Development of a high throughput system for screening anti-prion molecules

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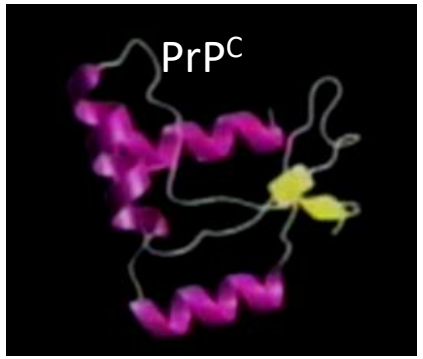
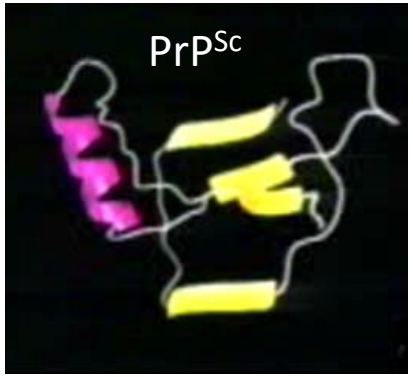
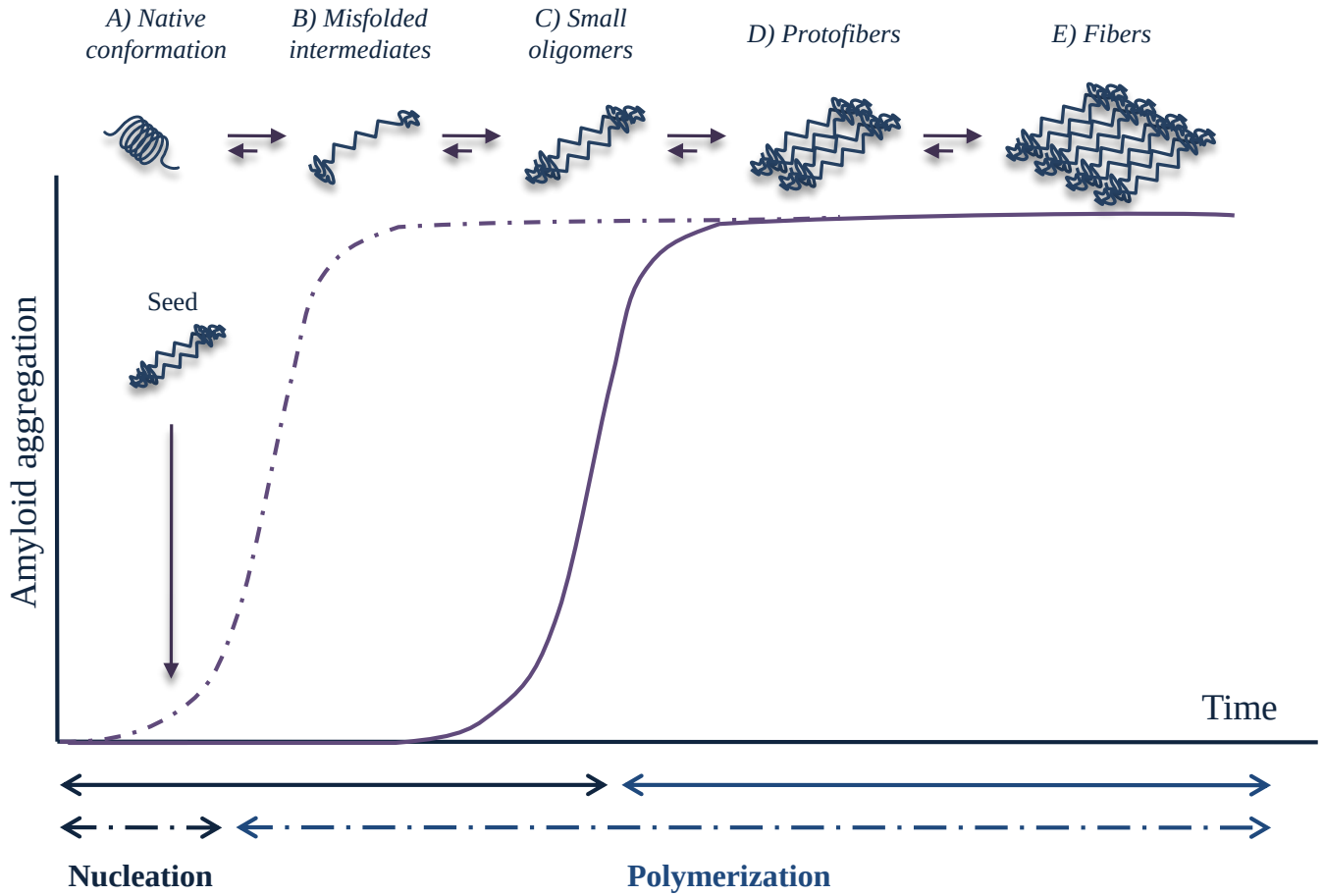
PrP misfolding as the causing agent of TSEs



High α-helix content
Low β-sheet content
Protease sensitive
Detergent soluble



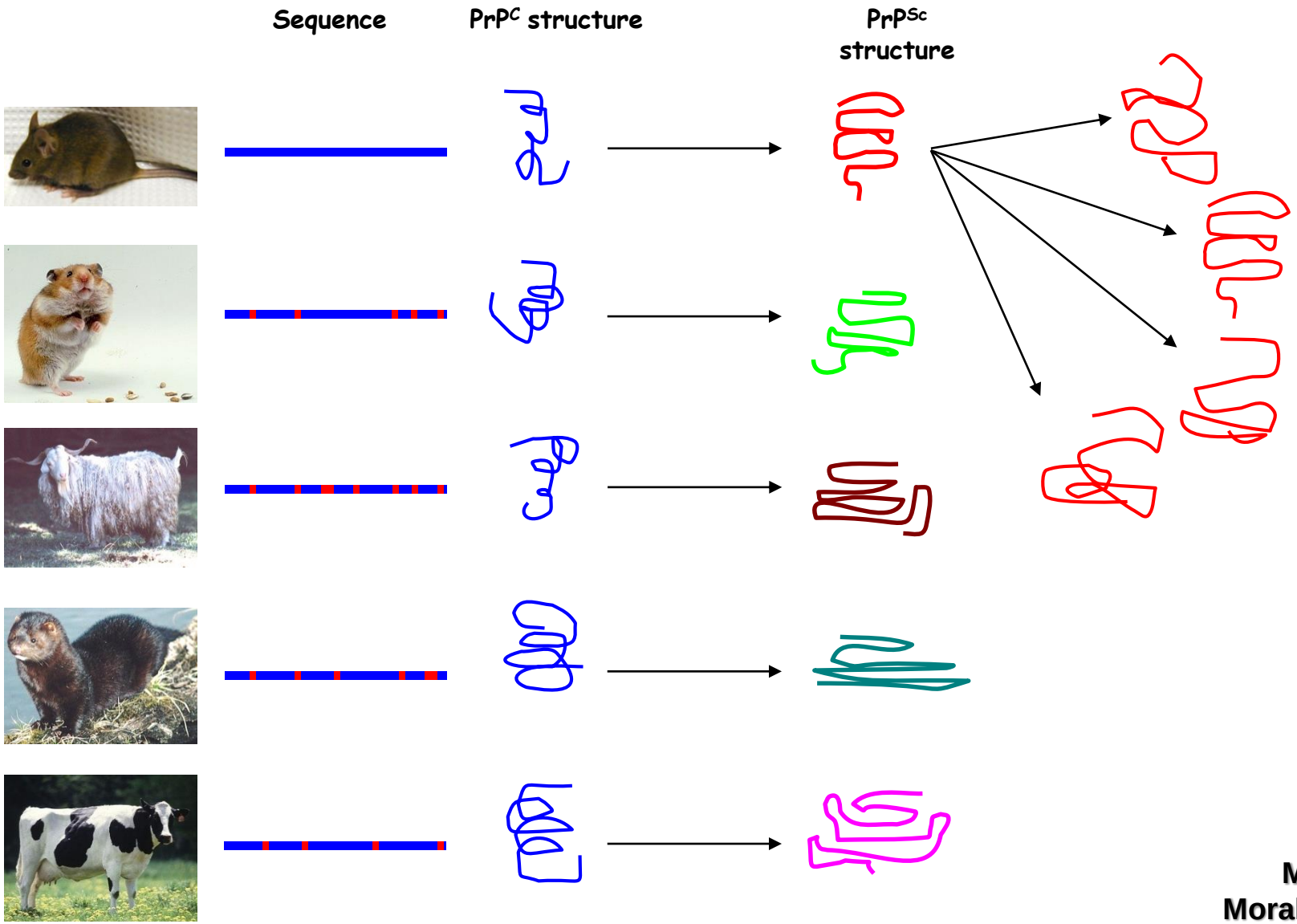
Low α-hélix content
High β-sheet content
Resistant to proteases
Detergent insoluble



Duran-Aniotz *et al*. (2013). In "Proteopathic Seeds and Neurodegenerative Diseases. M. Jücker and Y. Christen (Ed.). Springer, 2013; pp. 71-86

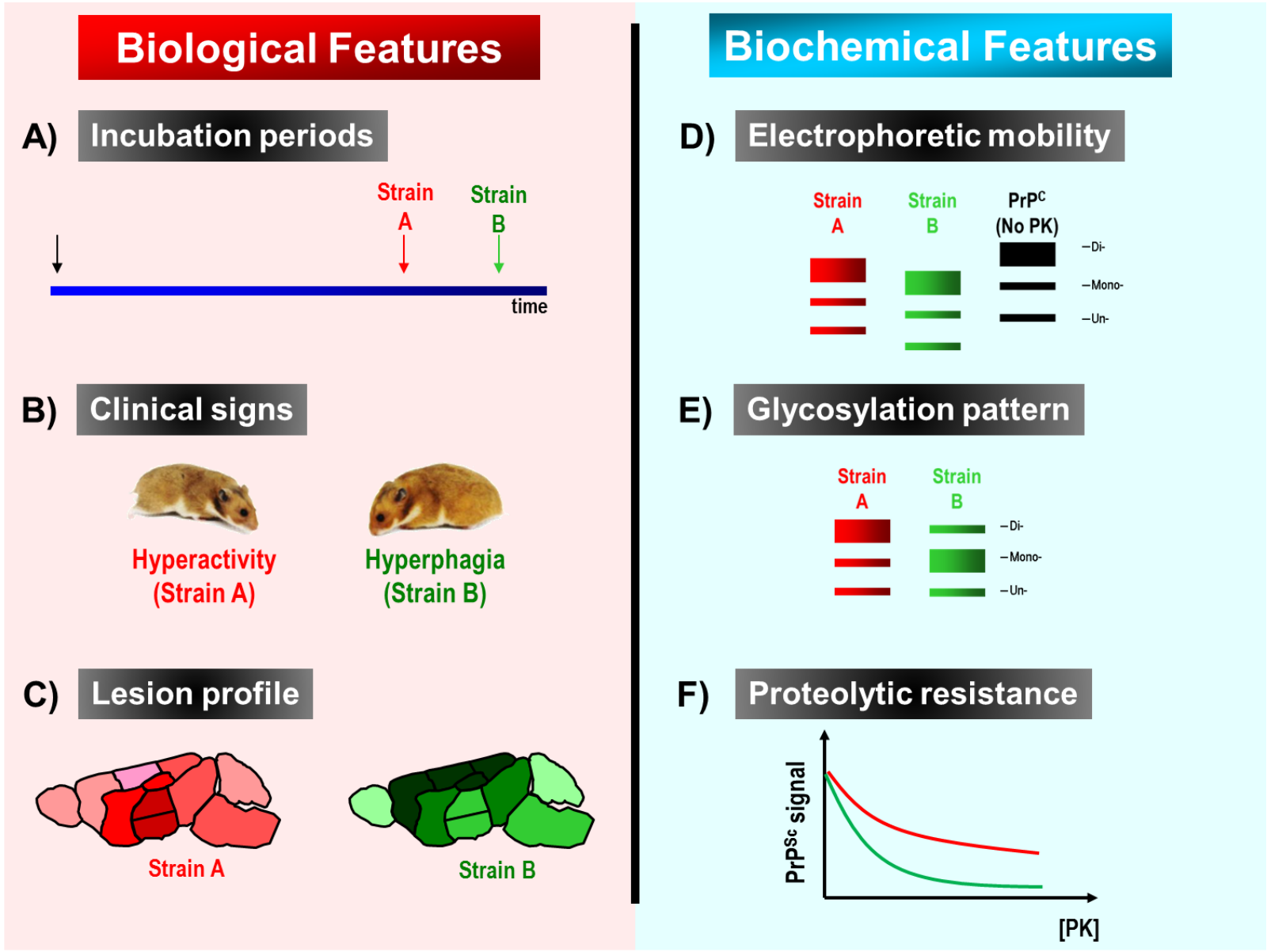
Prion strains

The main difference between prion strains lies in the conformation that PrP^{Sc} acquire.



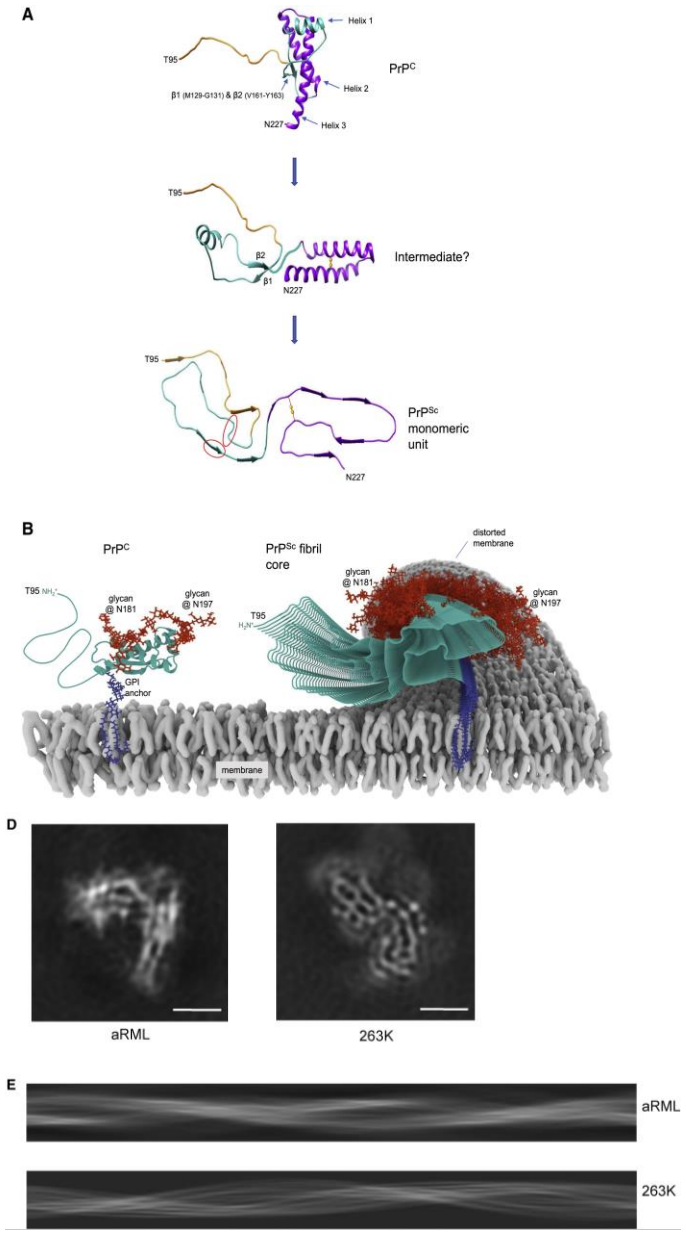
Morales *et al*. (2007) BBActa.
Morales. (2017) PLOS Pathogens

Prion strains features



Morales. (2017) PLOS Pathogens

Prion strains diversity and drug efficacy



Prion strain 1

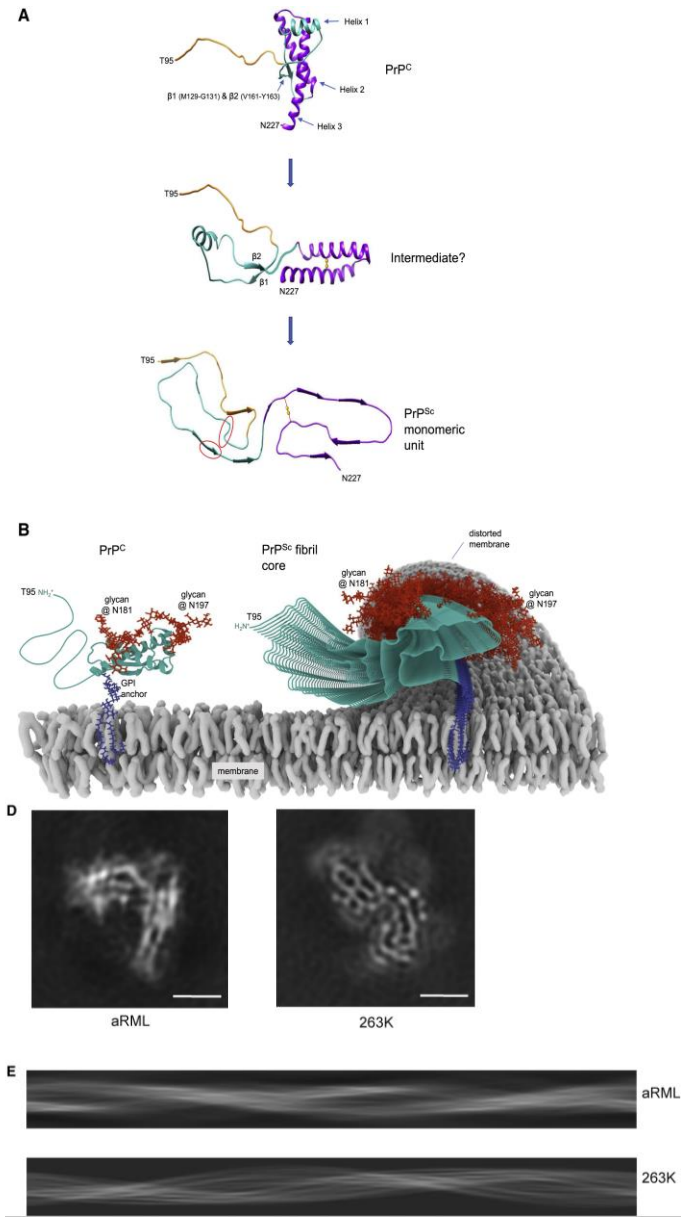
Anti-prion molecule A

Prion strain 2

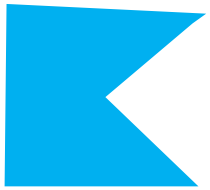
Anti-prion molecule A

Kraus *et al*. (2021) Mol. Cell

Prion strains diversity and drug efficacy



Kraus *et al*. (2021) Mol. Cell



Prion strain 1

Anti-prion
molecule A

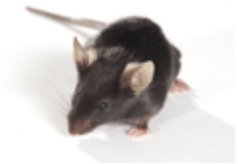
Prion strain 2

Anti-prion
molecule A

Experimental strategies used in prion reserach

Central event → **PrP conversion and aggregation**

Bioassays

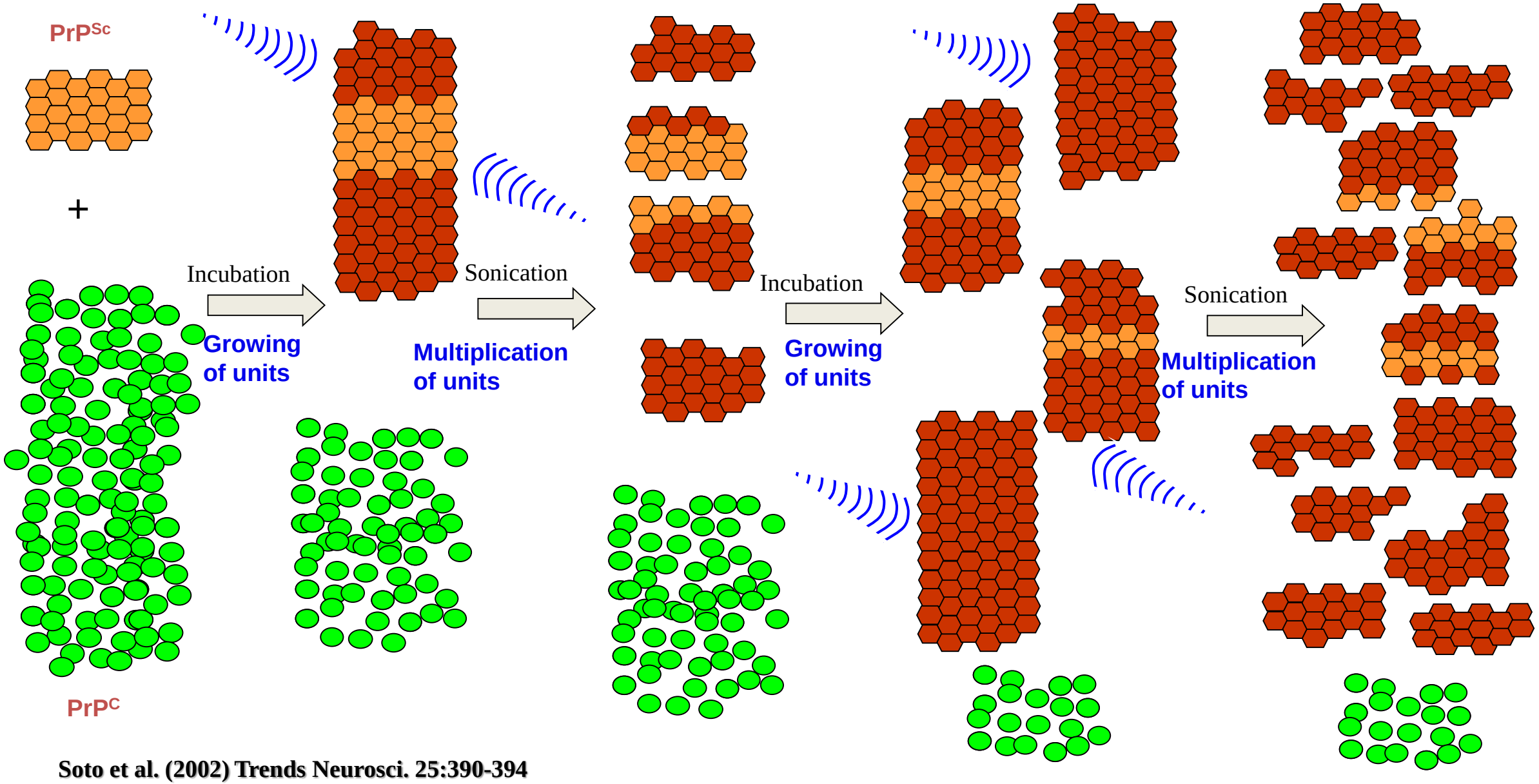


- Analysis of pathological features of the disease
- Transgenic models allow the interaction between different species
- Long incubation periods. Expensive

Cell cultures

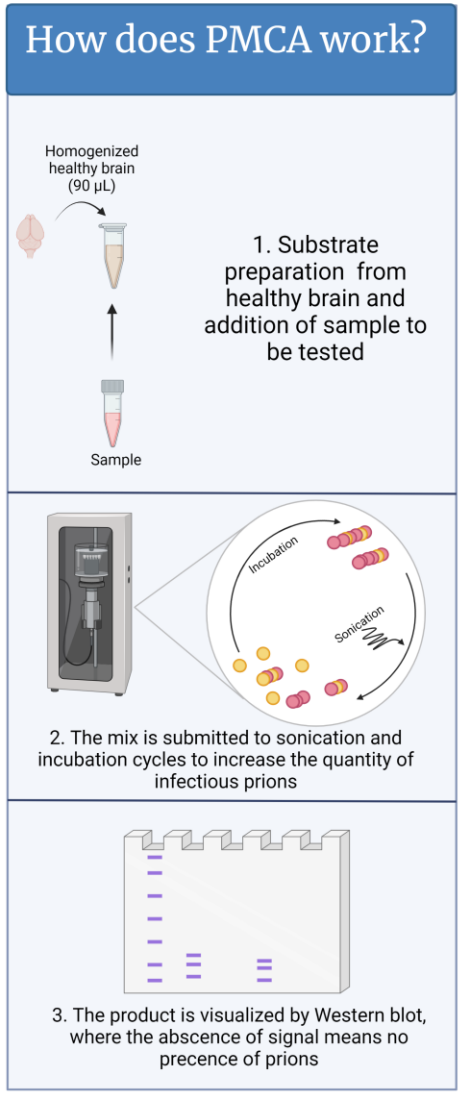
- Easy to maintain and fast propagation
- Easy to standarize
- Difficult to infect
- They do not propagate all prion strains

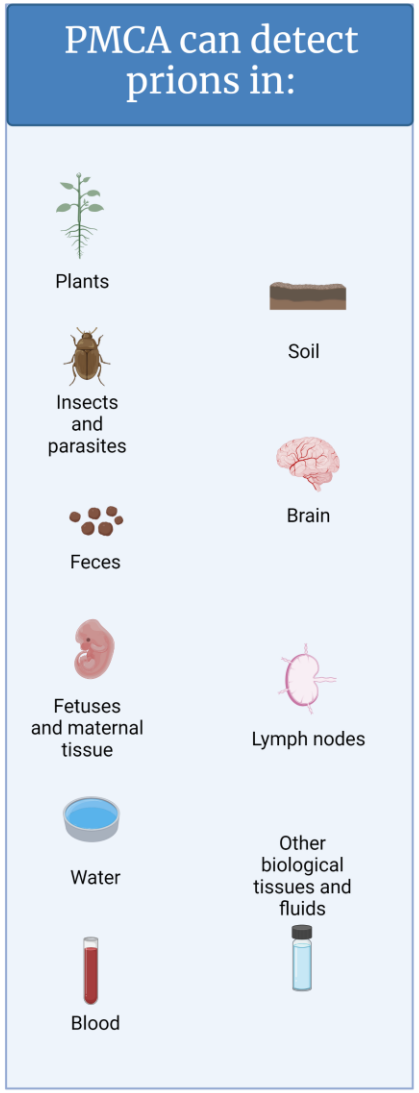
Protein Misfolding Cyclic Amplification (PMCA) (1)



Soto et al. (2002) Trends Neurosci. 25:390-394

Protein Misfolding Cyclic Amplification (PMCA) (2)





	PMCA
Platform	PCR Tubes
Volume	100µL
# of Teflon Beads	3 Beads
Number of Sonication Passages	3 Passages
Sonication Time	6 days
Volume of Sample Used for Analysis	19µL
Signal Analysis	Western Blot
Total Time	**8 days**

Do, Benavente *et al*. In preparation

Problem. **Prion diseases are caused by different prion strains. This may be a problem when identifying anti-prion drugs.**

Goal. **To develop an *in vitro* screening system to identify strain specific anti-prion molecules.**

Aims.

- **Specific Aim 1: Standardization of a PMCA platform to screen drug libraries.**
- **Specific Aim 2: Identify molecules active against deer and human prions using the modified PMCA method.**

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PMCA adaptation to a 96 well plate format for different prion strains (1)

	PMCA	96wp-PMCA
Platform	PCR Tubes	96-Well Plate
Volume	100µL	50µL
# of Teflon Beads	3 Beads	2 Beads
Number of Sonication Passages	3 Passages	1 Passage
Sonication Time	6 days	1 day
Volume of Sample Used for Analysis	19µL	5µL
Signal Analysis	Western Blot	Dot Blot
Total Time	**8 days**	**2 days**

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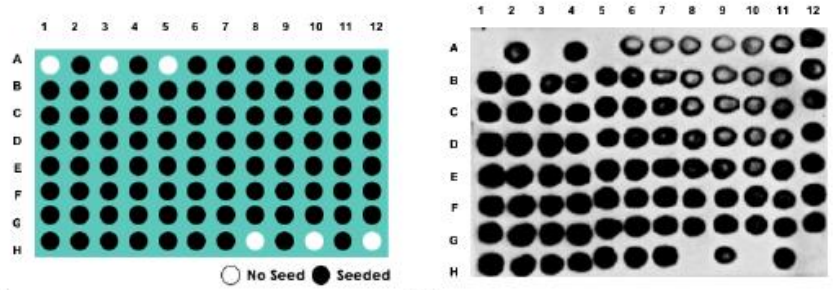
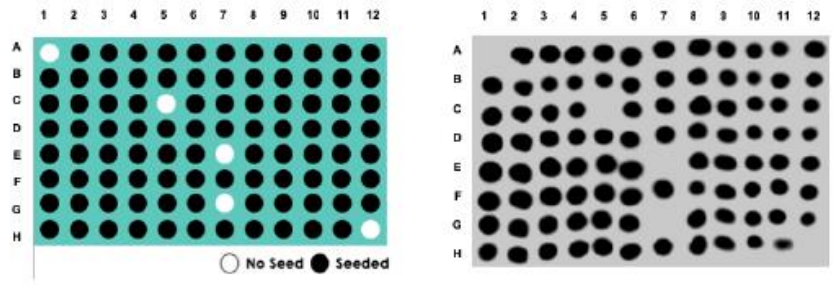
PMCA adaptation to a 96 well plate format for different prion strains (2)

Mouse

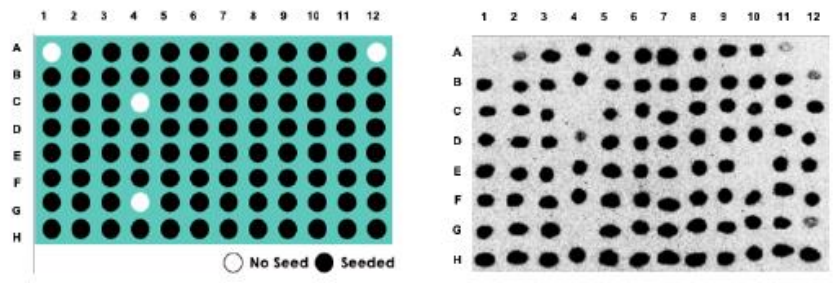
A RML



B 301C

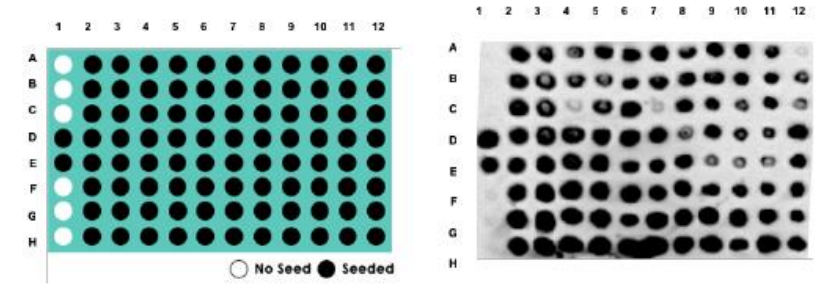


C ME7

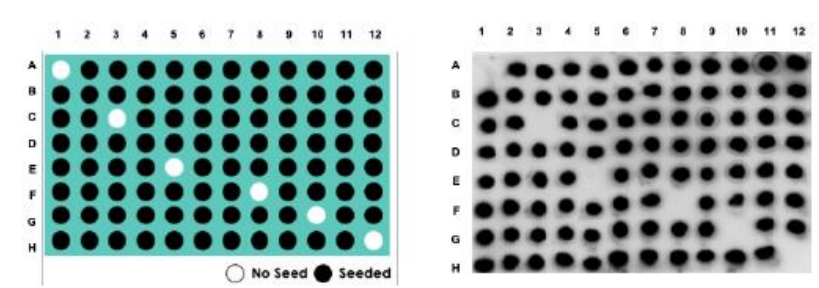


Syrian hamster

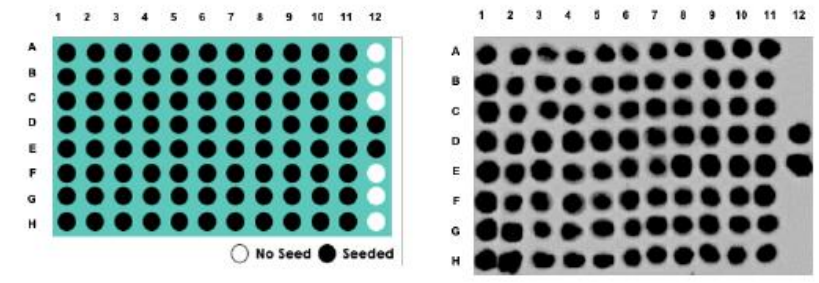
A HY



B SSLOW



C 263K



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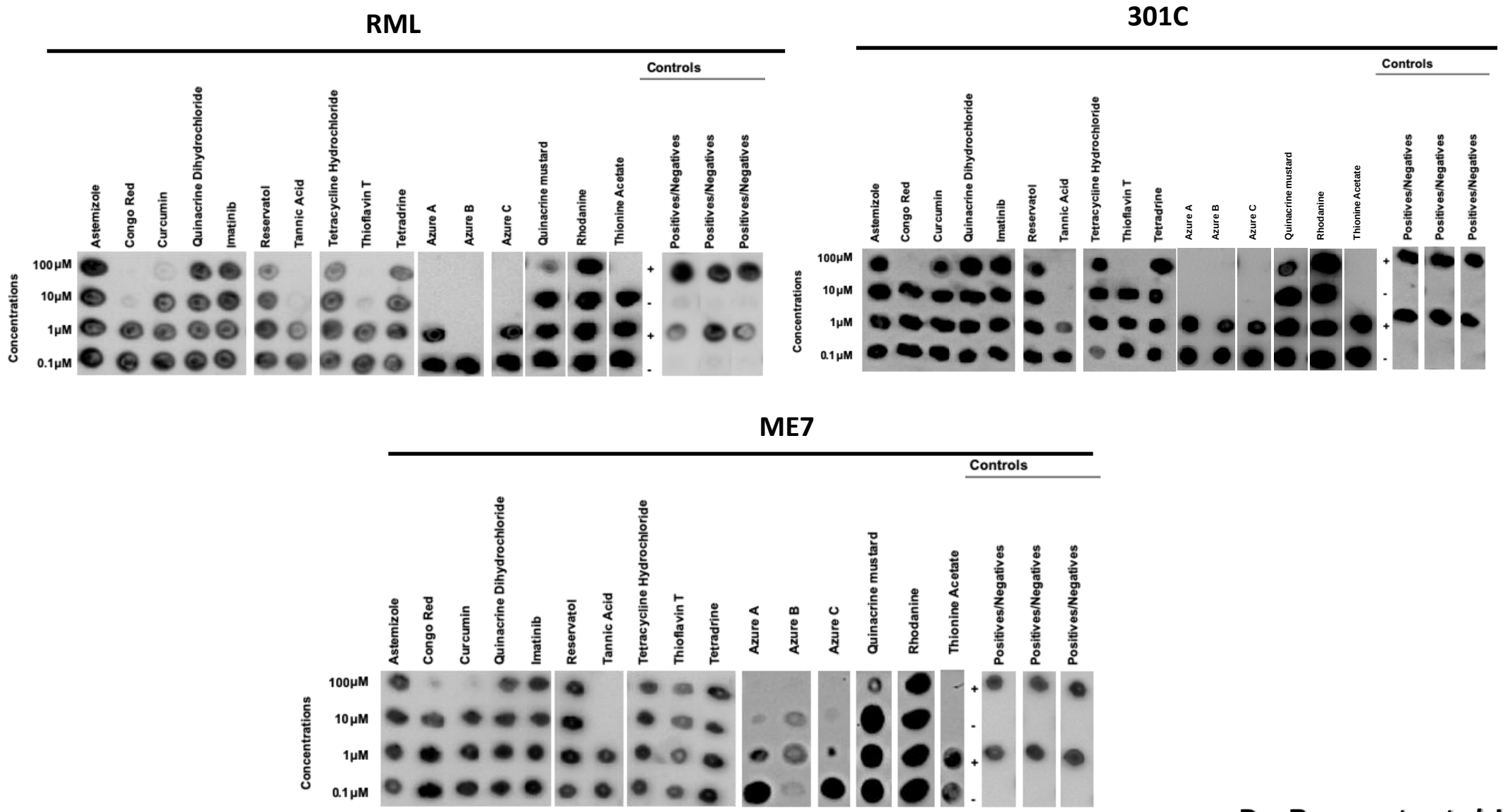
Screening of a small compound library on mouse prion strains (1)



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Screening of a small compound library on mouse prion strains (2)

Table 2. Lowest Inhibition Concentration for Mouse Prion Strains

		RML		301C		ME7	
		DMSO	Ethanol	DMSO	Ethanol	DMSO	Ethanol
Anti-Prion Molecules	Astemizole	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Congo Red	10μM	N/A	100μM	N/A	No Inhibition	N/A
	Curcumin	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Quinacrine Dihydrochloride	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Imatinib	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Reservatol	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Tannic Acid	10μM	10μM	10μM	10μM	10μM	10μM
	Tetracycline Hydrochloride	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Thioflavin T	10μM	10μM	100μM	100μM	No Inhibition	No Inhibition
	Tetradrine	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
Anti-Amyloid Molecules	Azure A	10μM	10μM	10μM	10μM	100μM	100μM
	Azure B	1μM	1μM	10μM	10μM	10μM	10μM
	Azure C	10μM	10μM	10μM	10μM	1μM	1μM
	Quinacrine mustard	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Rhodanine	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Thionine Acetate	100μM	100μM	10μM	100μM	10μM	10μM

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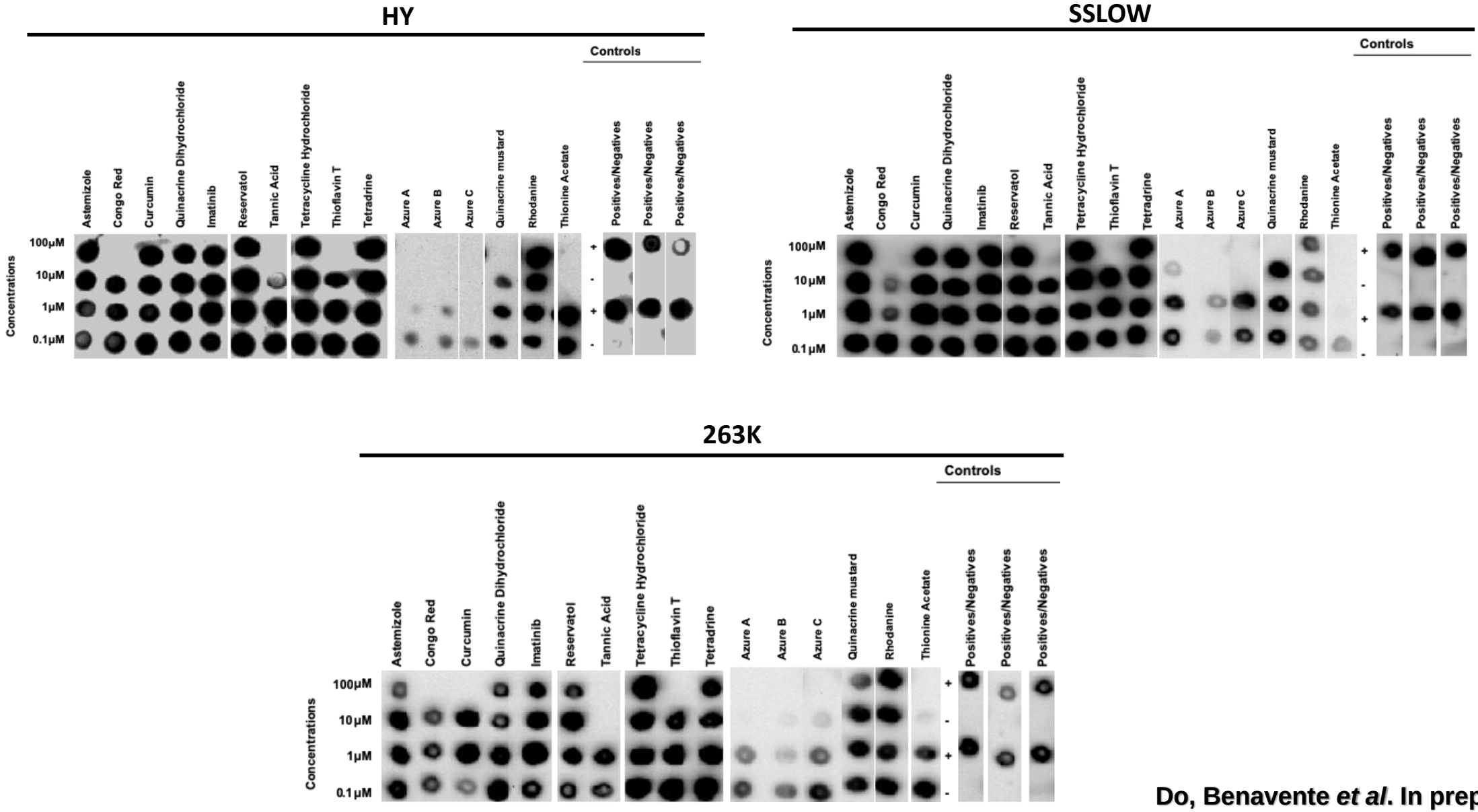
Screening of a small compound library on hamster prion strains (1)



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Screening of a small compound library on hamster prion strains (2)

Table 3: Lowest Inhibitor Concentrations for Hamster Strains

		HY		SSLOW		263K	
		DMSO	Ethanol	DMSO	Ethanol	DMSO	Ethanol
Anti-Prion Molecules	Astemizole	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Congo Red	100µM	N/A	100µM	N/A	100µM	N/A
	Curcumin	No Inhibition	100µM	No Inhibition	No Inhibition	100µM	100µM
	Quinacrine Dihydrochloride	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Imatinib	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Reservatol	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Tannic Acid	100µM	10µM	10µM	10µM	10µM	10µM
	Tetracycline Hydrochloride	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Thioflavin T	100µM	100µM	100µM	100µM	100µM	100µM
	Tetradrine	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
Anti-Amyloid Molecules	Azure A	10µM	10µM	100µM	100µM	10µM	10µM
	Azure B	10µM	10µM	10µM	10µM	10µM	10µM
	Azure C	1µM	1µM	10µM	10µM	10µM	10µM
	Quinacrine mustard	100µM	100µM	100µM	100µM	100µM	100µM
	Rhodanine	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition
	Thionine Acetate	10µM	10µM	100µM	100µM	100µM	100µM

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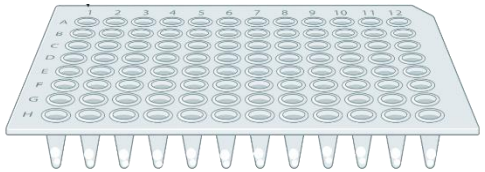
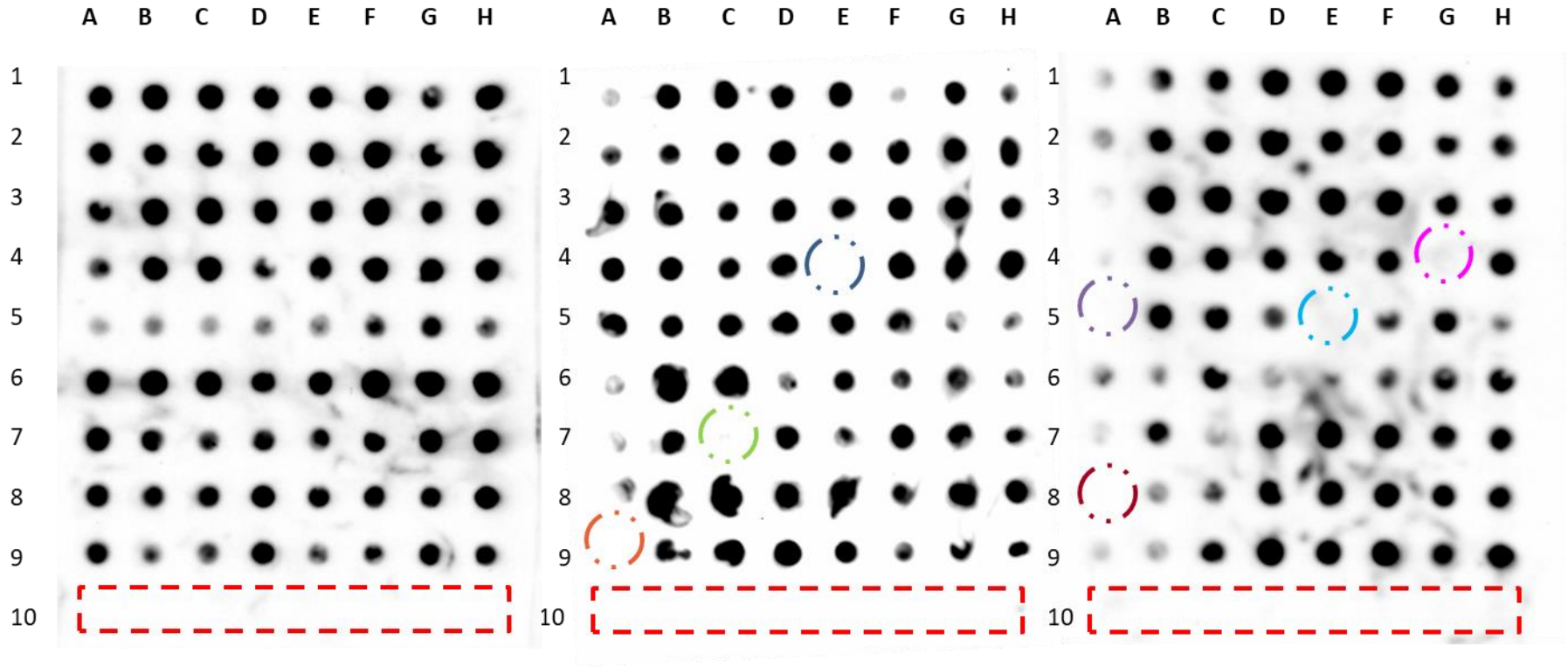
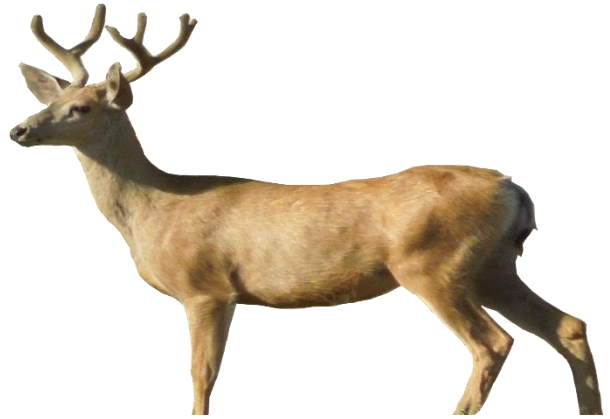
Screening of a compound library on chronic wasting disease (CWD) prions



- Library of >1,600 compounds
- Tested at 100 µM





At present, over 800 compounds have been tested and 24 hits have been identified.

Do, Benavente *et al*. In preparation

Current Progress.

- We successfully modified the PMCA technology to increase throughput (validated in six experimental prion strains).
- This PMCA system confirmed the prion strain-specific effect of several previously described anti-prion and anti-amyloid molecules.
- We initiated the screening of a larger compound library to identify molecules active against prion strains affecting deer.

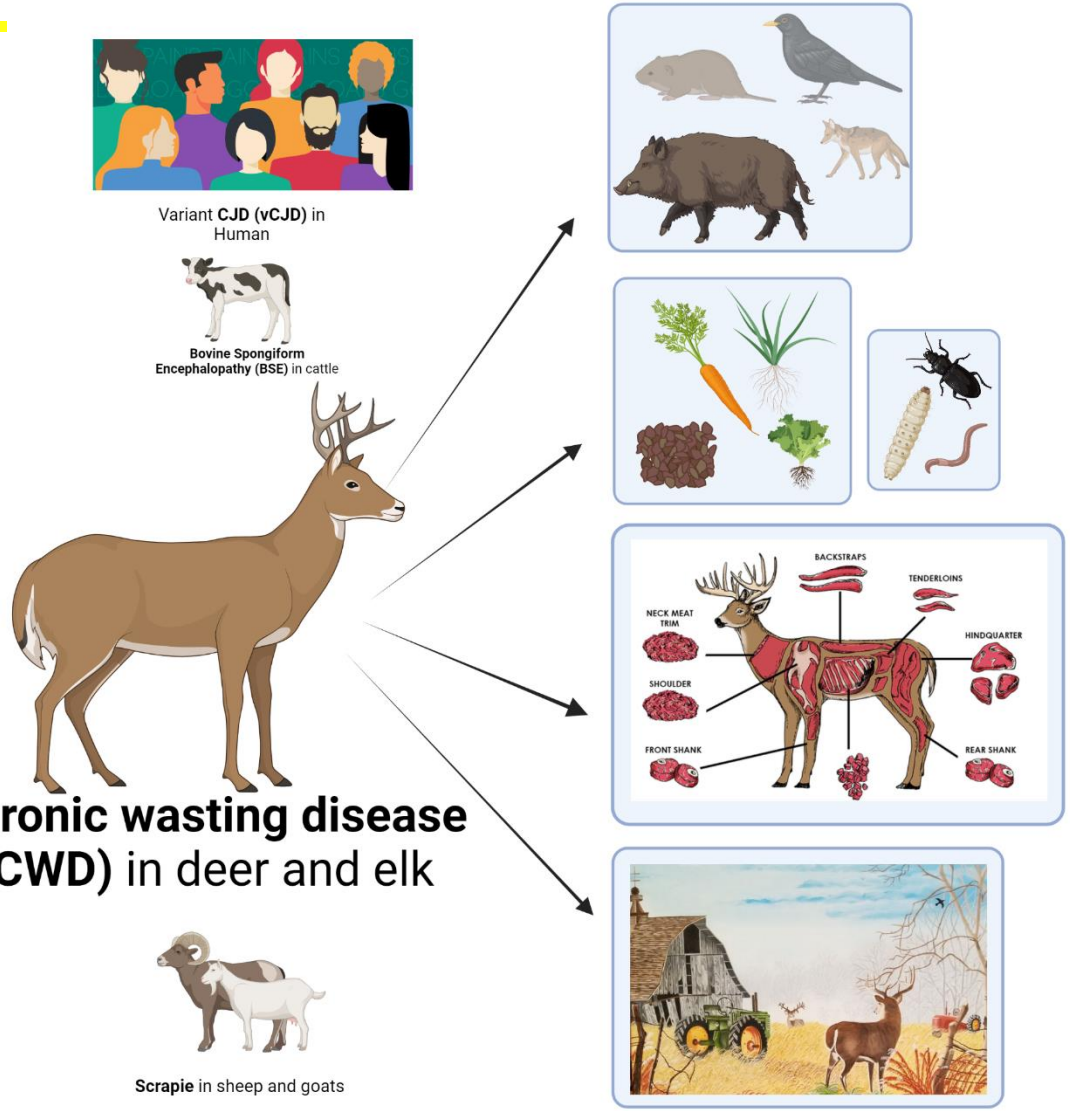
Future Plans.

- Conclude screening for deer prion strains.
- Conduct secondary analyses on hit compounds to increase chances of success in pre-clinical tests.
- Evaluate compound library in human prion strains.
- Efforts will be made to automatize this technique and set it up on 384 well plates.

Prion Projects

Transmissible Spongiform Encephalopathies (TSEs)

Group of **transmissible, progressive, and invariably fatal neurodegenerative diseases** for which there is **no effective treatment or cure.**



Potential transmission of infectious prions in animals co-existing with CWD infected cervids.

Identification of novel vectors of CWD transmission.

Assesment of prion content in processed meats and identification of prions entering the human food chain.

Establishment of novel methodologies for *pre-mortem* animal diagnosis and environmental screening.

National Institute of Allergy and Infectious Diseases

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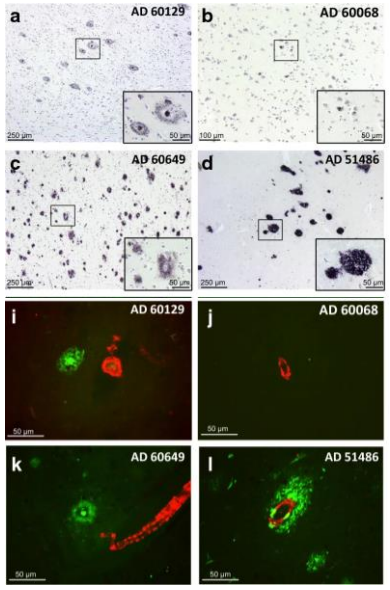
Supporting Families Affected by Prion Disease

United States Department of Agriculture

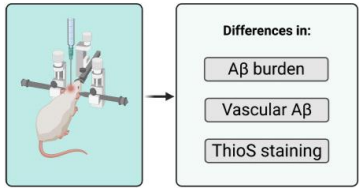
USGS science for a changing world

Alzheimer's Projects

Aβ strains and their role in AD clinical subtypes



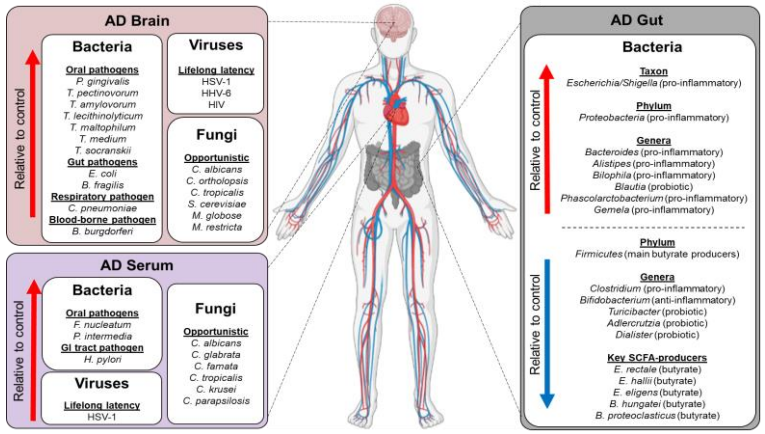
Differential amyloid pathology in patients



Induction of different pathology in mouse models

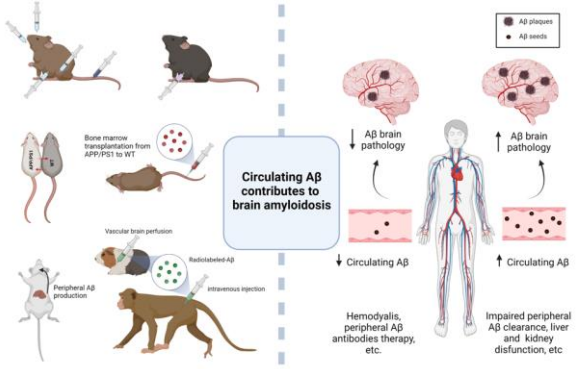
Long-term goal: Aβ strain-specific classification of Alzheimer's disease subtypes. This will lead to personalized diagnosis, prognosis and therapy.

Microbial infections (sepsis, meningitis, COVID-19) as potential risk factors for



Long-term goal: Evaluate the likelihood of bacterial infections to lead to Alzheimer's disease in the long term. This will allow for early interventions to delay or eliminate the chances to get Alzheimer's disease.

Role of peripheral Aβ in brain amyloidosis



Long-term goal: Understand the contribution of peripheral tissues and blood to Alzheimer's disease. This may open non-invasive avenues for diagnosis and treatment.

Other AD projects at the Morales' Lab

- Role of bacterial amyloids in the progression of Alzheimer's and Parkinson's pathologies.
- Amyloid-contaminated surgical tools and risks for iatrogenic infections.
- Alzheimer's pathology in the eye: mechanistic and diagnostic implications.
- Use of blood from younger individuals as means to decelerate aging: implications for Alzheimer's disease.

NIH National Institute on Aging

NIH National Institute of Allergy and Infectious Diseases

alzheimer's association®

TEXAS Alzheimer's Research and Care Consortium
a Darrell K Royal Texas Alzheimer's Initiative

Aknowledgments

2023 Morales's lab members:

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- Celso Catumbela
- Paulina Soto
- María-José Liberona
- Rebeca Benavente
- Catalina Valdes
- Reece McGinn

Funding:

NIH National Institute of Allergy and Infectious Diseases

CJD FOUNDATION CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC. *Supporting Families Affected by Prion Disease*

USDA United States Department of Agriculture

USGS science for a changing world

NIH National Institute on Aging

alzheimer's association

TEXAS Alzheimer's Research and Care Consortium a Darrell K Royal Texas Alzheimer's Initiative

UTHealth The University of Texas Health Science Center at Houston | McGovern Medical School

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Supporting Families Affected by Prion Disease

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