Development of NanoString Gene Expression Assays for Studying Frataxin-sensitive Gene Markers in Clinical Samples

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Cell Penetrant Peptide Provides a flexible solution to Deliver Frataxin (FXN) to Patients with Friedreich's Ataxia

- **Friedreich's ataxia (FA):**
- Is a rare, progressive, multi-symptom neurodegenerative genetic disease and simultaneous cardiac dysfunction
- Results from a reduced expression of the mitochondrial protein, frataxin (FXN), a major contributor to mitochondria functioning
- Is a debilitating disease that presents in mid-childhood and affects the functioning of multiple organs and systems
- Has no medical treatment options approved for patients with FA, to date
- Larimar is developing a Cell Penetrant Peptide-based therapy to deliver FXN
- **High variability in patients FXN reduction levels results in non-uniform clinical presentation and progression**
- **Biomarker approach is needed and actively sought-after to objectively assess interventional benefits**
- **There is no approved validated biomarker**

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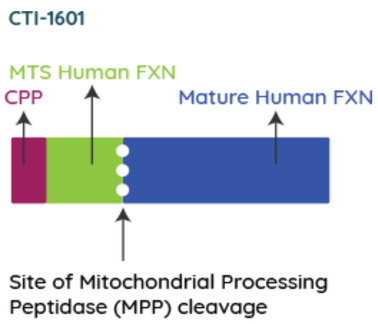
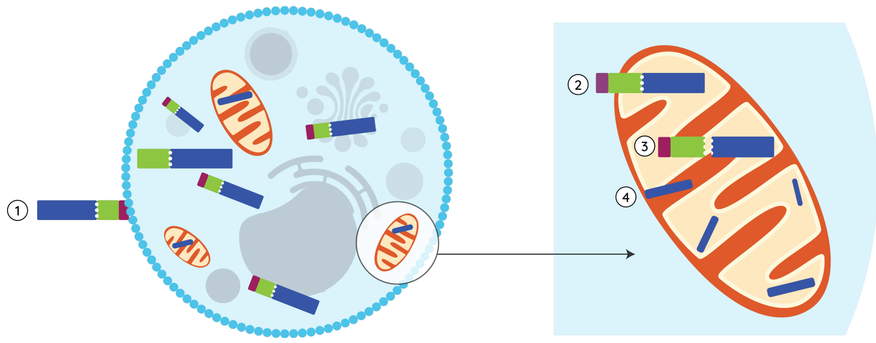
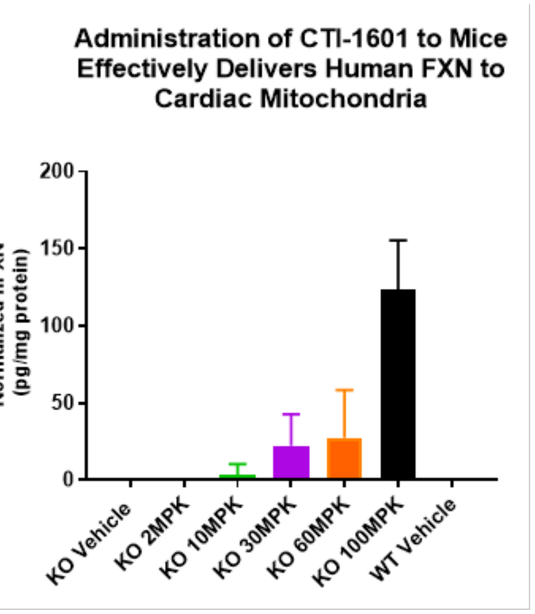
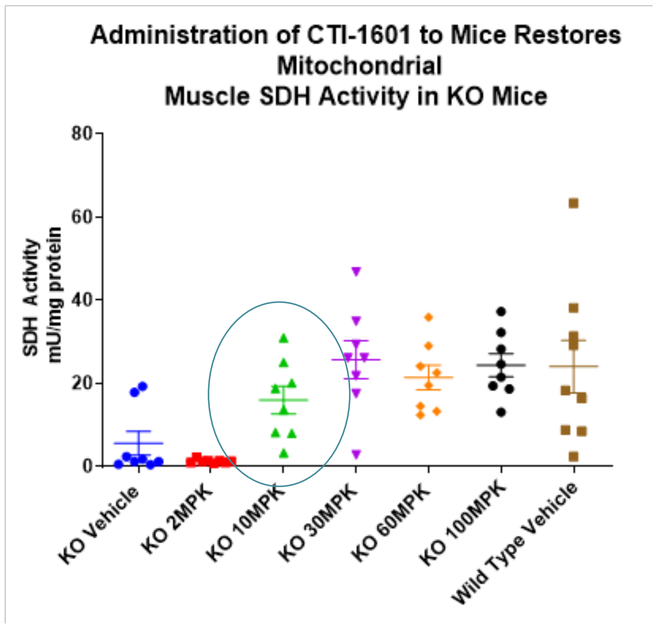
CTI-1601 Effectively Delivers Mature and Functional Frataxin to Cells and Organs in KO mice



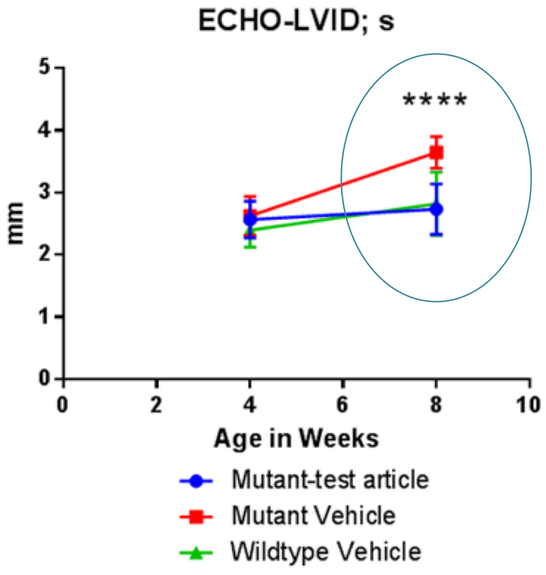


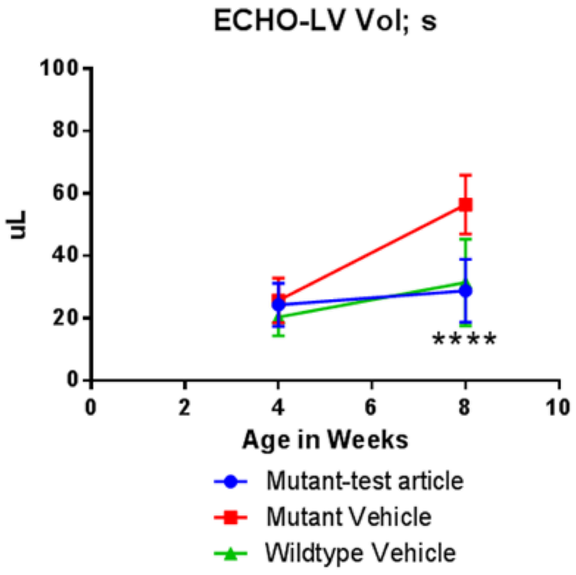
Administration of CTI-1601 to mice





Anesthetized echocardiography assessment in treated mice





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Gene Expression-Based Biomarker Strategy Defining Frataxin Sensitive Gene Markers- FSGMs

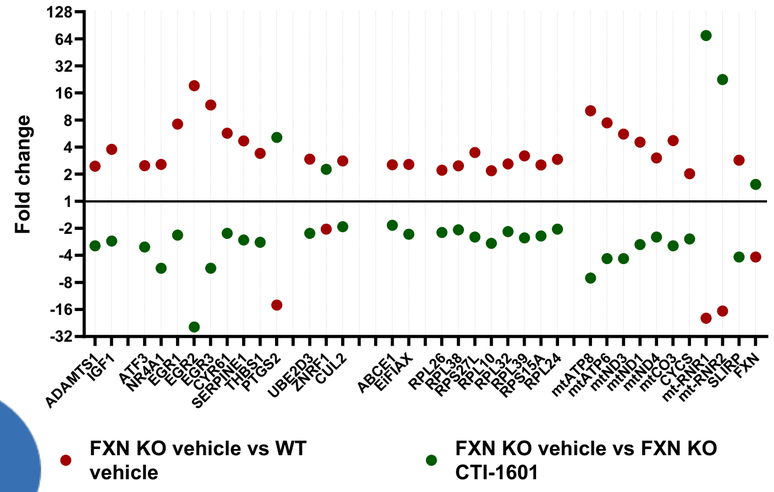
Mouse models

Confirm in patient-derived cells

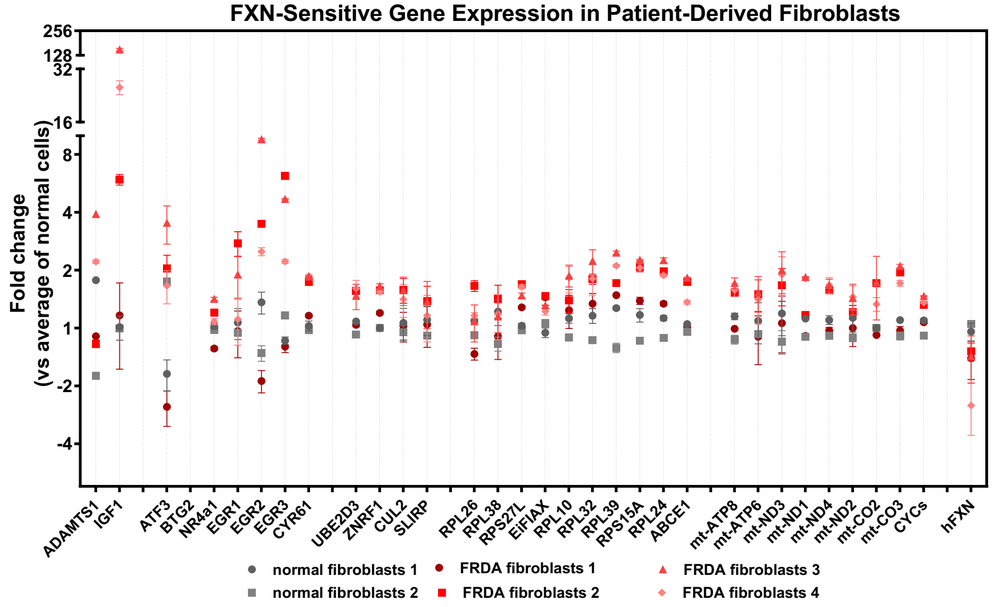
Confirm in patient specimen

- Tissue selection
- Trans-sectional and longitudinal variability

FXN-Sensitive Gene Markers are Inversely Regulated by lack of FXN and Treatment with CTI-1601



FXN-Sensitive Gene Expression in Patient-Derived Fibroblasts



Over 150 genes that are sensitive to cellular levels FXN were identified that could serve as biomarkers

Expression levels and sensitivity to FXN of FSGMs in human tissue is unknown

Limited amount of RNA in patient specimen could limit the use of FSGMs as biomarker approach

- Assessing expression levels of 150 genes by qRT-PCR would require 11ug RNA
- Yield from buccal swab 300ng-1.8ug

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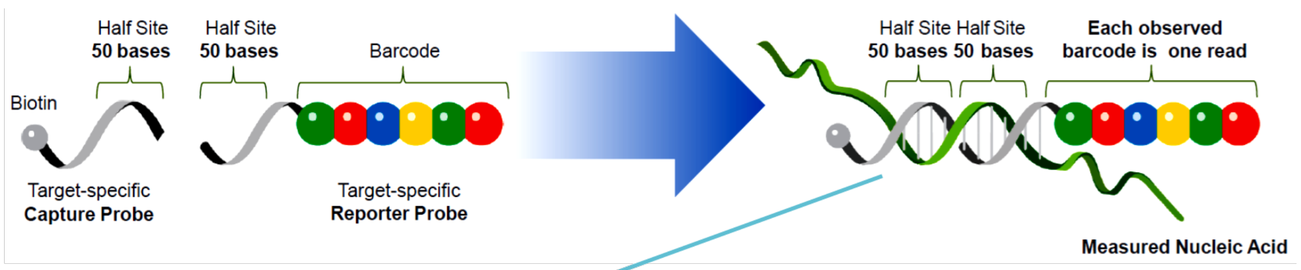
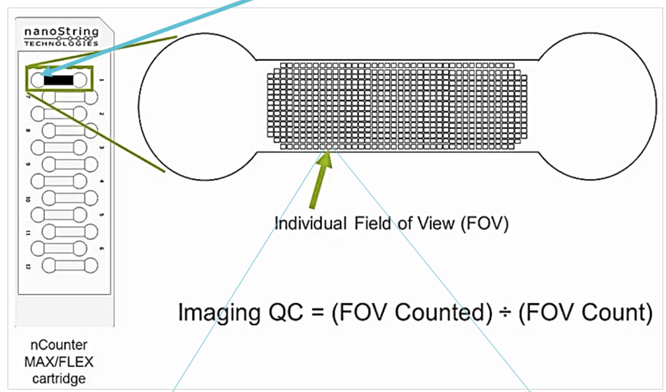
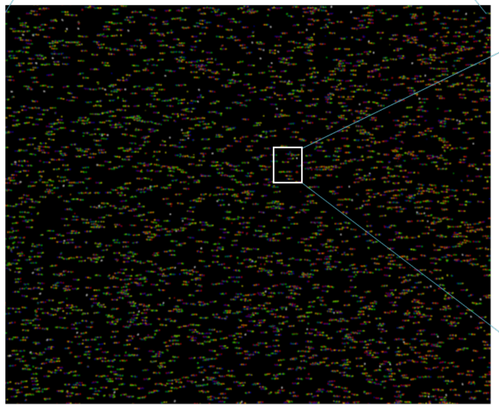
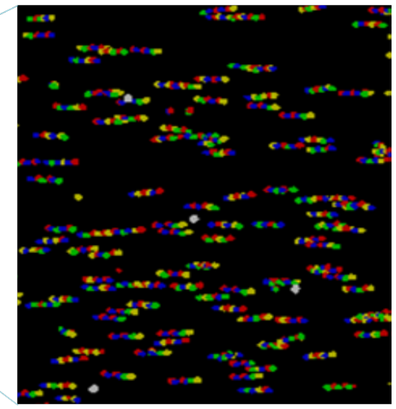
Use of Nanostring Technology as an alternative to qRT-PCR

- Comparability to qRT-PCR
- Explore adequacy in the absence of clear regulatory guidance around the use of gene expression biomarkers
- Sensitivity to Codeset (Target gene + house-keeping gene) composition
- Intra- and inter- sample variability
- Effect of RNA quality
- Linearity
- Workflow to minimize variability
- P&A and reproducibility on clinical samples
- Feasibility in clinical setting

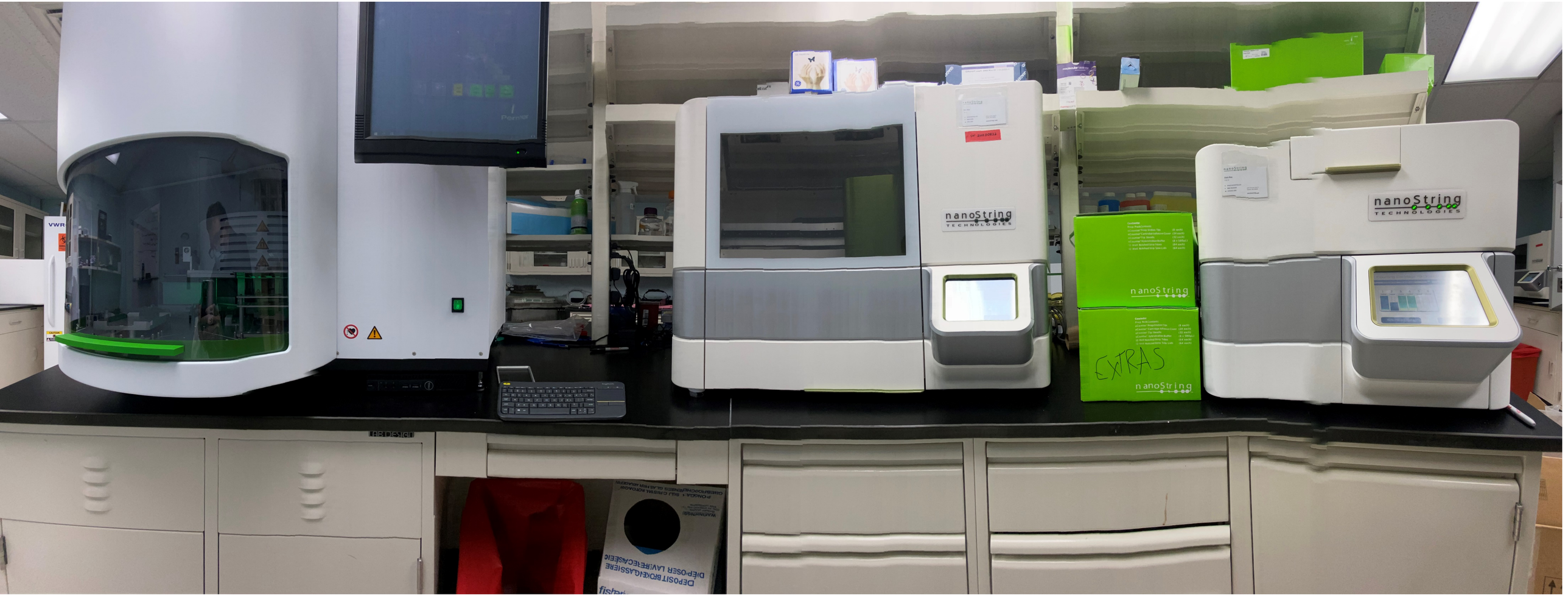








	A	B	C	D	E	F	G
1	Probe Name	Class Name	% Samples above	Avg Count	%CV	20211004_202109	20211004_202109
2	POS_A	Positive	0	62956.39	20.26	72520	69624
3	POS_B	Positive	0	20960.81	20.47	24628	23442
4	POS_C	Positive	0	4850.6	21.02	5755	5430
5	POS_D	Positive	0	1285.52	18.95	1495	1430
6	POS_E	Positive	0	235.58	25.79	317	254
7	POS_F	Positive	0	116.48	20.75	120	123
8	NEG_A	Negative	0	15.33	40.07	17	11
9	NEG_B	Negative	0	17.81	36.15	28	21
16	ACTB	Housekeeping	100	17257.51	42.59	20363.7	12729.6
17	AP2A2	Housekeeping	100	83.43	23.74	129	120.72
18	ATP6V0C	Housekeeping	100	1937.35	41.78	1862.62	1429.38
19	ATRN	Housekeeping	37.5	22.59	40.04	21.3	23.67
20	ATXN2	Housekeeping	89.58	70.84	57.92	87.95	37.24
21	Asxl1	Housekeeping	8.33	18.65	26.36	21.3	23.67
22	Atp1a1	Housekeeping	4.17	19.07	34.6	21.3	23.67
23	ABCE1	Endogenous	25	21.57	39.27	21.3	23.67
24	ADNP	Endogenous	93.75	70.35	35.41	104.56	51.37
25	ATF3	Endogenous	97.92	344.83	66.09	319.56	282.54
26	ATF4	Endogenous	100	633.69	39.67	653.77	477.75



Tandem Automated Tissue RNA Extraction-Gene Expression Analysis

Hybridization
65°C 18 hour (2X)
+RT

Two or more NanoString Prep Stations

NanoString Digital Analyzer
6 cartridges/24 hours

Use of PE Chemagics automated liquid Handler to Extract RNA

Up 72 samples per day-up to 770 genes
~360 /weeks

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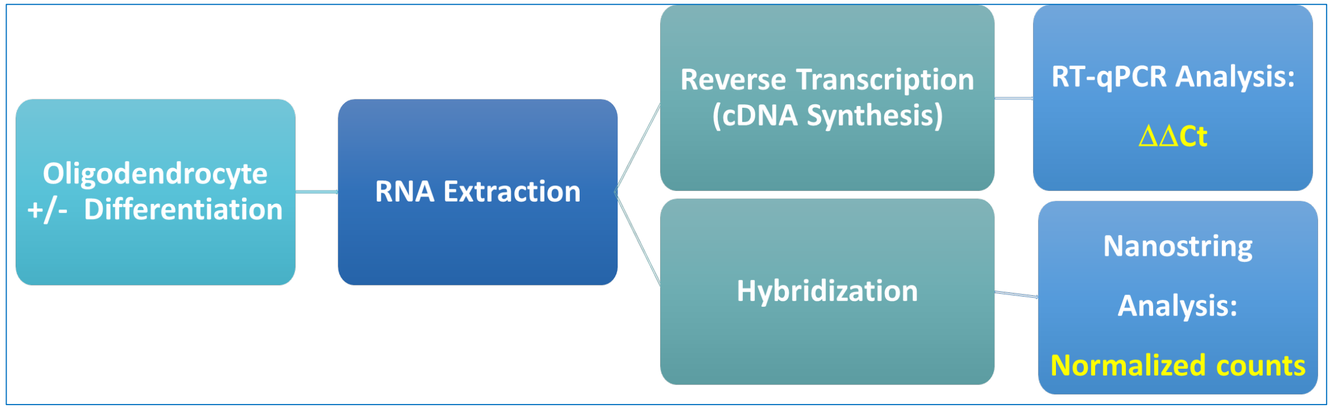
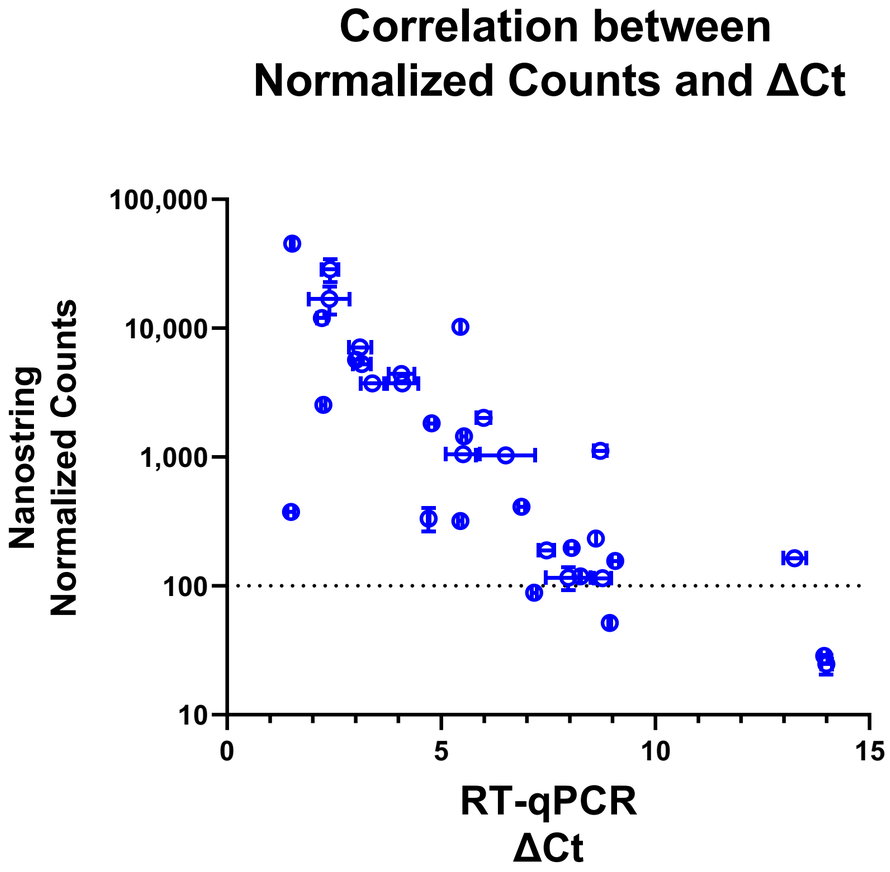
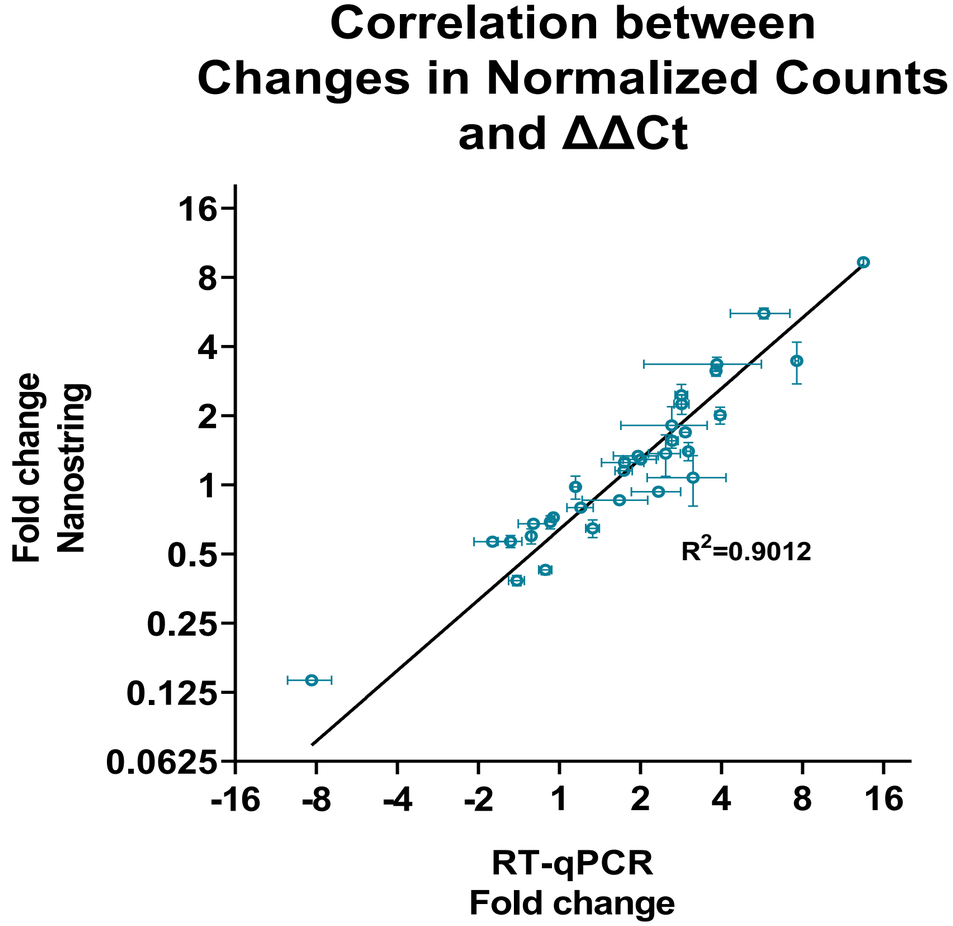
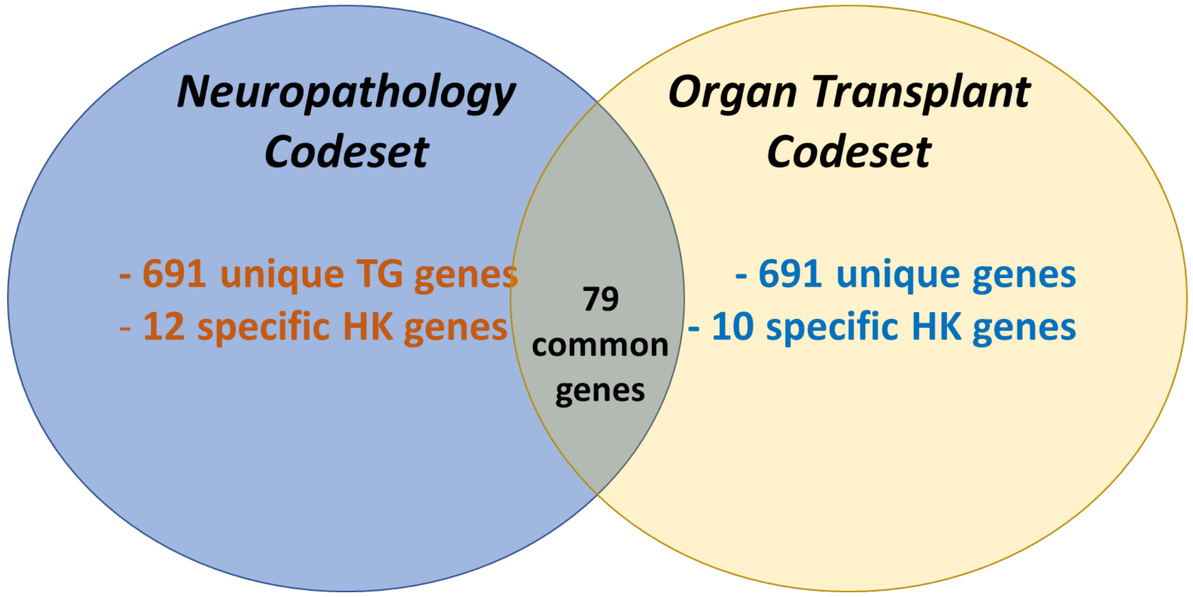
Gene expression and Changes of Gene Expression Levels: *qRT-PCR vs. Nanostring*

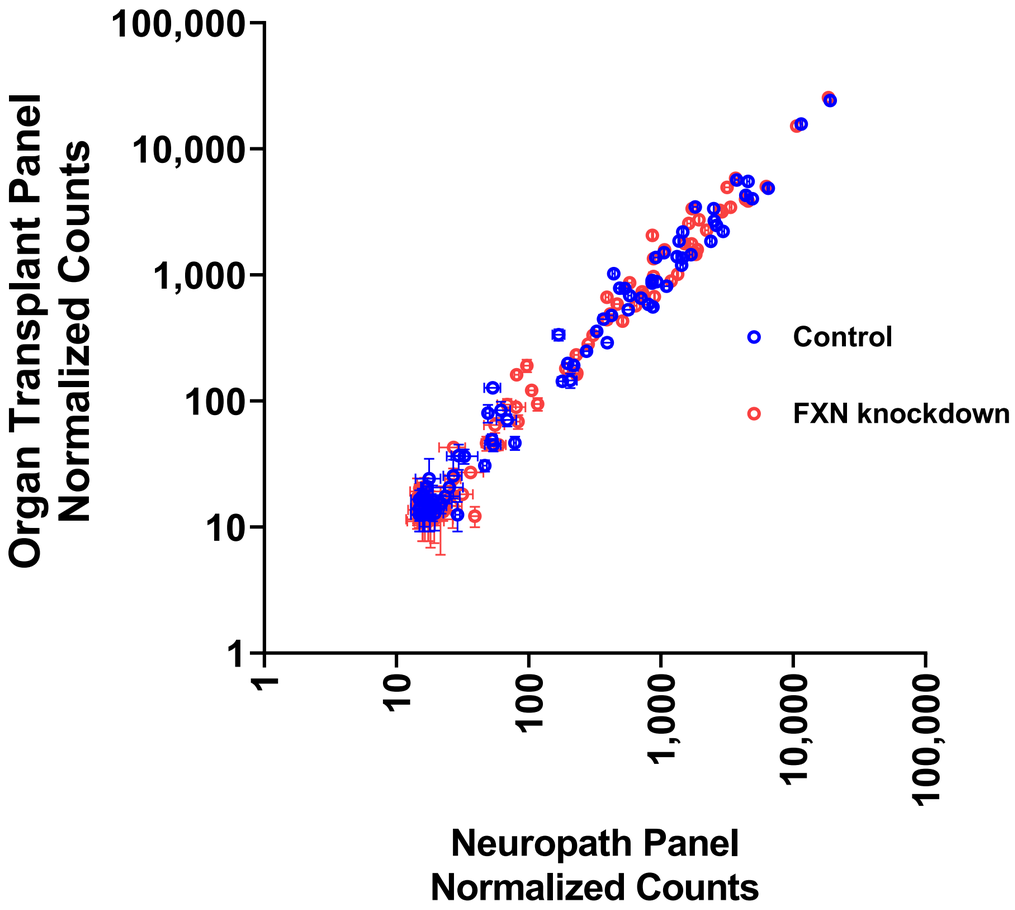


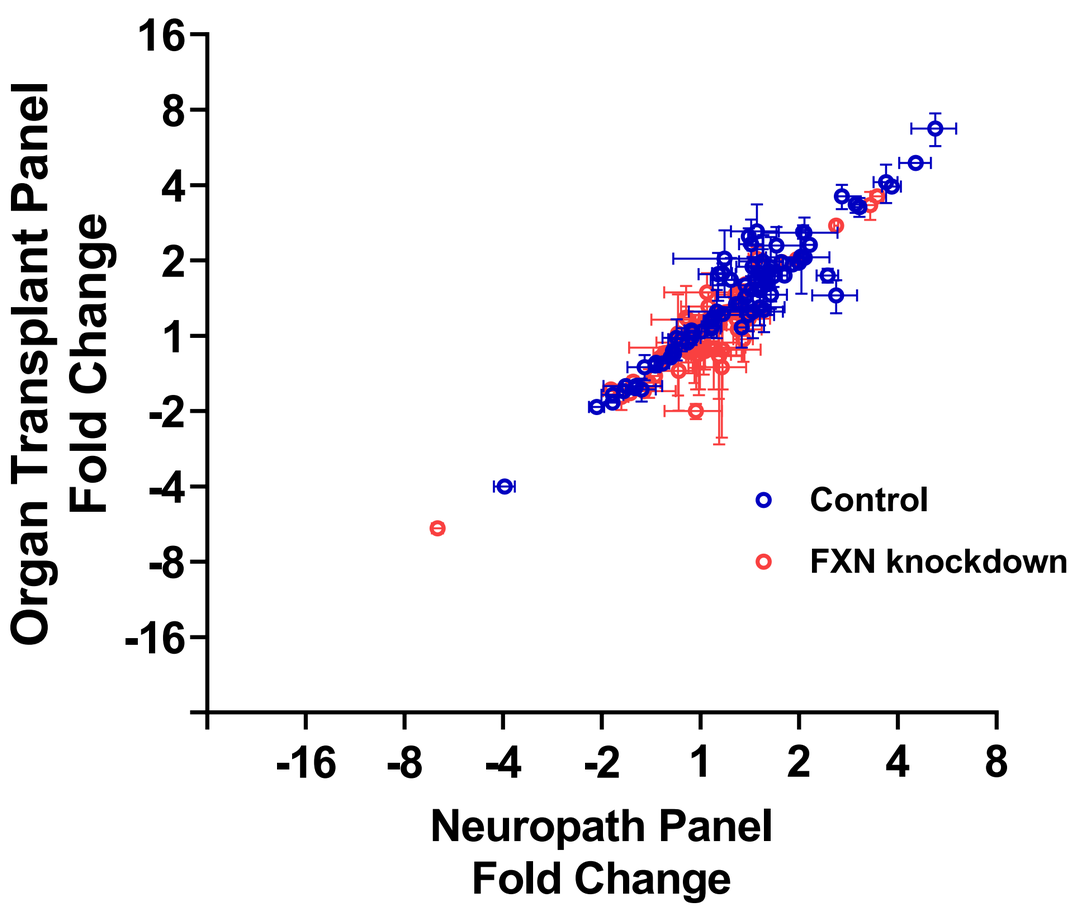




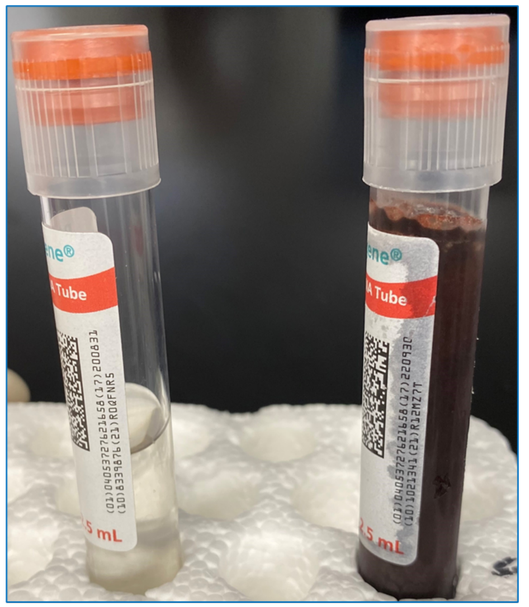
Effect of Codeset Composition on Gene Expression Quantitation and Normalization





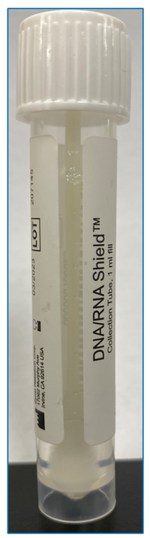


Specimen Collection and Handling



PAXgene Blood RNA Tubes

- **Collection device**
  - Blood Cells collected in PAXgene RNA tubes – 762165 PreAnalytix Qiagen
    - Exact volume essential
  - Buccal Cells collected in ZymoResearch Buccal Swab Collection kits – R1107-E ZymoResearch
    - Mouth rinse, 4 minutes swapping performed by operator
- **Storage**
  - Whole blood samples (when frozen/stored correctly)
    - 11+ years (long term storage) = -80°C
    - 3 days = Room temperature (~20°C)
  - Buccal cell samples
    - Indefinitely = -20°C to -80°C
    - >1 month = Room temperature (~20°C)
- **RNA isolation**
  - Chemagic RNA Blood 2.4K Kit H24 = CMG-1084 is used to extract RNA from 2 whole blood replicate tubes per sample and up to 2 buccal swab replicates per sample for a maximum of 24 samples per day.
- **RNA Quantification and QC**
  - Duplicate nanodrop quantification
    - average concentration [ng/uL]
    - average A260/A280 ratio.
  - RNA concentration above 50ng/ul is the sole influencing factor



ZymoResearch Buccal Swab

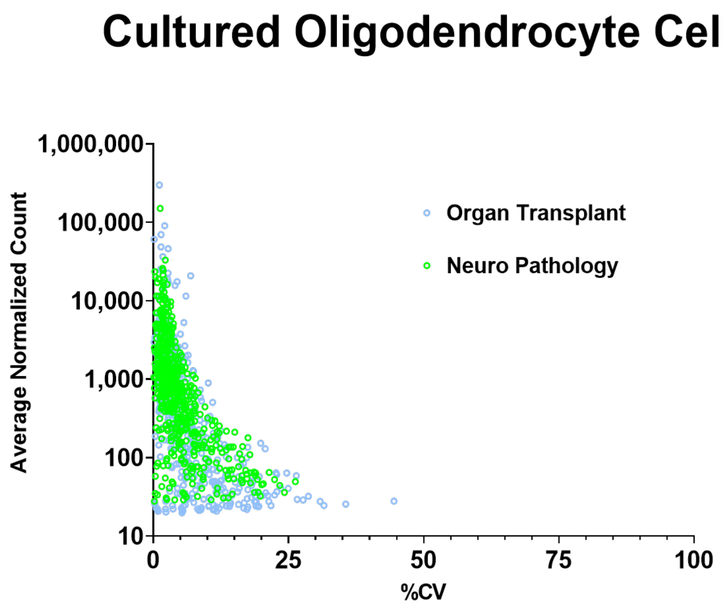
Assay and Patient Variability

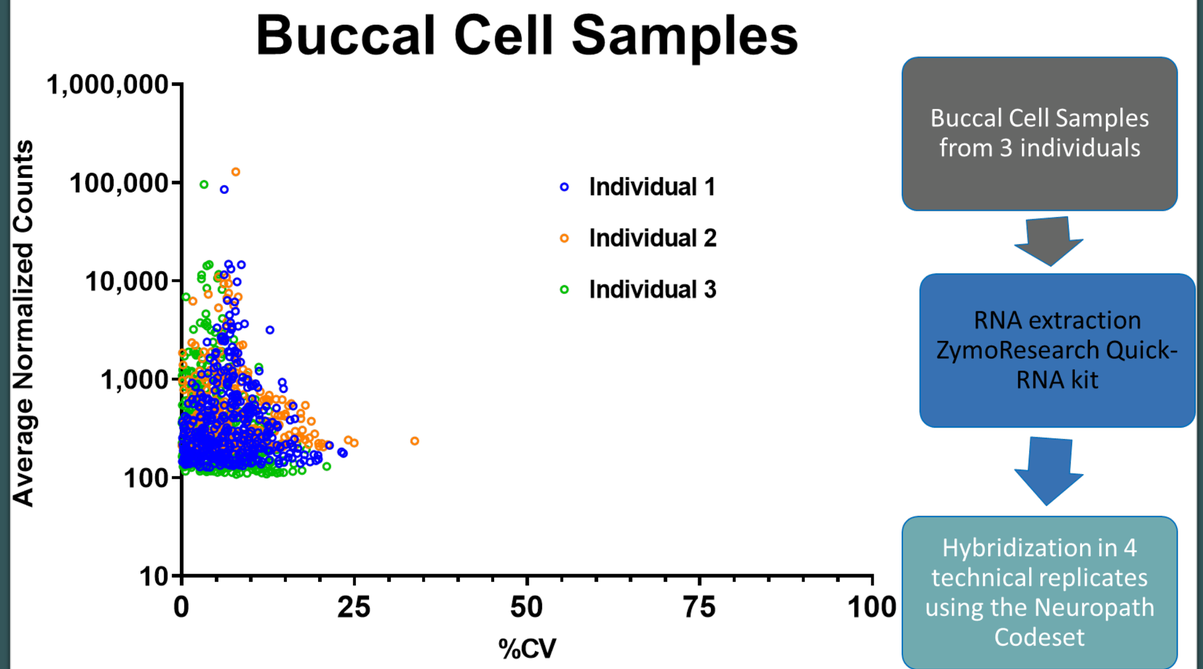
WT Oligodendrocyte cells cultured in duplicate

RNA extraction of both treatment conditions manually using Qiagen RNeasy Kit

Hybridization:

Three technical replicates to ***two*** Codesets



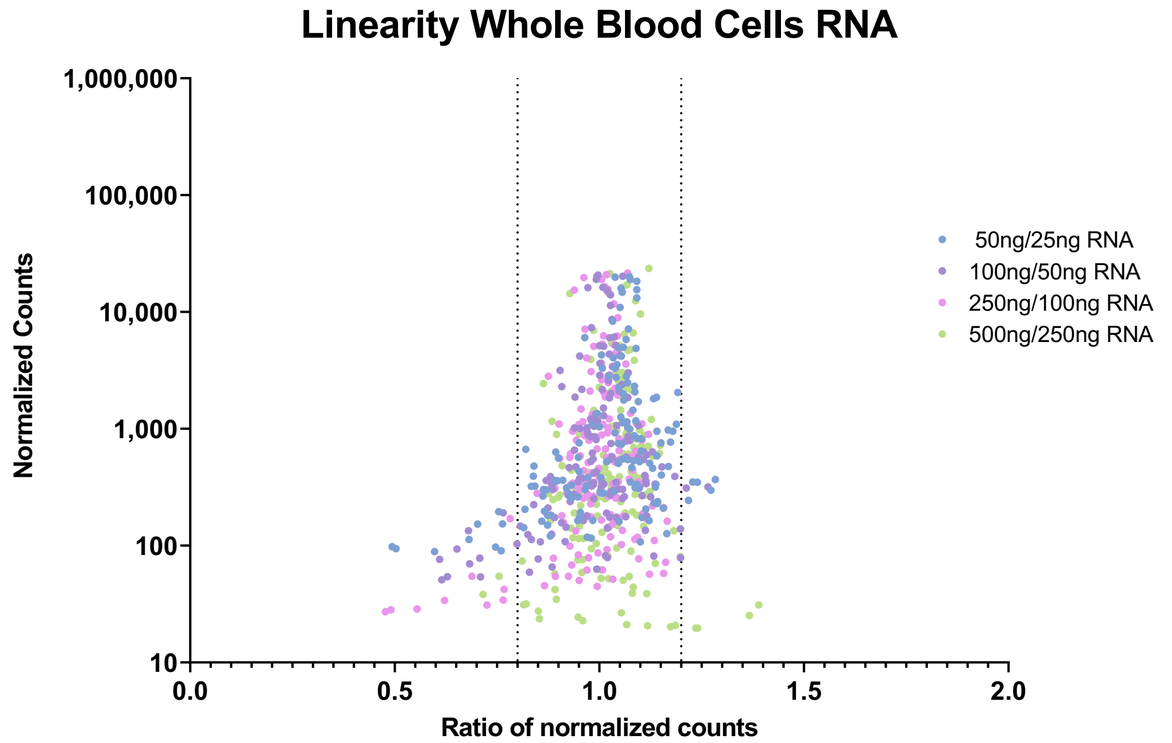


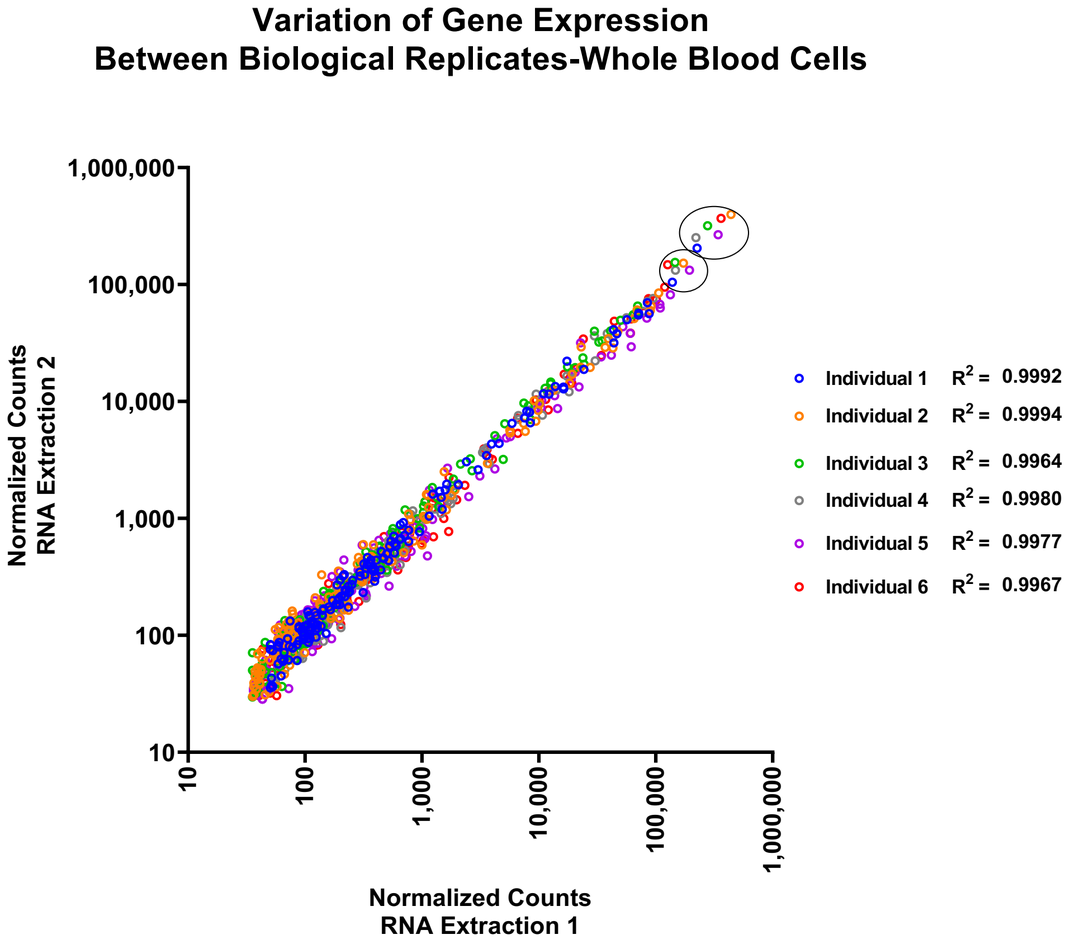
Buccal Cell Samples from 3 individuals

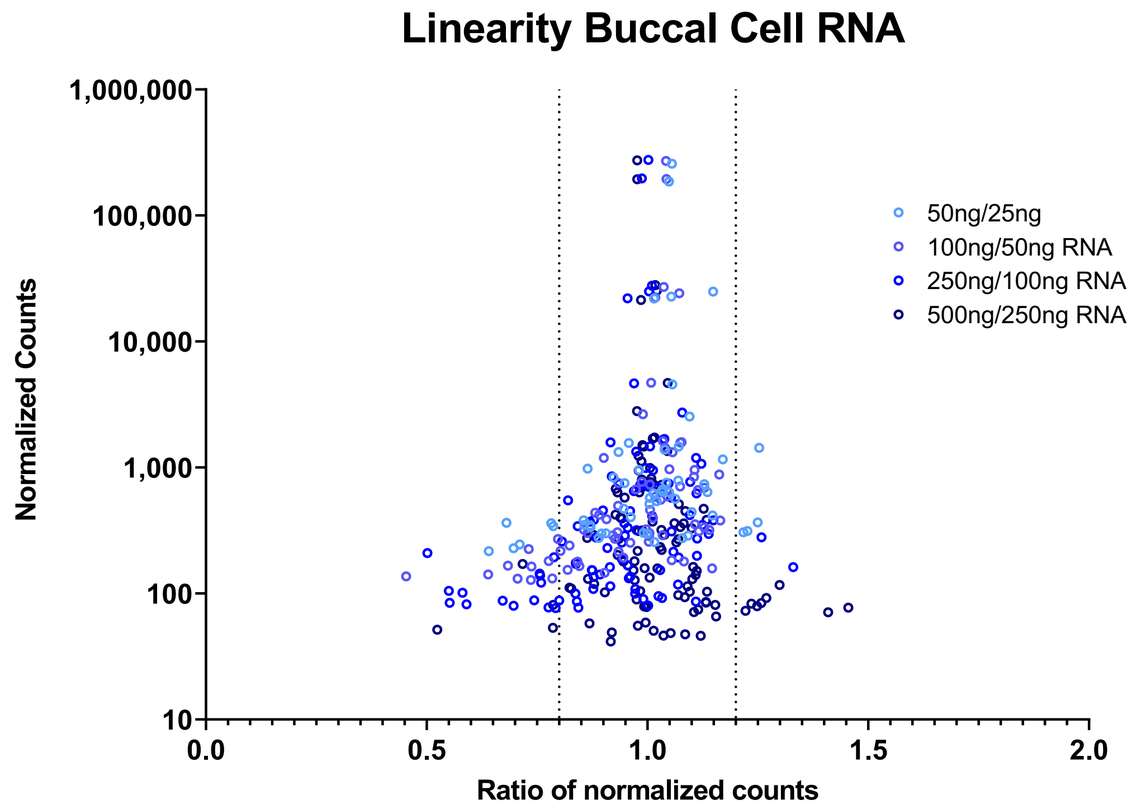
RNA extraction ZymoResearch Quick-RNA kit

Hybridization in 4 technical replicates using the Neuropath Codeset

Assay and Patient Variability







Larimar Clin-1601-102 Multiple Ascending Dose Study

Treatment Schedules for Each Cohort

Cohort 1 (25 mg; n = 8)

13-day Treatment Period

1	2	3	4	5	6	7	8	9	10	11	12	13	14

= Administration of CTI-1601 (n=6) or placebo (n=2)
= No Administration

Cohort 2 (50 mg; n = 9)

13-day Treatment Period

1	2	3	4	5	6	7	8	9	10	11	12	13	14

= Administration of CTI-1601 (n=7) or placebo (n=2)
= No Administration

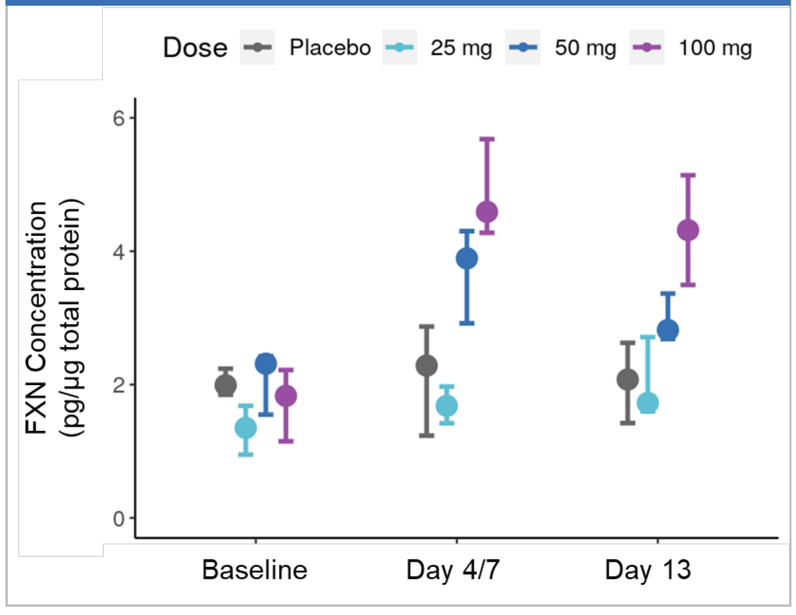
Cohort 3 (100 mg n = 10)

13-day Treatment Period

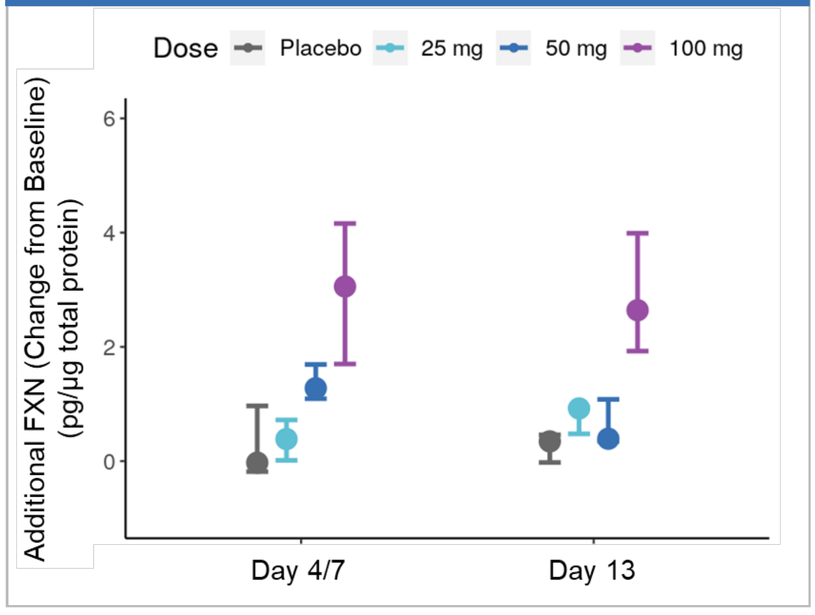
1	2	3	4	5	6	7	8	9	10	11	12	13	14

= Administration of CTI-1601 (n=3) or placebo (n=3)
= No Administration

FXN* Levels By Dose Group (Buccal Cells)

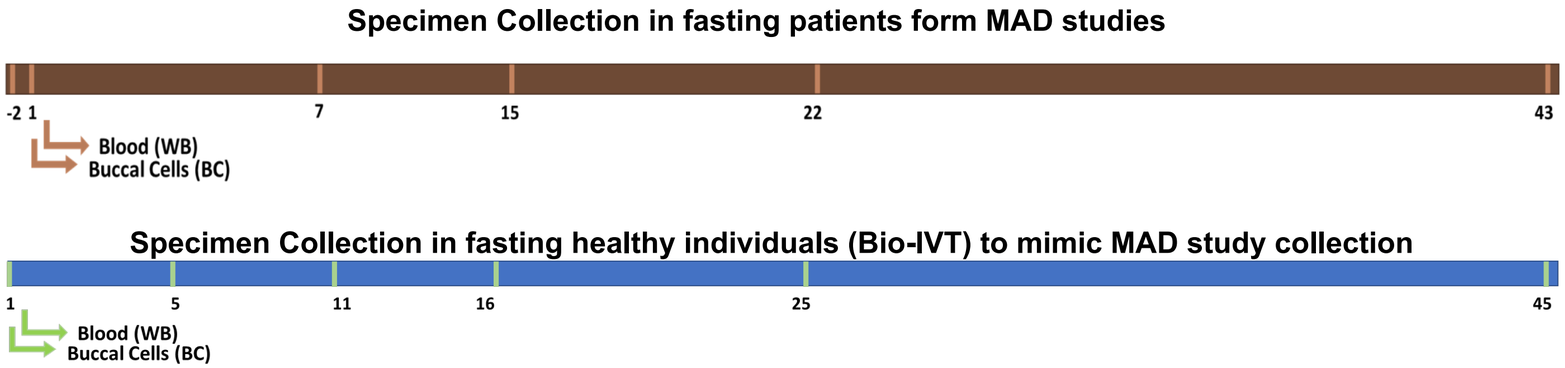


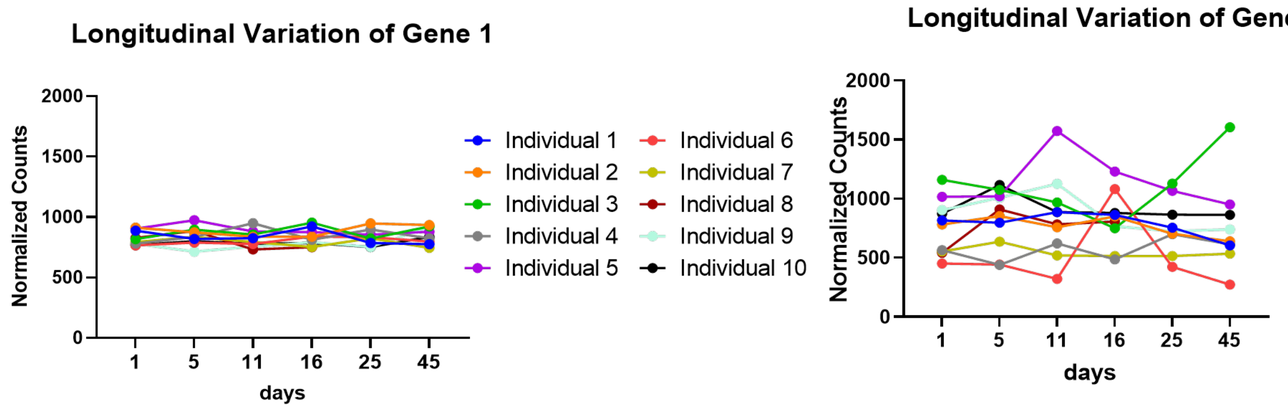
FXN* Change from Baseline By Dose Group (Buccal Cells)

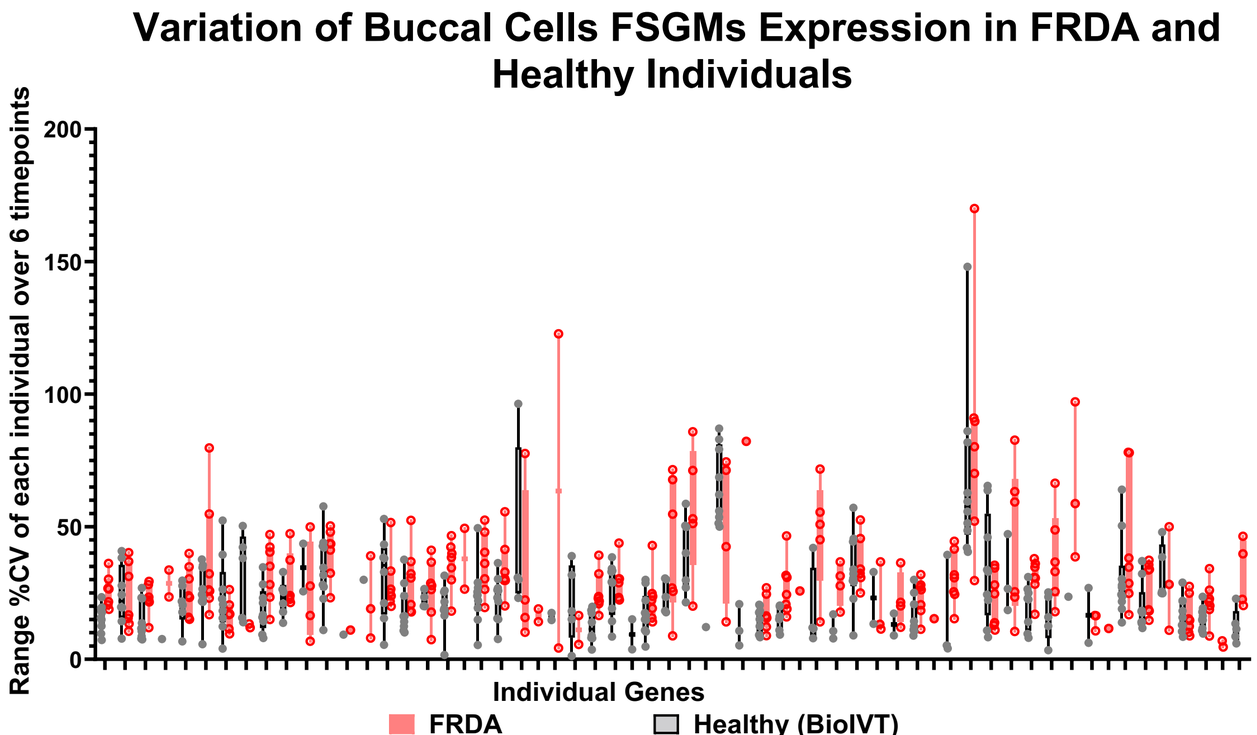


Specimen Collection in fasting pre-dose patients

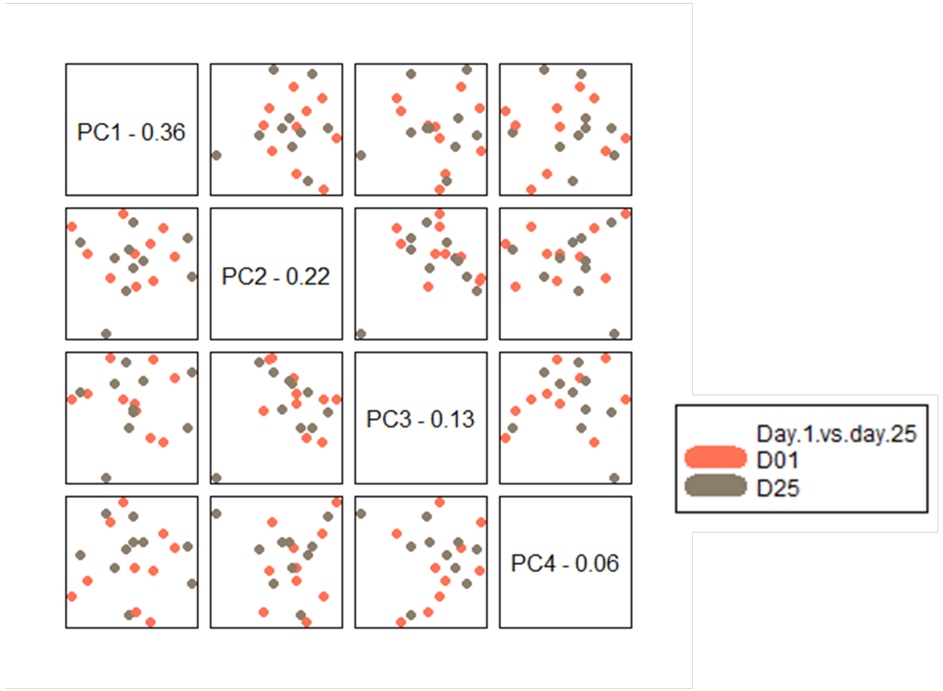
Gene Expression Analysis Discriminates Between Healthy Volunteers and FRDA Patients

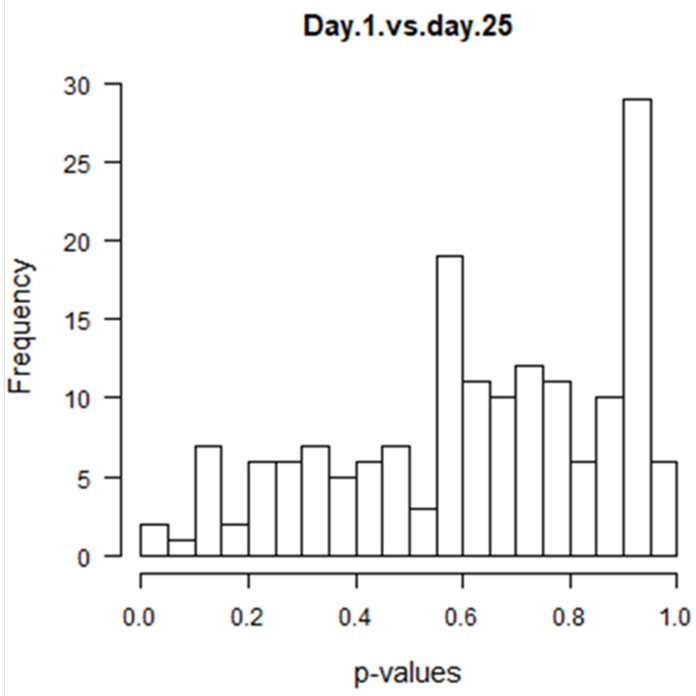




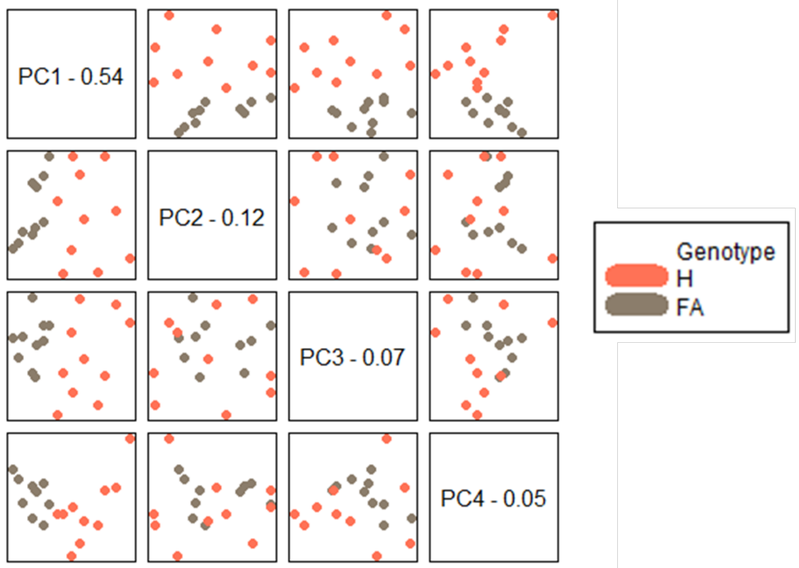


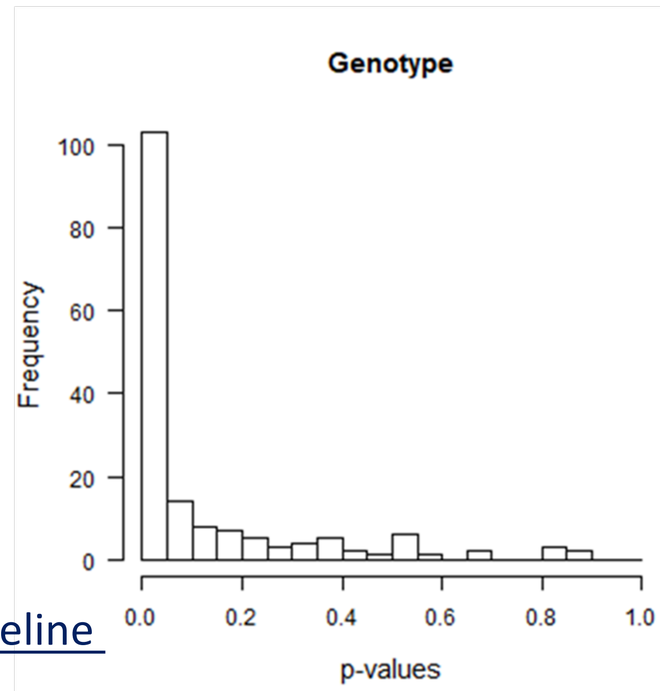
Gene Expression Analysis Discriminates Between Healthy Volunteers and FA Patients Populations





Healthy Volunteer Baseline vs Day 25





Healthy Volunteer Baseline vs FA Baseline

Conclusions

- Gene expression analysis can be a viable option for biomarker strategy in rare and orphan diseases associated with proteins that have no clear biological function
- Codeset approach constitutes a good alternative to genome wide analysis provided the target gene selection process is thorough, including determining robustness of gene expression in accessible tissue(s).
- Workflow is simple and can be performed with 1.5 FTE
- Data show tissue- and gene-specific variability of expression
- Data suggest that technology is robust enough and that its associated bioinformatics can discriminate between clinical populations
- Our data suggest that tissue gene expression analysis for biomarker discovery and evaluation in clinical context is achievable
- Regulatory guidance is scarce

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Thank you

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