FACULTY OF HEALTH AND MEDICAL SCIENCES
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Master's thesis

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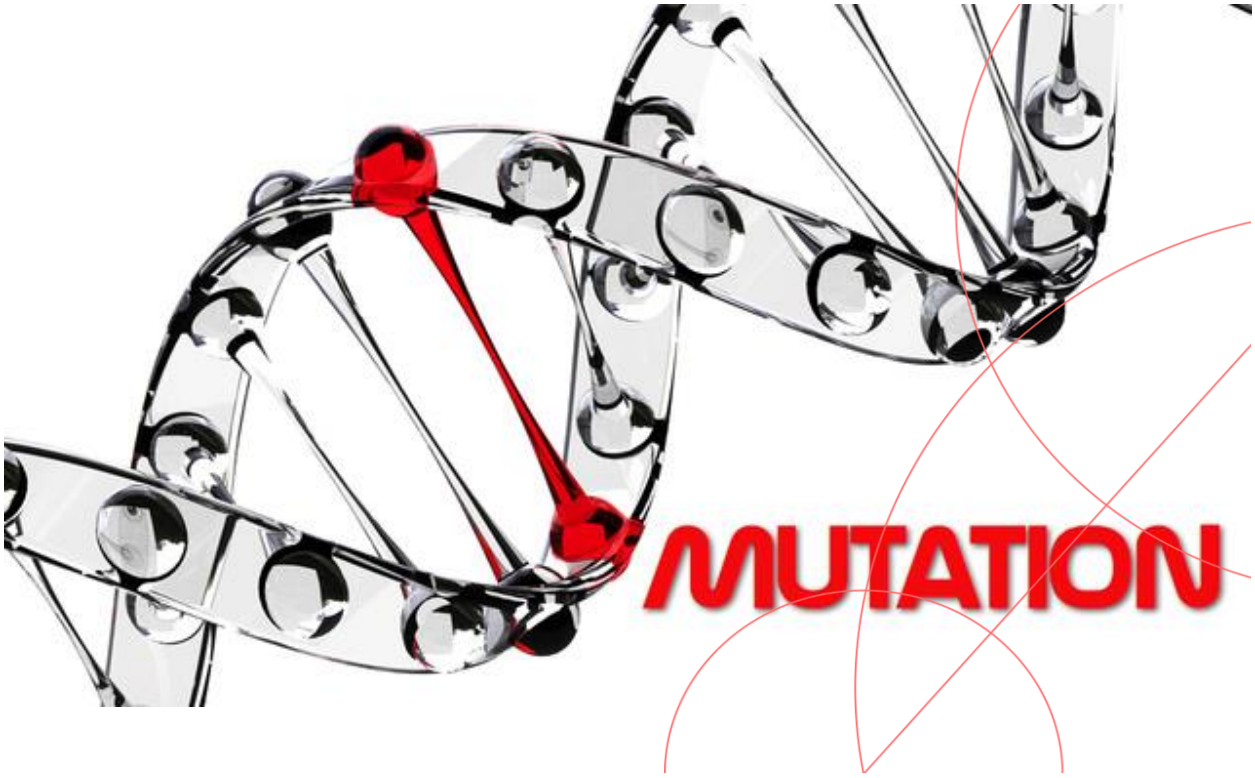
Sequencing and Electrophysiological Characterization of the Repolarizing KCNQ1 Channel Found in the Equine Heart



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Front page illustration: a graphic model of a DNA double helix highlighting a mutation site adapted from (IO9 2013)

Preface

This project is my master's thesis done as part of the master in veterinary medicine at the University of Copenhagen. The project is also done in close collaboration with the Ph-D project of Philip J. Pedersen. In the broader perspective the goal of the project is to help identify reasons for cardiac death in horses and to gain better understanding of the ion channels and their function in the equine heart. This will be done by mapping the I_{kr} and I_{ks} channels including their sub units, and expressing them in *Xenopus* oocytes to measure their function and responses to certain potentially pro-arrhythmic drugs.

In this particular study the KCNQ1 and KCNE1 and 2 are in focus as they were the ones that the most information could be ascertained about through PCR.

I would like to give thanks to the laboratory technicians of the department, and special thanks to my supervisors Dan A. Klærke, and Philip J. Pedersen who were always willing to help and give advice and along with all members of the department create a good work atmosphere.

And to my pregnant wife and my daughter: All my love for enduring my frequent absence during this project.

Abstract

Sudden death (SD) in horses is a great problem in equine athletics. In up to 22% of SD there is never found an underlying pathological cause, raising the question if there is a molecular basis to these cases of SD. In this study PCR was utilized to find parts of the equine sequences of KCNQ1, and KCNE1. The entire equine CDS of KCNQ1, KCNH2, KCNE1 and KCNE2 (with a few assumptions) was then individually analyzed by comparison to their human counterparts via BLAST, both nucleotide and protein sequences. It was especially investigated if there were any registered human LQTS mutations present in the equine sequences. KCNQ1 contained 3 protein dissimilarities identical to human mutations and a few more at same sites. KCNE1 also had human mutations present. KCNH2 had only one protein dissimilarity 1bp adjacent to a registered human mutation linked to LQT2. KCNE2 had only 3 such adjacencies. The conclusion to this is that the equine I_{Ks} current might be different from the human, whereas the I_{Kr} might be a good human homologue. The mutation analysis also gives good reason to believe there might be an equine form of LQTS, and emphasizes the need for drug studies for equine specific drugs.

KCNQ1, KCNE1 and KCNE2 were synthesized from the data we had, and attempted sub cloned in to a p-Xoom vector. This was however not achieved within the duration of this project. Therefor expression in *Xenopus* oocytes was not achieved either.

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Theoretical Background

Sudden Death in Horses

Sudden Death (SD) is a well-known problem in equine athletics. A retrospective study has been made to identify the causes of sudden death during or soon after exercise. A great proportion, up to 22% had no apparent cause despite a thorough post mortem pathological examination. (Lyle, Turley et al. 2010, Lyle, Uzal et al. 2011)

One study found histopathological similarities in 5 SD horses. 3 lesions were apparent in all 5 cases: Atrial myocardial fibrosis near the SA node, sclerotic vascular changes of the AV and SA node arteries, and fibroplastic and/or fibrotic lesions in the upper portion of the interventricular septum. These lesions were comparable with the fact that they showed cardiac symptoms at death. One horse even had perimortal ECG readings showing supra ventricular premature complexes and ventricular premature complexes up until ventricular fibrilation and subsequent death. However the mechanism for these changes, and the other unexplained equine exercise related deaths demand further research. (Kiryu, Machida et al. 1999)

A reason for the missing cause of death in many cases of SD in horses is not only due to the fact that no pathological anomalies are found, in most cases it is due to no post mortem examination at all. The data processed in the articles is mainly derived from racing associations' databases, and not all racing jurisdictions require mandatory post mortem examinations. This does however not invalidate the data given in this study in any way. Furthermore in cases where post mortem examination is performed the necropsy will only detect macroscopic changes, and perform histology when indicated or suspected. In the cases of arrhythmogenic heart failure there may not be any macro- or histo-pathological anomalies, so to do molecular testing, to dig deeper, it is necessary to understand the underlying electrophysiological mechanisms of the equine action potential.

The Cardiac Action Potential

The heart functions by sequentially triggering different ion channels through a single heartbeat; this sequence is known as the action potential. The action potential can be divided into 5 phases (0-4) depending on what channels are active (fig1). Phase 0 is characterized by the rapid influx of sodium which depolarizes the cell membrane from approximately -90 to > +20mV. Phase 1 is a quick partial repolarization, a result of outward flow of potassium. Phase 2 also known as the plateau phase is an equilibrium between several channels, notably inward calcium, outward potassium and calcium sodium exchange channels. Phase 3 initiates as the inward calcium flow ends leaving the outward potassium flow dominate

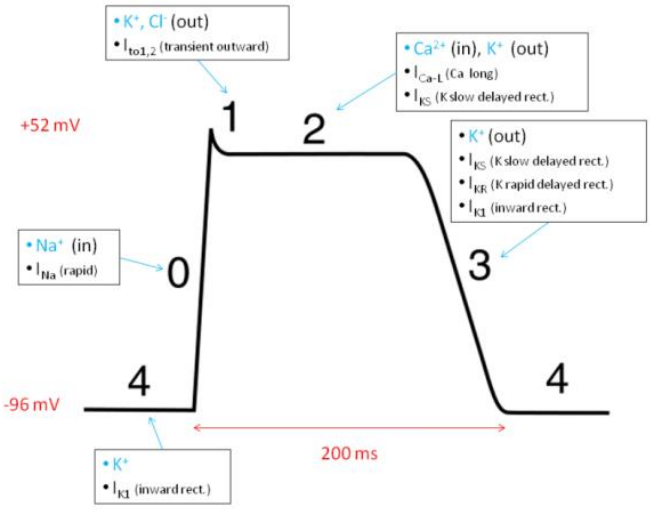


Figure 1: An overview of the channels involved at the different phases in the cardiac action potential. Adapted from Wikimedia commons

returning the cell to its resting potential of -90mV, thus starting Phase 4 where inward rectifier potassium channels keep the myocyte at its resting potential. (Nerbonne and Kass 2005)

Each channel in the heart cell membrane serves a specific purpose in the action potential. The I_{Ks} and I_{Kr} channels are responsible for the transport of potassium ions out of the cell in the phases 2-3, and their main constituents are KCNQ1 and KCNH2 respectively. (Nerbonne and Kass 2005)

Channel Structure and Composition

Most Potassium channels consist of 4 identical protein sub units comprising a homotetramer, and generally exist in 2 types based on the number of trans-membrane helices each subunit contains. The channels KscA(*Streptomyces Lividans*) which has 2 trans-membrane helices, and Shaker(*Drosophila Melanogaster*), 6 helices, lay the foundation for most of the knowledge there is about potassium channels. Shaker is the one most often compared to, since mammalian potassium channels have 6 trans-membrane helices. (Doyle, Morais et al. 1998) In addition, certain K channels, the socalled two-pore-channels consist of two subunits with each 4 transmembrane segments.

Potassium channels have what is known as a signature sequence of 8 amino acids (TXXTXGYG), where X is a random amino acid. This is also the selectivity filter which is responsible for the channels' extremely high affinity for potassium ions. In one study mutagenesis was used to examine how changes in the signature sequence would affect the channels kinetics. Some amino acid substitutions were tolerated without changing the affinity remarkably, while others ruined the function of the channel. (Heginbotham, Lu et al. 1994)

KCNQ1 and KCNE1

KCNQ1, formerly known as KvLQT1, was originally found and named due to its implication in the hereditary disease complex of Long QT Syndrome (LQTS). LTQS was at the time known to come in chromosome linked variants. KCNQ1 was thus found as the Chromosome-11 associated LQTS gene through positional cloning. KCNQ1 is classified as a voltage dependent delayed rectifier and is active during phase 2-3. (Wang, Curran et al. 1996)

KCNQ1 only forms the α sub unit of I_{Ks}. For it to yield the same current and function it needs to be co-expressed with the β sub unit made up from the gene KCNE1. Together they form the functional KCNQ1 channel, which is partly responsible for repolarization of the cardiac action potential (Sanguinetti, Curran et al. 1996)

A study has shown that the co-expression of these two sub units, at least in *Xenopus Laevus* oocytes, is transitory in the sense that KCNE1 presence in the cell membrane dwindles over time. The study described it as a "kiss and go" effect that 10 days in to the experiment left the α+β oocytes' electrical currents indistinguishable from the α oocytes' suggesting that the β sub unit is synthesized and transported to the outer cell membrane separately and may be involved in regulation of the KCNQ1 function. (Poulsen and Klaerke 2007)

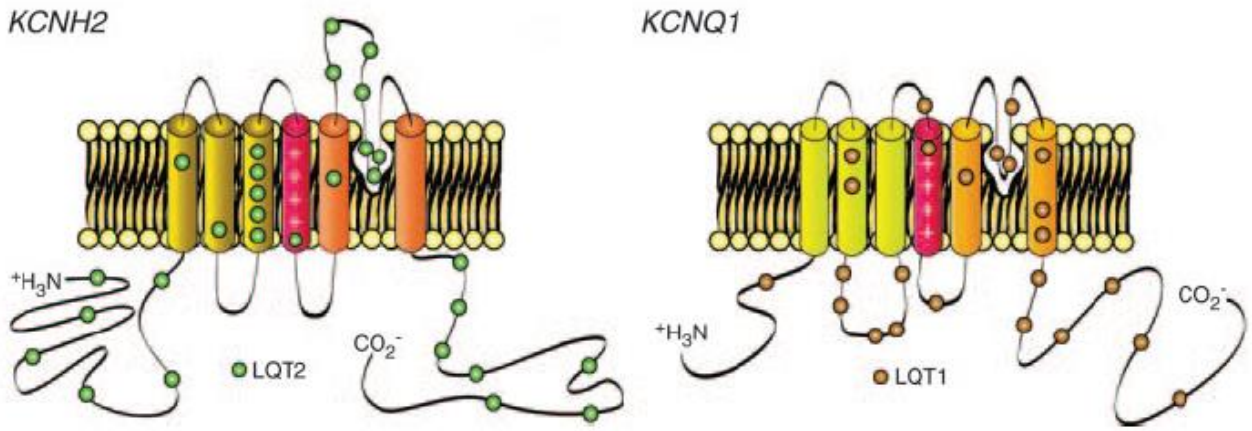


Figure 2: A simplified schematic of the α sub units of I_{Kr} and I_{Ks} left to right. The colored dots mark mutation sites related to LQT2 and LQT1 respectively as adapted from (Nerbonne and Kass 2005)

KCNH2 and KCNE2

KCNH2 is the main component of the I_{Kr} current and is also known as the Ether-a-go-go Related Gene or ERG, in humans this becomes hERG. (Warmke and Ganetzky 1994) KCNH2 is, like KCNQ1, a voltage dependent delayed rectifier, but mostly active in Phase 3. The reason for the later activation is that KCNH2 has a dual gating mechanism, which consists of an activation gate and an inactivation gate positioned on the intra- and extracellular sides of the membrane respectively. Both gates must be open for potassium current to flow. The gates react to the same changes in membrane potential but in different ways. The activation gate opens on depolarization and closes on repolarization, and vice versa for the inactivation gate. However the inactivation gate responds faster to the change in membrane potential opening it a fraction of time before the activation gate can close. This potentiates the already ongoing repolarization during phase 3 of the action potential. (Witchel, Milnes et al. 2002) The β sub unit KCNE2 is thought to be coexpressed with the ERG channel in order to regulate the I_{Ks} current. (Nerbonne and Kass 2005)

KCNH2 was until its discovery known as Chromosome-7 linked LQTS gene and is like KCNQ1 partly responsible for the repolarization of the cardiac action potential.

Long QT Syndrome

LQTS is a condition that puts a patient at significant risk of syncope and/or sudden death. It is diagnosed weighted on multiple criteria of which a long QT interval is one. The diagnosis is also based on patient and/or family history of syncope or sudden death. Another ECG finding relevant for the diagnosis of LTQS is Torsades de Pointes arrhythmias. LQTS comes both as an acquired form and a congenital. The acquired form is usually due to illicit or prescribed drug use, ischemic injury or other mechanical malformations in the heart. The congenital form is related to 12 different genes (LQT1 -12). Of these genes KCNH2 and KCNQ1 are the most important. (Hedley, Jorgensen et al. 2009)

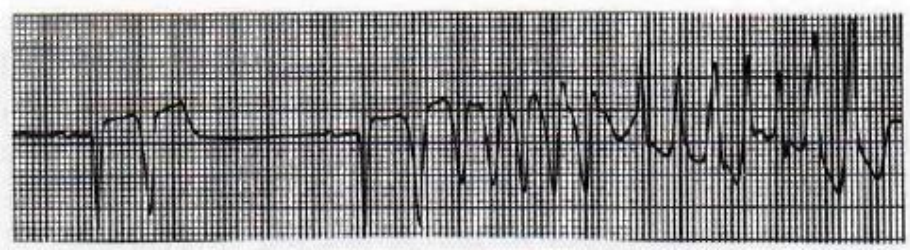


Figure 3: An ECG showing Torsades de Pointes adapted from (Tan, Hou et al. 1995)

In humans, one study claims that KCNQ1 accounts for more than 50% of the congenital cases of LQTS, LQT1. (Wang, Curran et al. 1996) KCNH2 is also related to a congenital form; LQT2, but is more known for its high sensitivity to a variety of drugs inducing an acquired LQT2.(Sanguinetti, Jiang et al. 1995) KCNH2 may account for up to 45% of congenital LTQS cases.(Splawski, Shen et al. 2000) The main focus of this project will be on these 2 genes as they are some of the major contributors to LQTS in the human population.

KCNE1 and KCNE2 are linked to the hereditary forms of LQTS: LQT5 and LQT6 respectively(Hedley, Jorgensen et al. 2009)

Both KCNQ1 and KCNH2 and their sub units of the KCNE family have been found in equine heart tissue via immunoblotting and RT-PCR. The same study measured electrophysiological currents similar to those of human homologues, suggesting that the repolarization mechanisms of the equine heart are also susceptible to LQTS.(Finley, Li et al. 2002)

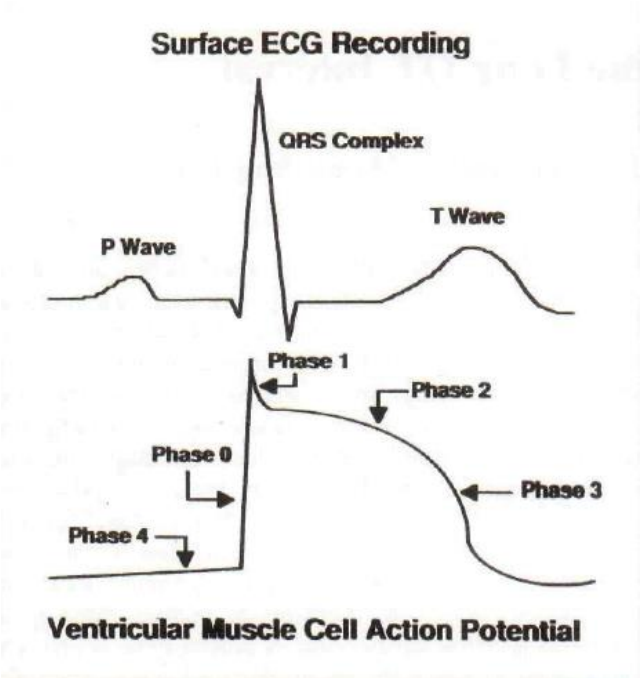


Figure 4: A graphic presentation showing the ECG and the action potential along the same time axis. Adapted from(Tan, Hou et al. 1995)

Methods

Bioinformatics

NCBI'S Basic Local Alignment Tool (BLAST) was used for all nucleotide and peptide searches pertaining to sequencing results, inter species comparison and primer design. http://blast.ncbi.nlm.nih.gov All BLAST's will have equine sequence as query and human sequence as subject.

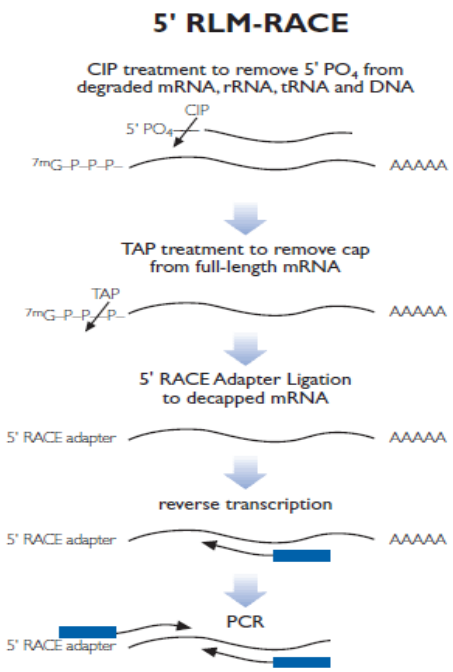
The NCBI database ClinVar: http://www.ncbi.nlm.nih.gov/clinvar and the web site: http://www.fsm.it/cardmoc/ were used for looking up registered human missense mutations related to KCNQ1, which were then compared to the equine sequence we had obtained. Frame shift and silent mutations were considered irrelevant to this study.

The TMHMM 2.0 tool: http://www.cbs.dtu.dk/services/TMHMM/ was used for predicting membrane topology of the found equine sequences so it could be compared to what is known about their human homologues.

Primers were designed using OligoCalc at: http://www.basic.northwestern.edu/biotools/oligocalc.html with the only modification from pre fixed settings that the concentration of primer is 500 nM instead of the 50 nM. The primers 67 and 68 (appendix I) were designed by me during this project and were designed to have an annealing temperature around 60°C, not be much longer than 20bp, as low GC% as possible, no more than 3 (2 is better) repeating C's or G's e.g. CCC or GGG and to end in a single G or C. All primers were tested for hairpin and auto-dimerization formation and judged usable if the pre fixed settings gave no matches for self-complementarity.

PCR

As this project was a continuation of work previously done by master student (Olander 2012), some information about the equine sequence of KCNQ1, KCNH2 and KCNE 1-4 was already available. It will be specified in the results section and relevant appendices of this project what data was found before and what was found during this project. The focus of this project was however, due to this, focused on finding the 5' CDS and UTR ends of the genes.



5' RACE PCR was therefore utilized to investigate these regions. The FirstChoice® RLM-RACE kit from Ambion® was used. This kit works by in vitro treating RNA harvested from equine myocardium just after euthanasia, removing the phosphorous cap at the 5' terminal of m-RNA and substituting it with a synthesized RNA adapter. This can then be reverse trans-scripted into cDNA, to which ready to use application oligonucleotide primers are included in the kit that fit the now present adapter. This means that it should only be mRNA with a complete 5' end that will be amplified using PCR, and that a reverse gene specific primer (GSP) is all that is needed since the forward primers are a known part of the adapter.

Figure 5: A schematic representation of the RLM-cDNA synthesis procedure. Adapted from the FirstChoice® kit. Calf Intestinal Phosphatase (CIP), Tobacco Acid Pyrophasphatase (TAP).

This was set up as a nested PCR meaning that first a PCR was set up with the 5' RACE outer primer of the kit and a GSP, using the adapted RNA as template. Then a second PCR was set up using the 5' RACE inner primer and another GSP slightly upstream from the first GSP, using the first PCR as template thus creating higher specificity for the wanted product. Due to empirical experience an additional technique was implemented to further specify the PCR. This was Touch Down (TD) PCR, in which the first 5 cycles the annealing temperature is set at 4°C above the expected and taken down 1°C each cycle through the first 5. The remaining cycles are the run at the expected annealing temperature (table 2).

The Phire Hot start II DNA Polymerase Kit (FS-122) from Thermo scientific was used for most of the PCR reactions. One reaction, the KCNQ1 results, was done with the FS-120 kit containing Phire I. All of the reactions were run on a Piko® Thermal cycler from Finnzymes®. The general composition of PCR solution is shown here in Table 1

Reaction Component	Ammount for a single 10µl reaction
dH2O	5,6 µl
5X Reaction buffer containing loading dye	2 µl
dNTP Mix	0,2 µl
Forward primer	0,5 µl
Reverse primer	0,5 µl
Template	1 µl
Polymerase enzyme	0,2 µl

Table 1: The general composition of a PCR solution. Used to prepare master mixes where primers and template were added individually to the reaction tubes.

Due to the empirical experience of my Councilor the standard PCR protocol was augmented, as previously mentioned, using the following adjustments the ones written in red being different from product manual guidelines:

Step	Temperature	Time	Notes
Initial denaturation	98°C	45s	
Denaturation	98°C	6s	Repeat 4 times, dropping extension temp. 1°C each time
Annealing	Primer specific +4°C	6s	
Extension	72°C	Dependent on expected length	
Denaturation	98°C	6s	Repeat 20 and 25 times for outer and inner reaction respectively
Annealing	Primer specific	6s	
Extension	72°C	Dependent on expected length	
Final extension	72°C	1min	Cooled to 4° immediately after this step

Table 2: a schematic view of the Nested TD PCR protocol utilized in this project, adapted from the technical manual of the FS-122 kit.

All gels were made with TBE buffer and 2% agarose since we were expecting shorter segments (<1000bp). The Gels were mixed with Ethidium Bromide for later UV Trans-lumination on a UVP MultiDoc It Digital Imaging Systems from AH Diagnostics. Gels were run at 60V corresponding to 4V per centimeter between the electrodes of the electrophoresis tub. The Phire® II kit had loading capabilities in the reaction buffer, but the reactions done with Phire® I were mixed with loading buffer on parafilm first. Each well was loaded with 1 µl of PCR reaction alongside a 100bp ladder from NEB®

Sequencing

Sequencing was done by Eurofinns MWG Operon sequencing department in Germany, the KCNQ1 results of this project was done on a cloned Vector insert. KCNE1 results were sent as unpurified PCR solutions having only been evaluated on a gel. This allowed for much faster responses and proved to be reliable enough that we went forward with the method.

Ligation, Transformation and Cloning:

Cloning was performed using the TOPO® TA cloning® kit from Invitrogen™. Prior to the ligation the PCR solution was incubated at 72°C for 10-15 minutes with the Taq-polymerase. This adds dA overhangs on the 3' strands of the PCR product which was left with blunt ends after the Phire-polymerase reaction. Thus enabling it to fit with the dT overhangs of the pCR™ 4-TOPO® vector. The *E. Coli* strain used was the One-Shot® TOP10. All liquid mediums and the agar plates were mixed with 100µg/ml ampicillin for vector selectivity.

The transformation was done by mixing PCR product and the vector. This mixture was then added to a vial of *E. Coli* and left to incubate on ice for about 30 minutes. The culture was then heat shocked at 42°C for

exactly 30 seconds and then immediately put on ice again. Soon after S.O.C. medium was added to the vial and it was left to incubate at 37°C semi horizontally at 200 RPM for an hour. Here after the suspension was plated on to LB agar plates and left over night at 37°C. The following day the colonies were singled out and transferred to another liquid LB suspension to be cultured for 16 hours. A glycerol stock was made of this to be stored at -80°.

Plasmid Purification:

Plasmid purification was performed with the GeneElute™ plasmid miniprep kit from Sigma-Aldrich. The procedure was done in accordance with the user guide, with the included recommendations for lastly eluting with 5mM Tris-HCl and reduced volume to optimize for sequencing use as seen in step 6 of figure 6.

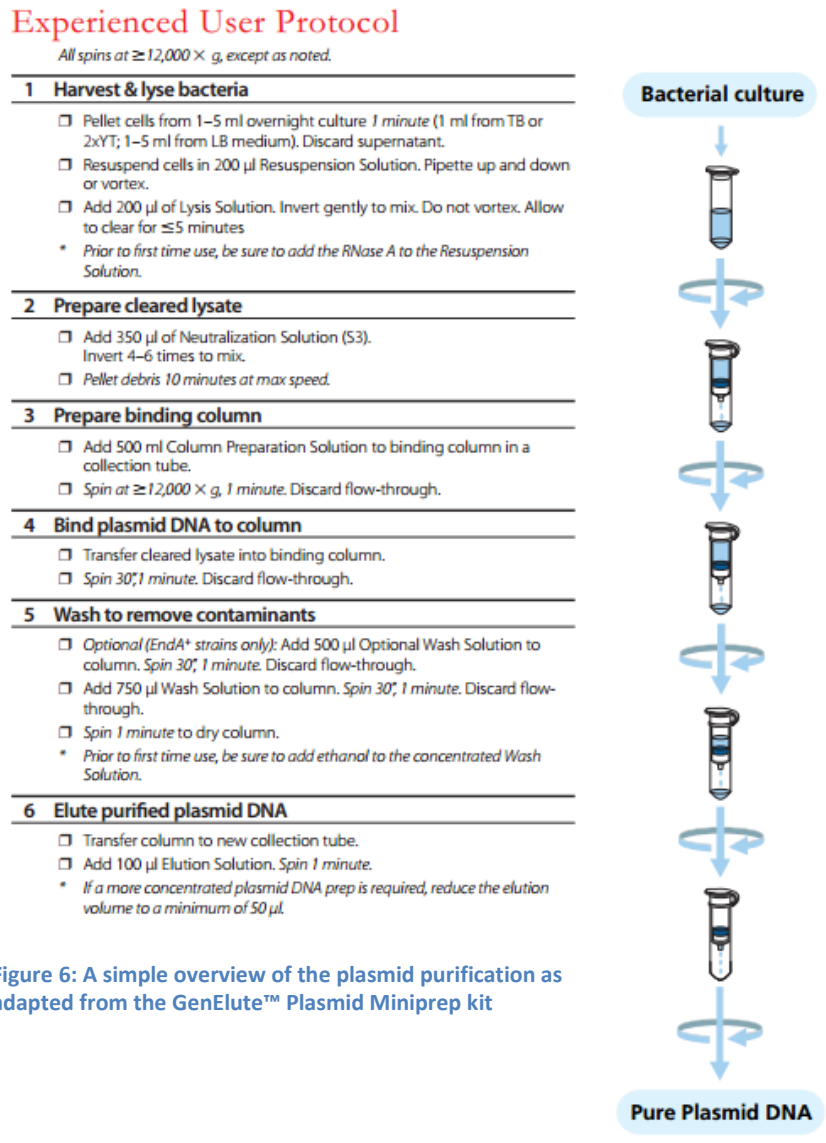


Figure 6: A simple overview of the plasmid purification as adapted from the GenElute™ Plasmid Miniprep kit

The purified eluent was messured on a NanoDrop™ 3300 Spectrophotometer from ThermoScientific

Gene Synthesis and Sub Cloning

The KCNQ1 (2050bp), KCNE1 (409bp) and -2 (391bp) Genes, as derived from the results, were ordered from www.Genescript.com on the 17-06-13 and were produced via their protocols, we ordered it to be provided in their pUC57-kan vector inserted at EcoR(I). For all 3 genes we added BStE(II) immediately downstream of the CDS so we could insert the sequence with directionality into the p-Xoom vector (5052bp). It was checked that none of the genes contained these restriction sites.

The restriction enzymes EcoRI and BstEII, both High Fidelity (HF) versions, were ordered from New England Biolabs® (NEB) and were used with CutSmart™ buffer (NEB).

The Genes were cut out using a double digest and the p-Xoom vector was linearized in the same way. The reaction was set up with 1µg DNA derived from measurements on the Nanodrop machine, 1µl of each enzyme (20.000u/ml), 3µl Cutsmart™ buffer, 3µl 10X Bovine Serum Albumine and dH2O added to a total of 30µl. These reactions were incubated at 37°C for 4 hours, then 5µl of 6X loading dye were added. The total

solution was loaded on a gel and run at about 100V. The KCNE genes were loaded on 2% agarose and the KCNQ1 and linearized vector were loaded on 1% agarose. The gels were then transluminated to evaluate the digestion and to visualize the bands in order to cut them out. This was done with a rectangular pipette transferring the gel piece to an 1,5ml eppendorf tube.

The DNA was extracted from the gel using the QIAquick® Gel Extraction Kit adapting some of the therein mentioned optimizations. These were leaving the PE-buffer atop the filter for 2 minutes prior to spinning the column to optimize for later salt sensitive reactions in the ligation. Also the final elution was done with 30µl 10mM Tris-HCL instead of 50µl to increase the DNA concentration.

The three ligation reactions were performed optimizing the molar ratio of insert to plasmid 3:1 not exceeding 100ng DNA, then adding 1µl T4 ligase enzyme, 2µl 10X T4 reaction buffer and adding dH2O to a total of 20µl per reaction. The mixture was left to incubate at room temperature for 2 hours and hereafter transferred to ice.

The Transformation was done using DH10B chemically competent *E. Coli*. 1µl of the ligation reaction was added to 100µl of DH10B culture thawed on ice from a -80°C stock, then left to incubate on ice for 30 minutes. Next step was heat shocking for exactly 45 seconds at 42°C on a heat block and then immediately transferring the tubes to ice again. Then 0,9ml of S.O.C. medium was added and the culture was left to incubate at 37°C and 225 Rpm vertically. The cultures were then spread on LB plates infused with 100µg/ml neomycin which the p-Xoom vector confers resistance to. Each culture had a plate with 50µl and 125µl spread onto it. These plates were left at 37°C overnight. Alongside, a negative control of the cut p-Xoom was attempted transformed as to have a background template for evaluating the plates the following day. This way we could evaluate if the vector had some self-ligating properties even though a double digest without compatible ends made it unlikely.

The plates which had growth had colonies selected from them which were resuspended in 1X PCR solution, without primers or enzymes, and spread on a grid plate. This PCR slur was then heat treated at 70°C for 5 minutes to destroy the cells and make the DNA accessible. It was then used as template for PCR to confirm the inserts were in the clones. For each reaction the same forward primer (IVTF appendix I) lying upstream of the insert was used with a gene specific reverse primer. All the reactions were done at the same time and run at 58°C since the gene specific primers used had all given results at this temperature. The extension time was set to 500bp or 5 seconds as the longest expected fragment would be around 390bp. These gels were also evaluated via translumination. The grid plate was checked the day after.

This is how far this project got before we would have moved on to in vitro transcription of the DNA into mRNA and used this to inject into *Xenopus* oocytes, to measure the currents via two-clamp electrode testing.

Results

PCR and Sequencing

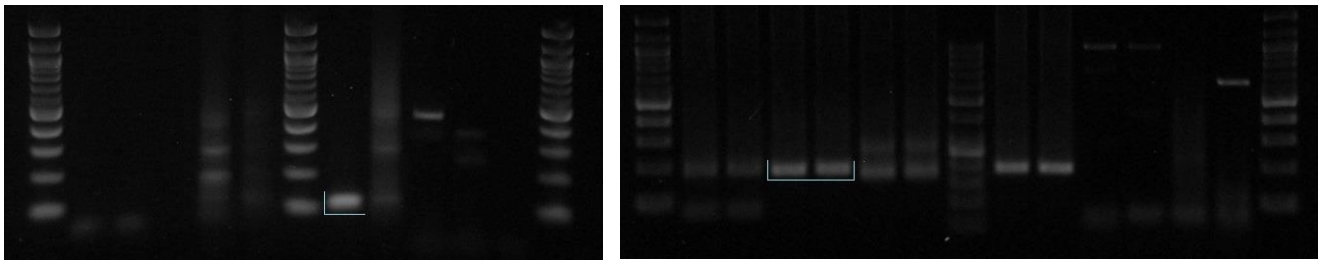


Figure 7: The first gel shows a product from KCNQ1 using primer 63 and 62 (appendix I) with the Ambion 5' outer and inner respectively, in a nested TD reaction with a length of ca. 120 bp; The second gel shows a product from KCNE1 using primer 68 and 67 (appendix I) with the Ambion 5' outer and inner respectively, in a nested TD reaction with a length of ca. 190 bp; Note: The middle ladder of the second gel was loaded erroneously so it is to be disregarded.

A roughly 120bp segment of KCNQ1 was amplified using primers 62 and 63 (appendix I) along with the Ambion 5' primers. The left over reaction was then given dA overhangs with the Taq-polymerase, ligated with the TOPO TA Vector and then transformed into DHB TOP10 chemically competent *E. Coli* cells. From these the plasmids were extracted in a mini-prep kit and shipped for sequencing yielding the following result not Showing vector:

5'-GCACTTCCAGCCCGTGGGGCGCTCGAGGAAGTT**GTA**GACGCGGCCTCCTCCCCGCCCAGGGCCGAGAGGAAGCG CTG-3'

The bold is where we assume the start sequence is and the underlined is the part of the equine sequence that was already known to us.

A roughly 190bp segment of KCNE1 was amplified using primers 68 and 67 (appendix I) along with the Ambion 5' primers. The left over reaction associated with the bands (figure 7) was pooled for a 17µl sample which was sent directly to sequencing yielding the following result:

5'-GTGCCCTGCGCTCGGCCAGCGCGGACCTCGCTGCACTGCTGCTCTCTCGGCGCCCCAAACCCGGACATTCCCTCTCC AGCAGTGTAACCTTGAAGCCCAGG**ATG**ATCCTGTCTAACACCACAGCTGTGATGCCCTTTCTGGCCAAGCTGT-3'

In the UTR of both results there were no in frame stop codons to definitely confirm the actual start codon. There were nor any start codons to suggest the contrary though. So we assume the start codons are the ones we have chosen.

During this project only PCR of KCNQ1 and KCNE1 yielded any new data. Attempts were made at amplifying 5' ends of KCNH2 and KCNE 2-4 as well however without luck. It was also attempted to amplify KCNQ1 as one continuous piece, also without luck.

Subcloning

The excision double digestion of the 3 genes gave 2 distinct bands of the expected lengths each (pUC-57 vector and insert). While the double digest to linearize the p-Xoom vector only showed the approximately 5kb band, even though it was attempted to visualize the 40bp excision between EcoRI and BSTEII to confirm the digestion.

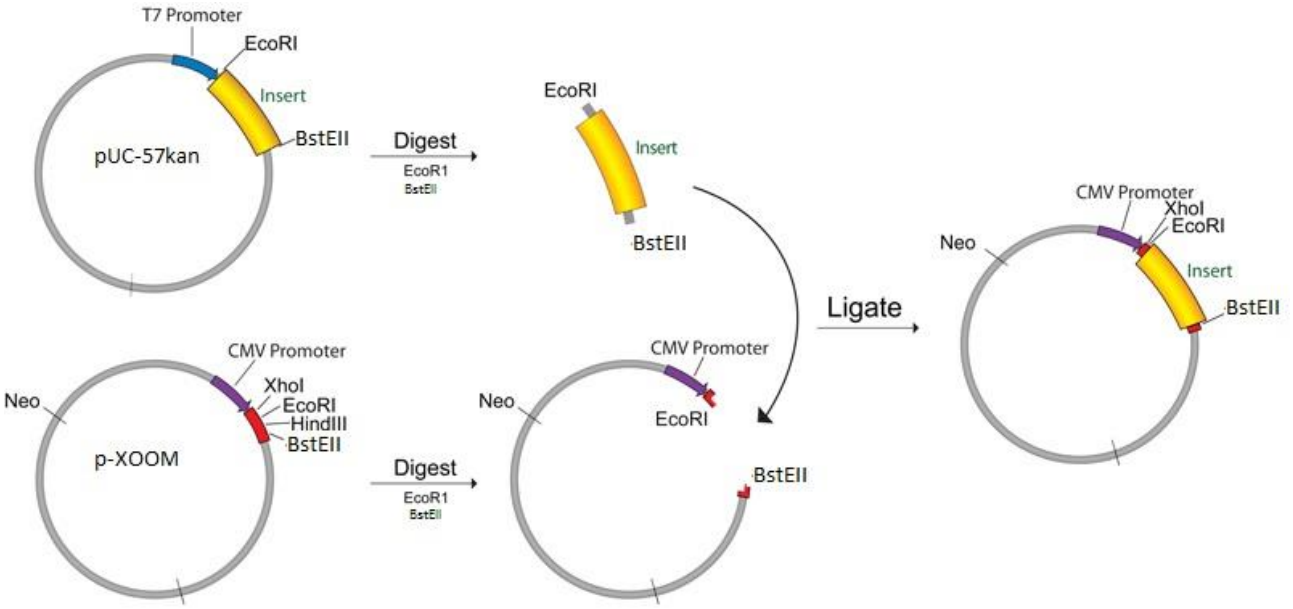


Figure 8: a graphic representation of the first steps of the subcloning process up until transformation. Adapted from (Addgene 2013)

During transformation the negative control or background plate with linearized p-Xoom did not have any growth over a 2 day period, this was done to see if the linearized vector had self-ligating properties. However the KCNE1 and -2 plates each had only 1 visible colony overnight and none further after another day. The uncut p-Xoom and KCNQ1 plates showed an abundance of colonies. 4 distinct KCNQ1, 2 p-Xoom and both KCNE colonies were selected for PCR testing and spread on a grid plate. The grid plate had growth in all but the 4 KCNQ1 grids.

The inserts of the final vector were quality tested via PCR with gene specific primers. This however yielded no visible bands aside from the ladder. When they should have showed single bands between 300 and 400bp

Gene Analysis

KCNQ1

A nucleotide comparison of our equine sequence against the human KCNQ1 CDS (NM_000218.2) shows 90% identities, and 100% overlap. There are 12 gaps, 6 in each species. The first 24 bp of the sequence is the part that has been deduced during this project (Blue appendix II). Due to the time constraints of this project, we have assumed that the ATG start sequence is actually there, since the sequencing results we received had the first 3 bp as GTA, making GTA→ATG (see 1. sequencing result page13) . In the sequencing result it was however also noted that the G and A were not highly probable (Turquoise appendix II). This was necessary in order to move on to oocyte expression so that we would have a complete CDS.

A protein comparison shows 91-93% similarity, the latter being % positives. 3 amino acid dissimilarities are identical to human mutations linked to LQT1 or cardiac arrhythmia (Red appendix II). Another 6 dissimilarities are directly at sites of human mutations but were not identical to the ones registered, and

finally 8 other dissimilarities are 1 amino acid adjacent of a registered human mutation (Yellow appendix II). 1 of the 6; and 1 of the 8 dissimilarities are inside a trans-membrane helix S2 and S1 respectively.

KCNH2

A nucleotide comparison of our equine sequence against the human KCNH2 CDS (NM_000238.3) shows 93% identities, and 100% overlap. There are 3 gaps, corresponding to a 3bp in frame deletion at 881bp of the equine sequence.

The protein comparison shows 98% similarity and only 1 dissimilarity is 1bp adjacent to a registered human mutation linked to LQT2 (Yellow appendix III). There are no dissimilarities in any of the trans-membrane helices.

KCNE1

A nucleotide comparison of our equine sequence against the human KCNE1 CDS (NM_000219.4) shows 85% identities, and 100% overlap. There are 2 gaps, one in each species.

The protein comparison shows 80-87% similarity. 1 amino acid dissimilarity is identical to a human mutation linked to LQT5 (Red appendix IV). Another 3 dissimilarities are directly at sites of human mutations but are not identical to the ones registered, and finally 8 other dissimilarities are 1 amino acid adjacent of a registered human mutation (Yellow appendix IV). One of the 3 dissimilarities is the only one inside the trans-membrane helix.

KCNE2

A nucleotide COMPARISON of our equine sequence against the human KCNE2 CDS (NM_172201.1) shows 87% identities, and 100% overlap. There are 0 gaps.

The protein COMPARISON shows 89-95% similarity. There are no dissimilarities directly at human mutation sites. There are 3 dissimilarities which are 1 amino acid adjacent of a registered human mutation (Yellow appendix V). There are no dissimilarities inside the trans-membrane helix.

Conclusion

Equine KCNQ1 and KCNE1 are overall comparable to their human counterparts, however there are many dissimilarities when BLAST'ed and several of these where at registered sites of human mutations linked to LQT1 and LQT6 respectively. Considering this it is likely that congenital LQTS may be found in the equine population. It also suggests that the entire I_{Ks} channel may have different kinetics from the human.

Considering KCNH2 there is very little difference from the human protein. And that only one dissimilarity was adjacent to a registered human mutation. The 98% protein similarity is somewhat higher than the other proteins, which is a noteworthy finding in itself. It cannot be contributed to this project however.

KCNE2 has only a few dissimilarities adjacent to registered human mutation sites. Otherwise there is good similarity. This combined with the knowledge of KCNH2 suggests that the I_{Kr} Channel complex is more like its human counterpart at least compared to I_{Ks}.

None of the genes have been definitely confirmed via in frame 5'-UTR stop codons.

Discussion

Animals have been used for models of human LQTS, in particular dogs used for medical trials. This is done by crushing part of the SA node and utilizing β blockade to induce bradycardia. This is of course done under anesthesia. Then the study drugs, in the case of this reference H1-antagonsitic antihistamines, are administered to see if they have detrimental effects on the ECG of the dog.(Weissenburger, Noyer et al. 1999)

These pharmacological conditions are not optimal since it cannot be known for sure whether β-blockade and or anesthesia interact with the test drug to either potentiate or negate any effects. Therefore a genetic approach to understanding the kinetics of the cardiac action potential in animals is important as it may lead to the finding of a spontaneous animal model for LQTS. Furthermore a broader understanding may lead to better in vitro testing capabilities as to reduce the number of test animals needed for research such as this.

One study has found spontaneous examples of acquired LQTS in dogs. (Campbell and Atwell 2002) Where in 39 cases dogs were admitted to the clinic suffering from tick toxicity (*Ixodes Holocyclus*) where they had ECG's done at admission, 24 hours later, at discharge, when clinically well and approximately 12 months later. These results showed that the dogs had prolonged QT intervals (corrected for heart rate) and abnormal T-wave morphology comparable to that of human LQTS. These ECG findings were present after clinical remission, but none had any ECG abnormalities 12 months after admission.

Another study have found congenital forms of LQTS in a canine population, I have however been unable to find the source which was mentioned to me by my advisor despite thorough searches on the OvidSP through our faculty library web page(Library 2013) so this will have to be confirmed.

This indicates that LQTS is a valid problem in animal populations, and we should therefor take care what drugs we use in animals. A study has tried to evaluate a few suspected sedatives with regard to echocardiographic evaluation. It was, among other things, found that romifidine and detomidine gave significantly reduced heart rate.(Buhl, Ersboll et al. 2007) This would be more severe if the horse already has an LQTS diathesis.

This problem of not knowing which drugs are detrimental to the equine heart was also a part of the master's thesis (Olander 2012) where she showed that Acepromazine could block the ERG channel expressed in *Xenopus* oocytes. Given time it would have been the goal of this project as well to try some of these suspected drugs in vitro on KCNQ1 with and without the KCNE1 subunit. Perhaps also try the KCNH2 experiments along with the KCNE2 subunit this time.

Analysis of KCNQ1's genomic structure indicates that it should not be expected to behave completely identical to the human channel. This emphasizes the need to screen drugs on a species level, and not to assume that human approved drugs have the same kinetics in horses. The next steps to express the channels and the subunits are an important step in this direction.

Analysis of KCNH2 on the other hand suggests that it might be a good human homologue. The high similarity might be explained by the channels dual gating mechanism and or its high sensitivity to drugs,

meaning that mutations are badly tolerated leading to high conservation. This is however my own speculation.

Another aspect of identifying the equine sequence is that it enables the possibility to screen the population for mutations and perhaps identify horses at risk of getting cardiac diseases, or post mortem identify possible reasons for sudden death. In the long run to improve the breeding of horses to perhaps eliminate carriers from the breeding programs. This is also a reason for debate as many horse owners may not be willing to start testing their horses out of fear of potentially losing the profitability of a valuable stallion or mare.

With regard to why the PCR quality control of our sub cloned vector failed to produce bands, it was not entirely clear if the forward primer IVT-forward (appendix I) was genuine. It was last used by a former Ph-D student whose data and notes we then had to interpret. Therefor after our failed attempt my last action in the laboratory, before committing to writing, was to order a new stock of the primer as well as a reverse primer downstream of the insert. So another attempt can be made.

Also during the sub cloning process, when no 40bp band was visualized at the linearization of the p-Xoom vector it might be due to such a small fragment easily diffusing through the gel making the concentration too low to see.

More research is needed to definitely confirm the 5’ ends of all four genes preferably by locating in frame stop codons in the 5’ UTR. Also the actual Start codon of KCNQ1 needs to be verified, as do the 5’ and 3’ ends of KCNE2. The 5’ RACE process seems good enough to do this the process just has to be tweaked some more.

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Appendices

Apendix I Primers used

Only the p-Xoom and Ambion primers were forward primers, the GSP's were reverse.

The user manual for the RACE kit specified that the primers were optimized for 60°C ±5°C

Ambion 5' RACE Primers:

Primer name	Sequence 5' – 3'	bp	Temperature	GC content	Sense strand
Inner	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGAT	34	72,77°C	56%	N/A
Outer	GCTGATGGCGATGAATGAACACTG	24	61,55°C	50%	N/A

KCNQ1:

Primer 62	CAGCGCTTCCTCTCGGCCCT	20	65,27°C	70%	AGGGCCGAGAGGAAGCGCTG
Primer 63	GAGAAGGGGCACTTCTTGGCCAGG	24	64,17°C	63%	CCTGGCCAAGAAGTGCCCCTTCTC

KCNE1:

Primer 67	ACAGCTTGGCCAGAAAGGGC	20	60,18°C	60%	GCCCTTTCTGGCCAAGCTGT
Primer 68	CGGGCTGGGCTAGACGTG	18	60,38°C	72%	CACGTCTAGCCCAGCCCG

KCNE2:

K2 Inner	GCTCAGCTGTCGTGTTCCTGCGC	23	66,88°C	65%	GCGCAGGAACACGACAGCTGAGC

p-Xoom:

IVTF	CAGAGCTCTCTGGCTAACTAGAG	23	61,49°C	52%	N/A

Apendix II KCNQ1

The Blue is what was found during this project, and the turquoise is putative.

Score	Expect	Identities	Gaps	Strand
2593 bits(1404)	0.0	1828/2037(90%)	12/2037(0%)	Plus/Plus

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Query  1    ATGCACCGGGCCTCCTCCCCGCCCAGGGCCGAGAGGAAGCGCTGGGGCTGGGGCCGCCTG  60
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Sbjct  1    ATGGCCGCGGCCTCCTCCCCGCCCAGGGCCGAGAGGAAGCGCTGGGGTTGGGGCCGCCTG  60

Query  61   CCGGGCGCCCGGCGGGGCAGCGCGGGCCTGGCCAAGAAGTGCCCCTTCTCCCTGGAGCTG  120
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Sbjct  61   CCAGGCGCCCGGCGGGGCAGCGCGGGCCTGGCCAAGAAGTGCCCCTTCTCGCTGGAGCTG  120

Query  121  GCTGAGGGCGGCCCGGCGGGCGGCGCGCTCTATGCGCCCATCGTgcctcccggcacccag  180
            || |||||||||||||||||||||||||||| ||||||||||    | ||||||  |||
Sbjct  121  GCGGAGGGCGGCCCGGCGGGCGGCGCGCTCTACGCGCCCATCG---CGCCCGGCGCCCCA  177

Query  181  gggcccgcgctccccgcgtctccggccccgccggccgcgcccccccgcagccgccgacctt  240
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Sbjct  178  GGTCCCGCGCCCCCTGCGTCCCCGGCCGCGCCCGCCGCGCCCCCAGTTGCCTCCGACCTT  237

Query  241  ggcccgcggccgccggtgagcctcgacccgcgcgTCTCCATCTACAGGACGCGCCGCCCG  300
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Sbjct  238  GGCCCGCGGCCGCCGGTGAGCCTAGACCCGCGCGTCTCCATCTACAGCACGCGCCGCCCG  297

Query  301  CTGCTCGCGCGCACCCACATCCAGGGCCGCGTCTACAACTTCCTCGAGCGCCCCACGGGC  360
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Sbjct  298  GTGTTGGCGCGCACCCACGTCCAGGGCCGCGTCTACAACTTCCTCGAGCGTCCCACCGGC  357

Query  361  TGGAAGTGCTTCGTCTACCACTTCGCAGTCTTCCTCATCGTCCTGGCCTGCCTCATCTTC  420
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Query  421  AGCGTGCTGTCTACCATCGAGCAGTATGTCACTCTGGCCACAGGGACCCTCTTCTGGATG  480
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Sbjct  418  AGCGTGCTGTCCACCATCGAGCAGTATGCCGCCCTGGCCACGGGGACTCTCTTCTGGATG  477

Query  481  GAGATCGTCCTGGTGGTGTTCTTTGGGACAGAGTACGCCGTCCGCCTCTGGTCAGCAGGC  540
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Sbjct  478  GAGATCGTGCTGGTGGTGTTCTTCGGGACGGAGTACGTGGTCCGCCTCTGGTCCGCCGGC  537

Query  541  TGCCGCAGCAAGTACGTGGGCATCTGGGGGCGGCTGCGCTTTGCCCGGAAGCCCATTTCC  600
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Sbjct  538  TGCCGCAGCAAGTACGTGGGCCTCTGGGGGCGGCTGCGCTTTGCCCGGAAGCCCATTTCC  597

Query  601  ATCATTGACCTCATTGTGGTTGTGGCCTCCATGGTTGTCCTCTGCGTGGGCTCCAAAGGG  660
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Sbjct  598  ATCATCGACCTCATCGTGGTCGTGGCCTCCATGGTGGTCCTCTGCGTGGGCTCCAAGGGG  657

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Query  781  CAGGAGCTGATAACCACCTTGTACATCGGCTTCCTGGGCCTCATCTTCTCCTCGTACTTC  840
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Query  901   GCAGATGCCCTTTGGTGGGGGGTGGTCACTGTCACCACCATTGGCTATGGAGACAAAGTG  960
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Sbjct  1915  ACCCAGCCCTGCGGCAGTGGCGGCTCCGTCGACCCTGAGCTCTTCCTGCCCAGCAACACC  1974

Query  1975  CTGCCCACCTACGAACAGCTGACCGTGCCCCACAGGGGCCCTGACGAGGGGTCCTGA  2031
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Sbjct  1975  CTGCCCACCTACGAGCAGCTGACCGTGCCCAGGAGGGGCCCCGATGAGGGGTCCTGA  2031
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Protein BLAST

A yellow color indicates a site where a human mutation is noted either in ClinVar or at http://www.fsm.it/cardmoc/ only ones that were 0 – 1bp adjacent from dissimilarities have been marked

Red indicates when said mutation is the same as the human

A green color indicates the membrane spanning helices as calculated from TMHMM 2.0 server

Score	Expect	Method	Identities	Positives	Gaps
1140 bits(2950)	0.0	Compositional matrix adjust.	614/678(91%)	631/678(93%)	4/678(0%)

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            M AASSPPRAERKRWGWGRLPGARRGSAGLAKKCPFSLELAEGGPAGGALYAPI P
Sbjct  1    MAAASSPPRAERKRWGWGRLPGARRGSAGLAKKCPFSLELAEGGPAGGALYAPIAPGAPG  60

Query  61   GPALPASPAPPAAPPAAADLGPRPPVSLDPRVSIYRTRRPLLARTHIQGRVYNFLERPTG  120
             PA PASPA PAAPP A+DLGPRPPVSLDPRVSIY TRRP+LARTH+QGRVYNFLERPTG
Sbjct  61   -PAPPASPAAPAAPPVASDLGPRPPVSLDPRVSIYSTRRPVLARTHVQGRVYNFLERPTG  119

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Query  181  CRSKYVGIWGRLRFARKPISIIDLIVVVASMVVLCVGSKGQVFATSAIRGIRFLQILRML  240
            CRSKYVG+WGRLRFARKPISIIDLIVVVASMVVLCVGSKGQVFATSAIRGIRFLQILRML
Sbjct  180  CRSKYVGLWGRLRFARKPISIIDLIVVVASMVVLCVGSKGQVFATSAIRGIRFLQILRML  239

Query  241  HVDRQGGTWRLLGSVVFIHRQELITTLYIGFLGLIFSSYFVYLAEKDAVNESGRVEFGSY  300
            HVDRQGGTWRLLGSVVFIHRQELITTLYIGFLGLIFSSYFVYLAEKDAVNESGRVEFGSY
Sbjct  240  HVDRQGGTWRLLGSVVFIHRQELITTLYIGFLGLIFSSYFVYLAEKDAVNESGRVEFGSY  299

Query  301  ADALWWGVVTVTTIGYGDKVPQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQQKQ  360
            ADALWWGVVTVTTIGYGDKVPQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQQKQ
Sbjct  300  ADALWWGVVTVTTIGYGDKVPQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQQKQ  359

Query  361  RQKHFNRQIPAAASLIQTAWRCYAAENPDSSTWEIYVRKPSRSHALLSPSPKPKKSAMVK  420
            RQKHFNRQIPAAASLIQTAWRCYAAENPDSSTW+IY+RK  RSH LLSPSPKPKKS +VK
Sbjct  360  RQKHFNRQIPAAASLIQTAWRCYAAENPDSSTWKIYIRKAPRSHTLLSPSPKPKKSVVVK  419

Query  421  KKKFKLDKDNGVSPGEKTLTVPHITCDLVSEERRPDHFLVESCDNSVKKSPTLLEVSTAH  480
            KKKFKLDKDNGV+PGEK LTVPHITCD   EERR DHF V+  D+SV+KSPTLLEVS  H
Sbjct  420  KKKFKLDKDNGVTPGEKMLTVPHITCD-PPEERRLDHFSVDGYDSSVRKSPTLLEVSMPH  478

Query  481  FMRTNSFAEDLDLEGETLLAPITHVSQLREHHRATIKVIRRMQYFVAKKKFQQARKPYDV  540
            FMRTNSFAEDLDLEGETLL PITH+SQLREHHRATIKVIRRMQYFVAKKKFQQARKPYDV
Sbjct  479  FMRTNSFAEDLDLEGETLLTPITHISQLREHHRATIKVIRRMQYFVAKKKFQQARKPYDV  538

Query  541  RDVIEQYSQGHLNLMVRIKELQRRLDQSIGKPSLFISGSEKSKDRGNNTIGARLNRVEDM  600
            RDVIEQYSQGHLNLMVRIKELQRRLDQSIGKPSLFIS SEKSKDRG+NTIGARLNRVED
Sbjct  539  RDVIEQYSQGHLNLMVRIKELQRRLDQSIGKPSLFISVSEKSKDRGSNTIGARLNRVEDK  598
```

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Query  601  VTQLDQRLVLITDMLHQLLSLHHGSPPGGRPPSGD-EAQVVQPCG-GGSTNPELFLPSNA  658
            VTQLDQRL LITDMLHQLLSLH GS PG   P  +  A + QPCG GGS++PELFLPSN
Sbjct  599  VTQLDQRLALITDMLHQLLSLHGGSTPGSGGPPREGGAHITQPCGSGGSVDPELFLPSNT  658

Query  659  LPTYEQLTVPHRGPDEGS  676
            LPTYEQLTVP RGPDEGS
Sbjct  659  LPTYEQLTVPRRGPDEGS  676
```

```
Equine 1    MDAASSPPRAERKRWGWGRLPGARRGSAGLAKKCPFSLELAEGGPAGGALYAPIVPPGTQGPALPASPAPPAAPPAAADL  80
Human  1    MAAASSPPRAERKRWGWGRLPGARRGSAGLAKKCPFSLELAEGGPAGGALYAPIAP-GAPGPAPPASPAAPAAPPVASDL  79

Equine 81   GPRPPVSLDPRVSIYRTRRPLLARTHIQGRVYNFLERPTGWKCFVYHFAVFLIVLACLIFSVLSTIEQYVTLATGTLFWM  160
Human  80   GPRPPVSLDPRVSIYSTRRPVLARTHVQGRVYNFLERPTGWKCFVYHFAVFLIVLVCLIFSVLSTIEQYAALATGTLFWM  159

Equine 161  EIVLVVFFGTEYAVRLWSAGCRSKYVGIWGRLRFARKPISIIDLIVVVASMVVLCVGSKGQVFATSAIRGIRFLQILRML  240
Human  160  EIVLVVFFGTEYVVRLWSAGCRSKYVGLWGRLRFARKPISIIDLIVVVASMVVLCVGSKGQVFATSAIRGIRFLQILRML  239

Equine 241  HVDRQGGTWRLLGSVVFIHRQELITTLYIGFLGLIFSSYFVYLAEKDAVNESGRVEFGSYADALWWGVVTVTTIGYGDKV  320
Human  240  HVDRQGGTWRLLGSVVFIHRQELITTLYIGFLGLIFSSYFVYLAEKDAVNESGRVEFGSYADALWWGVVTVTTIGYGDKV  319

Equine 321  PQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQQKQRQKHFNRQIPAAASLIQTAWRCYAAENPDSSTWEIYVRKP  400
Human  320  PQTWVGKTIASCFSVFAISFFALPAGILGSGFALKVQQKQRQKHFNRQIPAAASLIQTAWRCYAAENPDSSTWKIYIRKA  399

Equine 401  SRSHALLSPSPKPKKSAMVKKKKFKLDKDNGVSPGEKTLTVPHITCDLVSEERRPDHFLVESCDNSVKKSPTLLEVSTAH  480
Human  400  PRSHTLLSPSPKPKKSVVVKKKKFKLDKDNGVTPGEKMLTVPHITCD-PPEERRLDHFSVDGYDSSVRKSPTLLEVSMPH  478

Equine 481  FMRTNSFAEDLDLEGETLLAPITHVSQLREHHRATIKVIRRMQYFVAKKKFQQARKPYDVRDVIEQYSQGHLNLMVRIKE  560
Human  479  FMRTNSFAEDLDLEGETLLTPITHISQLREHHRATIKVIRRMQYFVAKKKFQQARKPYDVRDVIEQYSQGHLNLMVRIKE  558

Equine 561  LQRRLDQSIGKPSLFISGSEKSKDRGNNTIGARLNRVEDMVTQLDQRLVLITDMLHQLLSLHHGSPPG-GRPPSGDEAQV  639
Human  559  LQRRLDQSIGKPSLFISVSEKSKDRGSNTIGARLNRVEDKVTQLDQRLALITDMLHQLLSLHGGSTPGSGGPPREGGAHI  638

Equine 640  VQPCG-GGSINPELFLPSNALPTYEQLTVPHRGPDEGS  676
Human  639  TQPCGSGGSVDPELFLPSNTLPTYEQLTVPRRGPDEGS  676
```

Figure 9: A protein BLAST without any marking only indicating where there are differences in amino acids.

Apendix III KCNH2

Score	Expect	Identities	Gaps	Strand
5055 bits(2737)	0.0	3233/3480(93%)	3/3480(0%)	Plus/Plus

```
Query  1     ATGCCGGTGCGGAGGGGCCACGTCGCGCCGCAGAACACCTTCCTGGACACCATCATCCGC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     ATGCCGGTGCGGAGGGGCCACGTCGCGCCGCAGAACACCTTCCTGGACACCATCATCCGC  60

Query  61    AAGTTTGAGGGCCAGAGCCGCAAGTTCATTATCGCCAACGCTCGGGTGGAGAACTGCGCC  120
             ||||||||||||||||||| ||||||||| ||||||||||||||||||||||||||||||
Sbjct  61    AAGTTTGAGGGCCAGAGCCGTAAGTTCATCATCGCCAACGCTCGGGTGGAGAACTGCGCC  120

Query  121   GTCATCTACTGCAACGACGGCTTCTGCGAGCTGTGCGGCTACTCGCGGGCCGAGGTGATG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121   GTCATCTACTGCAACGACGGCTTCTGCGAGCTGTGCGGCTACTCGCGGGCCGAGGTGATG  180

Query  181   CAGCGGCCCTGCACCTGCGACTTCCTGCACGGgccgcgcacgcagcgccgcgccgccgcg  240
             ||||| ||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  181   CAGCGACCCTGCACCTGCGACTTCCTGCACGGGCCGCGCACGCAGCGCCGCGCTGCCGCG  240

Query  241   cagatcgcgcaGGCCTTGCTGGGCGCCGAGGAGCGCAAAGTGGAGATCTCCTTCTACCGG  300
             |||||||||||||  |||||||||||||||||||||||||| ||| ||||||||||||
Sbjct  241   CAGATCGCGCAGGCACTGCTGGGCGCCGAGGAGCGCAAAGTGGAAATCGCCTTCTACCGG  300

Query  301   AAGGATGGGAGCTGCTTCCTGTGCCTGGTGGATGTGGTGCCCGTGAAGAACGAGGATGGG  360
             || |||||||||||||||| || ||||||||||||||||||||||||||||||||||||
Sbjct  301   AAAGATGGGAGCTGCTTCCTATGTCTGGTGGATGTGGTGCCCGTGAAGAACGAGGATGGG  360

Query  361   GCTGTCATCATGTTCATCCTCAACTTCGAGGTGGTGATGGAGAAGGACATGGTGGGGTCC  420
             ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  361   GCTGTCATCATGTTCATCCTCAATTTCGAGGTGGTGATGGAGAAGGACATGGTGGGGTCC  420

Query  421   CCGGCCCGGGACACCAATCACCGTGGccccccACTAGCTGGCTGGCCACAGGTCGGGCC  480
             ||||| |  |||||||| ||||| |||||||||| |||||||||||| |||| || |||
Sbjct  421   CCGGCTCATGACACCAACCACCGGGGCCCCCCCACCAGCTGGCTGGCCCCAGGCCGCGCC  480

Query  481   AAGACCTTCCGCCTGAAGTTGCCTGCGCTGCTGGCCTTGACAGCGCGGGAGTCGACAGTG  540
             |||||||||||||||||| |||| ||||||||||  |||| || ||||||||| | |||
Sbjct  481   AAGACCTTCCGCCTGAAGCTGCCCGCGCTGCTGGCGCTGACGGCCCGGGAGTCGTCGGTG  540

Query  541   CGGCCAGGTGGCGCGGGCAGCACGGGGGCCCCCGGGGCTGTGGTGGTGGACGTGGACCTG  600
             ||| | || ||||||||| || |||| ||||| ||||| ||||||||||||||||||||
Sbjct  541   CGGTCGGGCGGCGCGGGCGGCGCGGGCGCCCCGGGGGCCGTGGTGGTGGACGTGGACCTG  600

Query  601   ACGCCTGCGGCGCCCAGCAGCGAGTCGCTGGCCCTGGACGAGGTGACAGCCATGGACAAC  660
             ||||| ||||| |||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  601   ACGCCCGCGGCACCCAGCAGCGAGTCGCTGGCCCTGGACGAAGTGACAGCCATGGACAAC  660

Query  661   CACGTGGCGGGGCTTGGGCCGGCGGAAGAGCGCCGCGCGCTGGTGGGCCCCGGCTCGCCG  720
             |||||||| ||||| ||||| ||||| ||||| || |||||||||| |||||||| |||
Sbjct  661   CACGTGGCAGGGCTCGGGCCCGCGGAGGAGCGGCGTGCGCTGGTGGGTCCCGGCTCTCCG  720

Query  721   CCCGCCTGTGCGCCCATCCCGCACCCGTCACCCCGGGCCCACAGCCTCAACCCCGACGCC  780
             |||  | | ||||||  || || ||| || |||||||| ||||||||||||||||||||
Sbjct  721   CCCCGCAGCGCGCCCGGCCAGCTCCCATCGCCCCGGGCGCACAGCCTCAACCCCGACGCC  780

Query  781   TCGGGCTCCAGCTGCAGCCTGGCCCGGACACGCTCCCGGGAGAGCTGTGCCAGCGTGCGC  840
             |||||||||||||||||||||||||||| |||||||| || ||||| ||||||||||||
Sbjct  781   TCGGGCTCCAGCTGCAGCCTGGCCCGGACGCGCTCCCGAGAAAGCTGCGCCAGCGTGCGC  840

Query  841   CGCGCCTCATCAGCGGATGACATCGAGGCCATGCGCACCGG---GCTGCCCCCACCGCCA  897
             |||||||| || || || |||||||||||||||||| ||||   ||||||||| || ||
Sbjct  841   CGCGCCTCGTCGGCCGACGACATCGAGGCCATGCGCGCCGGGGGTGCTGCCCCCGCCACCG  900
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Query  898   CGCCATGCCAGCACAGGGGCCATGCACCCCCTGCGCAGCGGCCTGCTTAACTCCACATCA  957
             ||||| |||||||| |||||||||||||| |||||||||||| |||| |||||||| ||
Sbjct  901   CGCCACGCCAGCACCGGGGCCATGCACCCACTGCGCAGCGGCTTGCTCAACTCCACCTCG  960

Query  958   GATTCGGACCTCGTGCGCTACCGCACCATTAGCAAGATTCCCCAAATCACCCTCAACTTT  1017
             || || ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  961   GACTCCGACCTCGTGCGCTACCGCACCATTAGCAAGATTCCCCAAATCACCCTCAACTTT  1020

Query  1018  GTGGACCTCAAGGGCGACCCCTTCCTGGCTTCGCCCACCAGTGACCGGGAGATCATAGCA  1077
             ||||||||||||||||||||||| |||||||||||||||||||| ||||||||||||
Sbjct  1021  GTGGACCTCAAGGGCGACCCCTTCTTGGCTTCGCCCACCAGTGACCGTGAGATCATAGCA  1080

Query  1078  CCCAAGATAAAGGAGCGGACCCACAATGTCACCGAGAAGGTCACCCAGGTCCTGTCTCTG  1137
             || |||||||||||||| |||||||||||||| |||||||||||||||||||||| |||
Sbjct  1081  CCTAAGATAAAGGAGCGAACCCACAATGTCACTGAGAAGGTCACCCAGGTCCTGTCCCTG  1140

Query  1138  GGTGCTGATGTGCTGCCGGAGTACAAGCTGCAGGCGCCACGCATCCACCGCTGGACCATC  1197
             || || || |||||||| |||||||||||||||| || ||||||||||||||||||||||
Sbjct  1141  GGCGCCGACGTGCTGCCTGAGTACAAGCTGCAGGCACCGCGCATCCACCGCTGGACCATC  1200

Query  1198  CTGCACTACAGCCCCTTCAAGGCCGTGTGGGACTGGCTCATCCTGCTGCTGGTCATCTAC  1257
             ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1201  CTGCATTACAGCCCCTTCAAGGCCGTGTGGGACTGGCTCATCCTGCTGCTGGTCATCTAC  1260

Query  1258  ACGGCCGTCTTCACGCCCTACTCGGCTGCCTTCCTGCTGAAGGAGACGGAAGAGGGCCCC  1317
             ||||| |||||||| |||||||||||||||||||||||||||||||||||| |||||
Sbjct  1261  ACGGCTGTCTTCACACCCTACTCGGCTGCCTTCCTGCTGAAGGAGACGGAAGAAGGCCCG  1320

Query  1318  CCGGCCACCGACTGTGGCTATGCCTGCCAGCCCCTGGCAGTGGTGGACCTCATCGTGGAT  1377
             || || ||||| |||||||| |||||||||| ||||| ||||||||||||||||||||
Sbjct  1321  CCTGCTACCGAGTGTGGCTACGCCTGCCAGCCGCTGGCTGTGGTGGACCTCATCGTGGAC  1380

Query  1378  ATCATGTTCATCGTGGACATCCTCATCAACTTCCGCACCACCTATGTCAATGCCAACGAG  1437
             |||||||||| |||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  1381  ATCATGTTCATTGTGGACATCCTCATCAACTTCCGCACCACCTACGTCAATGCCAACGAG  1440

Query  1438  GAGGTGGTCAGCCACCCTGGCCGCATCGCCGTCCACTACTTCAAGGGCTGGTTCCTCATC  1497
             |||||||||||||||| |||||||||||||||||||||||||||||||||||||||||||
Sbjct  1441  GAGGTGGTCAGCCACCCCGGCCGCATCGCCGTCCACTACTTCAAGGGCTGGTTCCTCATC  1500

Query  1498  GACATGGTGGCTGCCATCCCCTTTGACCTGCTCATCTTCGGTTCTGGCTCTGAGGAGCTG  1557
             |||||||||||| |||||||||| |||||||||||||||| ||||||||||||||||||
Sbjct  1501  GACATGGTGGCCGCCATCCCCTTCGACCTGCTCATCTTCGGCTCTGGCTCTGAGGAGCTG  1560

Query  1558  ATCGGGCTCCTGAAGACGGCGCGGCTGCTGCGACTGGTGCGCGTCGCACGGAAGCTGGAC  1617
             |||||||| |||||||| |||||||||||||| |||||||||| || ||||||||||
Sbjct  1561  ATCGGGCTGCTGAAGACTGCGCGGCTGCTGCGGCTGGTGCGCGTGGCGCGGAAGCTGGAT  1620

Query  1618  CGCTACTCGGAGTACGGGGCAGCGGTGCTCTTCCTGCTCATGTGCACCTTTGCGCTCATC  1677
             |||||||| |||||||| || || ||||| ||| |||||||||||||||||||||||||
Sbjct  1621  CGCTACTCAGAGTACGGCGCGGCCGTGCTGTTCTTGCTCATGTGCACCTTTGCGCTCATC  1680

Query  1678  GCGCACTGGCTGGCTTGCATCTGGTACGCCATCGGCAACATGGAGCAGCCGCACATGGAC  1737
             |||||||||| || |||||||||||||||||||||||||||||||||| |||||||||
Sbjct  1681  GCGCACTGGCTAGCCTGCATCTGGTACGCCATCGGCAACATGGAGCAGCCACACATGGAC  1740

Query  1738  TCCCGCATCGGCTGGCTGCACAACCTGGGCGACCAGATCGGCAAGCCCTACAACAGCAGT  1797
             || |||||||||||||||||||||||||||||||| ||||| ||||||||||||||
Sbjct  1741  TCACGCATCGGCTGGCTGCACAACCTGGGCGACCAGATAGGCAAACCCTACAACAGCAGC  1800

Query  1798  GGCCTGGGTGGCCCGTCCATCAAGGACAAGTATGTCACGGCCCTCTACTTCACCTTCAGC  1857
             |||||||| ||||| |||||||||||||||||||| ||||| ||||||||||||||||||
Sbjct  1801  GGCCTGGGCGGCCCCTCCATCAAGGACAAGTATGTGACGGCGCTCTACTTCACCTTCAGC  1860
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Query  1858  AGCCTCACTAGCGTGGGCTTCGGCAATGTCTCCCCCAACACCAACTCAGAGAAGATCTTC  1917
             ||||||||  ||  ||||||||||||||  |||||  ||||||||||||||||||||||||||||
Sbjct  1861  AGCCTCACCAGTGTGGGCTTCGGCAACGTCTCTCCCAACACCAACTCAGAGAAGATCTTC  1920

Query  1918  TCCATTTGTGTCATGCTCATTGGCTCCCTCATGTACGCCAGCATCTTTGGCAACGTGTCA  1977
             |||||  ||  ||||||||||||||||||||||||||  ||  ||||||||  ||||||||||
Sbjct  1921  TCCATCTGCGTCATGCTCATTGGCTCCCTCATGTATGCTAGCATCTTCGGCAACGTGTCG  1980

Query  1978  GCCATCATCCAGCGGCTATACTCGGGCACAGCCCGCTACCACACGCAAATGCTCCGGGTG  2037
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Sbjct  1981  GCCATCATCCAGCGGCTGTACTCGGGCACAGCCCGCTACCACACACAGATGCTGCGGGTG  2040

Query  2038  CGGGAGTTCATCCGCTTCCACCAGATCCCTAACCCGCTGCGCCAGCGCCTTGAGGAGTAT  2097
             ||||||||||||||||||||||||||||  ||  ||||||||||||||  ||||||||
Sbjct  2041  CGGGAGTTCATCCGCTTCCACCAGATCCCCAATCCCCTGCGCCAGCGCCTCGAGGAGTAC  2100

Query  2098  TTCCAGCACGCCTGGTCCTACACCAACGGCATCGACATGAACGCGGTGCTGAAGGGCTTC  2157
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Sbjct  2101  TTCCAGCACGCCTGGTCCTACACCAACGGCATCGACATGAACGCGGTGCTGAAGGGCTTC  2160

Query  2158  CCGGAGTGCCTGCAGGCAGACATCTGCCTGCACCTGAACCGCTCGCTGCTGCAACATTGC  2217
             ||  ||||||||||||||  ||||||||||||||||||||||||||  ||||||||  ||  |||
Sbjct  2161  CCTGAGTGCCTGCAGGCTGACATCTGCCTGCACCTGAACCGCTCACTGCTGCAGCACTGC  2220

Query  2218  AAGCCCTTCCGAGGGGCCACCAAAGGCTGCCTGCGGGCCCTGGCCATGAAGTTCAAGACG  2277
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Sbjct  2221  AAACCCTTCCGAGGGGCCACCAAGGGCTGCCTTCGGGCCCTGGCCATGAAGTTCAAGACC  2280

Query  2278  ACACACGCACCGCCAGGGGACACGCTGGTGCACGCCGGGGACCTGCTCACCGCCCTCTAC  2337
             |||||  ||||||||||||||||||  ||||||||  ||  ||||||||||||||||||||  |||
Sbjct  2281  ACACATGCACCGCCAGGGGACACACTGGTGCATGCTGGGGACCTGCTCACCGCCCTGTAC  2340

Query  2338  TTCATCTCCCGGGGCTCCATCGAGATCCTGCGGGGCGATGTCGTCGTGGCCATCCTGGGG  2397
             ||||||||||||||||||||||||||||||||||||||||  ||||||||||||||||||||||
Sbjct  2341  TTCATCTCCCGGGGCTCCATCGAGATCCTGCGGGGCGACGTCGTCGTGGCCATCCTGGGG  2400

Query  2398  AAGAATGACATCTTCGGAGAGCCTCTGAACCTGTATGCGCGGCCTGGCAAGTCCAATGGG  2457
             ||||||||||||||  ||  ||||||||||||||||||||  ||||||||||||||  ||  |||
Sbjct  2401  AAGAATGACATCTTTGGGGAGCCTCTGAACCTGTATGCAAGGCCTGGCAAGTCGAACGGG  2460

Query  2458  GATGTGCGGGCCCTCACCTACTGCGACCTGCACAAGATCCACCGGGACGACCTGCTGGAG  2517
             ||||||||||||||||||||||  |||||  ||||||||||  ||||||||||||||||||
Sbjct  2461  GATGTGCGGGCCCTCACCTACTGTGACCTACACAAGATCCATCGGGACGACCTGCTGGAG  2520

Query  2518  GTGCTGGACATGTACCCCGAGTTCTCCGACCACTTCTGGTCCAGCCTGGAGATCACCTTC  2577
             ||||||||||||||||  ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521  GTGCTGGACATGTACCCTGAGTTCTCCGACCACTTCTGGTCCAGCCTGGAGATCACCTTC  2580

Query  2578  AACCTTCGAGACACCAACATGATCCCCGGCTCTCCCGGCAGCACAGAGCTGGAGGGCGGC  2637
             |||||  |||||  ||||||||||||||  |||||  ||||||||  ||  |||  |  |||||  |||
Sbjct  2581  AACCTGCGAGATACCAACATGATCCCGGGCTCCCCCGGCAGTACGGAGTTAGAGGGTGGC  2640

Query  2638  TTCAACCGGCAACGCAAGCGCAAGCTGTCCTTCCGCAGACGCACCGACAAGGACCCGGAA  2697
             ||||  ||||||||||||||||||  ||||||||||||  |||||  |||||||||  ||||
Sbjct  2641  TTCAGTCGGCAACGCAAGCGCAAGTTGTCCTTCCGCAGGCGCACGGACAAGGACACGGAG  2700

Query  2698  CAgccaggggaggtgtcggccttggggccgggccgggcgggggcagggcccagtagccgg  2757
             ||||||||||||||||||||||||||||||||||||||||||||||||||||  |||||||||
Sbjct  2701  CAGCCAGGGGAGGTGTCGGCCTTGGGGCCGGGCCGGGCGGGGGCAGGGCCGAGTAGCCGG  2760

Query  2758  ggccggccaggggggcccgtggggggAAAGCCCGTCCAGTGGCCCCTCCAGCCCTGAGAGC  2817
             ||||||||  |||||  ||||||||||  ||||||||||||||||||||||||||||||||||
Sbjct  2761  GGCCGGCCGGGGGGGCCGTGGGGGGAGAGCCCGTCCAGTGGCCCCTCCAGCCCTGAGAGC  2820
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Query  2818  AGTGAGGATGAGGGCCCAGGCCGCAGCTCCAGCCCCCTCCGCCTGGTGCCCTTCTCCAGC  2877
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Sbjct  2821  AGTGAGGATGAGGGCCCAGGCCGCAGCTCCAGCCCCCTCCGCCTGGTGCCCTTCTCCAGC  2880

Query  2878  CCCAGGCCCCCCGGAGAGCCGCCGGGTGGGGAGCCCCTGATTGAGGACTGCGAGAAGAGC  2937
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Sbjct  2881  CCCAGGCCCCCCGGAGAGCCGCCGGGTGGGGAGCCCCTGATGGAGGACTGCGAGAAGAGC  2940

Query  2938  AGTGACACATGTAACCCGCTGTCAGGCGCCTTCTCGGGAGTGTCCAACATCTTCAGCTTC  2997
             || ||||| || ||||| ||||||||||||||| ||||||||||||| |||||||||
Sbjct  2941  AGCGACACTTGCAACCCCCTGTCAGGCGCCTTCTCAGGAGTGTCCAACATTTTCAGCTTC  3000

Query  2998  TGGGGGGATAGTCGGGGCCGCCAGTACCAGGAGCTGCCTCGCTGccccgccccgccccc  3057
             |||||||| |||||||||||||||||||||||| ||||| |||||||||||| |||||
Sbjct  3001  TGGGGGGACAGTCGGGGCCGCCAGTACCAGGAGCTCCCTCGATGCCCCGCCCCCACCCCC  3060

Query  3058  AGCCTCCTCAACATCCCTCTTTCCAGCCCTGGCCGGCGGCCCCGGGGCGATGTGGAGAGC  3117
             ||||||||||||||| || |||||||| || ||||||||||||||| |||||||||
Sbjct  3061  AGCCTCCTCAACATCCCCCTCTCCAGCCCGGGTCGGCGGCCCCGGGGCGACGTGGAGAGC  3120

Query  3118  AGGCTGGACGCCCTTCAGAGGCAGCTTAACAGGCTGGAGACGCGGCTGAGTGCAGACATG  3177
             |||||||| ||||| ||| | ||||| |||||||||||||| ||||||||||||||||||
Sbjct  3121  AGGCTGGATGCCCTCCAGCGCCAGCTCAACAGGCTGGAGACCCGGCTGAGTGCAGACATG  3180

Query  3178  GCCACCGTCCTGCAGCTACTGCAGAGACAGATGACACTGGTCCCTCCAGCCTACAGTGCT  3237
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Sbjct  3181  GCCACTGTCCTGCAGCTGCTACAGAGGCAGATGACGCTGGTCCCGCCCGCCTACAGTGCT  3240

Query  3238  GTGACCACCCCGGGGGCCCGGCCCCACCTCCACCTCCCCTCTCCTGCCTGTCAGCCCCATC  3297
             |||||||||||||||| |||||||| ||||| ||||| ||  |||| ||||||||| ||
Sbjct  3241  GTGACCACCCCGGGGCCTGGCCCCACTTCCACATCCCCGCTGTTGCCCGTCAGCCCCCTC  3300

Query  3298  CCCACTCTCACCCTGGATTCGCTTTCTCAGGTTTCCCAGTTCATGGCGTGCGAGGAGCTC  3357
             ||||| |||||| |||| |||||||||||||||||||||||||||||| ||||||||
Sbjct  3301  CCCACCCTCACCTTGGACTCGCTTTCTCAGGTTTCCCAGTTCATGGCGTGTGAGGAGCTG  3360

Query  3358  CCTCCGGGGGCCCCAGAGCTTCCCCAAGACGGCCCCACTCGACGCCTCTCCCTGCCGGGC  3417
             || |||||||||||||||||||||||| |||||||| |||||||||||||| ||||||
Sbjct  3361  CCCCCGGGGGCCCCAGAGCTTCCCCAAGAAGGCCCCACACGACGCCTCTCCCTACCGGGC  3420

Query  3418  CAGCTGGGGGCCCTCACCTCCCAGCCCCTGCACAGACACGGCTCAGACCCGGGCAGTTAG  3477
             |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  3421  CAGCTGGGGGCCCTCACCTCCCAGCCCCTGCACAGACACGGCTCGGACCCGGGCAGTTAG  3480
```

Protein BLAST

The TMHMM gave only 4 highly probable helices both for human and equine proteins. There were no dissimilarities between the first and the last trans membrane helix in the entire segment, 408bp to 661bp so the graphic is left out.

Score	Expect	Method	Identities	Positives	Gaps
2208 bits(5721)	0.0	Compositional matrix adjust.	1138/1159(98%)	1145/1159(98%)	1/1159(0%)

```
Query  1    MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVM  60
            MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVM
Sbjct  1    MPVRRGHVAPQNTFLDTIIRKFEGQSRKFIIANARVENCAVIYCNDGFCELCGYSRAEVM  60

Query  61   QRPCTCDFLHGPRTQRRAAAQIAQALLGAEERKVEISFYRKDGSCFLCLVDVVPVKNEDG  120
            QRPCTCDFLHGPRTQRRAAAQIAQALLGAEERKVEI+FYRKDGSCFLCLVDVVPVKNEDG
Sbjct  61   QRPCTCDFLHGPRTQRRAAAQIAQALLGAEERKVEIAFYRKDGSCFLCLVDVVPVKNEDG  120

Query  121  AVIMFILNFEVVMEKDMVGSPARDTNHRGPPTSWLATGRAKTFRLKLPALLALTARESTV  180
            AVIMFILNFEVVMEKDMVGSPA DTNHRGPPTSWLA GRAKTFRLKLPALLALTARES+V
Sbjct  121  AVIMFILNFEVVMEKDMVGSPAHDTNHRGPPTSWLAPGRAKTFRLKLPALLALTARESSV  180
```

```
Query  181   RPGGAGSTGAPGAVVVDVDLTPAAPSSESLALDEVTAMDNHVAGLGPAEERRALVGPGSP  240
             R GGAG  GAPGAVVVDVDLTPAAPSSESLALDEVTAMDNHVAGLGPAEERRALVGPGSP
Sbjct  181   RSGGAGGAGAPGAVVVDVDLTPAAPSSESLALDEVTAMDNHVAGLGPAEERRALVGPGSP  240

Query  241   PACAPIPHPSPRAHSLNPDASGSSCSLARTRSRESCASVRRASSADDIEAMRTG-LPPPP  299
             P  AP   PSPRAHSLNPDASGSSCSLARTRSRESCASVRRASSADDIEAMR G LPPPP
Sbjct  241   PRSAPGQLPSPRAHSLNPDASGSSCSLARTRSRESCASVRRASSADDIEAMRAGVLPPPP  300


Query  300   RHASTGAMHPLRSGLLNSTSDSDLVRYRTISKIPQITLNFVDLKGDPFLASPTSDREIIA  359
             RHASTGAMHPLRSGLLNSTSDSDLVRYRTISKIPQITLNFVDLKGDPFLASPTSDREIIA
Sbjct  301   RHASTGAMHPLRSGLLNSTSDSDLVRYRTISKIPQITLNFVDLKGDPFLASPTSDREIIA  360

Query  360   PKIKERTHNVTEKVTQVLSLGADVLPEYKLQAPRIHRWTILHYSPFKAVWDWLILLLVIY  419
             PKIKERTHNVTEKVTQVLSLGADVLPEYKLQAPRIHRWTILHYSPFKAVWDWLILLLVIY
Sbjct  361   PKIKERTHNVTEKVTQVLSLGADVLPEYKLQAPRIHRWTILHYSPFKAVWDWLILLLVIY  420

Query  420   TAVFTPYSAAFLLKETEEGPPATDCGYACQPLAVVDLIVDIMFIVDILINFRTTYVNANE  479
             TAVFTPYSAAFLLKETEEGPPAT+CGYACQPLAVVDLIVDIMFIVDILINFRTTYVNANE
Sbjct  421   TAVFTPYSAAFLLKETEEGPPATECGYACQPLAVVDLIVDIMFIVDILINFRTTYVNANE  480

Query  480   EVVSHPGRIAVHYFKGWFLIDMVAAIPFDLLIFGSGSEELIGLLKTARLLRLVRVARKLD  539
             EVVSHPGRIAVHYFKGWFLIDMVAAIPFDLLIFGSGSEELIGLLKTARLLRLVRVARKLD
Sbjct  481   EVVSHPGRIAVHYFKGWFLIDMVAAIPFDLLIFGSGSEELIGLLKTARLLRLVRVARKLD  540

Query  540   RYSEYGAAVLFLLMCTFALIAHWLACIWYAIGNMEQPHMDSRIGWLHNLGDQIGKPYNSS  599
             RYSEYGAAVLFLLMCTFALIAHWLACIWYAIGNMEQPHMDSRIGWLHNLGDQIGKPYNSS
Sbjct  541   RYSEYGAAVLFLLMCTFALIAHWLACIWYAIGNMEQPHMDSRIGWLHNLGDQIGKPYNSS  600

Query  600   GLGGPSIKDKYVTALYFTFSSLTSVGFGNVSPNTNSEKIFSICVMLIGSLMYASIFGNVS  659
             GLGGPSIKDKYVTALYFTFSSLTSVGFGNVSPNTNSEKIFSICVMLIGSLMYASIFGNVS
Sbjct  601   GLGGPSIKDKYVTALYFTFSSLTSVGFGNVSPNTNSEKIFSICVMLIGSLMYASIFGNVS  660

Query  660   AIIQRLYSGTARYHTQMLRVREFIRFHQIPNPLRQRLEEYFQHAWSYTNGIDMNAVLKGF  719
             AIIQRLYSGTARYHTQMLRVREFIRFHQIPNPLRQRLEEYFQHAWSYTNGIDMNAVLKGF
Sbjct  661   AIIQRLYSGTARYHTQMLRVREFIRFHQIPNPLRQRLEEYFQHAWSYTNGIDMNAVLKGF  720

Query  720   PECLQADICLHLNRSLLQHCKPFRGATKGCLRALAMKFKTTHAPPGDTLVHAGDLLTALY  779
             PECLQADICLHLNRSLLQHCKPFRGATKGCLRALAMKFKTTHAPPGDTLVHAGDLLTALY
Sbjct  721   PECLQADICLHLNRSLLQHCKPFRGATKGCLRALAMKFKTTHAPPGDTLVHAGDLLTALY  780

Query  780   FISRGSIEILRGDVVVAILGKNDIFGEPLNLYARPGKSNGDVRALTYCDLHKIHRDDLLE  839
             FISRGSIEILRGDVVVAILGKNDIFGEPLNLYARPGKSNGDVRALTYCDLHKIHRDDLLE
Sbjct  781   FISRGSIEILRGDVVVAILGKNDIFGEPLNLYARPGKSNGDVRALTYCDLHKIHRDDLLE  840

Query  840   VLDMYPEFSDHFWSSLEITFNLRDTNMIPGSPGSTELEGGFNRQRKRKLSFRRRTDKDPE  899
             VLDMYPEFSDHFWSSLEITFNLRDTNMIPGSPGSTELEGGF+RQRKRKLSFRRRTDKD E
Sbjct  841   VLDMYPEFSDHFWSSLEITFNLRDTNMIPGSPGSTELEGGFSRQRKRKLSFRRRTDKDTE  900

Query  900   QPGEVSALGPGRAGAGPSSRGRPGGPWGESPSSGPSSPESSEDEGPGRSSSPLRLVPFSS  959
             QPGEVSALGPGRAGAGPSSRGRPGGPWGESPSSGPSSPESSEDEGPGRSSSPLRLVPFSS
Sbjct  901   QPGEVSALGPGRAGAGPSSRGRPGGPWGESPSSGPSSPESSEDEGPGRSSSPLRLVPFSS  960

Query  960   PRPPGEPPGGEPLIEDCEKSSDTCNPLSGAFSGVSNIFSFWGDSRGRQYQELPRCPAPAP  1019
             PRPPGEPPGGEPL+EDCEKSSDTCNPLSGAFSGVSNIFSFWGDSRGRQYQELPRCPAP P
Sbjct  961   PRPPGEPPGGEPLMEDCEKSSDTCNPLSGAFSGVSNIFSFWGDSRGRQYQELPRCPAPTP  1020

Query  1020  SLLNIPLSSPGRRPRGDVESRLDALQRQLNRLETRLSADMATVLQLLQRQMTLVPPAYSA  1079
             SLLNIPLSSPGRRPRGDVESRLDALQRQLNRLETRLSADMATVLQLLQRQMTLVPPAYSA
Sbjct  1021  SLLNIPLSSPGRRPRGDVESRLDALQRQLNRLETRLSADMATVLQLLQRQMTLVPPAYSA  1080

Query  1080  VTTPGPGPTSTSPLLPVSPIPTLTLDSLSQVSQFMACEELPPGAPELPQDGPTRRLSLPG  1139
             VTTPGPGPTSTSPLLPVSP+PTLTLDSLSQVSQFMACEELPPGAPELPQ+GPTRRLSLPG
Sbjct  1081  VTTPGPGPTSTSPLLPVSPLPTLTLDSLSQVSQFMACEELPPGAPELPQEGPTRRLSLPG  1140
```

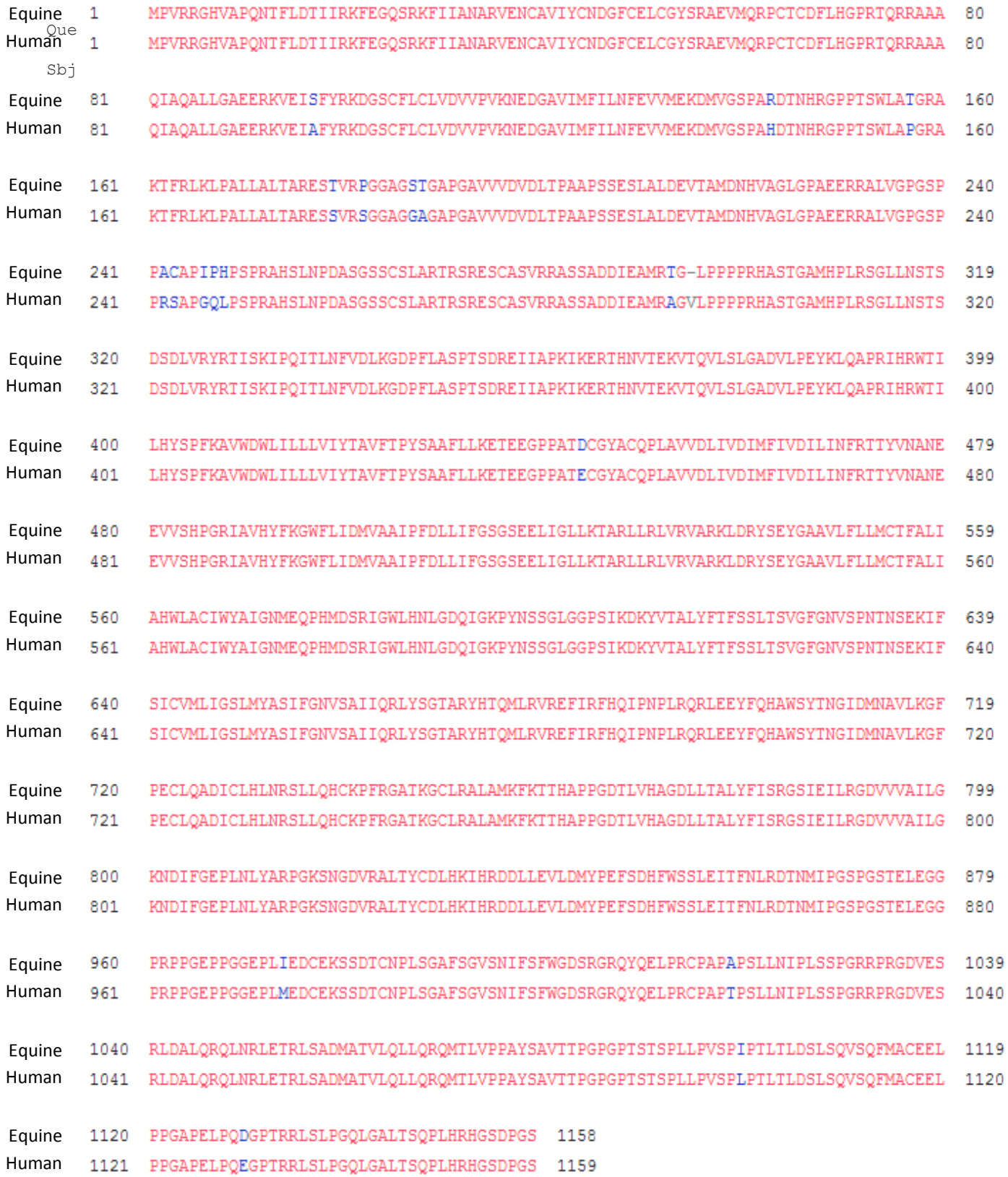

Figure 10: A protein BLAST without any marking only indicating where there are differences in amino acids.

Apendix IV KCNE1

Score	Expect	Identities	Gaps	Strand
399 bits(216)	6e-116	333/391(85%)	2/391(0%)	Plus/Plus

```
Query  1    ATGATCCTGTCTAACACCACAGCTGTGATGCCCTTTCTGGCCAAGCTGTGGCAGGGGACA  60
            ||||||||||||||||||||||| |||| |||||||||| ||||||||||||||| ||||
Sbjct  1    ATGATCCTGTCTAACACCACAGCGGTGACGCCCTTTCTGACCAAGCTGTGGCAGGAGACA  60

Query  61   GTTCAACAGGGCAGCAACACGTCTAGCCCAGCCCGCAGGTCCCCCAGCAACGAG-GACGG  119
            ||||| |||||  |||||| |||  |||  ||||||||||||||| ||| | || |||||
Sbjct  61   GTTCAGCAGGGTGGCAACATGTCGGGCCTGGCCCGCAGGTCCCCCCGCAGC-AGTGACGG  119

Query  120  CAAGCTTGAGGCACTCTACATTCTCATGGTGCTTGGCTTCTTCGGCTTCTTCACCCTGGG  179
            |||||| ||||| |||||| | |||||||| || || ||||||||||||||||||||||
Sbjct  120  CAAGCTGGAGGCCCTCTACGTCCTCATGGTACTGGGATTCTTCGGCTTCTTCACCCTGGG  179

Query  180  CATCATGCTGAGTTACATCCGCTCCAAGAAGCTGGAGCACTCCCACGACCCATTCAATGT  239
            |||||||||||| ||||||||||||||||||||||||||||  |||||||||||| ||
Sbjct  180  CATCATGCTGAGCTACATCCGCTCCAAGAAGCTGGAGCACTCGAACGACCCATTCAACGT  239

Query  240  GTACATCGAGTCTGACACCTGGCAGGAGAAGGACAAGAAGTACTTCCAGTCCCGGATTCT  299
             |||||||||| ||  ||||||| ||||||||||    ||  ||||| ||||| | ||
Sbjct  240  CTACATCGAGTCCGATGCCTGGCAAGAGAAGGACAAGGCCTATGTCCAGGCCCGGGTCCT  299

Query  300  GGAGAGCTACAGGGCGTGTTATGTCATTGAAAACGAGCTGGCTGTGGAACAGCCAGGCAC  359
            |||||||||||||| |||| |||||| |||||||| | |||||  | ||||| ||   |||
Sbjct  300  GGAGAGCTACAGGTCGTGCTATGTCGTTGAAAACCATCTGGCCATAGAACAACCCAACAC  359

Query  360  ATACCTTCCTGAGATGGACCCTTCATCATGA  390
            | |||||||||||| | | |||||  |||||
Sbjct  360  ACACCTTCCTGAGACGAAGCCTTCCCCATGA  390
```

Protein BLAST

Score	Expect	Method	Identities	Positives	Gaps
222 bits(566)	5e-80	Compositional matrix adjust.	103/128(80%)	112/128(87%)	0/128(0%)

```
Query  1    MILSNTTAVMPFLAKLWQGTVQQGSNTSSPARRSPSNEDGKLEALYILMVLGFFGFFTLG  60
            MILSNTTAV PFL KLWQ TVQQG N S  ARRSP + DGKLEALY+LMVLGFFGFFTLG
Sbjct  1    MILSNTTAVTPFLTKLWQETVQQGGNMSGLARRSPRSSDGKLEALYVLMVLGFFGFFTLG  60

Query  61   IMLSYIRSKKLEHSHDPFNVYIESDTWQEKDKKYFQSRILESYRACYVTENELAVEQPGT  120
            IMLSYIRSKKLEHS+DPFNVYIESD WQEKDK Y Q+R+LESYR+CYV+EN LA+EQP T
Sbjct  61   IMLSYIRSKKLEHSNDPFNVYIESDAWQEKDKAYVQARVLESYRSCYVVENHLAIEQPNT  120

Query  121  YLPEMDPS  128
            +LPE  PS
Sbjct  121  HLPETKPS  128
```

```
Equine 1    MILSNTTAVMPFLAKLWQGTVQQGSNTSSPARRSPSNEDGKLEALYILMVLGFFGFFTLGIMLSYIRSKKLEHSHDPFNV  80
Human  1    MILSNTTAVTPFLTKLWQETVQQGGNMSGLARRSPRSSDGKLEALYVLMVLGFFGFFTLGIMLSYIRSKKLEHSNDPFNV  80

Equine 81   YIESDTWQEKDKKYFQSRILESYRACYVIENELAVEQPGTYLPEMDPSS  129
Human  81   YIESDAWQEKDKAYVQARVLESYRSCYVVENHLAIEQPNTHLPETKPSP  129
```

Figure 11: A protein BLAST without any marking only indicating where there are differences in amino acids.

Apendix V KCNE2

The first 7bp and the last 3bp (turquoise) are putative based on the predicted equine sequence XM_001494194.1

Score	Expect	Identities	Gaps	Strand
459 bits(508)	7e-134	325/372(87%)	0/372(0%)	Plus/Plus

```
Query  1    ATGCCCACTTTATCCAATTTGACACAGACCCTGGAAGATGTCTTCAAAAAGATTTTTATT  60
            ||| | ||||||||||||||| |||||||| |||||||| ||||||  || ||||||||||
Sbjct  1    ATGTCTACTTTATCCAATTTCACACAGACGCTGGAAGACGTCTTCCGAAGGATTTTTATT  60

Query  61   ACCTATATGAACAATTGGCGCAGGAACACGACAGCTGAGCAAGAGGCCCTGCAAGCTAAA  120
            || |||||| |||||||||||  |||||| ||||||||||||||||||| ||||| |||
Sbjct  61   ACTTATATGGACAATTGGCGCCAGAACACAACAGCTGAGCAAGAGGCCCTCCAAGCCAAA  120

Query  121  GTGGACGCTGAGAATTTCTACTATGTCATCTTGTACCTTATGGTGATGATTGGAATGTTC  180
            || || |||||||| ||||||||||||||| ||||||| ||||||||||||||||||||
Sbjct  121  GTTGATGCTGAGAACTTCTACTATGTCATCCTGTACCTCATGGTGATGATTGGAATGTTC  180

Query  181  TCTTTCATCATTGTAGCCATCCTGGTGAGCACGGTGAAATCCAAGCGACGAGAACACTCC  240
            |||||||||||  || ||||||||||||||||  |||||||||||  ||||  |||||||||
Sbjct  181  TCTTTCATCATCGTGGCCATCCTGGTGAGCACTGTGAAATCCAAGAGACGGGAACACTCC  240

Query  241  AACGACCCCTACCACCAGTACATCGTAGAGGACTGGCAAGAGAAATACAGGAGTCAAATT  300
            || ||||||||||||||||||| |||||||||||||  || ||  ||||  |||  |||||
Sbjct  241  AATGACCCCTACCACCAGTACATTGTAGAGGACTGGCAGGAAAAGTACAAGAGCCAAATC  300

Query  301  TTGAATCTAGAGGAACCAAAGGCCACCATCCACAAGAACATTAGTGCAACCGAGTTCCAG  360
            |||||||||||  |||  | |||||||||||||  ||||||||  ||||  |  |  ||||  |
Sbjct  301  TTGAATCTAGAAGAATCGAAGGCCACCATCCATGAGAACATTGGTGCGGCTGGGTTCAAA  360

Query  361  ATGTCGCCTTGA  372
            |||||  ||  |||
Sbjct  361  ATGTCCCCCTGA  372
```

Protein BLAST

Score	Expect	Method	Identities	Positives	Gaps
234 bits(596)	1e-84	Compositional matrix adjust.	110/123(89%)	117/123(95%)	0/123(0%)

```
Query  1    MPTLSNLTQTLEDVFKKIFITYMNNWRRNTTAEQEALQAKVDAENFYYVILYLMVMIGMF  60
            M TLSN TQTLEDVF++IFITYM+NWR+NTTAEQEALQAKVDAENFYYVILYLMVMIGMF
Sbjct  1    MSTLSNFTQTLEDVFRRIFITYMDNWRQNTTAEQEALQAKVDAENFYYVILYLMVMIGMF  60

Query  61   SFIIVAILVSTVKSKRREHSNDPYHQYIVEDWQEKYRSQILNLEEPKATIHKNISATEFQ  120
            SFIIVAILVSTVKSKRREHSNDPYHQYIVEDWQEKY+SQILNLEE KATIH+NI A  F+
Sbjct  61   SFIIVAILVSTVKSKRREHSNDPYHQYIVEDWQEKYKSQILNLEESKATIHENIGAAGFK  120

Query  121  MSP  123
            MSP
Sbjct  121  MSP  123
```

```
Equine  1    XXXLSNLTQTLEDVFKKIFITYMNNWRRNTTAEQEALQAKVDAENFYYVILYLMVMIGMFSFIIVAILVSTVKSKRREHS  80
Human   1    MSTLSNFTQTLEDVFRRIFITYMDNWRQNTTAEQEALQAKVDAENFYYVILYLMVMIGMFSFIIVAILVSTVKSKRREHS  80

Equine  81   NDPYHQYIVEDWQEKYRSQILNLEEPKATIHKNISATEFQMSP  123
Human   81   NDPYHQYIVEDWQEKYKSQILNLEESKATIHENIGAAGFKMSP  123
```

Figure 12: A protein BLAST without any marking only indicating where there are differences in amino acids.