

KINEXUS BIOINFORMATICS

**KINETWORKS™**

## SERVICES INTRODUCTION

This presentation demonstrated the power of the Kinetworks™ signal transduction protein profiling services to uncover significant research results in a cost effective and efficient manner.





**FINDING  
THINGS  
BLOODY  
DIFFICULT?**

The mapping of cell signalling systems and establishing their linkages to normal and disease-related processes will be one of the most challenging and fruitful research endeavors in this decade. As more investigators adopt a systems biology approach in uncovering important signalling proteins in their experimental model systems of interest, we believe our unique Kinetworks™ proteomics services could be instrumental in identifying many new research leads.



# Kinetworks™ Solutions



**DOES IT SEEM  
LIKE A JUNGLE  
OUT THERE?**

Our Kinetworks™ signal transduction protein profiling services provide accurate and quantitative information about the expression levels and phosphorylation states of hundreds of proteins with thematic screens that utilize the best commercial antibodies available. Our Kinetworks™ services are unrivalled for speed, sensitivity, accuracy and quantitation. For the equivalent cost of a couple of antibodies, we can track from 18 to 36 target proteins for you.





**TRACK OVER  
230 KINASES, 44  
PHOSPHATASES  
+ 330 PHOSPHO-  
SITES**

We are able to process extracts from biopsy specimens as well as isolated cells from humans and rodents. Some specific applications of our services include the identification of disease markers and drugable targets, characterization of drug leads, and validation of appropriate animal models of human disease. Simply send us the tissue or cell lysates and you will get your results back within 4 weeks.



**TRACK OVER  
120 CELL  
CYCLE,  
STRESS AND  
APOPTOSIS  
PROTEINS**

When you retain Kinexus, you benefit from more than 16 years and \$8.0 million of investment in perfecting our proteomics services.

We can save you time and money!

We invite you to try us out and join our growing list of more than 1700 hundred highly satisfied clients.



# Customer Support

- We are eager to learn from our clients about how we can best serve their research needs.
- Our website at [www.kinexus.ca](http://www.kinexus.ca) features detailed information about our services, including a downloadable pdf that has everything you need to use our Kinetworks™ services.
- Only after we perform our services are clients invoiced. Payments may be made by cheque or credit card.

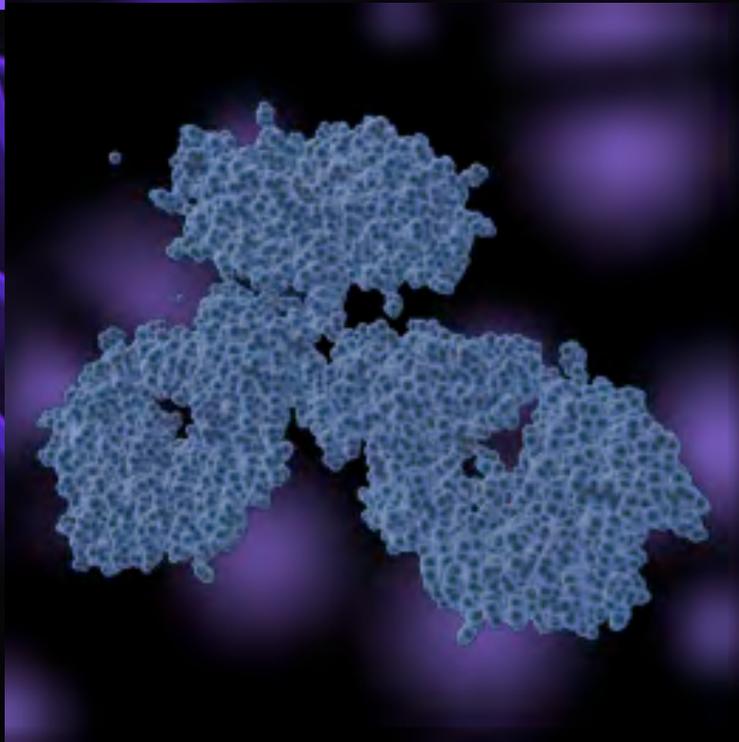


## Customer Support

- Our friendly and knowledgeable technical service representatives are committed to providing accurate and timely responses to client queries.
- Call toll free in North America 1-866-KINEXUS (546-3987) or overseas at 1-604-323-2547 Ext. 10 to talk to us from 8:30 am to 5:30 pm PST.
- Clients may also contact us anytime through the Internet at [info@kinexus.ca](mailto:info@kinexus.ca).



## Validated Antibodies



- The use of highly validated antibodies is a critical differentiating feature of the Kinetworks™ services.
- Kinexus has tested in-house more than 6000 antibodies, which have been sourced from over 25 major commercial suppliers, to ensure potency and specificity.
- We utilize over 877 pan-specific and phosphorylation site-specific antibodies in our Kinetworks™ screens.



## Antibody Testing



- We test our antibodies in at least 20 different experimental model systems.
- About 75% of the commercial antibodies that we rigorously examine fail our tests.
- The antibodies used in our Kinetworks™ screens are selected to perform in human, mouse and rat. However, these screens can often provide useful information across very diverse species.



## Sample Handling



- Clients prepare their cell and tissue lysates for SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The equipment and reagents needed for sample preparation are routinely available in most laboratories.
- Clients send their boiled lysates in SDS-PAGE sample buffer to Kinexus by Federal Express Courier. Our fees include these courier costs.



## Sample Handling



- To ensure efficient processing through Canadian Customs, we provide clear instructions in the Customer Information Package pdf that is downloadable from the Kinexus website.
- Upon receipt of client samples, they are immediately bar-coded and then stored at greater than  $-80^{\circ}\text{C}$  until they can be analyzed.



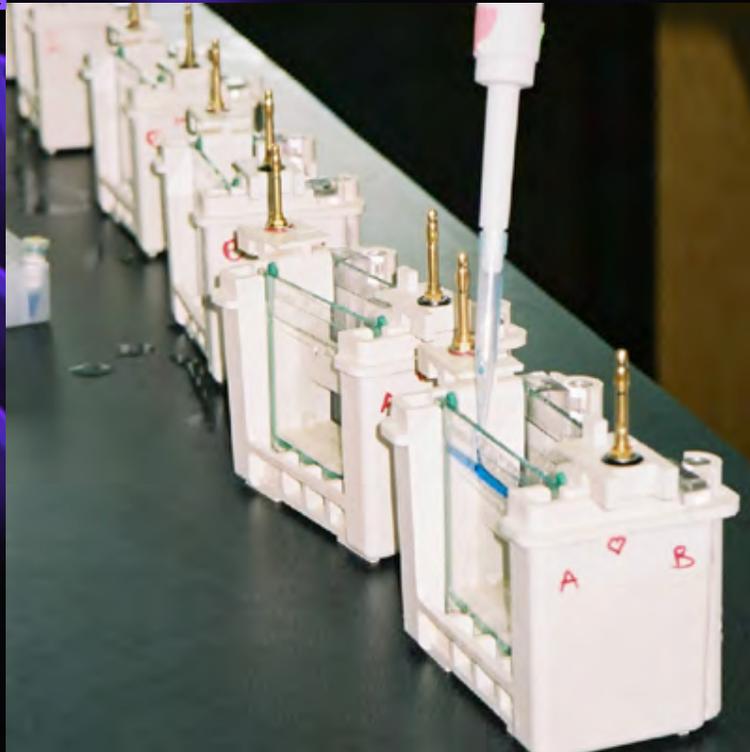
## Casting SDS-PAGE Gels



- Kinexus uses mini-SDS-PAGE for resolving the proteins in the cell/tissue samples provided by clients. This permits immunoblotting analysis with as little as 350  $\mu\text{g}$  of cell/tissue lysate protein.
- To ensure high reproducibility and comparison of multiple samples, standardized conditions and procedures in the preparation and operation of SDS-PAGE are rigidly followed.



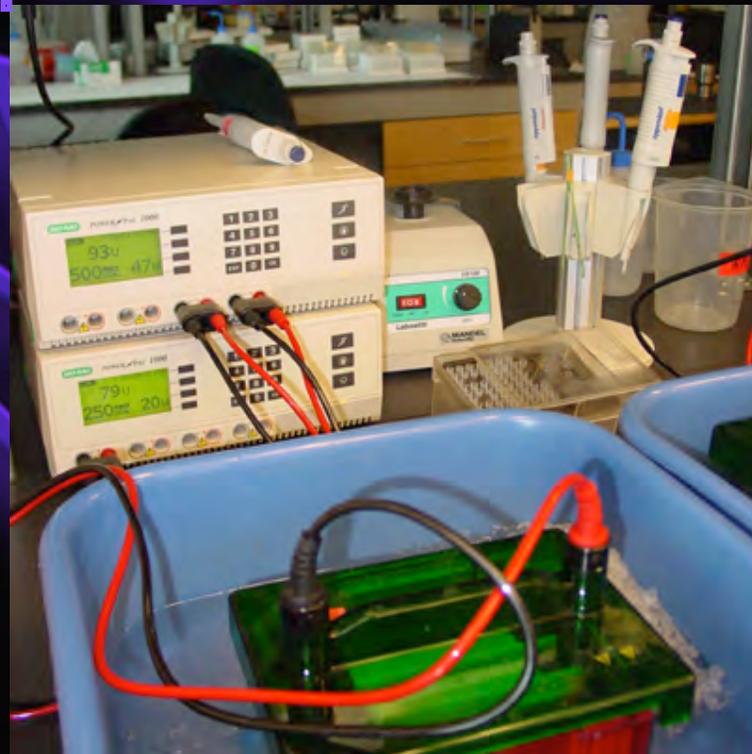
# Application of Samples



- Essentially all of the cell/tissue lysate sample that is provided by clients is applied in one lane that spans the width of the SDS-PAGE gel. Molecular weight marker proteins are deposited on both side of the SDS-PAGE gel to ensure accurate assignment of molecular masses of lysate proteins.



# SDS-PAGE Separation



- To permit maximum resolution of target proteins, SDS-PAGE is performed until after lower molecular weight proteins have eluted from the gel. The gels are formulated with a lower ratio of bisacrylamide to acrylamide. These conditions maximize the band-shift phenomena that occurs when many proteins undergo phosphorylation.



## Ponceau Staining of Gels



- To ensure that sufficient protein has been provided in the cell/tissue lysates, and that the electrophoretic transfer of proteins from the SDS-PAGE gel onto a nitrocellulose membrane has occurred efficiently, the membrane is probed for protein with Ponceau stain. If insufficient protein is detected, the client is immediately notified and queried whether the Kinetworks™ analysis should be continued.



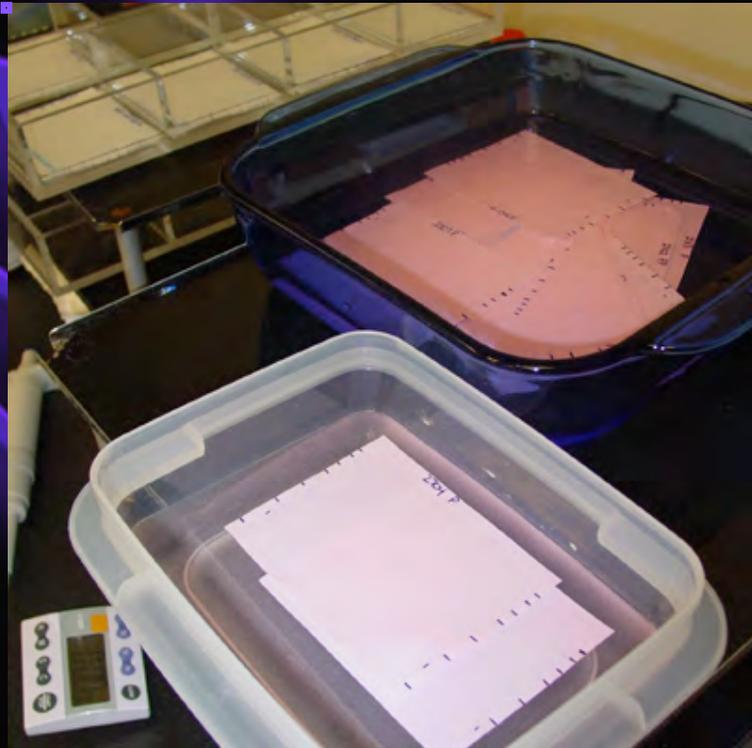
## Application of Antibodies



- For incubation with a panel of 18 or more primary antibodies, we use a multiblotting apparatus that features 20 separate slot chambers. No more than 3 antibodies are incubated in the same lane to minimize the risk of a cross-reacting band co-migrating with a target protein band. The antibody mixes have been very carefully optimized in at least 20 model systems to avoid overlap of any immunoreactive proteins.



## Addition of ECL Reagent



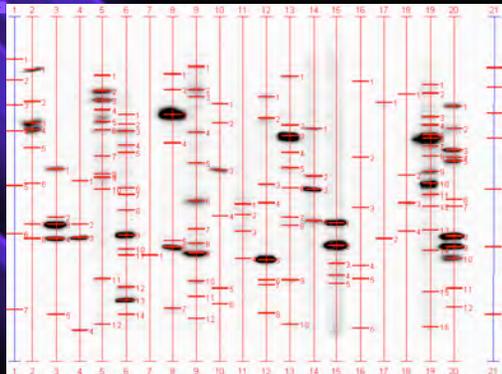
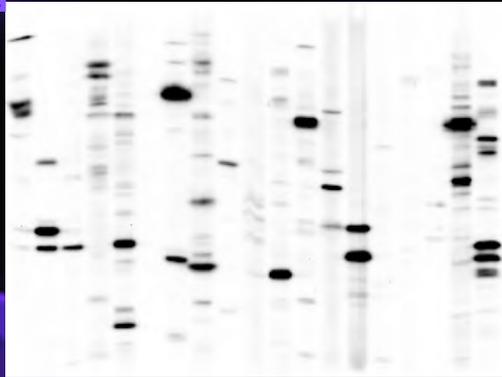
- After incubation with the primary antibodies at 4°C overnight and then for 30 minutes at room temperature with a panel of secondary antibodies, the immunoblot is incubated with enhanced chemoluminescence ECL-plus reagent. This ensures high sensitivity detection of sub-nanogram levels of the target proteins.





- The ECL signal produced on the immunoblot is detected using a Bio-Rad FluorS Max Multi-Imager with a 16-bit camera. In our experience, this is more sensitive than x-ray film, and with the Bio-Rad Quantity One software program, we are able to quantitate the levels of immunoreactivity over a 2000-fold range of linearity. The reproducibility of this analysis ranges from 80 to 99% for similar samples.





- The resulting Kinetworks™ immunoblot is typically very data-rich with information. The locations of the immunoreactive target bands permits their accurate identification. Part of the Kinetworks™ Report to clients includes TIFF files of the immunoblots both with and without all of the lane and band numbers indicated.



## Assignment of Band Identification

- The identification of target proteins on immunoblots is initially performed using an in-house software program by technicians that are highly experienced with the analysis of hundreds of Kinetworks™ immunoblots. At least 2 technicians and scientists separately review the assignments of target proteins for each immunoblot.
- A Kinetworks™ Report in an Excel spreadsheet is then generated.



# Kinetworks™ Reports

## KINETWORKS™ PROTEIN KINASE A SCREEN (KPKS-1.0A) REPORT

University of British Columbia, Dr. Steven Pelech, Order 365, Sample D167 CNT

ScanID	GelType	Lane	Band	ProteinName	Abbrev	Type	~ MW	Rel. Qty	Trace Qty	CPM	Norm. CPM
4824	KPKS-1.0A	2	1	Unclassified			178	1.0	246	106	131
4824	KPKS-1.0A	2	2	<b>v-raf murine oncogene homolog B1 (92)</b>	RAFB	PSTK	88	4.1	979	423	<b>523</b>
4824	KPKS-1.0A	2	3	Unclassified			71	1.5	369	159	197
4824	KPKS-1.0A	2	4	Unclassified			42	22.3	5324	2298	2846
4824	KPKS-1.0A	2	5	Unclassified			41	16.9	4045	1746	2162
4824	KPKS-1.0A	2	6	Unclassified			23	19.0	4538	1959	2425
4824	KPKS-1.0A	3	1	Unclassified			77	2.2	621	268	332
4824	KPKS-1.0A	3	2	<b>Protein kinase B alpha (57)</b>	PKBa	PSTK	57	2.9	820	354	<b>438</b>
4824	KPKS-1.0A	3	3	<b>Extracellular regulated kinase 1 (41)</b>	ERK1	PSTK	41	30.4	8634	3727	<b>4615</b>
4824	KPKS-1.0A	3	4	<b>Extracellular regulated kinase 1 (40)</b>	ERK1	PSTK	40	35.0	9932	4287	<b>5309</b>
4824	KPKS-1.0A	3	5	<b>Extracellular regulated kinase 2 (37)</b>	ERK2	PSTK	39	12.5	3554	1534	<b>1900</b>

- An example of a portion of a Kinetworks™ Report in Microsoft Excel.



# Kinetworks™ Reports

## KINETWORKS™ PROTEIN KINASE A SCREEN (KPKS-1.0A) COMPARISON REPORT

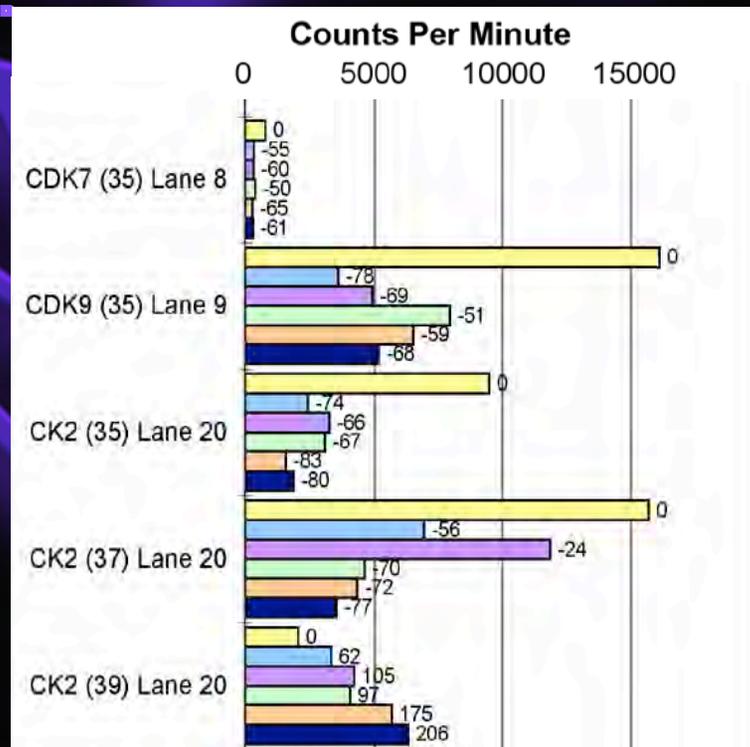
University of British Columbia, Dr. Steven Pelech, Order 365

<b>Normalized Data Comparison</b> <b>Of Normalized Counts per Minute</b> For The Following Samples				<i>Kinexus ID</i>	2319	2320	2321	2322	2323	2324
				<i>Scan ID</i>	4820	4824	4827	4831	4834	4837
				<i>Sample Number</i>	#1	#2	#3	#4	#5	#6
				<i>Sample Name</i>	NC1 CNT	D167 CNT	D167 G27	D168 CNT	D168 G27	D168 VacA
				<i>Normalization</i>	1	1	1	1	1	1
<b>FULL NAME OF PROTEIN</b>	<b>ABBREVIATION</b>	<b>LANE</b>	<b>TYPE</b>	<b>Norm C.P.M.</b>						
3-phosphoinositide dep. kinase 1 (PKB kinase) (56)	PDK1	4	PSTK				147	234	228	
Cancer Osaka thyroid oncogene (Tpl2) (53)	COT	19	PSTK	2642	2626	2205	1165	1102	1222	
Casein kinase 2 (35)	CK2	20	PSTK	9531	2446	3284	3130	1586	1889	
Casein kinase 2 (37)	CK2	20	PSTK	15757	6994	11901	4673	4387	3562	
Casein kinase 2 (39)	CK2	20	PSTK	2083	3368	4274	4108	5734	6371	
Cyclin-dependent kinase 5 (28)	CDK5	6	PSTK	2044	2947	1755	2241	2017	1173	
Cyclin-dependent kinase 7 (35)	CDK7	8	PSTK	797	355	320	402	281	309	
Cyclin-dependent kinase 9 (35)	CDK9	9	PSTK	16186	3632	4973	8005	6574	5227	
DNA-activated protein kinase (460)	DNAPK	14	PSTK	4314			628	1348	436	
Extracellular regulated kinase 1 (40)	ERK1	3	PSTK	2000	5309	3318	4306	5786	6456	
Extracellular regulated kinase 1 (41)	ERK1	3	PSTK	1417	4615	1528	1878	1253	1447	
Extracellular regulated kinase 2 (37)	ERK2	3	PSTK	1352	1900	808	4147	5442	3531	

- An example of a portion of a Kinetworks™ Comparison Report.



# Kinetworks™ Report



- Kinetworks™ Report includes TIFF images of the immunoblots, an Excel spreadsheet with quantitation of all detected proteins, a Comparison Table of multiple samples with data normalization and graphical representation of the Kinetworks™ results in publication ready format.
- The Kinetworks™ Report is delivered by e-mail within 4 weeks of receipt of client samples. Upon request, a hard copy or CD-ROM version of the results can be mailed to clients.



## Customer Follow up

Abbr.	Kinase Name	Protein Ref.Sequ	OMIM Record	Locus Link #
BMX	Bone marrow X kinase	NP_001712	300101	660
Btk	Bruton agammaglobulinemia tyrosine kinase	NP_000052	300300	695
CaMK1	Calmodulin-dependent kinase 1	NP_003647	604998	8536
CaMKK	Calmodulin-dependent kinase kinase	NP_006540	n/a	10645
CaMK4	Calmodulin-dependent kinase 4	NP_001735	114080	814
Cdk1	Cyclin-dependent kinase 1 (cdc2)	NP_001777	116940	983
Cdk2	Cyclin-dependent kinase 2	NP_001789	116953	1017
Cdk4	Cyclin-dependent kinase 4	NP_000066	123829	1019
Cdk5	Cyclin-dependent kinase 5	NP_004926	123831	1020
Cdk6	Cyclin-dependent kinase 6	NP_001250	603368	1021
Cdk7	Cyclin-dependent kinase 7	NP_001790	601955	1022
Cdk9	Cyclin-dependent kinase 9	NP_001252	603251	1025
CK1d	Casein kinase 1 delta	NP_001884	600864	1453
CK1e	Casein kinase 1 epsilon	NP_001885	600863	1454
Ck2a	Casein kinase 2 alpha	NP_001887	115442	1459
COT	Cancer Osaka thyroid (Tpl2)	NP_005195	603259	1326
Csk	c-SRC tyrosine kinase	NP_004374	124095	1445
DAPK	Death associated protein kinase 1	NP_004929	600831	1612
DNA-PK	DNA-activated protein kinase	NP_008835	600899	5591
Erk1	Extracellular regulated kinase 1	AAA36142.1	601795	5595
Erk2	Extracellular regulated kinase 2	NP_002736	176948	5594
Erk3	Extracellular regulated kinase 3	NP_002739	602904	5597
Erk6	Extracellular regulated kinase 6	NP_002960	602399	6300
FAK	Focal adhesion kinase	NP_005598	600758	5747

- When clients have reviewed the Kinetworks™ Reports for their samples, they can follow up on the leads provided by visiting the Kinexus website and using the tables that list the target proteins in the Kinetworks™ screens to link to internet sites with more detailed information about these proteins.
- Upon request, we will also provide information about those antibodies that revealed significant changes in target proteins.





- For a modest additional fee, Kinexus can also prepare presentation and publication ready Powerpoint slides of Kinetworks™ results for clients.



# Standard Kinetworks™ Services

KINEXUS BIOINFORMATICS

## Assay

## Description

## Product Code

Phosphoproteins

Tracks 33-40 known phosphoproteins with phosphorylation site-specific antibodies

KPSS 1.3,  
10.1 to 12.1



# Customized Kinetworks™ Services

## Assay

## Description

## Product Code

Multi Antibody

Choose from over 650 signal proteins to create a custom screen optimized for your model system

KCPS 1.0

Multi Sample

Choose up to 3 different signal transduction proteins of diverse MW to track in up to 8 different tissue/cell samples on the same blot

KCSS 1.0

Antibody Testing

Kinexus will test your antibodies against our panels of tissues and cell lines

In Vivo



## Kinexus Clients

- Over 10,000 commercial Kinetworks™ screens performed
- Over 1700 clients in:
  - Biopharmaceutical companies
  - Government
  - Academia
- Over 35% repeat rate within 1 year of using Kinetworks™ services
- Over 94% of our clients rank our services from good to excellent



## Contact Information



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KINEXUS BIOINFORMATICS

# LISTENING TO CELLS TO SILENCE DISEASE

