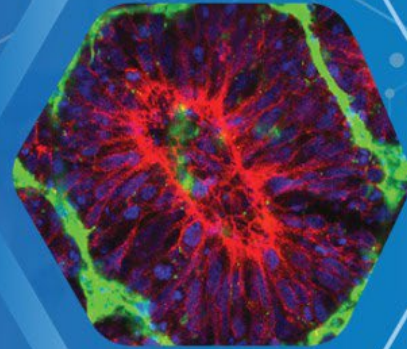
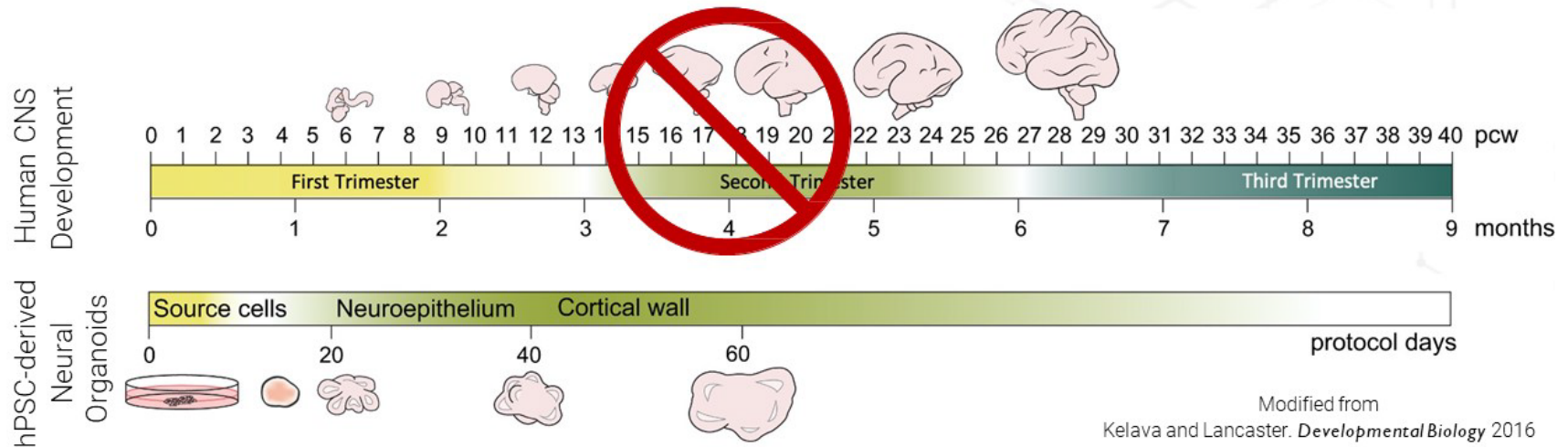




RosetteArray™ Platform for
Developmental Neurotoxicity
(DNT) Screening



Embryonic brain and spinal cord development is critical & delicate



- Neural organoid technology models CNS morphogenesis in vitro.
 - Reproducibility of tissue structure, cellular composition, and connectivity
 - Not high-throughput due to complex post-hoc analysis of variable 3D tissue



Our solution: Bioengineer Human Neural Organoid Morphogenesis

Chick Embryo (HH10)

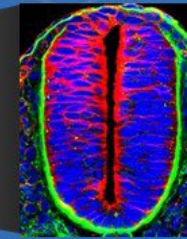


In Vivo

Transverse



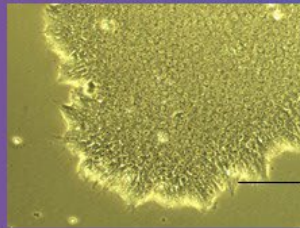
(Gilbert SF, *Developmental Biology* 2006)



N-cadherin / Laminin
/ Nucleus

(By Gwenvael LeDreau et al.-
Instituto de Biología Molecular de Barcelona-CSIC)

Human Pluripotent
Stem Cells (hPSCs)

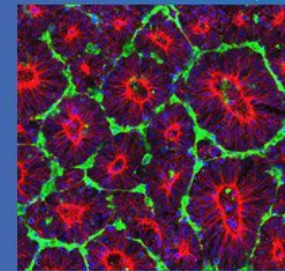


In Vitro

2D/3D
Neural Differentiation



Neural Stem Cells (NSCs)



N-cadherin / Laminin
/ Nucleus

(Gavin Knight, SOBRM)

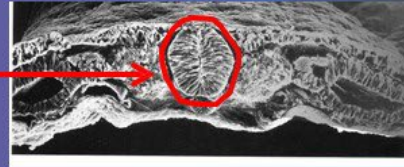
Our solution: Bioengineer Human Neural Organoid Morphogenesis

Chick Embryo (HH10)

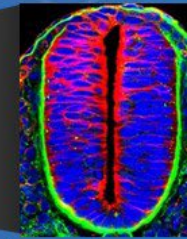


In Vivo

Transverse



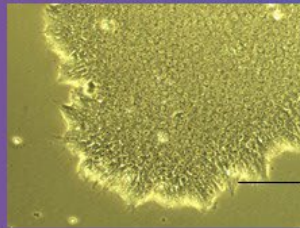
(Gilbert SF, *Developmental Biology* 2006)



M-cadherin / Nucleus / Laminin

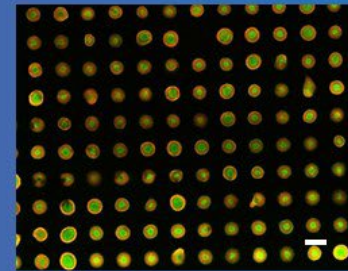
(By Gwenvael LeDreau et al. - Instituto de Biología Molecular de Barcelona-CSIC)

Human Pluripotent Stem Cells (hPSCs)



In Vitro

Micropatterned Substrate



M-cadherin / Nucleus / Nucleus

Scale Bar- 375µm

Knight GT, Lundin BF, Iyer N, Ashton LMT, Sethares WA, Willett RM, Ashton RS. *eLife* (2018)

Patent Application
No. 16/044236

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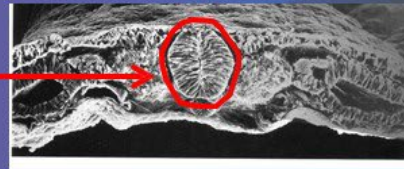
Our solution: Bioengineer Human Neural Organoid Morphogenesis

Chick Embryo (HH10)

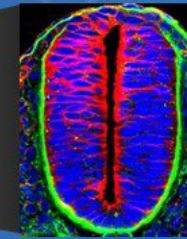


In Vivo

Transverse



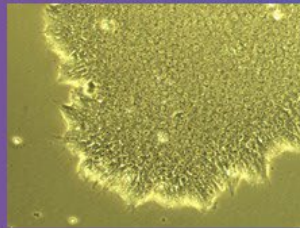
(Gilbert SF, *Developmental Biology* 2006)



M-cadherin / Nucleus
Laminin

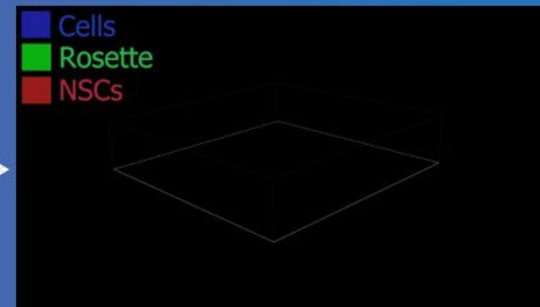
(By Gwenvael LeDreau et al. -
Instituto de Biología Molecular de Barcelona-CSIC)

Human Pluripotent
Stem Cells (hPSCs)



In Vitro

Micropatterned
Substrate



Knight GT, Lundin BF, Iyer N, Ashton LMT, Sethares WA, Willett RM, Ashton RS. *eLife* (2018)

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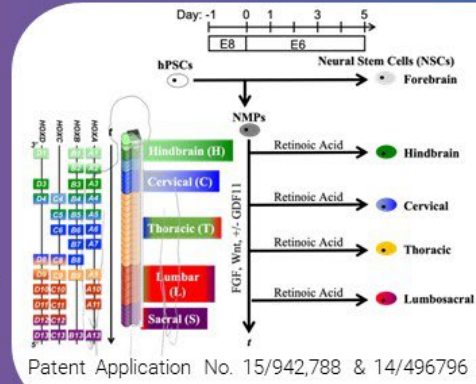


RosetteArray™ platform for qHTS of human DNT

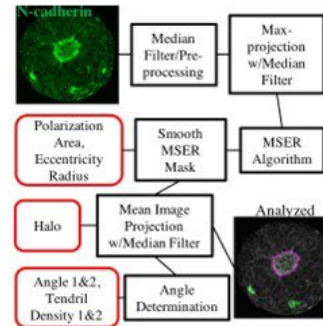
Lippmann ES, Estevez-Silva MC, Ashton RS. *Stem Cells* 32 (2014)

Lippmann ES, Williams CE, Estevez-Silva MC, Coon JJ, and Ashton RS. *Stem Cell Reports* 4 (2015)

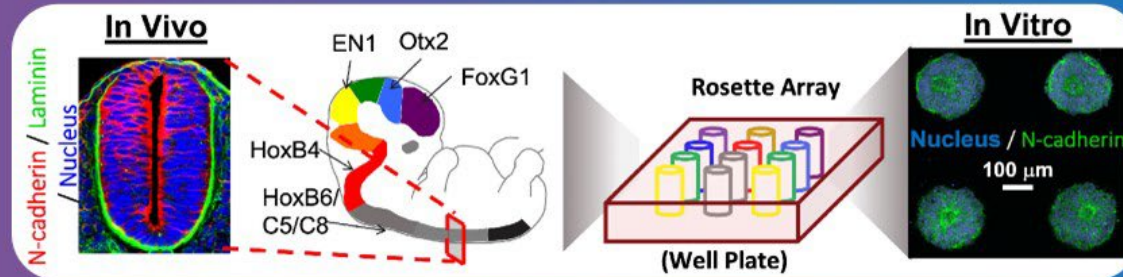
Iyer NR, Shin J, et al. *Sci. Adv.* 8 (2022)



HT Image Analysis



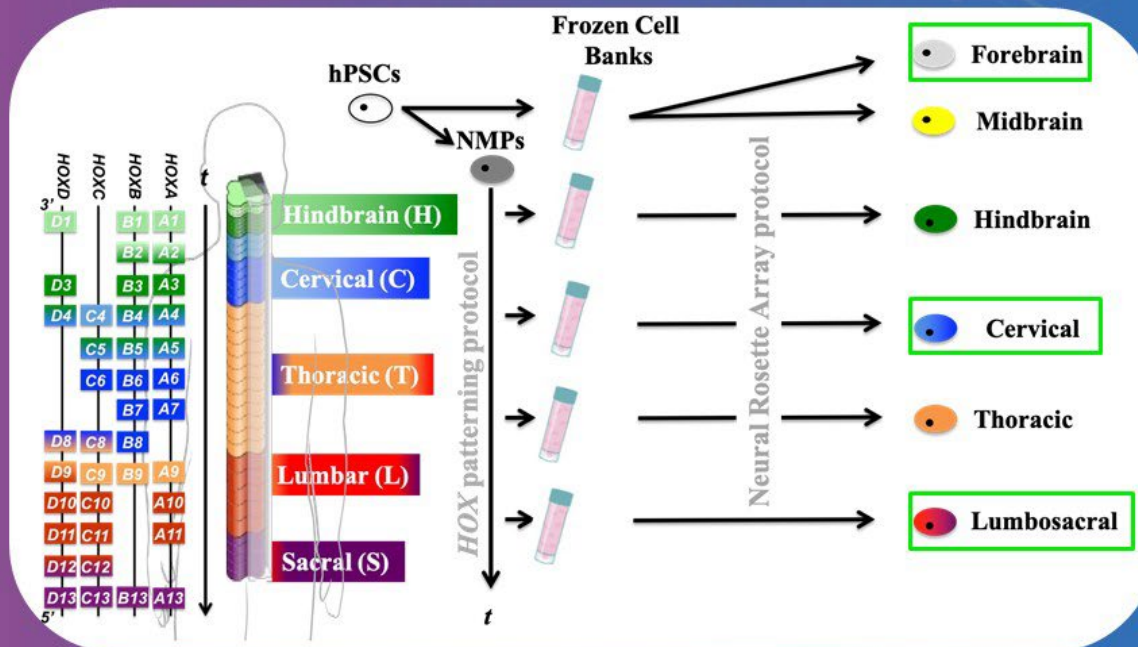
Knight GT, Lundin BF, Iyer N, Ashton LMT, Sethares WA, Willett RM, Ashton RS. *eLife* 7 (2018)



Patent Application No. 16/044236



RosetteArray™ platform for qHTS of human DNT



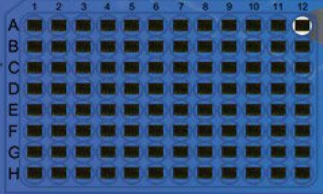
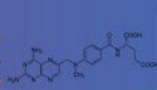
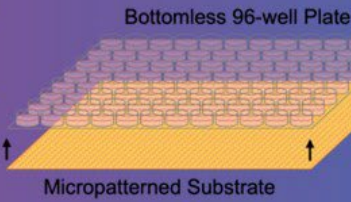
- 5-7 day culture period

Quantitative Image Analysis:

- Cell viability
- Cell proliferation
- Neural Differentiation
- CNS Morphogenesis/Rosette formation

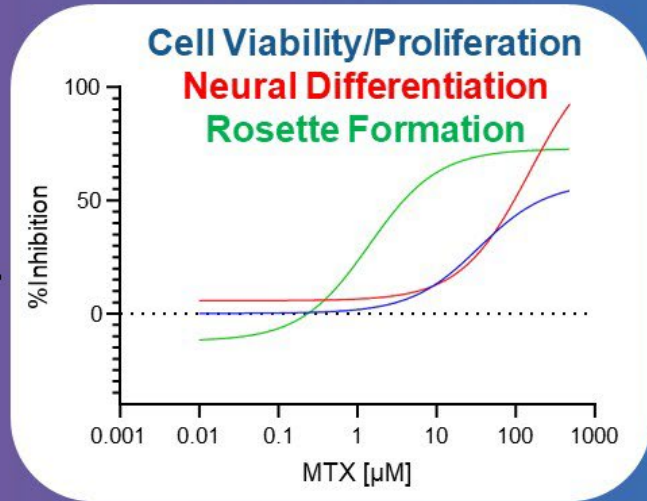
RosetteArray™ platform for qHTS of human DNT

Cryopreserved progenitors



x 3840 per 96-well plate

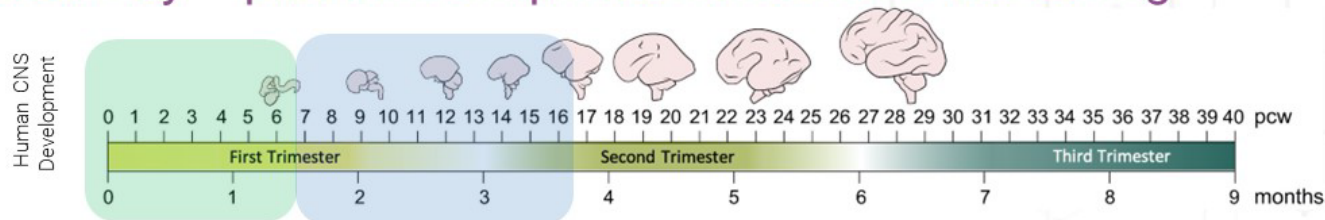
Mechanistic Insight
(not just toxicity)



Neurosetta, LLC
Tissue detection
image processing
software



RosetteArray™ platform simplifies human DNT screening



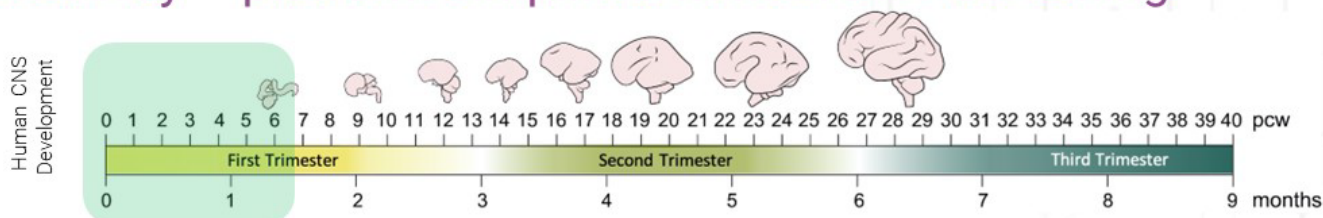
Current qHTS DNT screening battery:

- Neural stem cell viability
- Neural stem cell proliferation
- Neural cell migration
- Zebrafish neurulation
- Neural differentiation/Morphogenetic patterning
- Neuronal differentiation/survival
- Neuronal activity and network formation
- Glia differentiation/survival
- Glial activation
- Oligodendrocyte differentiation/survival
- Oligodendrocyte myelination
- Blood brain barrier formation

Future qHTS DNT screening battery:

- Human RosetteArray™ Technology
 - Scalable and Quantitative
 - Single Imaging Readout!
- Neuronal differentiation/survival
- Neuronal activity and network formation
- Glia differentiation/survival
- Glial activation
- Oligodendrocyte differentiation/survival
- Oligodendrocyte myelination
- Blood brain barrier formation

RosetteArray™ platform simplifies human DNT screening



- Works with DMSO solvent up to 0.1% (i.e., 1:1000 dilution)
- Excellent reproducibility
 - Forebrain RosetteArray: $86.3\% \pm 9.06$ (stdv) single rosette emergence efficiency
 - Cervical Spinal RosetteArray: $73.3\% \pm 12.6$ (stdv) single rosette emergence efficiency
 - Lumbosacral Spinal: $93.8\% \pm 4.10$ (stdv) single rosette emergence efficiency
- Integration of human metabolism for developmental neurotoxicity (DNT) screening
- 29 compound screen of positive and negative controls and pesticides: 96% sensitivity and 100% specificity.
- Unique dose-response profiles for Forebrain versus Spinal Rosette Array assays

Want to access the RosetteArray™ solution?

Conduct human Developmental Neurotoxicity (DNT) Screening in quantitative high-throughput.

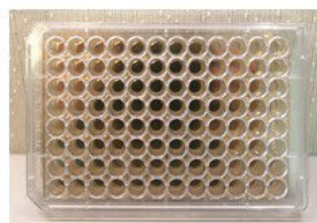
- Products:
 - Human DNT Screening as contract research
 - Micropatterned well plates and Human DNT screening kits.
- \$1.7M STTR Grant from NIEHS
 - Scale RosetteArray platform, use iPSCs
 - 100 compound screen

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Screening for chemical or drug safety:

- ❖ Contact: customerservice@neurosetta.com



Conduct RosetteArray screen in-house:

- ❖ Purchase micropatterned well plates (96-, 24-, or 6-well) at www.neurosetta.com
- ❖ Kit with cells, media, and well-plates will be available soon.





Protecting human brain and
spinal cord development

Questions?

