

## The AnaBios Advantage

- High-quality human dorsal root ganglia cells
- Ethically-consented donor samples
- Functional, translational tissue samples

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AnaBios is one of the only contract research organizations in the United States with access to a vast network of hospitals with human tissue and intact human dorsal root ganglia (DRG) from consented donors. We have more than 10 years of experience procuring ethically-sourced human tissue samples processed utilizing proprietary methods to maximize success in experimentation involving proteomics, metabolomics and gene expression analysis. These specialized DRG tissue samples are ideally suited for supporting scientific research and drug discovery in therapeutic areas involving pain and itch.

## HUMAN DRG TISSUE & CELLS FOR *IN VITRO* ASSAYS

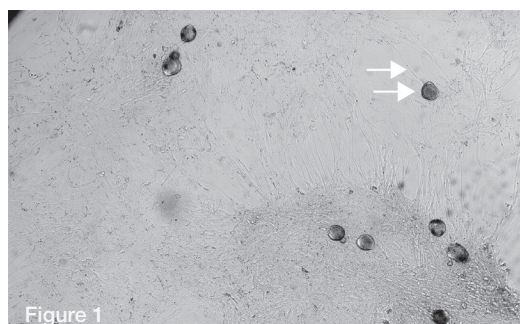


Figure 1

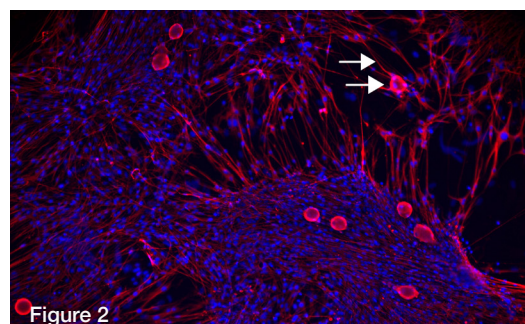


Figure 2

AnaBios recovers DRG (from thoracic level to sacral) from individual donors. The bilateral pair are recovered per vertebra level. Our DRG are either preserved in RNAlater by freezing or by fixation; or they are enzymatically dissociated into neuronal cultures (Figures 1 & 2). Figure 1 shows a brightfield image, while Figure 2 is an image of an immunostained neuronal marker (Beta III Tubulin in red, nuclei dye Hoechst33342 in blue). The top arrow shows a neuronal cell body while the bottom arrow identifies a neurite from that neuron. The nuclei from the numerous population of supporting glial cells are identified by Hoechst33342 stain. The neuronal cultures are fixed for immuno=cytochemistry assays or the cultures are used for testing compounds with in vitro electrophysiology assays.

# VALIDATION OF HUMAN DRG NEURONS

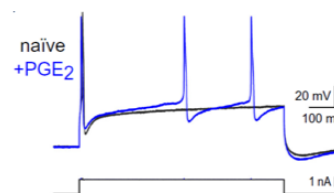
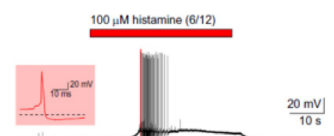
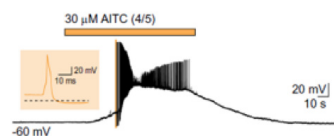
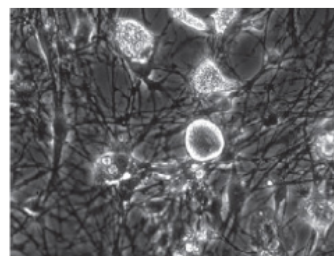
Functional Parameter	Measured Outcome	Significance
Resting membrane potential	$-70 \text{ mV} < V_m < -60 \text{ mV}$	<ul style="list-style-type: none"> <li>Physiological range</li> <li>Normal function of mitochondria and ionic pumps</li> </ul>
Action potential generation	Cells fire action potentials at $0.1 - 20 \text{ Hz}$	<ul style="list-style-type: none"> <li>Cells are electrically active and exhibit the physiological frequency range</li> </ul>
Reponse to known nociceptors	Heat, capsaicin, cold, mustard oil	<ul style="list-style-type: none"> <li>Cells are fully functional as nociceptive neurons</li> </ul>
Response to known pruritogens	Histamine, chloroquine, Bam	<ul style="list-style-type: none"> <li>Cells are fully responsive to irritants</li> </ul>
Sensitization by inflammatory agents	Sensitization can be induced by $\text{PGE}_2$ , bradykinin, ATP	<ul style="list-style-type: none"> <li>Normal pathological response attest to the integrity of the relevant biochemical pathways</li> </ul>

## HIGH-QUALITY TISSUE

AnaBios offers high-quality human DRG neurons ethically-sourced from consenting donors. Our human tissue samples are processed utilizing proprietary methods to maximize preservation of physiological function and success in experimentation involving functional end points or gene expression analysis, proteomics and metabolomics.

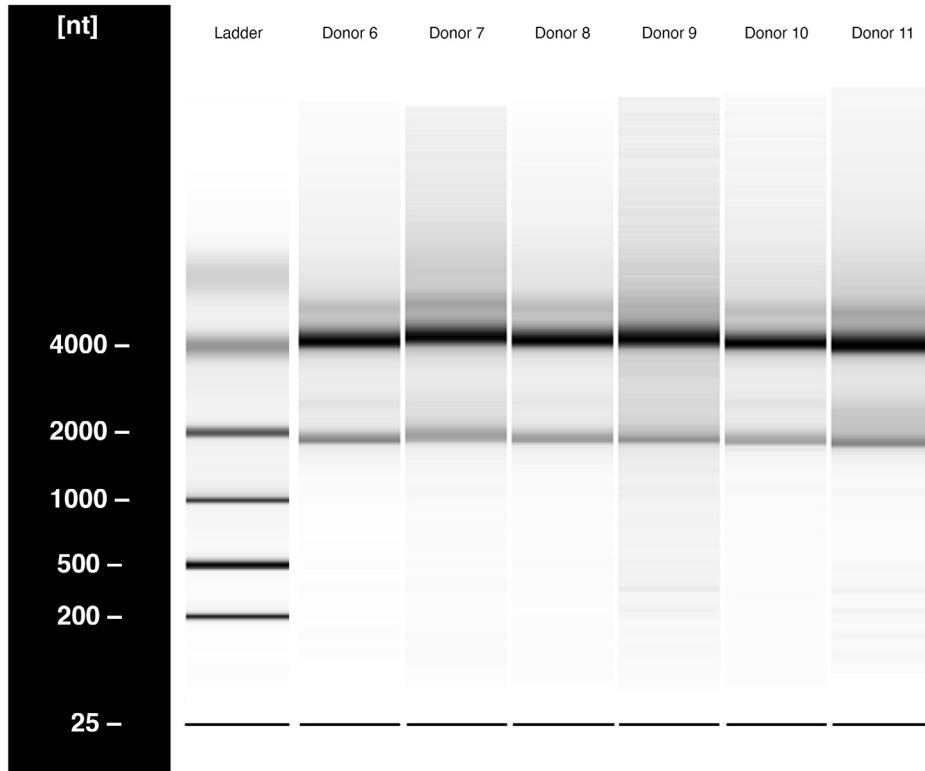
AnaBios offers normal and diseased human DRG tissue and provides demographic details, including sex, age, race and body mass index.

For more information about our human tissue samples, please email [info@anabios.com](mailto:info@anabios.com) or call (868) 366-8608.



**PICTURED:** Membrane voltage traces from isolated human DRG neurons patch clamped in current clamp mode. TRPA1 agonist (AITC) activated spontaneous action potential (AP) firing in a high fraction of DRG neurons (4/5, upper trace); and the pruritogen, histamine activated AP firing in 50% of DRG neurons (6/12, middle trace). The bottom trace shows the impact of inflammatory mediators,  $\text{PGE}_2$  and bradykinin on AP discharge. In DRG neurons from healthy individuals never exhibit secondarily evoked AP's in response to current stimulation, while a subset of DRG neurons exposed to 5 minutes of  $\text{PGE}_2/\text{BK}$  show continued firing in the presence of these molecules.

# HUMAN DRG TISSUE RIN SCORES



RNA integrity is an important quality parameter in gene expression studies and is key to ensuring isolation of high-quality RNA for drug discovery and academic research. RNA was extracted and isolated from flash frozen DRG tissue samples to determine its RNA integrity number (RIN) using an Agilent Bioanalyzer.

The image to the left shows the gel electrophoresis of the extracted RNA from the human DRG tissue. Bands for 18S and 28S ribosomal RNA are clear, indicating no RNA degradation. All RIN values are greater than seven, which indicates that RNA is intact and was not degraded during the tissue recovery process.

## PUBLICATIONS

1. Hordeaux et al. (2020) **Science Trans Med** MicroRNA-mediated Inhibition of Transgene Expression Reduces Dorsal Root Ganglion Toxicity by AAV Vectors in Primates <https://doi.org/10.1126/scitranslmed.aba9188>

2. Ray et al. (2018) **Pain** Comparative Transcriptome Profiling of the Human and Mouse Dorsal Root Ganglia: An RNA-seq-based Resource for Pain and Sensory Neuroscience Research <https://doi.org/10.1097/j.pain.0000000000001217>

3. Schwaid et al. (2018) **Comparison of the Rat and Human Dorsal Root Ganglion Proteome** <https://doi.org/10.1038/s41598-018-31189-9>

4. Shiers et al. (2020) **Quantitative Differences in Neuronal Subpopulations Between Mouse and Human Dorsal Root Ganglia Demonstrated with RNAscope In Situ Hybridization** <https://doi.org/10.1097/j.pain.0000000000001973>

5. Nickolls et al. (2020) **Cell Reports** Transcriptional Programming of Human Mechanosensory Neuron Subtypes from Pluripotent Stem Cells <https://doi.org/10.1016/j.celrep.2019.12.062>

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