

Elucidating the factors required for propagating human CJD prions

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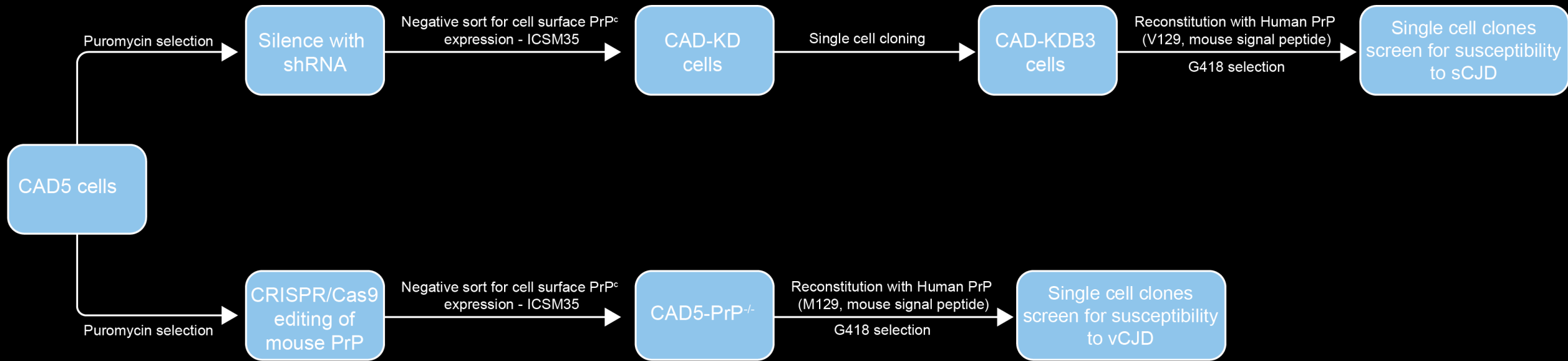
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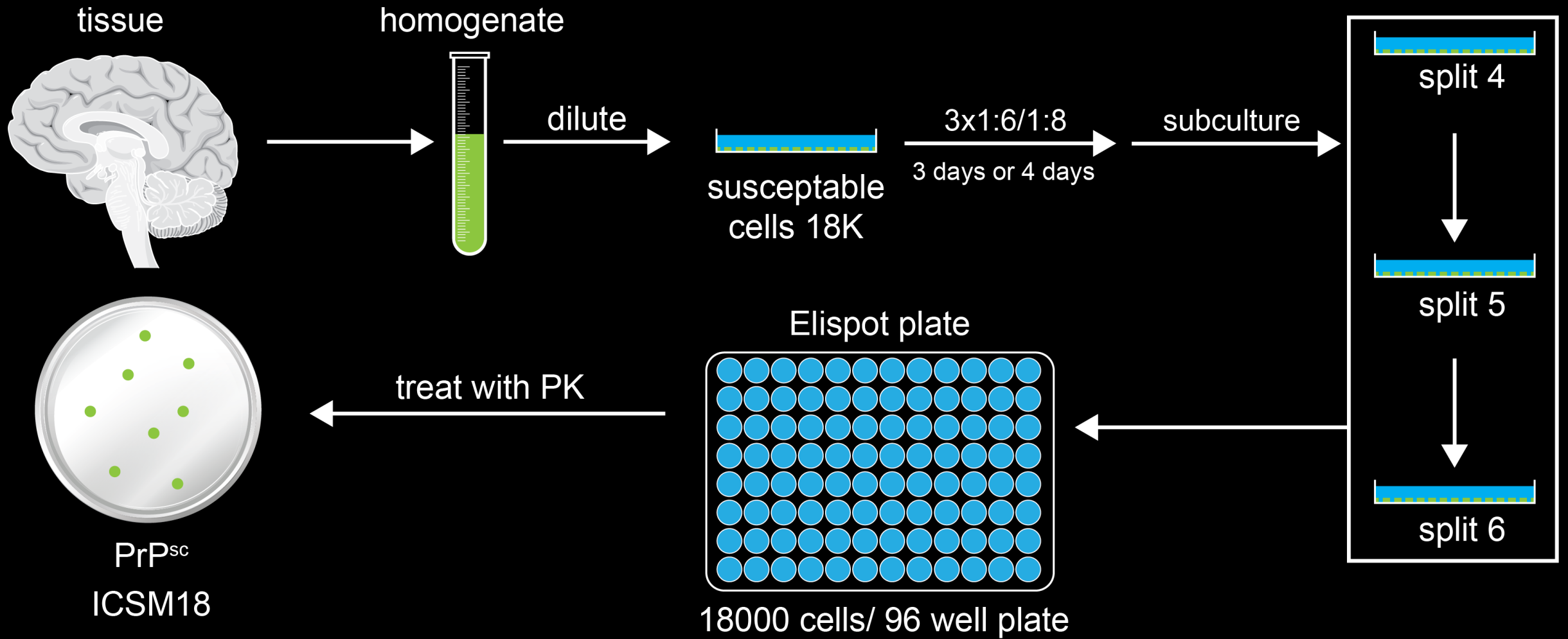
Background to research

- Cell models have been invaluable for *in vitro* studies of many complex processes and diseases
- Cell models capable of propagating mouse and other animal prions, enabling their accurate quantification have led to important advances in understanding prion biology
- Establishing cell-based models of human prion infection and propagation has been an important, yet elusive, goal of the prion field for decades.
- The major obstacle to developing cell-based models of human prion infection and propagation has been the lack of knowledge of the cellular factors that are required for prion propagation in addition to the human prion protein.

Development of cells susceptible to CJD prions: silencing followed by reconstitution



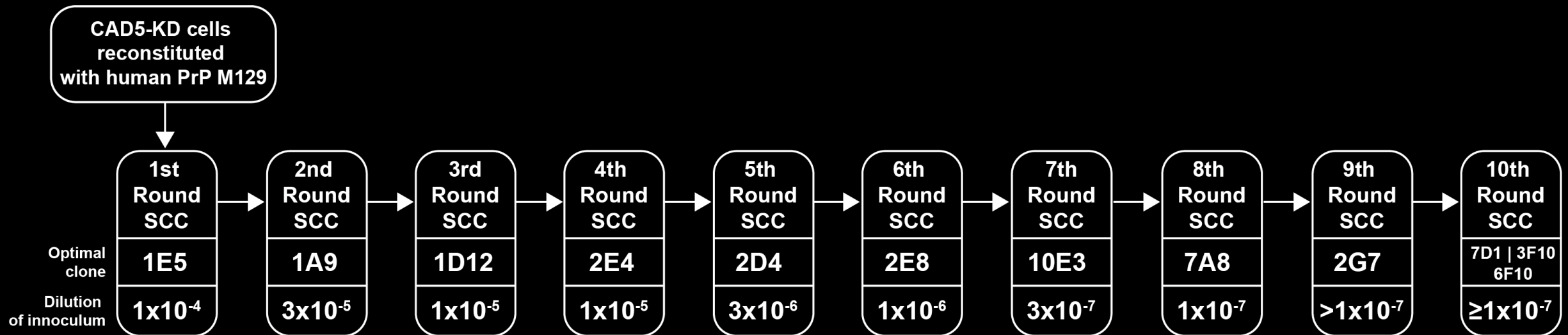
Human Prion Assay



Development of cells susceptible to variant CJD prions

- Cofactors required in addition to PrP are not known.
- Recapitulate strategy used to develop mouse models of human prion disease
- Individual lines are selective for CJD type

PRNP Codon genotype	Susceptible	
	variant CJD	sporadic CJD
V129	-	+ (T3 MV, T3VV, T2VV)
M129	+ (T4MM, T4MV)	-



Primary Transmission of vCJD infectivity in FVB/N mice after propagation in CAD5 KO-1D12 cells

Group 12

Group 8

Group 10

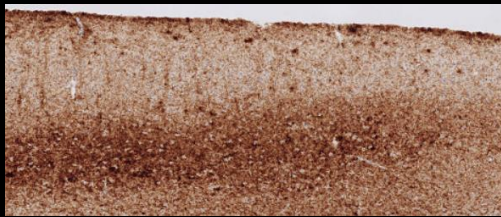
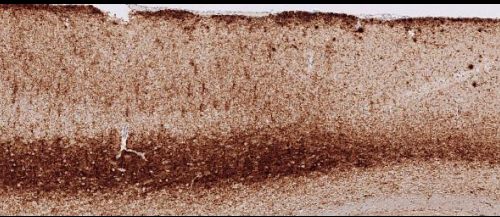
129 MM PDG 5063

CAD5 MSSM129
cell line 1D12 clone

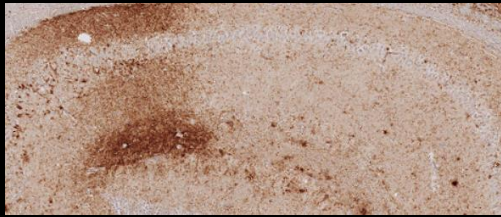
CRISPR/Cas9 CAD5KD



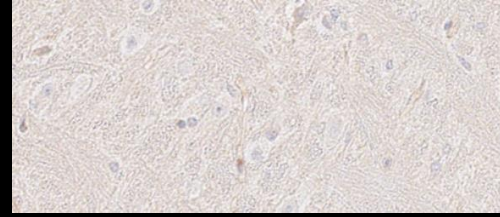
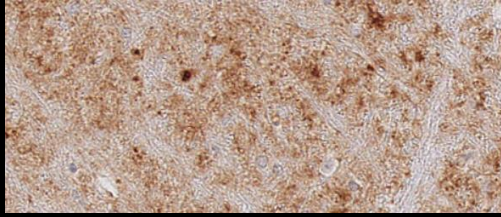
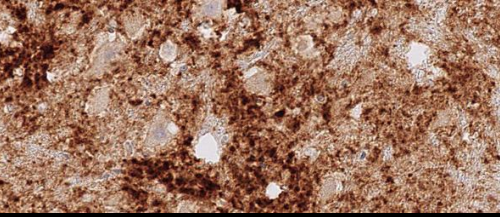
Cortex



Hippocampus



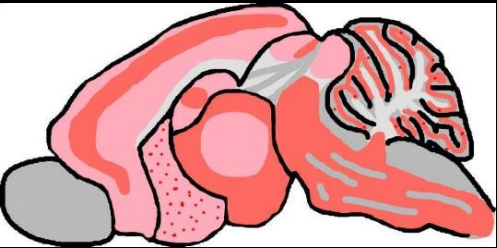
Midbrain



Primary Transmission of vCJD infectivity in FVB/N mice after propagation in CAD5 KO-1D12 cells

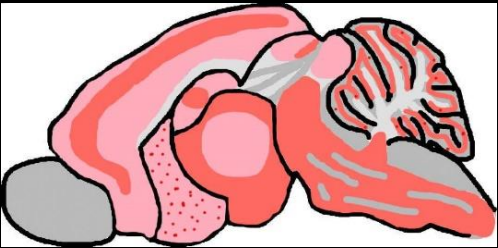
Group 11

129MM PDG 3041



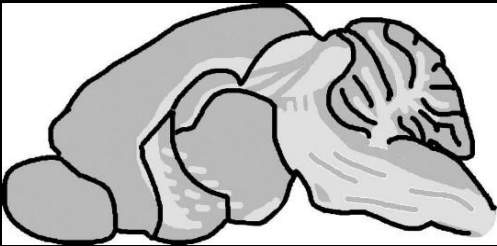
Group 7

CAD5 PrP KO MSSM129
cell line 1D12 clone

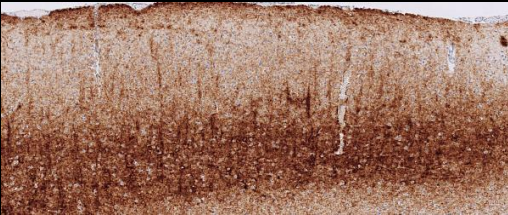


Group 9

CRISPR/Cas9 CAD5KO



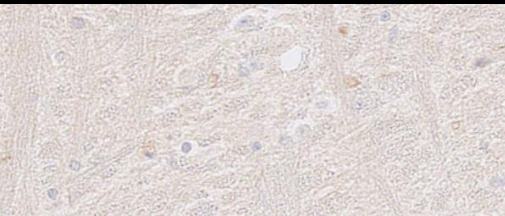
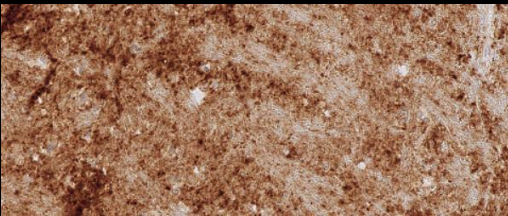
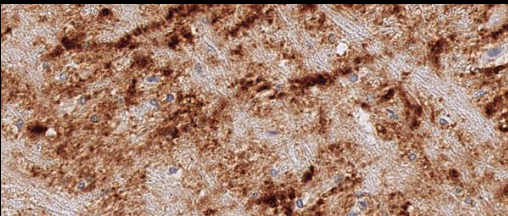
Cortex



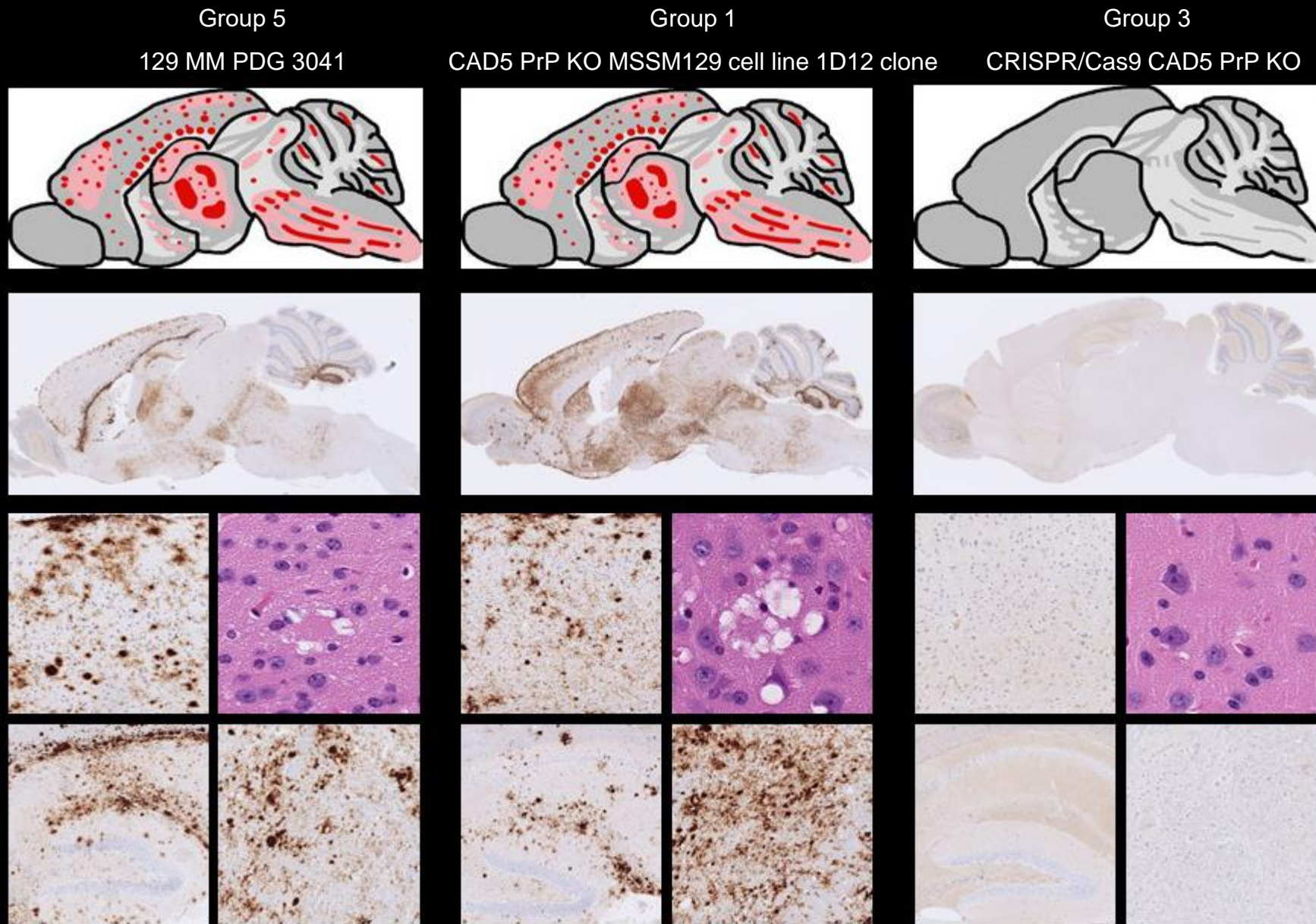
Hippocampus



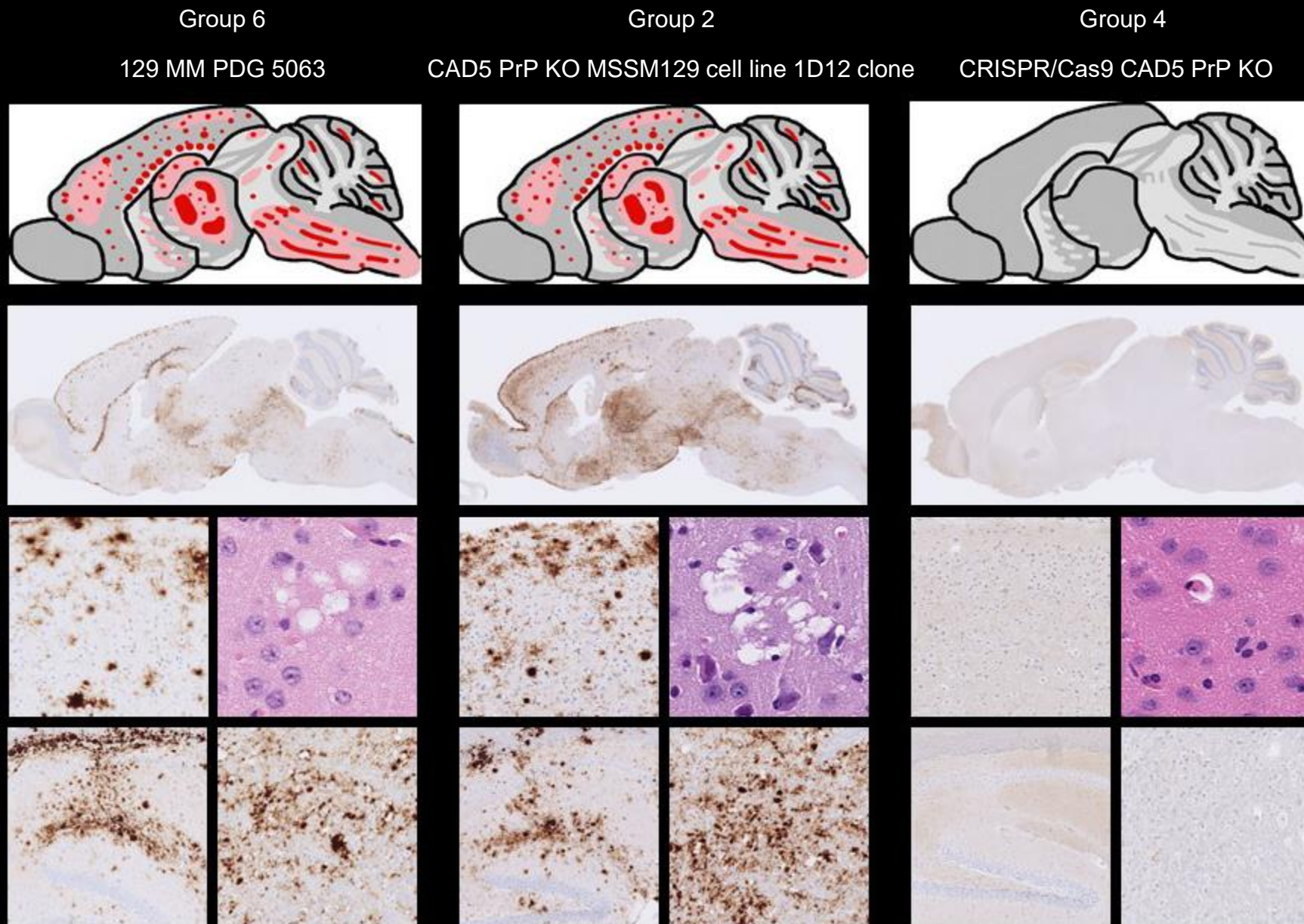
Midbrain



Primary Transmission of vCJD infectivity in Tg (HuPrP M129^{+/+}Prnp^{-/-})-35 (Tg35) mice after propagation in CAD5 KO-1D12 cells



Primary Transmission of vCJD infectivity in Tg (HuPrP M129^{+/+}Prnp^{-/-})-35 (Tg35) mice after propagation in CAD5 KO-1D12 cells



CAD5 KO- 7A8 cells are susceptible to increasing dilutions of infectious brain homogenates

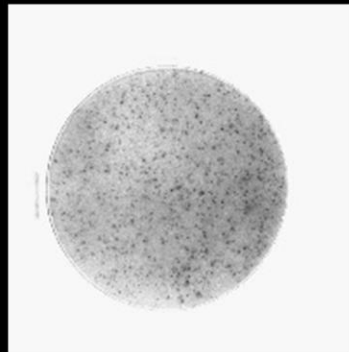
1×10^{-4}



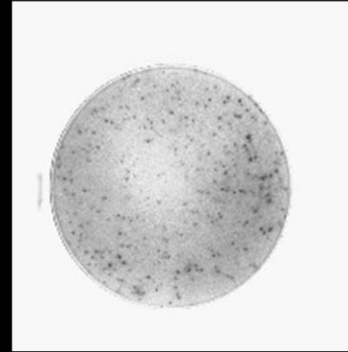
3×10^{-5}



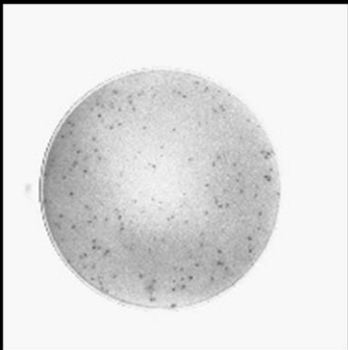
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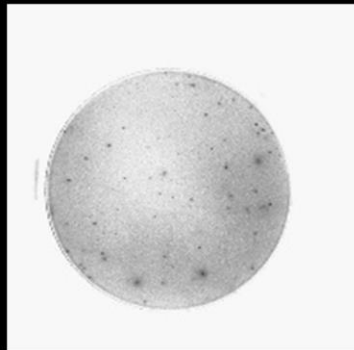
3×10^{-6}



1×10^{-6}



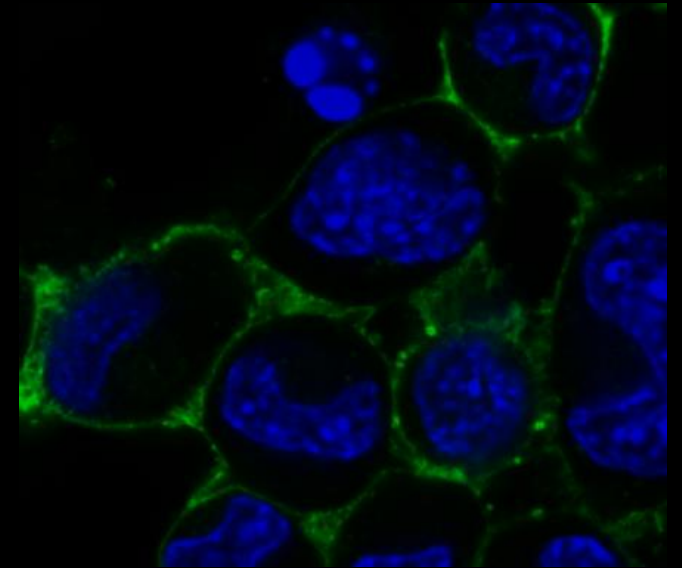
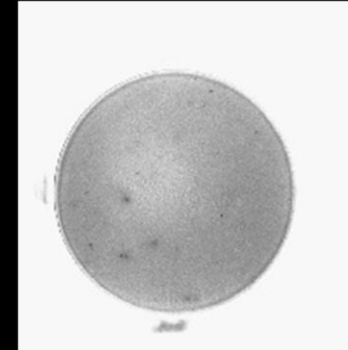
3×10^{-7}



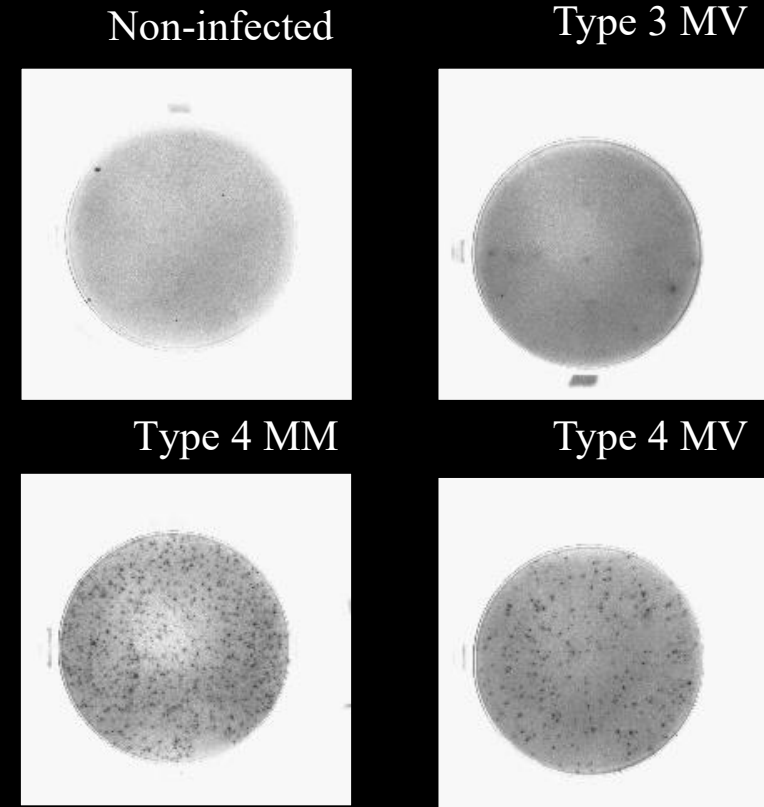
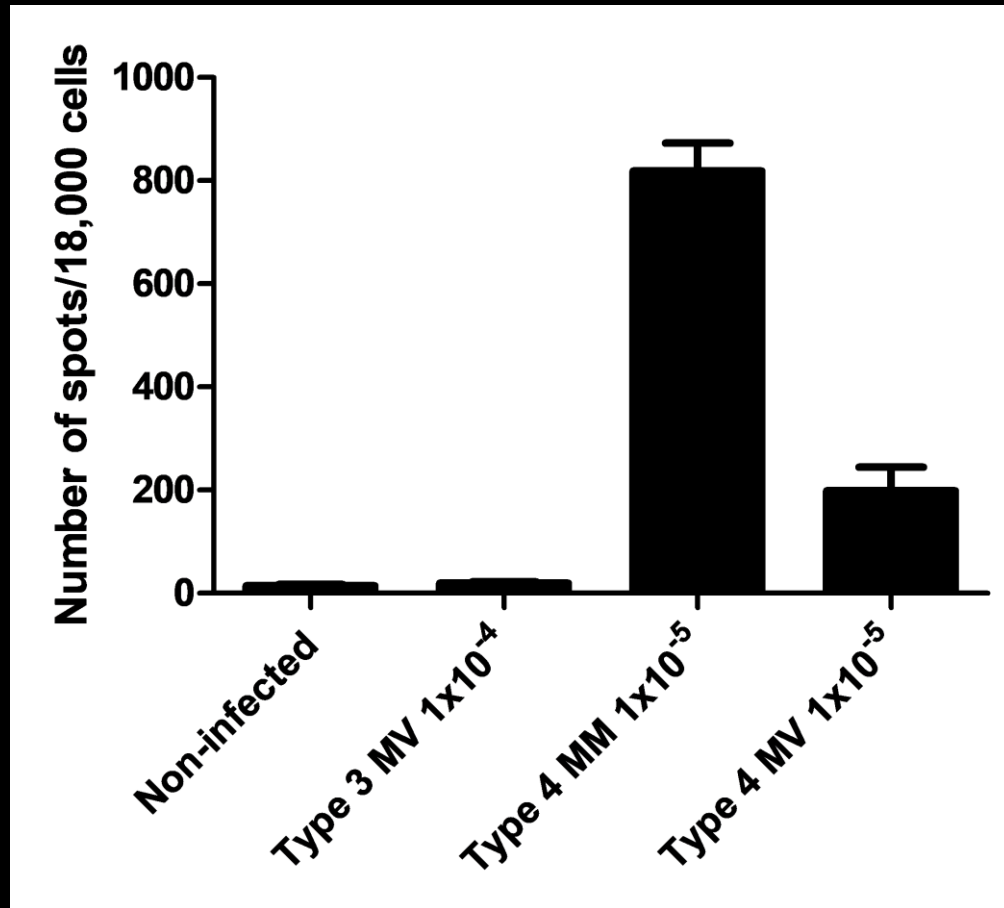
1×10^{-7}



3×10^{-8}

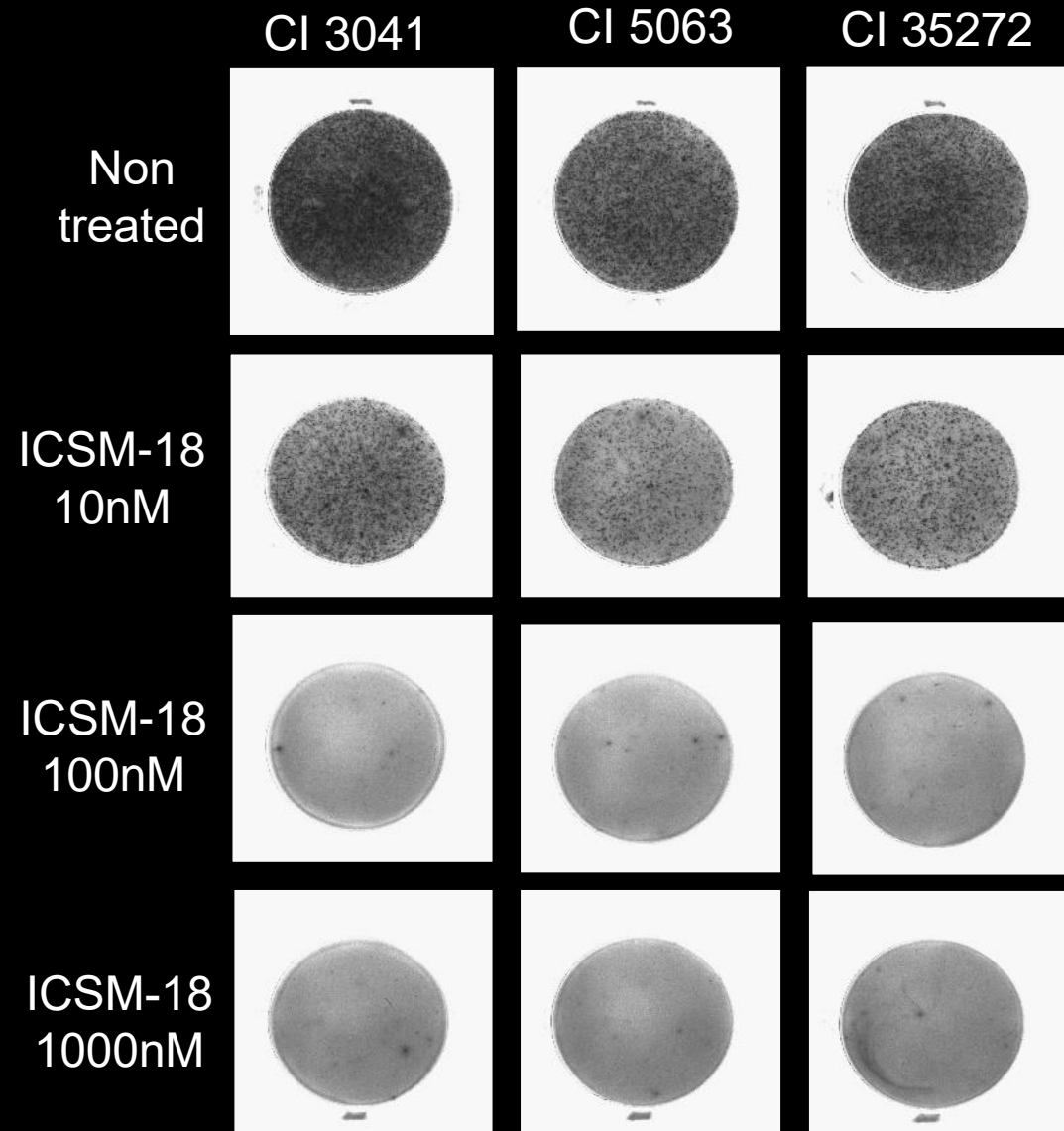


CAD5-7A8 cells propagate variant CJD prions but not sporadic CJD prions

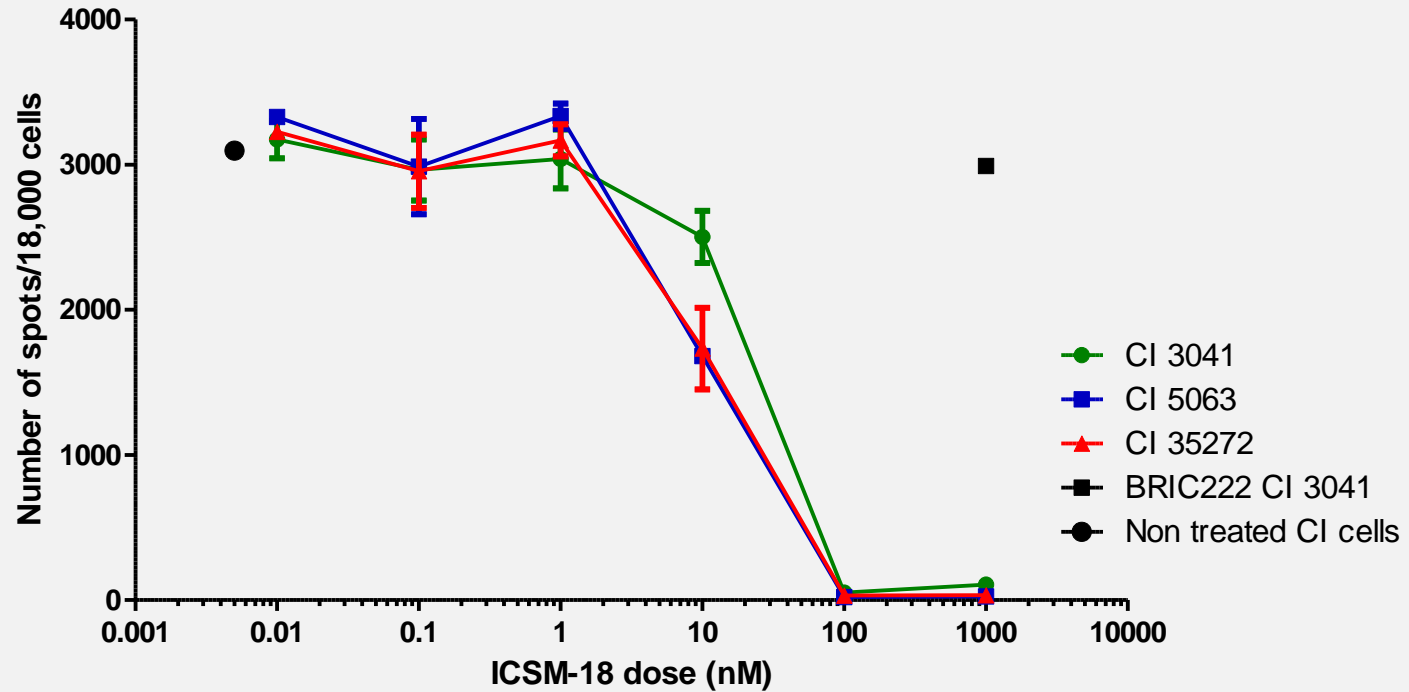


Cells chronically infected with variant CJD prions can be cured

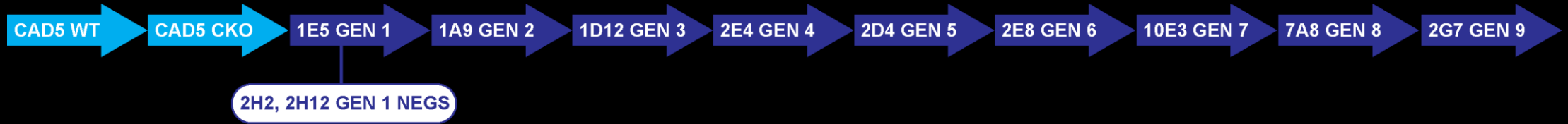
- Cells chronically infected with sporadic CJD prions can also be cured with ICSM18.
- Test decontamination procedures.
- Small molecule screens for compounds capable of curing.



Dose dependent curing of cells chronically infected with vCJD prions



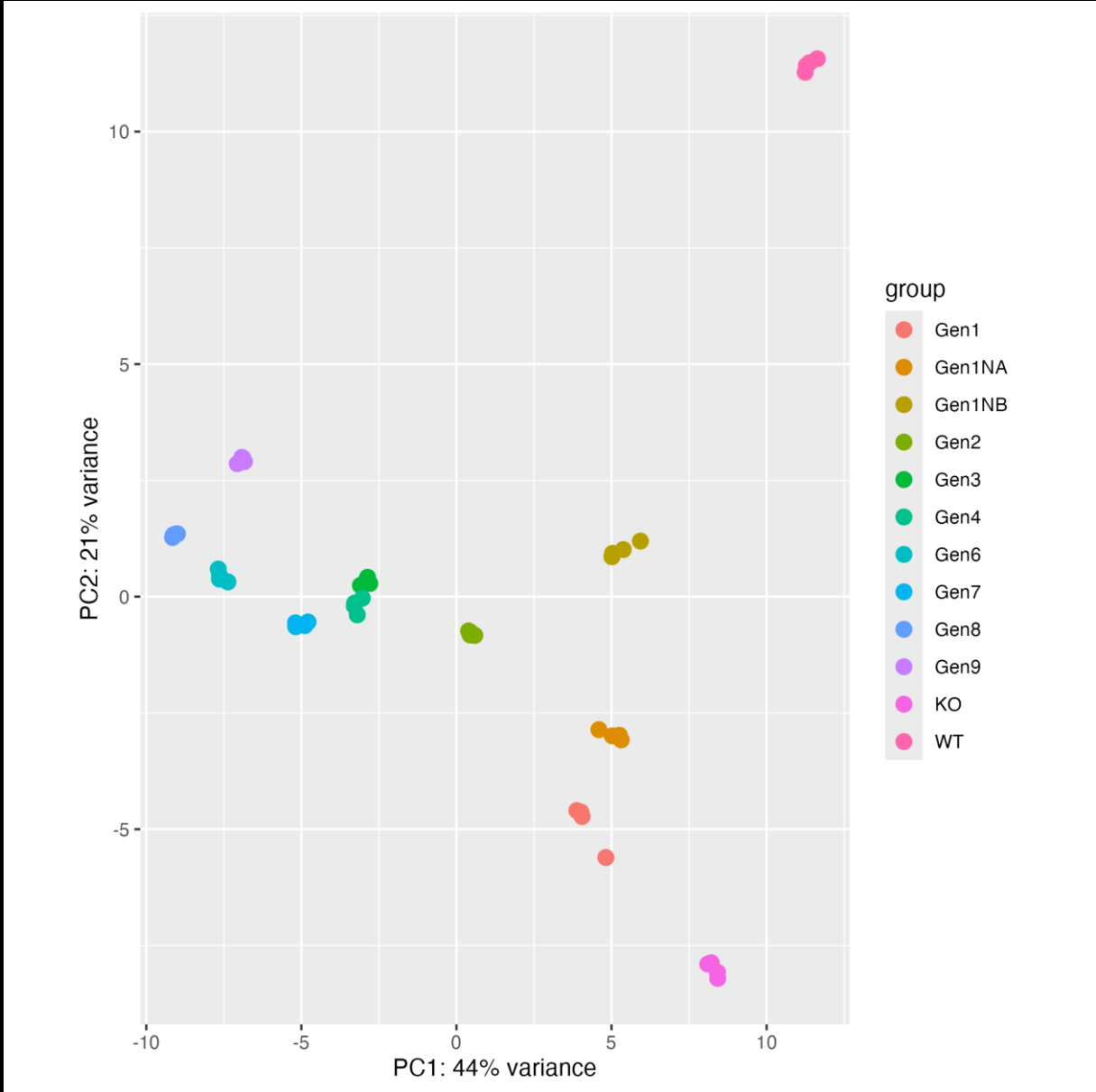
vCJD LINES



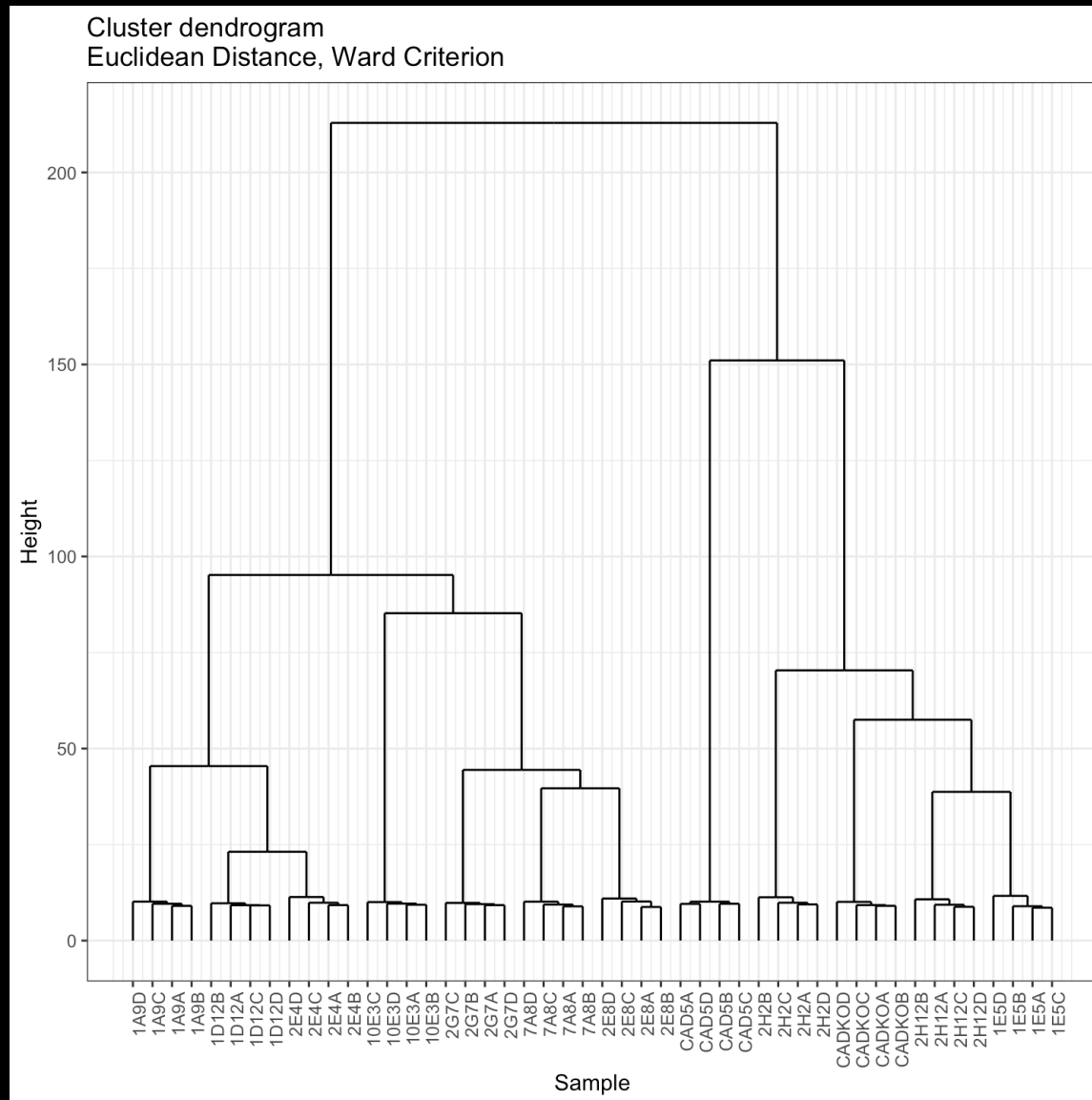
sCJD LINES



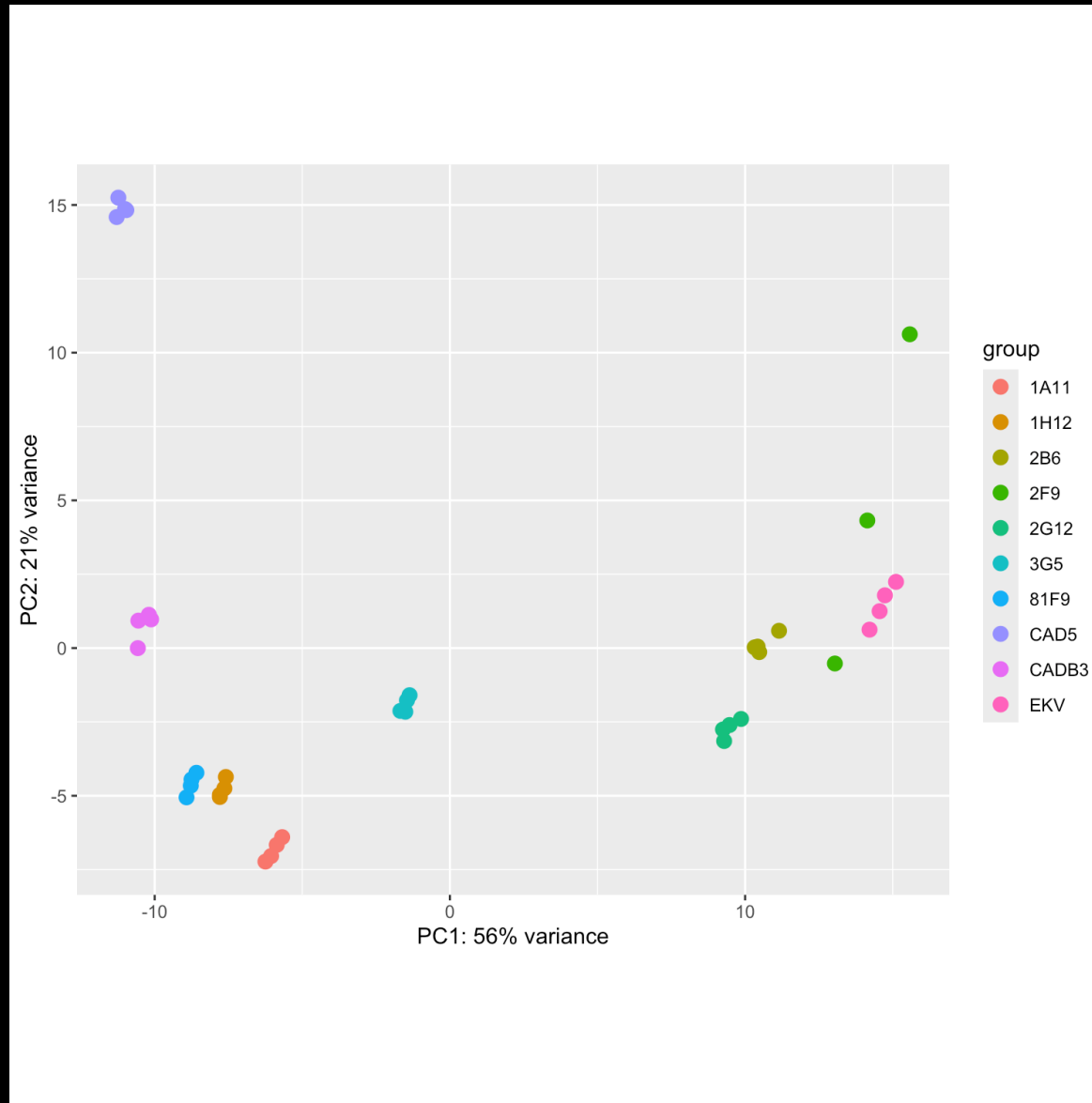
Principle component plot for vCJD cell lines



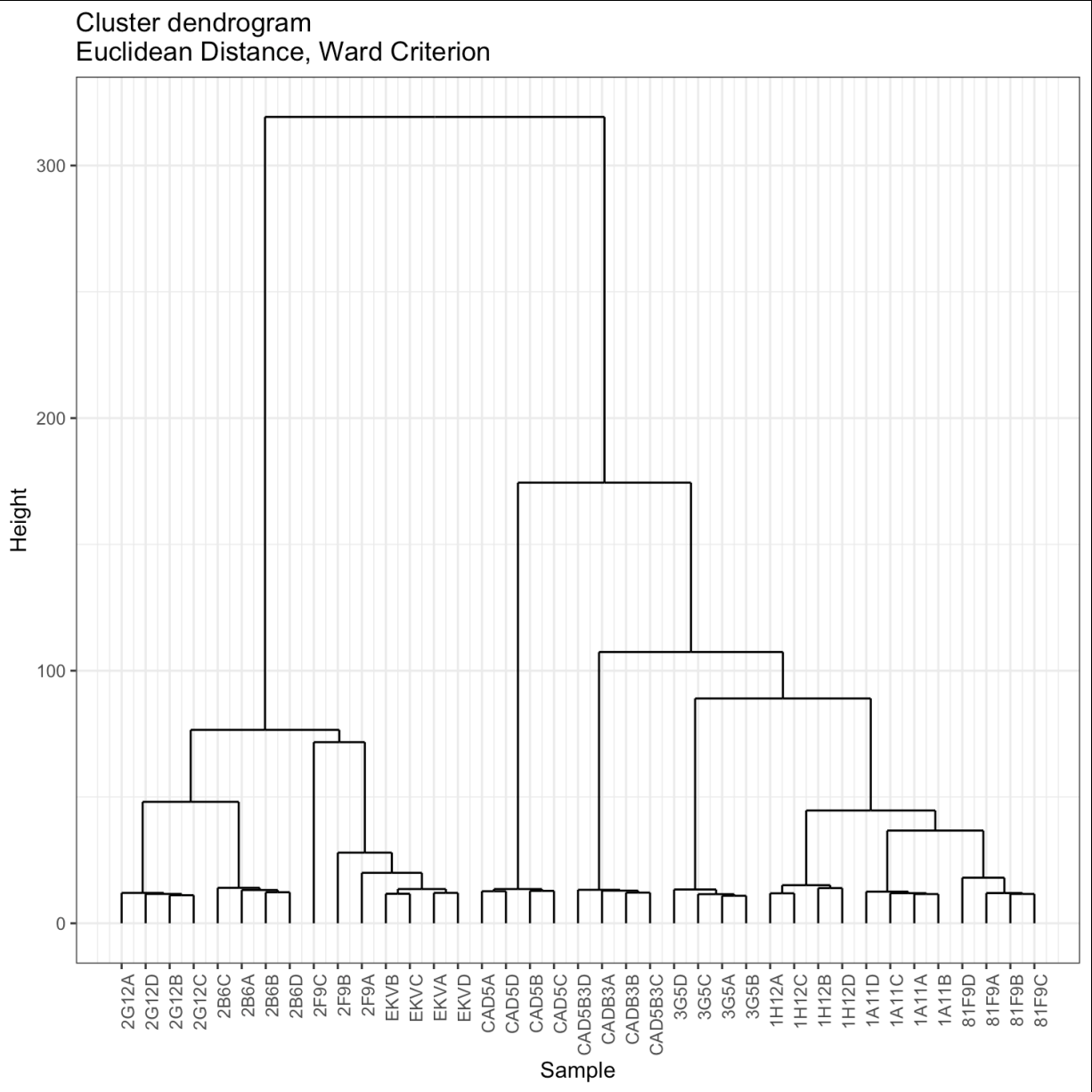
Cluster dendrogram of vCJD cell lines



Principle component plot for sCJD cell lines



Cluster dendrogram for sCJD cell lines



Summary

- Developed dividing cells susceptible to:
 - variant CJD – 10^7 fold dilution of brain homogenate.
 - sporadic CJD – 10^5 fold dilution of brain homogenate.
- Developed human prion assay for infectious vCJD and sCJD prions.
- Cells propagate *bona fide* CJD prions – mouse bioassay.
- Developed chronically infected cells – retain infectivity upon freeze/ thaw.
- Chronically infected cells can be cured.
- RNA-seq and whole genome sequencing undertaken to identify changes that correlate with increasing susceptibility.

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