRKY Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C1 Pb01PKA-C1 PKACA PRKXCB PRKX PKX	<pre>QDGYYAIKILHKADVIKLKQVEHVENEVRILAAVSGHPFIJSLISTSDDQNLYMLDYCEGGEVSTELRRAMRFSERTSGYYAAEIVLILEFLHEVHGV NQEFYAIKULKAQVVMKQVEHTHDERRULQVK-HPFLITLSGYPFDQVKILYWMDFVEGGEVSTELRRSQFPPHPVAKFYAAEVTILLEFLHEVHGV NQEFYAIKVLKKAQVVMKQVEHTHDERRULQVK-HPFLITLSGYPFDQVKILYWMDFVEGGEVSTELRRSQFPPHPVAKFYAAEVTILLEFLHEVHGV IYRDLKPENLLIDQQGYLQVTDFGFAKKVKGR-TWILGGTPEYLAPEIILSKGYNKADDWALGVLIYEMAAGYPFFDD-QPIGIYEKIVSGKVFPSH HRDLKPENLLIDQQGYLQVTDFGFAKKVKGR-TWILGGTPEYLAPEIILSKGYNKADDWALGVLIYEMAAGYPFFDD-QPIGIYEKIVSGKVFPSH HRDLKPENLLIDQQGYLQVTDFGFAKKVKGR-TWILGGTPEYLAPEIILSKGYNKADDWALGVLIYEMAAGYPFPDD-NPFGIYQKILAGKIFPSH HRDLKPENLLIDQGGYLQVTDFGFAKKVDDR-TWILGGTPEYLAPEVIQSKGHGRAVDWALGULIYEMAGYPFPDD-NPFGIYQKILAGKIFPSH HRDLKPENLLIDAGGHIKLTDFGFAKKLVDR-TWILGGTPEYLAPEVIQSKGHGRAVDWALGULIYEFIVGQPPFPDD-NPFGIYQKILAGKIFPSH AYRDLKPENILLDAEGHIKLTDFGFAKKLVDR-TWILGTPEYLAPEVIQSKGHGRAVDWALGULIYEFIVGQPPFPDG-NPFGIYQKILAGKIFPSH AYRDLKPENILLDAEGHIKLTDFGFAKKLVDR-TWILGGTPEYLAPEVIQSKGHGRAVDWALGULIYEFIVGQPPFPDG-NPFGIYQKILAGKIFPN AYRDLKPENILLDAEGHIKLTDFGFAKKLVDR-TWILGGTPEYLAPEVINSGHGANJOWALGULIYEFIVGQPPFPDG-NPFGIYQKILAGKIFPN YYRDLKPENILLDAEGHIKLTDFGFAKKLVDR-TWILGGTPEYLAPEVINSGHGNAJGVLIYEFIVGQPPFPDGSPMNUFYENI IKCRKYPSY YYRDLKPENILLDAEGHIKLTDFGFAKKUVDI-TWILGGTPEYLAPEVINKGHGLAJDWALGULIYEFIVGQPPFNDGSPMNIYENI IKCRKYPSY YYRDLKPENILLDAEGHIKLTDFGFAKSUVDI-TWILGGTPEYLAPEVSSKGYMSVDWAIGGULIYEFIVGQPPFNDGSPMNIYENI IKCRKYPSY YYRDLKPENILLDAEGHIKLTDFGFAKSUVDI-TWILGTPEYLAPEVSSKGYMSVDWAIGGULIYEFIVGQPPFNDGSPMNIYENI IKCRKYPSY YYRDLKPENILLDAEGHIKLTOFGFAKSUVKDI-TWILGTPEYLAPEVSKGKGNSVDWAIGGULIYEFIVGQPPFNDGSPMNIYENI IKCRKYPSY YYRDLKPENILLDAEGHIKLTOFGFAKSUVKDI-TWILGTPEYLAPEYSSKGYMSVDWAIGGULIYEFIVGQPPFNDGSPMNIYENI IKCRVKYPSY YYRDLKPENILLDAEGHIKUNGYGIKHKWFATTDWIAIYQRVEAPFIPEKSG-SGDTSNDDYEEEEIRYSINEKC</pre>
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Pb18PKA-C2   PRKACA   PRKACB   PRKACG   PRKX   PRKY   Pb01PKA-C1   Pb03PKA-C2   Pb03PKA-C2   Pb18PKA-C1   Pb18PKA-C2   PRKX   PRKACA   PRKACA   PRKACA   PRKACA   PRKACC   PPKACC   PPKACA   PRKACB   PRKX   PRKY   Pb01PKA-C1   Pb18PKA-C1   Pb18PKA-C2   PRKACA   PRKACB   PRKACA   PRKACA   PRKACA   PRKACC   Pb18PKA-C1   Pb01PKA-C2   Pb03PKA-C1   Pb01PKA-C2   Pb03PKA-C1   Pb03PKA-C1   Pb03PKA-C1   Pb03PKA-C1   Pb03PKA-C1   Pb18PKA-C1   Pb18PKA-C1   Pb18PKA-C2	QDGYVAIKILHKADVIKLKQVEHVRNERMAQSK-HVRLCATSDDQVKILYMLDVCGGGELF5LLRAHRSSERTSQFYAAEIVLILEFLHEVHGV NQRFYAIKVLKKAQVKHKQVEHVDERMAQSK-HVRLCATPEILISISTESDDQVKILYMVBUFVGGGELF5LLKKSQRFPPHVKARFYAAEVILLEFYLHEVHG I'NDLKPENLLDQQGYIQYTDGFAKKVKGR-TWRLCATPEILDESLISKGYNKAVDWWALGVLIYMAAGYPPFFAD-QPIQIYKKVSGKVRFPSH HKDLKPENLLDDQQGYIQYTDGFAKKVKGR-TWRLCATPEYLAPEILLSKGYNKAVDWWALGVLIYMAAGYPPFFAD-QPIQIYKKVSGKVRFPSH HKDLKPENLLDDQQGYIQYTDGFAKKVKGR-TWRLCATPEYLAPEILSKGYNKAVDWWALGULIYMAAGYPPFFAD-QPIQIYKKVSGKVRFPSH HKDLKPENLLDDQGYLQYTDGFAKKVKGR-TWRLCATPEYLAPEILSKGYNKAVDWWALGULIYMAAGYPPFFAD-QPIQIYKKVSGKVRFPSH HKDLKPENLLDDQGYLQYTDGFAKKVKGR-TWRLCATPEYLAPEILSKGYNKAVDWWALGULIYMAAGYPPFFAD-QPIQIYKKVSGKVRFPSH HKDLKPENLLDDQGYLQYTDGFAKKVKGR-TWRLCATPEYLAPEILSKGYNKAVDWWALGULIYMAGGPFPFYD-NPFGIYGKLAGKLYFPH VYRDLKPENLLDDRGHIKLTDGFAKKUVDR-TWRLCATPEYLAPEVIGSKGHGRAVDWWALGULIFENLSGFPFFDD-NPFGIYGKLAGKLYFPH VYRDLKPENLLDDRGHIKLTDGFAKKVVDI-TWRLCGTPEYLAPEVIGSKGHGRAVDWWALGULIFETVQOPPFHQD-NFMGIYSGUGKLFFVN VYRDLKPENLLDDRGHIKLTDGFAKKEVKDI-TWRLCGTPEYLAPEVISSKGNMSVDWWSLGILIFENLCGFTPFHOGGSSMKIYENIGKLFFVN VYRDLKPENLLDDRGHKKUTDFGFAKEVKDI-TWRLCGTPEYLAPEVISSKGNMSVDWWSLGILIFENLCGFTPFHOGGSSMKIYENIGKLFFVN VYRDLKPENLLDDRGHKKUTDFGFAKEVKDI-TWRLCGTPEYLAPEVISSKGNMSVDWWSLGILIFENLCGFTPFHOGGSSMKIYENIGKLFFVN VYRDLKPENLLDDRGHKKUTDFGFAKGUWSSRTYTLCGTPEYLAPEVISSKGNMSVDWWSLGILIFENCGCFTPFFVDGGSSMKIYENIGKKFY YYNDLKPENLLDDRGHKKUTDFGFAKGUWSSKGNNGVGVSSKGNUSVDWSLGILIFENCCGFTPFVDGGSSMKIYENIGKKFY YYNDLKPENLLDRGGSDWKNDFFKSUNGI-TWRLCATPEYLAPEVIHNSGHGLAVDWSLGULIFENUGCGFTPFVDGGSSMKIYENIGKLFFVN YYNDLKPENNLLDDRGHKKUTDGFAKGUWSSKGNNGVGVSSWDWSLGILIFFNCCGFTPFVDGSSMCHIYENIKKTVENYSY '***********************************

S1 Figure: Multiple alignments of the *Paracoccidioides* and human PKA-C subunits. This analysis was performed using the t-coffee program at http://www.ebi.ac.uk/Tools/msa/tcoffee/. The N-terminus present only in PbPKA-C2 subunits is highlighted in yellow. The "\*", "." and ":" indicate the conserved residues. Observe that human and *Paracoccidioides*PKA-C are highly divergent both in the N- and C-terminus (highlighted in color).

# Predicted cellular localization of the *Paracoccidioides* PKA subunits

Subcellular localization data gives important clues to understanding protein function. The cellular localization of PKA is decisive in determining which substrates are phosphorylated. The *in silico* prediction suggests that *Paracoccidioides* PKA subunits can be found in more than one subcellular location, as shown in table2. It seems that PKA-C1 subunits act mainly in the cytosol, while the PKA-C2 is transported to the nucleus. Psort II prediction also states a possibility that these subunits might be placed in other subcellular location, like mitochondria and secretory vesicles. However, these data need to be experimentally confirmed.

So far, there is only one study demonstrating the location of a PKA subunit in *Paracoccidioides*. It has been demonstrated that Pb01PKA-C2 goes to the nucleus where it phosphorylates transcription factors, altering the expression of genes that control the morphological changes in this fungus [14].

Several studies have demonstrated that the localization of PKA subunits may change depending on the external stimulus and also over lifecycle [25,26]. For instance, in *S. cerevisiae* cells cultured in the presence of glucose, during exponential growth, both Bcy1 and Tpk2 are localized in the nucleus, whereas Tpk1 and Tpk3 display a mixed pattern of nucleo-cytoplasmic localization; but in cells grown on glycerol and during stationary phase, the PKA subunits exhibited a cytoplasmic localization [26].

In multicellular organisms, the localization of PKA holoenzymes depends on the interaction with A kinase anchoring proteins (AKAPs). These proteins target PKA to specific subcellular locations, conferring spatio-temporal control of PKA signaling [27]. Nevertheless, AKAP proteins have not been identified in fungi and, so, the knowledge of the spatio-temporal control of PKA in these organisms is restricted.

#### **Protein domains in the PKA subunits**

The protein domains were scanned in the PROSITE database and the results are depicted in figure 2. The *Paracoccidioides* PKA subunits present the canonical domains both for the PKA-R and the PKA-C subunits.

Each PKA-R subunit presents two tandem copies of the cyclic nucleotide monophosphate-binding (CNMP) domain at the carboxyl terminus. The CNMP-binding is a structural domain of about 120 residues present in proteins that bind the cyclic nucleotides cAMP or cGMP. Prior to activation, PKA isoforms are maintained in an

inactive state by the formation of an heterotetrameric complex consisting of two PKA-C bound to two PKA-R subunits. The cooperative binding of two cAMP molecules to the CNMP-binding domains mediate the allosteric activation of the holoenzyme by releasing the PKA-C subunits [28].

All the Paracoccidioides PKA-C subunits present 2 protein domains: a protein kinase and an AGC-kinase Cterminal domain. The protein kinase domain is a structurally conserved protein domain involved in the transfer of the gamma phosphate group to amino acid residues in proteins. This event results in conformational changes that turns on/off many cellular processes, like cycle progression, transcription, metabolism, cell cytoskeletal rearrangement, apoptosis, and differentiation [29]; The AGC-kinase C-terminal domain, where AGC stands for cAMP-dependent, cGMP-dependent and protein kinase C protein kinase family, is found in several protein kinases. AGC kinase proteins exhibit three conserved phosphorylation sites that regulate their function. These sites serve as phosphorylation-regulated switches to control both intra- and inter-molecular interactions [30] Although both PbPKA-C1 and PbPKA-C2 present the same domains, differences in the expression pattern, protein activity and subcellular localization may confer specificity of PKA signaling to different stimulus and environmental conditions.

## Comparative alignment of human and *Paracoccidioides* PKA subunits

Multiple sequence analysis was used to create a branched tree that displays the relationship between Paracoccidioides and human PKA catalytic subunits (figure 3). This analysis clearly indicates a distinction between PKA in these two organisms. The multiple alignments shows that, although there is a moderate degree of conservation between the human and *Paracoccidioides* PKA subunits, a significant difference in both the N-and C-terminus can be observed (S1 figure). This divergence between human and Paracoccidioides PKA protein sequence, coupled with the central role that this protein plays in many cellular processes, makes PKA an attracting target for development of antifungal drugs. To date, there are few studies on the Paracoccidioides PKA function and interactions. Further work is required to identify and characterize the expression, activity and cellular targets of PKA in these fungi.



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#### References

- 1. Hanks SK, Quinn AM, Hunter T (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241(4861): 42-52.
- Taylor SS, Kim C, Cheng CY, Brown SH, Wu J, et al. (2008) Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. Biochim Biophys Acta 1784(1): 16-26.
- 3. Turnham RE, Scott JD (2015) Protein kinase A catalytic subunit isoform PRKACA; History, function and physiology. Gene 577(2): 101-108.
- Taskén K, Skålhegg BS, Taskén KA, Solberg R, Knutsen HK, et al. (1997) Structure, function, and regulation of human cAMP-dependent protein kinases. Adv Second Messenger Phosphoprotein Res 31: 191-204.
- Soberg K, Jahnsen T, Rognes T, Skalhegg BS, Laerdahl JK (2013) Evolutionary paths of the cAMP-dependent protein kinase (PKA) catalytic subunits. PLoS One 8: e60935.
- 6. Toda T, Cameron S, Sass P, Zoller M, Wigler M (1987) Three different genes in S. cerevisiae encode the

catalytic subunits of the cAMP-dependent protein kinase. Cell 50(2): 277-287.

- Pan X, Heitman J (1999) Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in Saccharomyces cerevisiae. Mol Cell Biol 19(7): 4874-4887.
- 8. Choi W, Dean RA (1997) The adenylate cyclase gene MAC1 of Magnaporthe grisea controls appressorium formation and other aspects of growth and development. Plant Cell 9(11): 1973-1983.
- 9. Gold S, Duncan G, Barrett K, Kronstad J (1994) cAMP regulates morphogenesis in the fungal pathogen Ustilago maydis. Genes Dev 8(23): 2805-2816.
- 10. Lee N, D'Souza CA, Kronstad JW (2003) Of smuts, blasts, mildews, and blights: cAMP signaling in phytopathogenic fungi. Annu Rev Phytopathol 41: 399-427.
- 11. Rocha CR, Schroppel K, Harcus D, Marcil A, Dignard D, et al. (2001) Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus Candida albicans. Mol Biol Cell 12(11): 3631-3643.
- 12. Zhao W, Panepinto JC, Fortwendel JR, Fox L, Oliver BG, et al. (2006) Deletion of the regulatory subunit of protein kinase A in Aspergillus fumigatusalters morphology, sensitivity to oxidative damage, and virulence. Infect Immun 74(8): 4865–4874.
- 13. Chen D, Janganan TK, Chen G, Marques ER, Kress MR, et al. (2007) The cAMP pathway is important for controlling the morphological switch to the pathogenic yeast form of Paracoccidioides brasiliensis. Mol Microbiol 65(3): 761-779.
- 14. Janganan TK, Chen G, Chen D, Menino JF, Rodrigues F, et al. (2015) A G $\beta$  protein and the TupA Co-Regulator Bind to Protein Kinase A Tpk2 to Act as Antagonistic Molecular Switches of Fungal Morphological Changes. PLoS One 10(9): e0136866.
- 15. Matute DR, McEwen JG, Puccia R, Montes BA, San-Blas G, et al. (2006) Cryptic speciation and recombination in the fungus Paracoccidioides brasiliensis as revealed by gene genealogies.Mol Biol Evol 23(1): 65-73.
- 16. Teixeira MM, Theodoro RC, de Carvalho MJ, Fernandes L, Paes HC, et al. (2009) Phylogenetic analysis reveals a high level of speciation in the

Paracoccidioides genus. Mol Phylogenet Evol 52(2): 273-283.

- 17. Bocca AL, Amaral AC, Teixeira MM, Sato PK, Shikanai-Yasuda MA et al. (2013) Paracoccidioidomycosis: ecoepidemiology, taxonomy and clinical and therapeutic issues. Future Microbiol 8(9): 1177-1191.
- Klein BS, Tebbets B (2007) Dimorphism and virulence in fungi. Curr Opin Microbiol 10(4): 314-319.
- Desjardins CA, Champion MD, Holder JW, Muszewska A, Goldberg J, et al. (2011) Comparative Genomic Analysis of Human Fungal Pathogens Causing Paracoccidioidomycosis. PLoS Genet 7(10): e1002345.
- 20. Muñoz JF, Gallo JE, Misas E, Priest M, Imamovic A, et al. (2014) Genome update of the dimorphic human pathogenic fungi causing paracoccidioidomycosis. PLoS Negl Trop Dis 8(12): e3348.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3): 403-410.
- 22. Di Tommaso P, Moretti S, Xenarios I, Orobitg M, Montanyola A, et al. (2011) T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. Nucleic Acids Res 39: W13-17.
- Sigrist CJ, de Castro E, Cerutti L, Cuche BA, Hulo N, et al. (2012) New and continuing developments at PROSITE. Nucleic Acids Res41(Database issue): D344-347.

- 24. Nakai K, Horton P (1999) PSORT: a program for detecting the sorting signals of proteins and predicting their subcellular localization. Trends Biochem Sci 24(1): 34-36.
- 25. Schaekel A, Desai PR, Ernst JF (2013) Morphogenesisregulated localization of protein kinase A to genomic sites in Candida albicans. BMC Genomics 14: 842.
- Tudisca V, Recouvreux V, Moreno S, Boy-Marcotte E, Jacquet M, et al. (2010) Differential localization to cytoplasm, nucleus or P-bodies of yeast PKA subunits under different growth conditions. Eur J Cell Biol 89(4): 339-348.
- 27. Edwards AS, Scott JD (2000) A-kinase anchoring proteins: protein kinase A and beyond. Curr Opin Cell Biol 12(2): 217-221.
- 28. Herberg FW, Taylor SS, Dostmann WR (1996) Active site mutations define the pathway for the cooperative activation of cAMP-dependent protein kinase. Biochemistry 35(9): 2934-2942.
- 29. Hanks SK, Quinn AM (1991) Protein kinase catalytic domain sequence database: Identification of conserved features of primary structure and classification of family members. Methods Enzymol 200: 38-62.
- 30. Parker PJ, Parkinson SJ (2001) AGC protein kinase phosphorylation and protein kinase C. Biochem Soc Trans 29(6): 860-863.