### Find a Gene Project Assignment

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#### A15866978

[Q1] Tell me the name of a protein you are interested in. Include the species and the accession number. This can be a human protein or a protein from any other species as long as it's function is known.

Name: p53

Accession: NP\_001263625.1

Species: Homo Sapiens

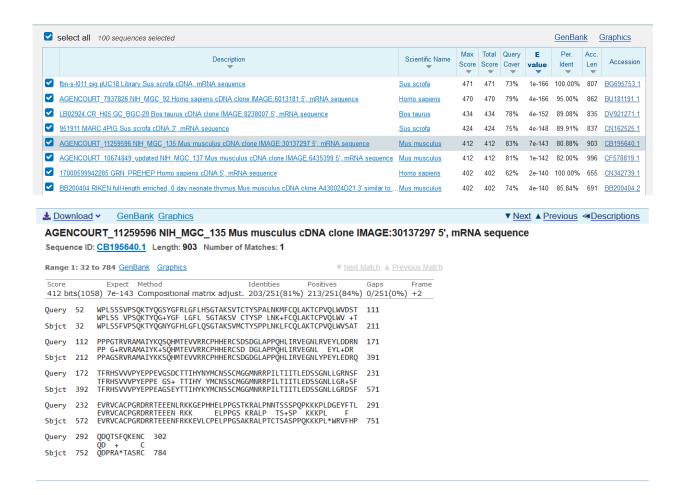
[Q2] Perform a BLAST search against a DNA database, such as a database consisting of genomic DNA or ESTs. The BLAST server can be at NCBI or elsewhere. Include details of the BLAST method used, database searched and any limits applied (e.g. Organism).

Method: tBlastn

Database: Expressed Sequence Tags (est)

Enter Query	Sequence							
Enter accession number(s), gi(s), or FASTA sequence(s) ? Clear Query subrange ?								
NP_001263625.1	From							
Or, upload file	Browse No file selected.							
Job Title	NP_001263625:cellular tumor antigen p53 isoform  Enter a descriptive title for your BLAST search ?							
Align two or m	ore sequences 🔞							
Choose Sear	rch Set							
Database	♦ Expressed sequence tags (est) ✓							
Organism Optional	Enter organism name or id—completions will be suggeste exclude Add organism  Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown							
Exclude Optional	☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences							
Limit to Optional Entrez Query	Sequences from type material  You Tube Create custom database							
Optional	Enter an Entrez query to limit search ?							

Match: Accession Number: CB195640.1



[Q3] Gather information about this "novel" protein. At a minimum, show me the protein sequence of the "novel" protein as displayed in your BLAST results from [Q2] as FASTA format (you can copy and paste the aligned sequence subject lines from your BLAST result page if necessary) or translate your novel DNA sequence using a tool called EMBOSS Transeq at the EBI. Don't forget to translate all six reading frames; the ORF (open reading frame) is likely to be the longest sequence without a stop codon. It may not start with a methionine if you don't have the complete coding region. Make sure the sequence you provide includes a header/subject line and is in traditional FASTA format

## Sequence from EMBOSS:

```
>CB195640.1_1 AGENCOURT_11259596 NIH_MGC_135 Mus musculus cDNA clone IMAGE:30137297 5', mRNA sequence WASGPCPSHSMAPVIFCPFSKNLPGQLWLPPGLPAVWDSQVCYVHVLSSPQ*AILPAGED VPCAVVGQRHTSSWEPCPRHGHLQEVTAHDGGRETLPPP*ALLRW*WPGSSPASYPGGRK FVSRVSGRQADFSPQRGGTL*ATRGRL*VYHHPLQVHV**LLHGGHEPPTYPYHHHTGRL QWEPSGTGQL*GSCLCLPWERPPYRRRKFPQKGSPLP*TAPRERKESAAHLHKRLSPAKE KTTLMESISPSRSAGVNGFEMFRGAEMRPLELKGMPMLQKESGRQQGASLQLTWKTKEKG Q
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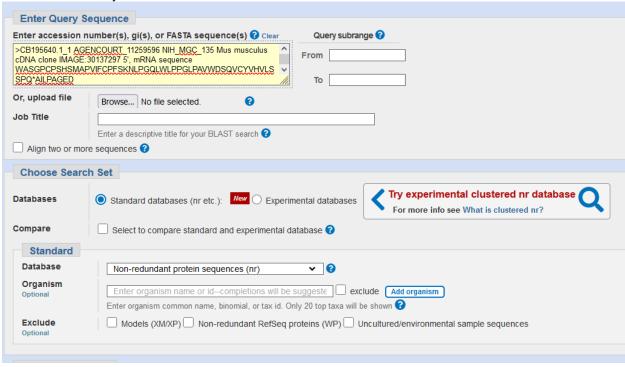
Name: cellular tumor antigen p53 isoform a [Mus musculus]

Species: Mus Musculus

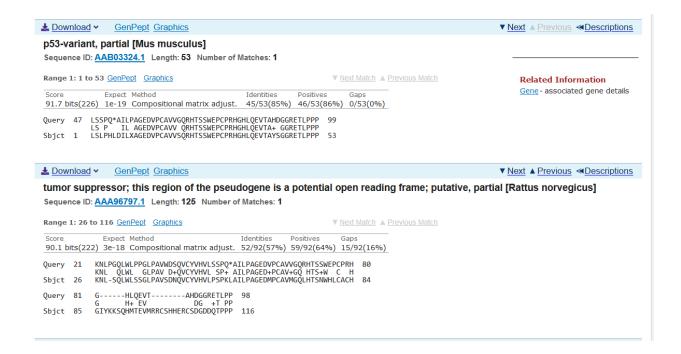
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
Myomorpha;
Muroidea; Muridae; Murinae; Mus; Mus.

[Q4] Prove that this gene, and its corresponding protein, are novel. For the purposes of this project, "novel" is defined as follows. Take the protein sequence (your answer to [Q3]), and use it as a query in a blastp search of the nr database at NCBI

A BlastP search against the NR database provided a result from mus musculus with 17% query cover and 84.91% identity







Q5] Generate a multiple sequence alignment with your novel protein, your original query protein, and a group of other members of this family from different species. A typical number of proteins to use in a multiple sequence alignment for this assignment purpose is a minimum of 5 and a maximum of 20 - although the exact number is up to you. Include the multiple sequence alignment in your report. Use Courier font with a size appropriate to fit page width.

### Labeled Sequences for Alignment

>Human P53 NP\_001263625.1 cellular tumor antigen p53 isoform i [Homo sapiens] MDDLMLSPDDIEQWFTEDPGPDEAPRMPEAAPPVAPAPAAPTPAAPAPASWPLSSSVPSQKTYQGSYGF RLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHH ERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRR PILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPK KKPLDGEYFTLQDQTSFQKENC

>House Mouse P53 Sequence from blast result CB195640.1\_1 AGENCOURT\_11259596 NIH\_MGC\_135 Mus musculus cDNA clone IMAGE:30137297 5', mRNA sequence WASGPCPSHSMAPVIFCPFSKNLPGQLWLPPGLPAVWDSQVCYVHVLSSPQAILPAGED VPCAVVGQRHTSSWEPCPRHGHLQEVTAHDGGRETLPPPALLRWWPGSSPASYPGGRK FVSRVSGRQADFSPQRGGTLATRGRLVYHHPLQVHVLLHGGHEPPTYPYHHHTGRL QWEPSGTGQLGSCLCLPWERPPYRRRKFPQKGSPLPTAPRERKESAAHLHKRLSPAKE KTTLMESISPSRSAGVNGFEMFRGAEMRPLELKGMPMLQKESGRQQGASLQLTWKTKEKG O

>Western Lowland Gorilla P53 XP\_018868682.2 cellular tumor antigen p53 isoform X2 [Gorilla gorilla] MGSSQTAFRVTAMEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQWFTEDP GPDEAPRMPEAAPPVAPAPAAPTPAAPAPASWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSP

ALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVE GNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRN SFEVRVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERF EMFRELNEALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD

>Chimpanzee P53 XP\_016786959.2 cellular tumor antigen p53 isoform X1 [Pan troglodytes]

METVSGSIGKAGPPPPHPNPSPLVETCGKRKFHGTDFLLLSFRLPENNVLSPLPSQAMDDLMLSPDDIEQ WFTEDPGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSV TCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQ HLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSG NLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQI RGRERFEMFRELNEALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD

>Bonobo P53 XP\_003810114.2 cellular tumor antigen p53 [Pan paniscus] MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQWFTEDPGPDEAPRMPEAA PRVAPAPAPAPAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKT CPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRN TFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGR DRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALEL KDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD

>Northern White-Cheeked Gibbon P53 XP\_030656345.1 cellular tumor antigen p53 [Nomascus leucogenys]

MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPEDIAQWFTEDPGPHEAPRMSEAA PPMAPAPAPTLAAPAPASWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKT CPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRN TFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGR DRRTEEENFHKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALEL KDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD

>Sumatran Orangutan P53 XP\_002827020.2 cellular tumor antigen p53 [Pongo abelii]

MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAVDDLLLSPDDIAQWFIEDPGPDEAPRMSEAA SPVDPAPAAPIPAAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKT CPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRN TFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGR DRRTEEENFRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALEL KDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD

Results obtained from MUSCLE

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

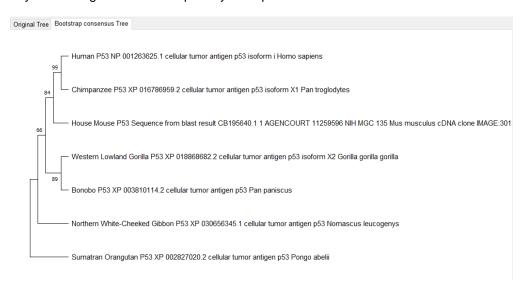
House	WASGPCPSHSMAP
Sumatran	MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAVDD
Western	MGSSQTAFRVTAMEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDD
Bonobo	MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDD
Northern	MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDD
Chimpanzee	METVSGSIGKAGPPPPHPNPSPLVETCGKRKFHGTDFLLLSFRLPENNVLSPLPSQAMDD
Human	MDD
	:

House VIFCP----FSKNLPGQ---LWLPPGLPAVWDSQVCYVHVLSSPQAILPAGEDVPCAV Sumatran LLLSPDDIAQWFIED-PGPDEAPRMSEAASPVDPAPAAPIPAAPAPAPSWPLSSSVPSQK

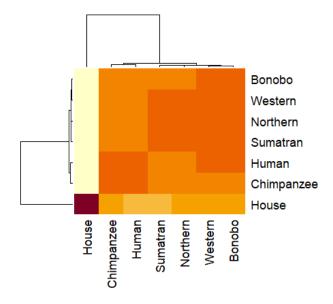
Western Bonobo Northern Chimpanzee Human	LMLSPDDIEQWFTED-PGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQK LMLSPDDIEQWFTED-PGPDEAPRMPEAAPRVAPAPAAPTPAAPAPAPSWPLSSSVPSQK LMLSPEDIAQWFTED-PGPHEAPRMSEAAPPMAPAPAAPTLAAPAPAPSWPLSSSVPSQK LMLSPDDIEQWFTED-PGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQK LMLSPDDIEQWFTED-PGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQK L***  * :: **  * :: **  * :: **
House Sumatran Western Bonobo Northern Chimpanzee Human	VGQRHTSSWEPCPRHGHLQEVTAHDGGRETLPPPALLRWWPGSSPASYP TYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPP TYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPP TYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPP TYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPP TYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPP TYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPP  1* *::: * *:::: * *:::: * *. *:::: * *. *:::: * *. *:::: * *. *::::: * *. *::::: * *. *::::: * *. *::::: * *. *::::: * *. *::::: * *. *:::::: * *. *:::::: *. *:::::: * *. *::::::: * *. *::::::::
House Sumatran Western Bonobo Northern Chimpanzee Human	GGRKFVSRVSGRQADFSPQRGGTLATRGRLVYHHPLQVHVLLHGGHEPPTYPYH GTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFR GTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFR GTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFR GTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFR GTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFR GTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFR * * . : . : : : : : : : : : : : : : : :
House Sumatran Western Bonobo Northern Chimpanzee Human	HHTGRLQWEPSGTGQLGSCLCLPWERPHSVVV-PYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVHSVVV-PYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVHSVVV-PYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVHSVVV-PYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVHSVVV-PYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVHSVVV-PYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVHSVVV-PYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEV
House Sumatran Western Bonobo Northern Chimpanzee Human	PYRRKFPQKGSPLPTAPRERKESAAHLHKRLSPAKEKTTLMESISPSRS RVCACPGRDRRTEEENFRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQI RVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQI RVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQI RVCACPGRDRRTEEENFHKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQI RVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQI RVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQI * * *
House Sumatran Western Bonobo Northern Chimpanzee Human	AGVNGFEMFRGAEMRPLELKGMPMLQKESGRQQGASLQLTWKTKEKG RGRERFEMFRELN-EALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPD RGRERFEMFRELN-EALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPD RGRERFEMFRELN-EALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPD RGRERFEMFRELN-EALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPD RGRERFEMFRELN-EALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPD
House Sumatran Western Bonobo Northern	Q- SD SD SD

Chimpanzee SD Human --

[Q6] Create a phylogenetic tree, using either a parsimony or distance-based approach. Bootstrapping and tree rooting are optional. Use "simple phylogeny" online from the EBI or any respected phylogeny program (such as MEGA, PAUP, or Phylip). Paste an image of your Cladogram or tree output in your report.



[Q7] Generate a sequence identity based heatmap of your aligned sequences using R. If necessary convert your sequence alignment to the ubiquitous FASTA format (Seaview can read in clustal format and "Save as" FASTA format for example). Read this FASTA format alignment into R with the help of functions in the Bio3D package. Calculate a sequence identity matrix (again using a function within the Bio3D package). Then generate a heatmap plot and add to your report. Do make sure your labels are visible and not cut at the figure margins.



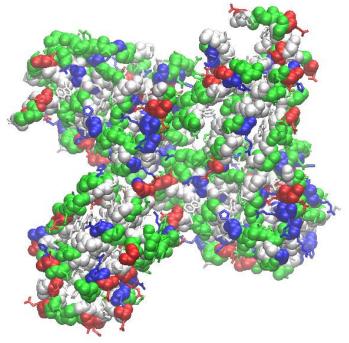
[Q8] Using R/Bio3D (or an online blast server if you prefer), search the main protein structure database for the most similar atomic resolution structures to your aligned sequences.

List the top 3 unique hits (i.e. not hits representing different chains from the same structure) along with their Evalue and sequence identity to your query. Please also add annotation details of these structures. For example include the annotation terms PDB identifier (structureId), Method used to solve the structure (experimentalTechnique), resolution (resolution), and source organism (source).

ID	Technique	Resolution	Source	e-value	Identity
6XRE_M	EM	4.6	Homo	0.00e00	98.663
			Sapiens		
6LHD_A	X-ray	2.499	Homo	1.70e-147	84.937
			Sapiens		
2XWC_A	X-ray	1.82	Homo	6.19e-80	57.143
			Sapiens		

[Q9] Generate a molecular figure of one of your identified PDB structures using VMD. You can optionally highlight conserved residues that are likely to be functional. Please use a white or transparent background for your figure (i.e. not the default black). Based on sequence similarity. How likely is this structure to be similar to your "novel" protein?

As the percent identity is almost 85% this structure is likely to be similar to our novel protein in some form.



[Q10] Perform a "Target" search of ChEMBEL (https://www.ebi.ac.uk/chembl/) with your novel sequence. Are there any Target Associated Assays and ligand efficiency data reported that may be useful starting points for exploring potential inhibition of your novel protein?

ChEMBEL displays one inhibition assay and no others. There is no ligand efficiency data.

https://www.ebi.ac.uk/chembl/g/#search\_results/all/query=%3ECB195640.1\_1%20AGENCOURT\_11259596%20NIH\_MGC\_135%20Mus%20musculus%20cDNA%20clone%20IMAGE%3A30137297%205%26%23x27%3B%2C%20mRNA%20sequence%20WASGPCPSHSMAPVIFCPFSKNLPGQLWLPPGLPAVWDSQVCYVHVLSSPQ\*AILPAGED%20VPCAVVGQRHTSSWEPCPRHGHLQEVTAHDGGRETLPPP\*ALLRW\*WPGSSPASYPGGRK%20FVSRVSGRQADFSPQRGGTL\*ATRGRL\*VYHHPLQVHV\*\*LLHGGHEPPTYPYHHHTGRL%20QWEPSGTGQL\*GSCLCLPWERPPYRRRKFPQKGSPLP\*TAPRERKESAAHLHKRLSPAKE%20KTTLMESISPSRSAGVNGFEMFRGAEMRPLELKGMPMLQKESGRQQGASLQLTWKTKEKG%20Q

The inhibition assay utilized CHO-K1 cells expressing human Kv1.5 sequences in order to test the expression of Kv1.5 across mediums.

Note: ChEMBL does not provide a reference journal for this assay.