Sarcometrics offers the following assays to quantify cardiac functional phenotype ex vivo.

Cardiac Slice Contraction-Relaxation Function: The experimental model system is the excitable cardiac slice prepared from mammalian left ventricle. Unlike other excitable preparations, sarcomere length can be established in this model system. Characteristics of isometric force transients (max force, max rate of force development, max rate of force decline, etc.) are quantified as measures of contraction-relaxation function and their dependence on sarcomere length, preload, and afterload including variable preload and afterload. In addition, cardiac slices will be used to generate force-length work loops, which mimic the pressure-volume work loops of a heart in vivo, at multiple sarcomere lengths. The dependence of contractility on sarcomere length, i.e., length-dependent activation also known as the Frank-Starling response in heart muscle, provides a measure of intrinsic contractility of the heart.

Cardiac Slice Intracellular Calcium Regulation: The experimental model system is the excitable cardiac slice prepared from mammalian left ventricle, loaded with CalRed AM, and exposed to excitation light at 490 nm. The ratio of emissions at 525 nm / 650 nm provides a measure of intracellular calcium concentration. Characteristics of intracellular calcium transients (max ratio, time to peak ratio, max rate of ratio development, max rate of ratio decline, etc.) quantify intracellular calcium regulation and can be used to distinguish the effects of disease, sex, intervention by drugs, intervention by known signaling agonists (e.g., isoproterenol), or gene therapies. This assay is often performed in conjunction with contraction-relaxation function.

Myofilament Force Production and Calcium Sensitivity: The experimental model system is demembranated mammalian myocardium, i.e., not excitable, that is ~120 um in diameter and 1 mm long. Sarcomere length can be visualized and used to establish starting muscle length, and isometric tension is recorded over multiple calcium concentrations (tension-pCa relationship)**.** The result indicates the maximum force per cross-sectional area of the muscle sample and the calcium sensitivity of the myofilaments.

Myosin Crossbridge Kinetics: Myosin crossbridge kinetics can be quantified in demembranated myocardium using sinusoidal analysis and a force-response to a quick stretch. Myosin crossbridge detachment rate and rates of force decline and redevelopment can be measured and used to distinguish the effects of disease, sex, intervention or gene therapy.

Cardiac Myocyte Contraction-Relaxation Function: The experimental model system is the excitable cardiac myocyte prepared from left ventricle. Characteristics of contraction-relaxation function include (max shortening, max rate of shortening, max rate of relaxation, etc.). Cardiac myocytes are especially useful for rapid detection of a drug candidate's dose response and cardiotoxicity with high sample counts, i.e., n=50-500.

Cardiac Myocyte Intracellular Calcium Regulation: The experimental model system is the excitable cardiac myocyte prepared from left ventricle. loaded with CalRed AM, and exposed to excitation light at 490 nm. The ratio of emissions at 525 nm / 650 nm provides a measure of intracellular calcium concentration. Characteristics of intracellular calcium transients (max ratio, time to peak ratio, max rate of ratio development, max rate of ratio decline, etc.) quantify intracellular calcium regulation and can be used to distinguish the effects of disease, sex, intervention by drugs, intervention by known signaling agonists (e.g., isoproterenol), or gene therapies. This assay is often performed in conjunction with contraction-relaxation function.

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Overview of animal use

- Mice and rats are fully anesthetized with isoflurane and euthanized by removing their hearts.
- Expect 4-8 usable cardiac slices from an adult mouse heart, 3-6 slices if mice are ~10-16 weeks old.
- Expect 6-12 usable cardiac slices from a rat heart.
- All aims can be performed using the same heart.

Deliverables

i) Raw data in tab-delimited text format readable in Excel.
ii) Detailed notes related to experimental conditions and performance.
iii) Representative raw data traces of isometric force transient, force-length work loops, and pressure transients for visual inspection of the effects of each genotype.
iv) Calculated parameter values provided in tab-delimited text format readable in Excel.
v) Results of statistical analyses as described above.
vi) Presentation by Principal Investigator of results and interpretations.

Personnel

Principal Investigator: Brad Palmer, Ph.D., has over 25 years experience designing and interpreting these types of measurements. He will design the experimental protocols, communicate with personnel and clients, perform data acquisition, perform data analysis including statistical analysis, and deliver results to client.

Research Associate: Lucy Pilcher, Ph.D., has over 5 years experience preparing these experimental models and performing these assays.

Technician: Greg Jacobs, A.S. has 1 year experience preparing and performing the experimental models using demembranated myocardium.