# Cloning, sequence identification and tissue transcription profile analysis of novel inwardly rectifying potassium channel *KCNJ12* gene from the Chinese water buffalo (*Bubalus bubalis*)

JOURNAL OF ANIMAL PLANT SCIENCES

Jin-long HUO<sup>1\*</sup>, Wei-zhen LI<sup>1\*</sup>, Yong-yun ZHANG<sup>2\*</sup>, Qiao-ling ZHANG<sup>3\*</sup>, Hai-long HUO<sup>4</sup>, Li-xian LIU<sup>4</sup>, Jing LENG<sup>1</sup>, Da-lin LI<sup>5</sup>, Yue-yun YUAN<sup>5</sup>, Feng YUAN<sup>1</sup>, Lian-jun LI<sup>1</sup>, Yong-wang MIAO<sup>1†</sup>

<sup>1</sup> Faculty of Animal Science and Technology, Yunnan Agricultural University, Kunming 650201, Yunnan, China <sup>2</sup> Teaching Demonstration Conten of the Pacie Festberiments of Agricultural Majors Yunnan, Agricultural University

- <sup>2</sup> Teaching Demonstration Center of the Basic Experiments of Agricultural Majors, Yunnan Agricultural University, Kunming 650201, Yunnan, China
- <sup>3</sup> College of Animal Science and Veterinary Medicine, Jilin University, Changchun 130062, Jilin, China
- <sup>4</sup> Department of Husbandry and Veterinary, Yunnan Vocational and Technical College of Agriculture, Kunming 650031, Yunnan, China
- <sup>5</sup> Domestic Animal Breeding and Crossbreed-improvement Station of Yunnan Province, Kunming 650021, Yunnan, China
- \* These authors contributed equally to this work

*†* Corresponding author. E-mail: yongwangmiao999@163.com & miaoyw1@ynau.edu.cn **Key words:** Water Buffalo; *KCNJ12* gene; tissue transcription profile; bioinformatics analysis

# 1 SUMMARY

Potassium inwardly-rectifying channel, subfamily J, member 12 (KCNJ12) plays a critical role in central ventilator chemosensitivity, and this role could possibly exhibit developmental changes. In the present study, water buffalo *KCNJ12* cDNA was cloned and characterized. This novel gene was then deposited into NCBI database and assigned to accession number KC011846 (amino acids accession number AGC91909). The complete open reading frame of water buffalo *KCNJ12* gene was 1281 bp encoding a KCNJ12 protein of 427 amino acids with a molecular weight of 48.44 kDa and a pI of 5.55. The putative proteins of KCNJ12, which are located in the cytoplasm (94.1%), contain a conserved domain of Ion\_trans\_2 superfamily and three potential transmembrane regions without N-terminal signal peptide, which indicates that KCNJ12 was non-secretory and membrane-embedded proteins. Similarity comparisons for amino acid sequences reveal that the water buffalo KCNJ12 protein shares 99.8%, 99.8%, 96.5%, 96.3%, 94.4% and 94.1% identity with that of *Bos taurus, Ovis aries, Pan troglodytes, Homo sapiens, Rattus norvegicus* and *Mus musculus*. The phylogenetic tree analysis revealed that the buffalo *KCNJ12* gene has a closer genetic relationship with the *KCNJ12* genes of *Bos taurus* and *Ovis* 

*aries* than with those of *Pan troglodytes*, *Homo sapiens*, *Rattus norvegicus* and *Mus musculus*. Real-time PCR analysis shows that water buffalo *KCNJ12* gene is expressed in various tissues, but at different levels. The expression levels of this gene are high in longissimus dorsi, fat, cerebrum, and heart, moderate in uterine wall, skin, lung, duodenum, oviduct, placenta, spleen, and mammary gland, non-expressed in liver, stomach, pancreas, and ovary. These data provide a foundation for further insight into this buffalo gene.

## 2 INTRODUCTION

The inwardly rectifying potassium current  $I_{K1}$  in cardiac myocytes appears to play an important role in stabilizing the resting membrane potential (RMP) and forming the late repolarization phase of the action potential (Shimoni et al., 1992; Lopatin & Nichols, 2001; Miake et al., 2003). The Kir2 (IRK) subfamily is expressed in many cell types including cardiac myocytes and neurons, and controls the excitability of these cells (Nichols and Lopatin, 1997). It is not surprising that there is functional evidence from transcriptional analysis in combination with comparisons of the biophysical characteristics of cloned Kir channels that members of the Kir2 subfamily provide the major component of cardiac IK1 (Dixon & McKinnon, 1994; Barry et al., 1995; Brahmajothi et al., 1996). In the rat brain, three different subunits of the Kir2 channel subfamily have been found and identified: Kir2.1 (Kubo et al., 1993), Kir2.2 (Takahashi et al., 1994), and Kir2.3 (Morishige et al., 1994). In guinea pig heart, Kir2.2 (potassium inwardly-rectifying channel, subfamily J, member 12, KCNJ12) have been discovered contribute to

#### 3 MATERIALS AND METHODS

**3.1** Animals and samples collection: Sixteen kinds of tissue samples, including the cerebrum, skin, heart, liver, spleen, lung, stomach, pancreas, duodenum, longissimus dorsi, fat, mammary gland, ovary, oviduct, uterine wall and placenta were

 $I_{K1}$  (Liu *et al.*, 2001). Recent studies displayed that, in the rat brainstem, a group of chemosensitive nuclei (e.g., locus coeruleus, raphe nucleus) expressed Kir2.2 in an age-dependent way, whereas other subtypes of Kir2: Kir2.1 and Kir2.3 were not (Karschin et al., 1996). This finding indicates that Kir2.2 may play a significant ventilatory part in central chemosensitivity; furthermore, the role could be exhibited in developmental changes in a large part. In Kaibar's study, they have identified human Kir2.2 gene in normal individuals that contained R285 (Arginine residue at position 285) in the deduced amino-acid sequence (Kaibara et al., 2002). Water buffalo are the important domestic animals in subtropical and tropical areas, in this study we firstly cloned the fulllength coding sequence of the water buffalo KCNJ12 gene, and subsequently did the bioinformatics analysis based on the data obtained, and finally examined their expression in 16 tissues by quantitative real-time PCR. These will provide a primary foundation for further research on this water buffalo gene.

collected from six adult female Binlangjiang buffalo and five adult female Dehong buffalo in Yunnan province, China. The samples were snap-frozen immediately in liquid nitrogen after buffalo had been slaughtered.

JOURNAL OF ANIMAL PLANT SCIENCES

**3.2 RNA isolation, cDNA synthesis:** The total RNA was extracted using the RNAiso Plus (TaKaRa, Dalian) according to the manufacturer's instructions. To remove genomic DNA contamination, total RNA was digested with RNase-free DNase I (Huo *et al.*, 2012; Deng *et al.*, 2013). Three micrograms of RNA were reverse transcribed with oligo (dT)<sub>18</sub> primer and M-MLV reverse transcriptase (Invitrogen, USA). The efficiency of reverse transcription was checked on 2% agarose gel electrophoresis stained with ethidium bromide.

3.3 Isolation of the water buffalo KCNJ12 gene: The KCNI12 sequences for cattle (accession no. NM\_001024690), rat (accession no. NM\_053981) and human (accession no. NM\_021012) were used to design a primer pair to amplify the complete coding sequence of KCNJ12 gene by using Primer Premier 5.0 software. The primer set was: 5'-TGCCCCCACCTCCTGGATGAC -3' (forward) and 5'- CTCAGATCTCAGACTCCCGT -3' (reverse). Reverse transcription-polymerase chain reaction (RT-PCR) was performed to isolate the water buffalo KCNJ12 using the pooled cDNAs from different tissues mentioned above. The 25  $\mu$ l reaction system was: 1  $\mu$ l (25 ng/ $\mu$ l) cDNA, 4  $\mu$ l 2.5 mM mixed dNTPs (TaKaRa, Dalian), 12.5 µl 2×GC buffer I (TaKaRa, Dalian), 0.5 µl 10 µM forward primer,  $0.5 \,\mu l \, 10 \,\mu M$  reverse primer,  $0.25 \,\mu l \, 5 \, U/\mu l$ Ex Taq HS DNA polymerase (5 U/µl, TaKaRa, Dalian), and 6.25 µl sterile water. The PCR program of KCNJ12 initially started with a 95 °C denaturation for 5 min, followed by 34 cycles of 94 °C / 30 s, 58 °C /45 s, 72 °C /2 min, then 72 °C extension for 5 min, finally 4 °C to terminate the reaction. The PCR products from water buffalo KCNJ12 cDNA were then cloned into pMD18-T vector (TaKaRa, Dalian) and sequenced bidirectionally with the commercial fluorometric method. At least ten independent clones were sequenced for each PCR product. The complete

coding sequence of the water buffalo *KCNJ12* gene has been deposited in the NCBI database and was assigned accession no. KC011846.

3.4 Bioinformatics analysis: Sequences were examined and edited by using the DNASTAR software. Sequence alignments were performed using online software in National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). The position and number of SNPs as well as corresponding haplotypes were exported with DNASTAR, Clustal X 1.83, EditPlus and MEGA 4.0 softwares. The base composition analysis was done by employing Mega version 4.0. Number of amino acids, theoretical pI, molecular weight, amino acid composition, total number of negatively charged residues, total number of positively charged residues, atomic composition, formula, total number of atoms, extinction coefficients, half-life, the N-terminal of the sequence, instability index, aliphatic index were predicted using ProtParam tool (http://web.expasy.org/protparam/). Signal peptide was predicted using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/). Protein sorting signals and localization sites were predicted using PSort II (http://psort.hgc.jp/). protein domains, families and functional sites was predicted using Prosite (http://prosite.expasy.org/). The protein conserved domains and alignment were analyzed using the Conserved Domain Architecture Retrieval Tool of BLAST at the NCBI server (http://www.ncbi.nlm.nih.gov/BLAST).

Transmembrane helices in proteins were also predicted by TMHMM Server version 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

Secondary structures of deduced amino acid sequences were predicted by SOPMA (http://npsapbil.ibcp.fr/). Protein hydrophobicity structure was predicted using ProtScale (http://us.expasy.org/cgibin/protscale.pl). Sequence similarity comparison and

the neighbor-joining phylogenetic trees were constructed based on KCNJ12 protein sequences by employing the Clustal X 1.83 and MEGA 4.0 and DNAMAN, which subsequently were edited manually. Statistical significance of groups within phylogenetic trees was evaluated using the bootstrap method with 10,000 replications. Web-based microRNA (miRNA) predicting programs were used to locate conserved potential miRNA targets: miRBase (http://www.mirbase.org/).

**3.5 Expression profile analysis by quantitative real-time PCR:** Quantitative real-time PCR was performed with Mastercycler ep realplex<sup>4</sup> (Eppendorf) using GoTaq<sup>®</sup> qPCR Master Mix (Promega) according to the manufacturer's instructions. We selected the housekeeping gene *18S* as the endogenous control. The control primers used

#### 4 **RESULTS**

# **4.1 PCR result of water buffalo** *KCNJ12* gene: The PCR products amplified from different tissue cDNAs for water buffalo *KCNJ12* gene were 1301 bp, (Fig. 1). This cDNA nucleotide sequence analysis using the BLAST software at NCBI server revealed that BMI *KCNJ12* gene was not homologous to any of the known water buffalo genes and it was then deposited into the GenBank database under accession No. KC011846.

**4.2 Physical and chemical characteristics of KCNJ12:** The coding region for the *KCNJ12* was 1284 bp encoded 427 amino acids. The *KCNJ12* CDS had an overall base composition of A 19.39% (249),

were: 5'- GGACATCTAAGGGCATCACAG -3' (forward) and 5'-AATTCCGATAACGAACGAGACT -3' (reverse) with a predicted amplicon size of 145 base pairs. The primers of KCNJ12 were: 5'-GGCAACCTACGCAAGAGC -3' (forward), 5'-CAGGATGGTGATGGGAGACA -3' (reverse) with a predicted amplicon size of 101 base pairs. Relative transcript quantification was performed using standard curves generated for 18S and KCNJ12 gene from a 2 fold serial dilution of cDNA. Heart sample cDNA was used to generate the standard curves. The amplification conditions were used the default setting. Optical data were collected at the end of each extension step, and relative expression of PCR products was determined by the 2-AACT method (Livak et al., 2001).

G 30.84% (396), T 18.77% (241) and C 31% (398). The complete CDS and the encoded amino acids were presented in Fig.2 The theoretical pI and the molecular weight of KCNJ12 are 5.55 and 48.44 kDa respectively. The protein contains twenty kinds of amino acids: 32 Ala (A) (7.5%), 30 Arg (R) (7.0%), 14 Asn (N) (3.3%), 24 Asp (D) (5.6%), 10 Cys(C) (2.3%), 15 Gln (Q) (3.5%), 34 Glu (E) (8.0%), 26 Gly (G) (6.1%), 12 His (H) (2.8%), 28 Ile (I) (6.6%), 37 Leu (L) (8.7%), 16 Lys (K) (3.7%), 13 Met (M) (3.0%), 25 Phe (F) (5.9%), 16 Pro (P) (3.7%), 26 Ser (S) (6.1%), 22 Thr (Γ) (5.2%), 5 Trp (W) (1.2%), 11 Tyr (Y) (2.6%), 31 Val (V) (7.3%).



**Figure 1:** RT-PCR result for BMI *KCNJ12* gene. M, DL2000 DNA marker; 1, PCR product.

Total number of negatively charged residues (Asp + Glu) is 58 and total number of positively charged residues (Arg + Lys) is 46. The protein contains 2158 Carbon (C), 3369 Hydrogen (H), 591 Nitrogen (N), 632 Oxygen (O), 23 Sulfur (S), formula is C<sub>2158</sub>H<sub>3369</sub>N<sub>591</sub>O<sub>632</sub>S<sub>23</sub>, total number of atoms is 6773, Ext. coefficient is 44515 (extinction coefficients are in units of M<sup>-1</sup> cm<sup>-1</sup>, at 280 nm measured in water), estimated half-life is 30 hours (mammalian reticulocytes, in vitro), the N-terminal of the sequence considered is M (Met), the instability index (II) is computed to be 43.84 and aliphatic index is 87.92. Submitting the KCNJ12 protein sequence to SignalP, the KCNJ12 protein has no N-terminal signal peptide and which is a non-secretory protein (Petersen et al., 2011). For subcellular localization analysis, the amino acid sequence was submitted to the PSORT II program, and Reinhardt's method showed water buffalo KCNJ12 was probably located in the cytoplasm with up to 94.1% probability (Nakai and Horton, 1999). Then putative protein was analyzed using Prosite (http://prosite.expasy.org/), five kinds sites were found, which were Protein kinase C phosphorylation sites (4-SgR-6, 38-TrR-40, 64-SqR-66, 354-TpR-356, 358-SaK-360), Casein kinase II phosphorylation sites (14-SseE-17, 15-SeeD-18, 74-TcvD-77, 284-SrqD-287, 358-SakD-361, 385-SrdE- 388, 414-TalE-417), N-myristoylation sites (178-GAimAK-183, 301-GMveAT-306); Tyrosine kinase phosphorylation site (236-RvteEge.Y-243) and cAMP- and cGMP-dependent protein kinase phosphorylation site (422-RReS-425). Examined using the Conserved Domain Architecture Retrieval Tool of NCBI Blast the at server (http://www.ncbi.nlm.nih.gov/BLAST) indicated that KCNJ12 contains two separated conserved domain-inward rectifier potassium channel Nterminal (from 2 to 46 amino acid residues) and inward rectifier potassium channel (from 47 to 383 amino acid residues), (Fig.2 and Fig.3). Transmembrane topology prediction made by TMHMM program (Moller et al., 2001) indicated that there were three transmembrane sequences found in the KCNJ12 (84-106AA, 126-148AA and 155-177AA) (Fig. 4). The prediction of secondary structure by SOPMA indicates that the deduced water buffalo KCNJ12 contains 147 alpha helices (34.43%), 85 extended strands (19.91%), 17 beta turns (3.98%) and 178 random coils (41.69%) (Fig. 5). The hydrophobic structure prediction of buffalo KCNJ12 by ProtScale indicated that KCNJ12 had the hydrophobic maximum 3.011 at 104th amino acids and the minimum -3.078 at 422th and 421th amino acids (Fig. 6)

	ROURNAL	V
- 4	Or	н
	ANIMAL	N
1		H
2	PLANT	Þ
	SCIENCES	E.
	A REAL PROPERTY OF A DESCRIPTION OF A DE	

1	ATG	ACT	GCG	тсо	GGC	CGC	ACA	AAC	ccc	TAC	AGO	ATC	GTG	тст	TCA	GAG	GAG	GAC	GGG	стб	CAC	стб	GTC	ACC	ATG	TCG	GGC	GCC	AAC	GGC
1	М	Т	A	S	G	R	Т	N	P	¥	S	Ι	V	S	S	Е	Е	D	G	L	н	L	V	Т	М	S	G	A	N	G
91	ттс	GGC	AAT	GGC	AAG	GTG	CAC	ACG	CGG	CGC	AGG	TGC	CGG	AAT	CGC	TTC	GTC	AAG	AAG	AAC	GGC	CAG	TGC	AAC	ATC	GAG	ттс	GCC	AAC	ATG
31	F	G	N	G	K	v	Н	Т	R	R	R	С	R	N	R	$\mathbf{F}$	V	К	К	N	G	Q	С	N	Ι	Е	$\mathbf{F}$	A	N	М
181 61	GAT D	GAG E	AAG K	TCG S	CAG 0	CGC R	TAC Y	CTG L	GCG A	GAC D	ATG M	TTC F	ACC T	ACG T	TGC C	GTG V	GAC D	ATC	CGC R	TGG W	CGC R	TAC Y	ATG M	CTG L	стс L	ATC I	TTC F	TCG S	CTG L	GCC A
																														_
271	TTC	стс	GCC	TCC	TGG	TTG	CTG	TTC	GGG	GTC	ATC	TTC	TGG	GTC	ATI	GCI	GTG	GCC	CAI	GGG	GAC	CTG	GAG	сст	GCC	GAG	GCC	CAC	GGC	CGC
91	F	L	A	S	W	L	L	F	G	V	Ι	F	₩	V	Ι	A	V	A	н	G	D	L	Е	Р	A	Е	A	н	G	R
361	ACG	CCG	TGC	GTG	CTG	CAG	GTG	CAT	GGC	ттс	ATG	GCG	GCC	ттс	сто	ттс	тсс	ATT	GAG	ACG	CAG	ACC	ACC	ATT	GGC	TAC	GGG	CTG	CGC	TGC
121	Т	Р	С	V	L	Q	V	Н	G	F	M	A	A	F	L	F	S	Ι	E	Т	Q	Т	Т	Ι	G	¥	G	L	R	с
451	GTG	ACC	GAG	GAG	TGC	CCG	GTG	GCG	GTG	TTC	ATG	GTG	GTG	GCG	CAG	тсс	ATC	GTG	GGC	TGC	ATC	ATC	GAC	тсс	ттс	ATG	АТТ	GGC	GCC	ATC
151	v	Т	Е	Е	с	Р	v	A	V	F	М	v	v	A	Q	S	Ι	v	G	с	Ι	Ι	D	S	F	M	Ι	G	A	I
					_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_					
541	ATG	GCC	AAG	ATG	GCG	CGG	CCC	AAG	AAG	CGT	GCA	CAG	ACG	CTG	CTA	TTC	AGC	CAC	AA1	GCG	GTG	GTG	GCG	CTG	CGT	GAC	GGC	AAG	стс	TGC
101		A	м	м	А	к	Р	ĸ	м	к	А	ų	1	г	г	г	3	л	м	А	v	v	A	г	к	<u> </u>	G	г	г	<u> </u>
631	стс	ATG	TGG	CGC	GTG	GGC	AAC	СТА	CGC	AAG	AGO	CAT	TTA	GTG	GAG	GCC	CAC	GTG	CGG	GCC	CAG	сто	ATC	AAG	ccc	CGG	GTC	ACG	GAG	GAG
211	L	М	₩	R	v	G	N	L	R	к	S	н	Ι	v	Е	A	н	v	R	A	Q	$\mathbf{L}$	Ι	к	Р	R	v	Т	Е	Е
701		0.20		3.00	000		0.20	0.00	3.00	0.20	- <b>75</b> m m					0.74	330			0.80	000		TTT /	0.000		T C T		3.00/2	a.ch	370
241	69C 6	GAG E	TAC V	T	р	L	DAU	СА6 П	T	D	T	D	V	.999. G	F	D	AAG K	יטטי ק	L	DAU	R		F	L	V V	5	P	T	T	T
		_	-	-	-	_	-	v	-	-	-	-	-	-	-	-		-	-	-	-	-	-	_	-	-	-	-	-	_
811	CTG	CAC	GAG	ATC	GAC	GAG	GCC	AGC	ССТ	CTG	TTT	GGC	ATC	AGC	CGG	CAG	GAC	CTG	GAA	ACG	GAT	GAC	TTC	GAG	ATC	GTT	GTC	ATC	TTG	GAG
271	L	н	Ε	Ι	D	Е	A	S	Р	L	F	G	Ι	S	R	Q	D	L	Е	Т	D	D	F	Е	Ι	V	V	Ι	L	E
901	GGC	атб	GTG	GAG	GCC	АСТ	GCC	атб	ACC	ACG	CAG	GCC	CGC	AGC	тсс	тас	стб	GCC	AAC	GAG	атс	стб	TGG	GGC	CAC	CGC	ттт	GAG	сст	GTC
301	G	M	v	Е	A	Т	A	М	T	Т	Q	A	R	S	s	¥	L	A	N	Е	Ι	L	₩	G	н	R	F	Е	Р	v
991 221	стс	TTT	GAG	GAG	AAA	AAC	CAG	TAC	AAG	ATC	GAC	TAC	TCG	CAT	TTC	CAC	AAG	ACG	TAC	GAG	GTG	CCA	TCC	ACA	.ccc	CGC	TGC	AGT	GCC	AAG
331		г	Е	Е	м	и	Ŷ	I	л	1	<b>D</b>	I	3	л	Г	л	г	1	I	Е	•	P	3	1	P	к	<u>с</u>	3	A	<u>м</u>
1081	GAC	СТА	GTG	GAG	AA	AAG	TTC	CTG	CTG	CCA	AGO	ACC	AAC	тсс	ттс	TGC	TAC	GAG	AAT	GAG	стб	GCC	ттс	CTG	AGC	CGC	GAC	GAG	GAG	GAC
361	D	$\mathbf{L}$	v	Е	ท	к	F	L	L	Р	S	Т	N	S	F	с	¥	Е	N	Е	$\mathbf{L}$	A	F	$\mathbf{L}$	s	R	D	Е	Е	D
1171	GAG	GTG	GAC	GGA	GAG	САА	GAC	AGC	стс	GGC	cco	CAG	GCC	AGG	CGC	GAC	ттт	GAC	AGG	CCR	CAG	GCC	GGC	ACA	GCC	стт	GAG	CAG	CGG	сст
391	E	v	D	G	Е	Q	D	S	L	G	P	Q	A	R	R	D	F	D	R	P	0	A	G	T	A	L	E	Q	R	P
						•						•									•							•		
1261	TAC	AGA	CGG	GAG	тст	GAG	ATC	TGA	L																					
471	I	к	к	Е	2	E	т	-																						

**Figure 2:** The complete CDS and its deduced amino acids of buffalo *KCNJ12* gene (GenBank accession number: KC011846). Asterisk denotes the stop codon. Two conserved domain sequences of inward rectifier potassium channel are underlined. Transmembrane sequences are shaded. Predicted microRNA target sites are boxed.





Figure 3: The putative domains of the protein encoded by KCNJ12



Figure 4: Prediction of transmembrane regions of buffalo KCNJ12 by TMHMM. abscissa axis, Amino acid position; vertical axis, probability; Red shows transmembrane, blue shows inside, pink shows outside of membrane.



Figure 5: The predicted secondary structure of the KCNJ12 protein by SOPMA. Alpha helices, extended strands, beta turns and random coils are indicated, respectively, with the longest, the second longest, the third longest and the shortest vertical lines.



**Figure 6:** The hydrophobicity structure prediction of buffalo KCNJ12 by ProtScale. Score>0, hydrophobic; score<0, hydrophilic

**4.3** Sequence polymorphism analysis: Further sequence alignment in Bovidae family revealed that buffalo *KCNJ12* CDS in this study had 99.3% and 98.0% identity with that of *Bos taurus* and *Ovis aries.* There was no polymorphism found in the coding region of the *KCNJ12* within buffalo. However, there

were three nucleotide differences for the *KCNJ12* gene between buffalo and other bovine species, which were synonymous. The c.354C, c.895T and c.918T in the *KCNJ12* CDS are unique in buffalo. The sequence variations of the *KCNJ12* haplotypes in buffalo and other bovine species were shown in Fig. 7.

																								44	111	111	111	111	111	111	111	111
																							1.0									
			111	333	333	333	333	333	333	444	444	555	555	555	555	555	666	666	777	777	888	888	999	00	000	000	000	111	111	111	111	222
	666	666	445	111	444	444	555	666	888	333	333	111	667	т	т	888	666	666	111	555	888	999	111	88	011	444	444	000	333	888	999	111
haplotype	123	456	890	678	345	678	234	456	234	012	678	123	890	123	789	345	456	789	234	789	678	567	678	45	901	234	567	234	567	678	567	012
Bubalus bubalis _H1(KC011846)	CAC	CTG	AAC	GCT	GCC	GAG	CAC	CCG	CAT	ATT	TAC	ATC	CGT	GCA	ACG	CTA	CAT	ATT	ACG	GTC	GTT	TTG	ACT	AA	CAG	ACG	TAC	CTG	AAT	CAA	CTC	AGG
Bos taurus_H1(NM_001024690)	.6.	I	I		I	A	I													A		C	6					I	•••	•••	•••	•••
Bos taurus_H2(DQ823214)		I	I		I	A	I								•••			•••		A	•••	C	6		•••			I		•••	•••	•••
Ovis aries _H1(XH_004012746)		•••	•••	6	•••		.GT	A	C	C	I	I	C	6	I	C	C	C	C		6	C	6	.6	A	A	I		C	6	I	C

**Figure 7:** Alignment of the coding region of the *KCNJ12* gene of Bovidae family. Numbering is scored relative to first nucleotide of coding sequence of *KCNJ12* in buffalo. Dots (·) denote identity with the buffalo reference sequence. The nucleotide variable sites between buffalo and other bovine species are shaded.

**4.4 Analysis of sequence identity and evolutionary relationships of buffalo KCNJ12:** The deduced protein sequence of buffalo KCNJ12 was

submitted to generate BLAST reciprocal best hits, and similarity comparison revealed that buffalo KCNJ12 protein has high homology with the KCNJ12 proteins of six other species: *Bos taurus* (99.8%), *Ovis aries* (99.8%), *Pan troglodytes* (96.5%), *Homo sapiens* (96.3%), *Rattus norvegicus* (94.4%) and *Mus musculus* (94.1%) (Fig. 8 and Fig. 9). To evaluate the evolutionary relationships of buffalo KCNJ12 with other species, then we constructed a phylogenetic tree using DNAstar, Cluster, MEGA and DNAMAN softwares

based on the KCNJ12 amino acid sequences. The phylogenetic tree analysis revealed that the buffalo *KCNJ12* gene has a closer genetic relationship with the *KCNJ12* genes of *Bos taurus* and *Ovis aries* than with those of *Pan troglodytes*, *Homo sapiens*, *Rattus norvegicus* and *Mus musculus* (Fig. 10).

	1	2	3	4	5	6	7		
1		99.8	99.8	96.5	96.3	94.4	94.1	1	Bubalus bubalis
2	0.2		99.5	96.3	96.0	94.1	93.9	2	Bos taurus
3	0.2	0.5		96.7	96.5	94.6	94.4	3	Ovis aries
4	3.6	3.8	3.4		99.8	96.5	95.6	4	Pan troglodytes
5	3.8	4.1	3.6	0.2		96.7	95.8	5	Homo sapiens
6	5.9	6.1	5.6	3.6	3.4		98.4	6	Rattus norvegicus
7	6.1	6.4	5.9	4.6	4.3	1.7		7	Mus musculus
	1	2	3	4	5	6	7		
	1 2 3 4 5 6 7	1 1   2 0.2   3 0.2   4 3.6   5 3.8   6 5.9   7 6.1   1 1	1 2   1 99.8   2 0.2 99.8   3 0.2 0.5   4 3.6 3.8   5 3.8 4.1   6 5.9 6.1   7 6.1 6.4	1 2 3   1 99.8 99.8   2 0.2 99.8   3 0.2 0.5   4 3.6 3.8 3.4   5 3.8 4.1 3.6   6 5.9 6.1 5.9   7 6.1 6.4 5.9   1 2 3 3	1 2 3 4   1 99.8 99.8 96.5   2 0.2 99.5 96.3   3 0.2 0.5 90.5 96.7   4 3.6 3.8 3.4 90.5   5 3.8 4.1 3.6 0.2   6 5.9 6.1 5.6 3.6   7 6.1 6.4 5.9 4.6   1 2 3 4 4.6	1 2 3 4 5   1 99.8 99.8 96.5 96.3   2 0.2 99.8 99.8 96.3 96.0   3 0.2 0.5 96.3 96.7 96.3   4 3.6 3.8 3.4 99.8 99.8   5 3.8 4.1 3.6 0.2 99.8   6 5.9 6.1 5.6 3.6 3.4   7 6.1 6.4 5.9 4.6 4.3   7 8.1 2.3 4 5	1 2 3 4 5 6   1 99.8 99.8 96.5 96.3 94.4   2 0.2 99.8 99.5 96.3 96.0 94.1   3 0.2 0.5 96.7 96.5 94.6   4 3.6 3.8 3.4 99.8 96.7 96.5 94.6   4 3.6 3.8 3.4 99.8 96.5 94.6   5 3.8 4.1 3.6 0.2 96.7 96.5 94.6   6 5.9 6.1 5.6 3.6 3.4 96.7 96.7   6 5.9 6.1 5.6 3.6 3.4 96.7   7 6.1 6.4 5.9 4.6 4.3 1.7   7 6.1 2 3 4 5 6	1 2 3 4 5 6 7   1 99.8 99.8 96.5 96.3 94.4 94.1   2 0.2 99.8 99.5 96.3 96.0 94.4 93.9   3 0.2 0.5 96.7 96.3 96.5 94.6 94.4   4 3.6 3.8 3.4 96.7 96.5 94.6 94.4   4 3.6 3.8 3.4 96.7 96.5 94.6 94.4   5 3.8 4.1 3.6 0.2 99.8 96.5 95.6   5 3.8 4.1 3.6 0.2 99.8 96.7 95.8   6 5.9 6.1 5.6 3.6 3.4 98.4   7 6.1 6.4 5.9 4.6 4.3 1.7 98.4   7 6.1 2.3 4 5 6 7	1 2 3 4 5 6 7   1 99.8 99.8 96.5 96.3 94.4 94.1 1   2 0.2 99.8 99.5 96.3 96.0 94.4 93.9 2   3 0.2 0.5 96.7 96.5 94.6 94.4 3   4 3.6 3.8 3.4 99.8 96.5 94.6 94.4 3   4 3.6 3.8 3.4 99.8 96.5 95.6 4   5 3.8 4.1 3.6 0.2 99.8 96.7 95.8 5   6 5.9 6.1 5.6 3.6 3.4 98.4 6   7 6.1 6.4 5.9 4.6 4.3 1.7 7   6 7 6.1 5.9 3.4 5 6 7

Percent Identity

Figure 8: Percent identify and divergence result of comparison of the deduced amino acid sequence from buffalo *KCNJ12* gene with those from *Bos taurus* (NP\_001019861), *Ovis aries* (XP\_004012795), *Pan troglodytes* (JAA00661), *Homo sapiens* (NP\_066292), *Rattus norvegicus* (NP\_446433) and *Mus musculus* (CAA56622). Upper matrix, percent identity; lower matrix, divergence.

Bubalus bubalis	MTASGRTNPYSIVSSEEDGL <mark>H</mark> LVTMSGANGFGNGKVHTRRRCRNRFVKKNGQCNIEFANMDEKSQRYLADMFT	73
Bos taurus	${\tt MTASGRTNPYSIVSSEEDGLR} IVTMSGANGFGNGKVHTRRCRNRFVKKNGQCNIEFANMDEKSQRYLADMFT}$	73
Ovis aries	MTASGRTNPYSIVSSEEDGL <mark>H</mark> LVTMSGANGFGNGKVHTRRRCRNRFVKKNGQCNIEFANMDEKSQRYLADMFT	73
Pan troglodytes	MT A <mark>ASRA</mark> NP YS IVSSEEDGL <mark>H</mark> LVTMSGANGFGNGKVHTRRRCRNRFVKKNGQCN IEFANMDEKSQRYLADMFT	73
Homo sapiens	MTA <mark>ASRA</mark> NPYSIVSSEEDGL <mark>H</mark> LVTMSGANGFGNGKVHTRRRCRNRFVKKNGQCNIEFANMDEKSQRYLADMFT	73
Rattus norvegicus	MTA <mark>AS</mark> RANPYSIVSSEEDGL <mark>H</mark> LVTMSGANGFGNGKVHTRRRCRNRFVKKNGQCNIEFANMDEKSQRYLADMFT	73
Mus musculus	MT A <mark>ASR</mark> ANPYS I VSSEEDGL <mark>H</mark> LVTMSGANGFGNGKVHTRRRCRNRFVKKNGQCN IEFANMDEKSQRYLADMFT	73
Bubalus bubalis	TCVDIRWRYMLLIFSLAFLASWLLFG <mark>V</mark> IFWVIAVAHGDLEPAE <mark>A</mark> HGRTPCV <mark>L</mark> QVHGFMAAFLFSIETQTTIGY	146
Bos taurus	TCVDIRWRYMLLIFSLAFLASWLLFG <mark>V</mark> IFWVIAVAHGDLEPAE <mark>A</mark> HGRTPCVL <mark>O</mark> VHGFMAAFLFSIETOTTIGY	146
Ovis aries	TCVDIRWRYMLLIFSLAFLASWLLFG <mark>V</mark> IFWVIAVAHGDLEPAE <mark>AR</mark> GRTPCV <mark>L</mark> QVHGFMAAFLFSIETQTTIGY	146
Pan troglodytes	TCVDIRWRYMLLIFSLAFLASWLLFG <mark>V</mark> IFWVIAVAHGDLEPAE <mark>GR</mark> GRTPCVMQVHGFMAAFLFSIETQTTIGY	146
Homo sapiens	TCVDIRWRYMLLIFSLAFLASWLLFG <mark>I</mark> IFWVIAVAHGDLEPAE <mark>GR</mark> GRTPCVMQVHGFMAAFLFSIETQTTIGY	146
Rattus norvegicus	TCVDIRWRYMLLIFSLAFLASWLLFG <mark>IIFWVIAVAHGDLEPAE<mark>GR</mark>GRTPCV<mark>L</mark>QVHGFMAAFLFSIETQTTIGY</mark>	146
Mus musculus	TCVDIRWRYMLLIFSLAFLASWLLFG <mark>IIFWVIAVAHGDLEPAE<mark>GR</mark>GRTPCV<mark>L</mark>QVHGFMAAFLFSIETQTTIGY</mark>	146
Bubalus bubalis	GLRCVTEECPVAVFMVVAOSIVGCIIDSFM <mark>I</mark> GAIMAKMARPKKRAOTLLFSHNAVVALRDGKLCLMWRVGNLR	219
Bos taurus	GLRCVTEECPVAVFMVVAOSIVGCIIDSFM <mark>I</mark> GAIMAKMARPKKRAOTLLFSHNAVVALRDGKLCLMWRVGNLR	219
Ovis aries	GLRCVTEECPVAVFMVVAOSIVGCIIDSFM <mark>I</mark> GAIMAKMARPKKRAOTLLFSHNAVVALRDGKLCLMWRVGNLR	219
Pan troglodytes	GLRCVTEECPVAVFMVVAQSIVGCIIDSFM <mark>I</mark> GAIMAKMARPKKRAQTLLFSHNAVVALRDGKLCLMWRVGNLR	219
Homo sapiens	GLRCVTEECPVAVFMVVAQSIVGCIIDSFM <mark>I</mark> GAIMAKMARPKKRAQTLLFSHNAVVALRDGKLCLMWRVGNLR	219
Rattus norvegicus	GLRCVTEECPVAVFMVVAQSIVGCIIDSFM <mark>I</mark> GAIMAKMARPKKRAQTLLFSHNAVVALRDGKLCLMWRVGNLR	219
Mus musculus	GLRCVTEECPVAVFMVVAQSIVGCIIDSFMNGAIMAKMARPKKRAQTLLFSHNAVVALRDGKLCLMWRVGNLR	219
Bubalus bubalis	KSHIVEAHVRAOLIKPRVTEEGEYIPLDOIDIDVGFDKGLDRIFLVSPITILHEIDEASPLFGISRODLETDD	292
Bos taurus	KSHIVEAHVRAQLIKPRVTEEGEYIPLDQIDIDVGFDKGLDRIFLVSPITILHEIDEASPLFGISRQDLETDD	292
Ovis aries	KSHIVEAHVRAQLIKPRVTEEGEYIPLDQIDIDVGFDKGLDRIFLVSPITILHEIDEASPLFGISRQDLETDD	292
Pan troglodytes	KSHIVEAHVRAQLIKPRVTEEGEYIPLDQIDIDVGFDKGLDRIFLVSPITILHEIDEASPLFGISRQDLETDD	292
Homo sapiens	KSHIVEAHVRAQLIKPRVTEEGEYIPLDQIDIDVGFDKGLDRIFLVSPITILHEIDEASPLFGISRQDLETDD	292
Rattus norvegicus	${\tt KSHIVEAHVRAQLIKPRVTEEGEYIPLDQIDIDVGFDKGLDRIFLVSPITILHEIDEASPLFGISRQDLETDD$	292
Mas musculus	KSHIVE A HVRAQLIK PRVTEEGEYIPLDQIDID VGFDKGLDRIFL VSPITILHEIDE ASPLFGIS RQDLETDD	292
Bubalus bubalis	FEIVVILEGMVEATAMTTQARSSYLANEILWGHRFEPVLFEEKNQYKIDYSHFHKTYEVPSTPRCSAKDLVEN	365
Bos taurus	FEIVVILEGMVEATAMTTQARSSYLANEILWGHRFEPVLFEEKNQYKIDYSHFHKTYEVPSTPRCSAKDLVEN	365
Ovis aries	FEIVVILEGMVEATAMTTQARSSYLANEILWGHRFEPVLFEEKNQYKIDYSHFHKTYEVPSTPRCSAKDLVEN	365
Pan troglodytes	FEIVVILEGMVEATAMTTQARSSYLANEILWGHRFEPVLFEEKNQYKIDYSHFHKTYEVPSTPRCSAKDLVEN	365
Homo sapiens	FEIVVILEGMVEATAMTTQARSSYLANEILWGHRFEPVLFEEKNQYKIDYSHFHKTYEVPSTPRCSAKDLVEN	365
Rattus norvegicus	FEIVVILEGMVEATAMTTQARSSYLANEILWGHRFEPVLFEEKNQYKIDYSHFHKTYEVPSTPRCSAKDLVEN	365
Mas musculus	FEIVVILEGMVEATAMTTQARSSYLANEILWGHRFEPVLFEEKNQYKIDYSHFHKTYEVPSTPRCSAKDLVEN	365
Bubalus bubalis	KFLLPSTNSFCYENELAFL <mark>S</mark> RDEEDE <mark>VDGEQ</mark> DSL <mark>GPQAR</mark> RDFDRPQAG.T <mark>ALEOR</mark> PYRRESEI	427
Bos taurus	KFLLP STNSFCYENELAFL <mark>S</mark> RDEEDE <mark>VDGEQ</mark> DSL <mark>GPQAR</mark> RDFDRPQA <mark>G.TALEQR</mark> PYRRESEI	427
Ovis aries	KFLLPSTNSFCYENELAFL <mark>S</mark> RDEEDE <mark>VDGEQ</mark> DSL <mark>GPQAR</mark> RDFDRPQAG.T <mark>ALEOR</mark> PYRRESEI	427
Pan troglodytes	KFLLPS <mark>A</mark> NSFCYENELAFL <mark>S</mark> RDEEDE <mark>ADGDO</mark> DGRSRDGLSPOARHDFDRLQAGGGVLEORPYRRESEI	433
Homo sapiens	KFLLP S <mark>A</mark> NSFCYENELAFL <mark>S</mark> RDEEDEA <mark>DGDO</mark> DGRSRDGLSPOARH <mark>DFDRLOAG</mark> G <mark>VLEOR</mark> PYRRESE I	433
Rattus norvegicus	KFLLPS <mark>A</mark> NSFCYENELAFL <mark>S</mark> RDEEDE <mark>VATO</mark> RD <mark>G</mark> RSPQ <mark>PEH</mark> DFDRLQASS <mark>GALE</mark> R.PYRRESEI	427
Mus musculus	KELLPSANSECVENELAFLTRDEEDEVSTORDY, RTPOPEHDEDRTOASSANLVR, PYRRESET	427

Figure 9: The alignment of the protein encoded by the buffalo KCNJ12 and other six kinds of KCNJ12 from *Bos taurus* (NP\_001019861), *Ovis aries* (XP\_004012795), *Pan troglodytes* (JAA00661), *Homo sapiens* (NP\_066292), *Rattus norvegicus* (NP\_446433) and *Mus musculus* (CAA56622).

## Journal of Animal & Plant Sciences, 2013. Vol.18, Issue 1: 2668-2681 Publication date 31/5/2013, http://www.m.elewa.org/JAPS; ISSN 2071-7024



Figure 10: The phylogenetic tree for several kinds of KCNJ12 protein from *Bubalus bubalis* (AGC91909), *Bos taurus* (NP\_001019861), *Ovis aries* (XP\_004012795), *Pan troglodytes* (JAA00661), *Homo sapiens* (NP\_066292), *Rattus norvegicus* (NP\_446433) and *Mus musculus* (CAA56622)

4.5 Location of potential miRNA targets: In this study, three Bos taurus microRNAs (bta-miR-2382-3p, bta-miR-248, bta-miR-2382-3p and btamiR-873) were found to have the target sites in the buffalo KCNJ12 CDS. They were 786cuuccuggugucucccaucac-806, 1231caggccggcacagcccuu-1095-1248 and caaguuccugcugccaagcacca -1117.

**4.6 Tissue transcription profile:** In order to examine the differential distributions of *KCNJ12* in

tissues of water buffalo, the relative mRNA expression levels of *KCNJ12* were evaluated by qPCR. *KCNJ12* mRNA was widely expressed in the tissues examined, being high in longissimus dorsi, fat, cerebrum and heart, moderate in uterine wall, skin, lung, duodenum, oviduct, placenta, spleen and mammary gland, non-expressed in liver, stomach, pancreas and ovary (Fig. 11).



**Figure 11:** Tissue transcription profile of buffalo *KCNJ12* gene. The *18S* expression level is used for the internal control. M, DL2000 DNA marker; 1, cerebrum; 2, skin; 3, heart; 4, liver; 5, spleen; 6, lung; 7, stomach; 8, pancreas; 9, duodenum; 10, longissimus dorsi; 11, fat ; 12, mammary gland; 13, ovary; 14, oviduct; 15, uterine wall; 16, placenta

Journal of Animal & Plant Sciences, 2013. Vol.18, Issue 1: 2668-2681 Publication date 31/5/2013, http://www.m.elewa.org/JAPS; ISSN 2071-7024

5 DISCUSSION

In this study, the full-length CDS of the KCNJ12 were obtained from buffalo cDNAs. The KCNJ12 CDS has 1284 nucleotides encoding a protein of 427 residues with a molecular weight of 48.44 kDa and a pI of 5.55. The KCNJ12 protein has two conserved domain of Inward rectifier potassium channel. Most protein functions are regulated by the modification of some amino acids in polypeptide chain, such as phosphorylation, acetylation, glycosylation and myristoylation, and so on. There were five kinds of putative functional (protein sites kinase С phosphorylation Π sites, casein kinase phosphorylation sites, N-myristoylation sites, tyrosine kinase phosphorylation site and cGMP-dependent protein kinase phosphorylation site ) found here in buffalo KCNJ12 protein, which suggests that the KCNJ12 protein may exert critical functional effects through these sites and their corresponding domains. The Sequence alignment revealed that KCNJ12 CDS was highly similarly among different bovine species, indicating functional conservation of the KCNJ12 within the Bovidae family. The evolutionary relationship based on the KCNJ12 amino acid sequences revealed that buffalo had closer genetic relationships with the Bovidae species. This implied that the buffalo KCNJ12 has minor divergence functionally with that of other Bovidae species and may have large function differences with other mammals. Therefore, the studying about buffalo KCNJ12 can be used as a reference for understanding

### 6 ACKNOWLEDGMENTS

This study was financially supported by the Natural Science Foundation Key Project of Yunnan Province, China (No. 2007C0003Z), the National Natural Science Foundation of China (No.30660024), the Applied and Basic Research Foundation of Yunnan

possible function of the KCNJ12 in other Bovidae species. MicroRNAs are small noncoding singlestranded RNA molecules of 17 to 24 nucleotides that can regulate gene expression by binding to or regulating the translation of their target mRNAs (Bartel 2004; Zeng et al. 2003). In the present study, three Bos taurus microRNAs have been found to have their corresponding target sites in the CDS of buffalo KCNJ12 gene by theoretical prediction. Further investigation is needed to confirm whether corresponding miRNA molecules can regulate the KCNI12 gene expressions in buffalo. Tissue transcription profile analysis showed that the KCNJ12 was widely expressed in tissues examined, but at different levels. It was expressed remarkably high in longissimus dorsi, fat, cerebrum and heart, moderate in uterine wall, skin, lung, duodenum, oviduct, placenta, spleen and mammary gland, non-expressed in liver, stomach, pancreas and ovary. From the tissue transcription profile analysis in our experiment, it can be seen that KCNJ12 gene was obviously differently expressed in some tissues. The suitable explanation for this is that at the same time those biological activities associated with the gene function was presented diversely in different tissues. In conclusion, we firstly isolated the buffalo KCNJ12 gene and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further insight into the buffalo gene.

Province, China (No. 2006C0034M and 2010ZC243) and the Foundation of Yunnan Department of Finance, China (Study on the germplasm characteristics of Binglangjiang water buffalo).

### 7 **REFERENCES**

- Barry DM, Trimmer JS and Merlie JP: 1995. Differential expression of voltage-gated K<sup>+</sup> channel subunits in adult rat heart. Relation to functional K<sup>+</sup> channels? *Circulation Research* 77: 361-369.
- Bartel DP: 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297
- Brahmajothi MV, Morales MJ and Liu S: 1996. In situ hybridization reveals extensive diversity of K<sup>+</sup> channel mRNA in isolated ferret cardiac myocytes. *Circulation Research* 78: 1083-1089.
- Deng MH, Huo JL and Zhu HS: 2013. Molecular cloning and tissue expression analyses of two novel pepper genes: heterotrimeric G protein beta 2 subunit and ArcA1. *Genetics and Molecular Research* 12: 1223-1231.
- Dixon JE and McKinnon D: 1994. Quantitative analysis of potassium channel mRNA expression in atrial and ventricular muscle of rats. *Circulation Research* 75: 252-260.
- Huo JL, Wang P and Zhao Y: 2012. Molecular cloning, mRNA expression and characterization of a novel FAIM1 gene from Chinese Banna Mini-pig Inbred Line (BMI). Journal of Animal and Veterinary Advances 11: 1080-1086.
- Kaibara M, Ishihara K, and Doi Y: 2002. Identification of human Kir2.2 (KCNJ12) gene encoding functional inward rectifier potassium channel in both mammalian cells and Xenopus oocytes. FEBS Letters 531: 250-254.
- Karschin C, Dissmann E, and Stühmer W: 1996. IRK(1-3) and GIRK(1-4) inwardly rectifying K<sup>+</sup> channel mRNAs are differentially expressed in the adult rat brain. *Journal of Neuroscience* 16: 3559-3570.

- Kubo Y, Baldwin TJ and Jan YN: 1993. Primary structure and functional expression of a mouse inward rectifier potassium channel. *Nature* 362:127-133.
- Liu GX, Derst C, and Schlichthörl G: 2001. Comparison of cloned Kir2 channels with native inward rectifier K<sup>+</sup> channels from guinea pig cardiomyocytes. *The Journal of Physiology* 532:115-26.
- Livak KJ and Schmittgen TD: 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C(T)) method. *Methods* 25: 402-408.
- Lopatin AN and Nichols CG: 2001. Inward rectifiers in the heart: an update on I (K1). *Journal of Molecular and Cellular Cardiology* 33: 625-638.
- Miake J, Marban E, and Nuss HB: 2003. Functional role of inward rectifier current in heart probed by Kir2.1 overexpression and dominant-negative suppression. *Journal of Clinical Investigation* 111: 1529-1536.
- Moller S, Croning MD and Apweiler R: 2001. Evaluation of methods for the prediction of membrane spanning regions. *Bioinformatics* 17: 646-653.
- Morishige KI, Takahashi N, and Jahangir A: 1994. Molecular cloning and functional expression of a novel brain-specific inward rectifier potassium channels. *FEBS Letters* 346: 251-256.
- Nakai K and Horton P: 1999. PSORT: a program for detecting the sorting signals of proteins and predicting their subcellular localization. *Trends in Biochemical Sciences* 24: 34-35.
- Nichols CG and Lopatin AN 1997. Inward rectifier potassium channels. *Annual Review of Physiology* 59, 171-191.

# Journal of Animal & Plant Sciences, 2013. Vol.18, Issue 1: 2668-2681 Publication date 31/5/2013, http://www.m.elewa.org/JAPS; ISSN 2071-7024

JOURNAL OF ANIMAL PLANT SCIENCES

- Petersen TN, Brunak S, and von Heijne G: 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nature Methods* 8: 785-786.
- Shimoni Y, Clark RB and Giles WR: 1992. Role of an inwardly rectifying potassium current in rabbit ventricular action potential. *The Journal* of *Physiology*, 448, 709–727.
- Takahashi N, Morishige KI, and Jahangir A: 1994. Molecular cloning and functional expression of cDNA encoding a second class of inward rectifier potassium channels in the mouse

brain. Jounal of Biological Chemistry 269: 23274-23279.

Zeng Y, Yi R, and Cullen BR: 2003 MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. *Proceedings* of the National Academy of Sciences 100: 9779-9784.