A Reintroduction to the Genomics Core

Who We Are and How We Can Help Your Research

Kevin Childs – Director Genomics Core

Who Am I?

- Assistant Professor Fixed Term in Department of Plant Biology
- Ph.D. Plant Physiology
- M.S. Computer Science
- Fifteen years genomics experience
- Interests
  - Plant abiotic gene networks
  - Genome annotation
  - Grass gene GC content
  - Codon usage and protein translation

Research Technology Support Facility (RTSF)

- Bioinformatics
- Flow Cytometry
- IVIS Imaging
- Mass Spectrometry
- Proteomics
- Genomics

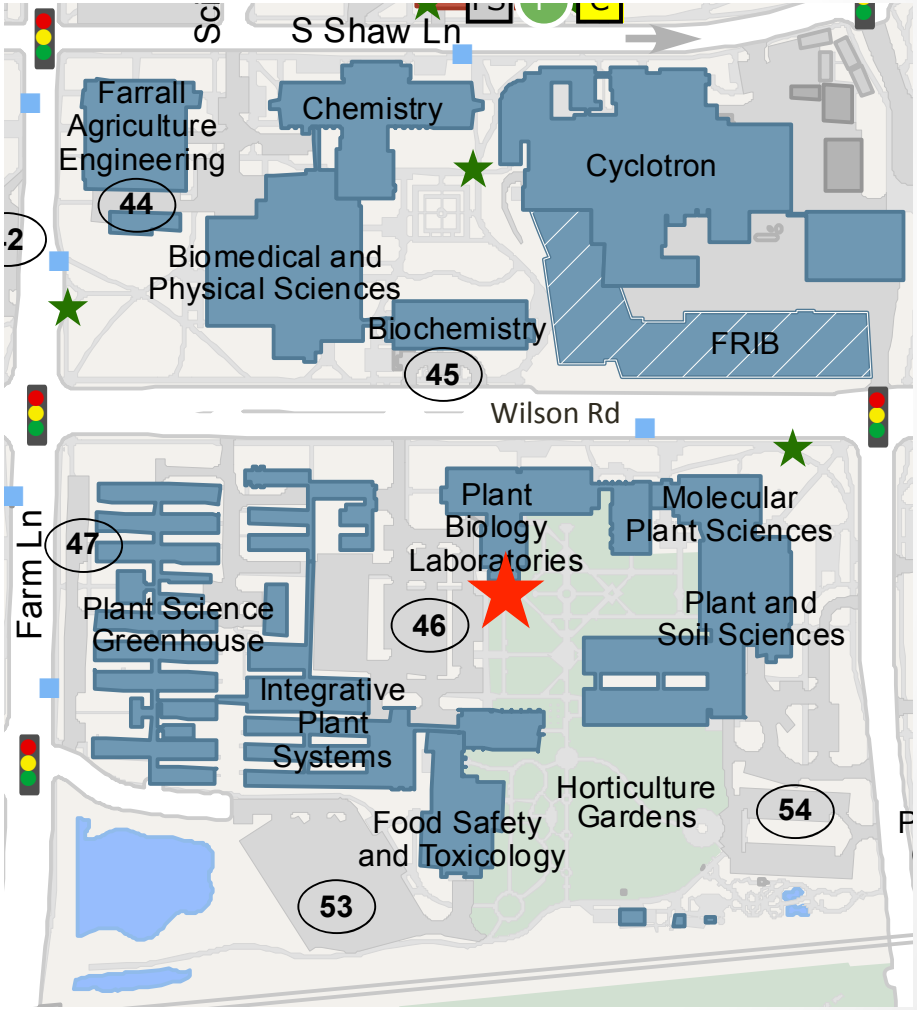
Genomics Core Staff

- Operations Manager
  - Emily Crisovan - gtsf@msu.edu
- Research Assistants
  - Shari Tjugum-Holland
  - Colleen Curry
  - Christi Harris
  - Melissa Borrusch
- Bioinformatics Specialist
  - Kevin Carr

Genomics Core Location

Plant Biology Laboratories
S18 and S20
(in the basement)

Sample drop off in
the refrigerator in the
hallway



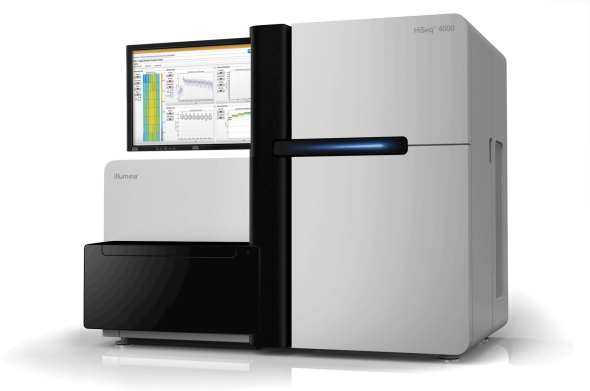
Overview of Services

- Next Generation Sequencing
- Sanger Sequencing
- RT-qPCR
- Probe-based quantification
- Liquid handling robots
- Miscellaneous instruments
- Self-serve instruments
- Workshops/Training
- Consulting

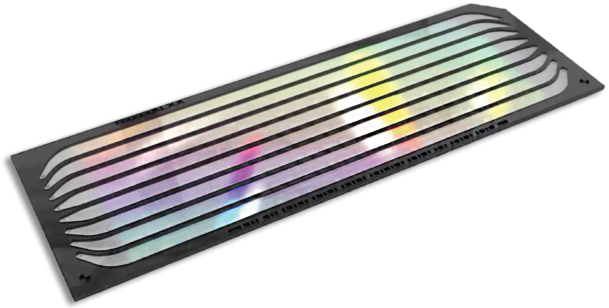
Next-Gen Library Preparation

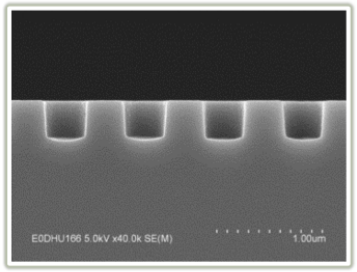
- DNA-seq libraries
  - Single end, paired end
  - Mate pair
  - Low input
  - Methylation-seq
- RNA-seq libraries
  - Stranded mRNA, total RNA, small RNA
  - Low input
  - Ribosome depletion
- Amplicon libraries
  - 16S, 18S, ITS

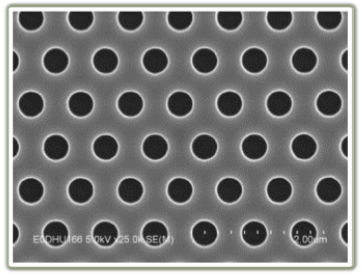
HiSeq 4000



- Most economical
- Eight lanes per flowcell
- Patterned flowcell
  - Biased towards small inserts
- SE50 and PE150 runs
- Typically 350 million reads per lane







NextSeq 500

- Less economical than the HiSeq
- One sample per flowcell
- Not a patterned flowcell
  - No biases
- Mid and High output flowcells
  - Mid output – 130 million reads
  - High output – 400+ million reads
  - SE75, SE150, PE35, PE75, PE150

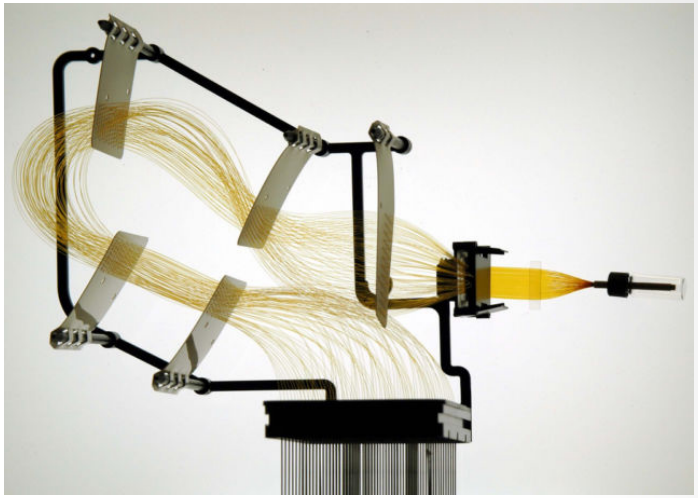
Two MiSeqs

- Least economical, but versatile
- One sample per flowcell
- Not a patterned flowcell
- v2 chemistry
  - Standard, micro, nano outputs
  - 1 to 12 million reads
  - SE50, PE150, PE250
- v3 chemistry
  - 22 million reads
  - SE150, PE75, PE300

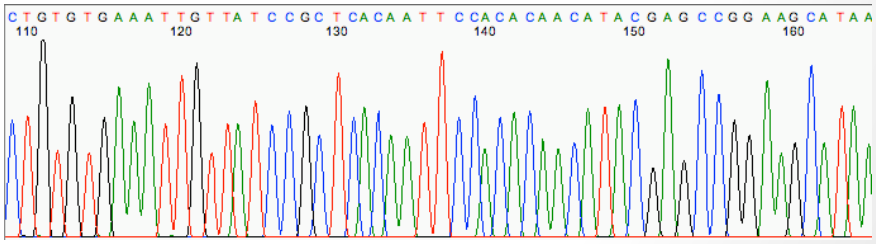
How Much Sequencing Do I Need??

- How many reads? How much coverage?
- Depends on several variables
  - Genome, transcriptome or something else?
  - How big is the genome?
  - How many genes?
  - What is your community?
- Which machine?
- How many lanes?
- Workshops this Fall

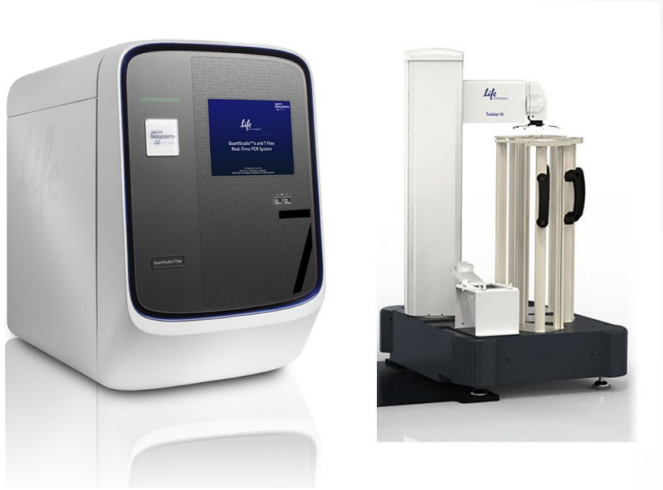
Sanger Sequencing



- ABI 3730xl sequencer
  - 96-capillary array
  - Sample loader holds 16 96-well plates
- Regular Sanger sequencing
  - Individual tubes (1 – 8)
  - 96-well plates (9 or more)
- Cell ID with Promega GenePrint for humans
- AFLP analysis
- SSR marker analysis



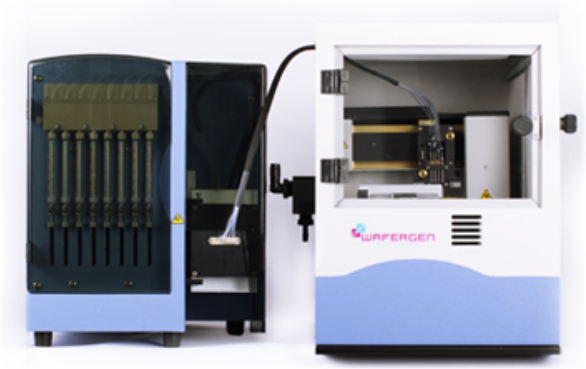
Real Time qPCR

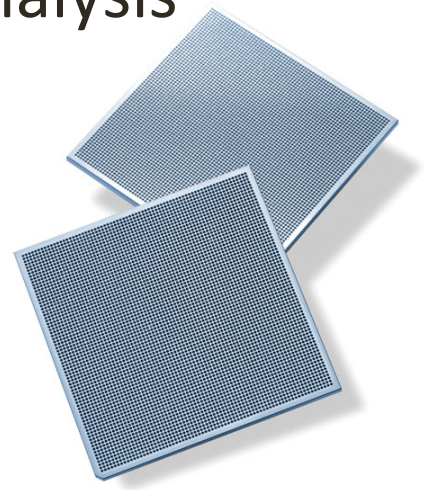


- QuantStudio 7
  - 384-well plates
  - Robotic plate handling
  - TaqMan probe assays
    - FAM, VIC, TAMRA
  - SYBR green assays
  - ROX normalization
  - Other dyes require calibration

Real Time qPCR

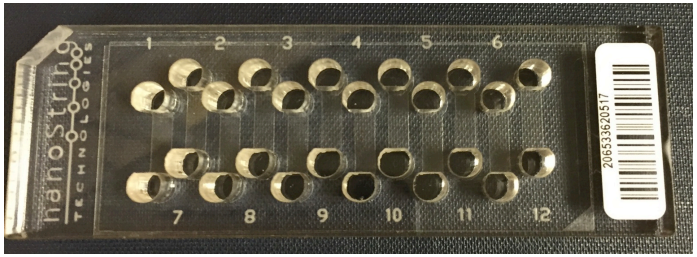
- SmartChip Analysis System
  - 5184-well chip
  - 100 nl wells
  - Loaded from 384-well plate
  - RT-qPCR
  - Copy number analysis

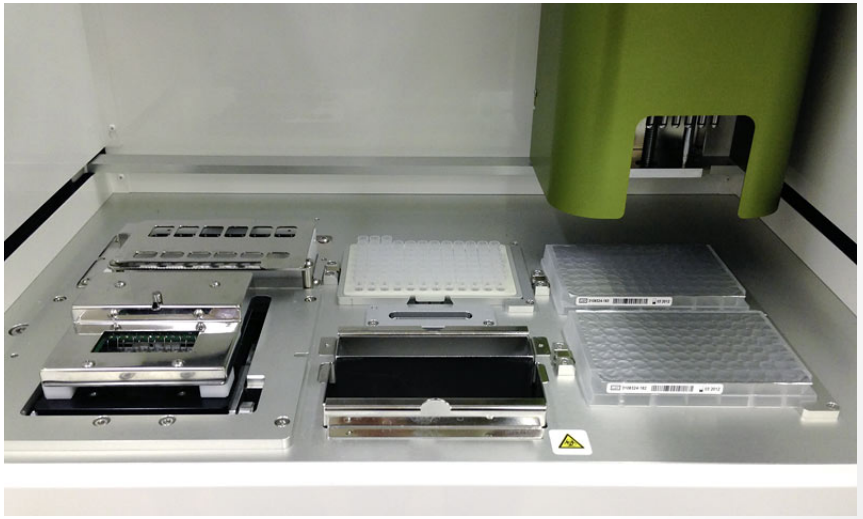




Assays	Samples
12	384
24	216
36	144
48	108
54	96
72	72
80	64
96	54
120	42
144	36
216	24
248	20
296	16
384	12

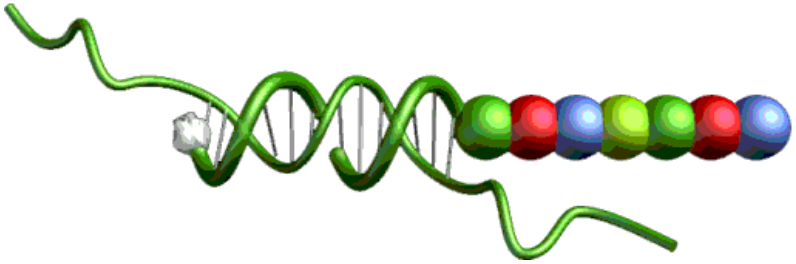
NanoString nCounter MAX

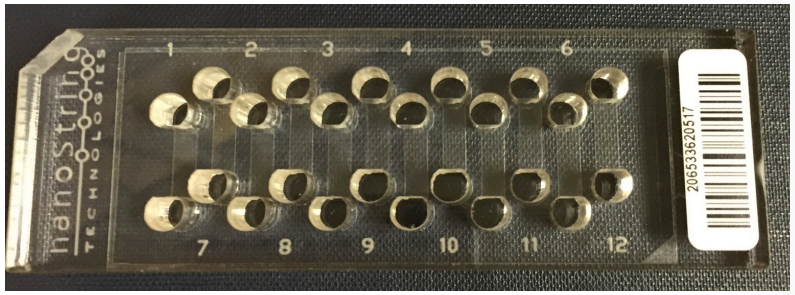


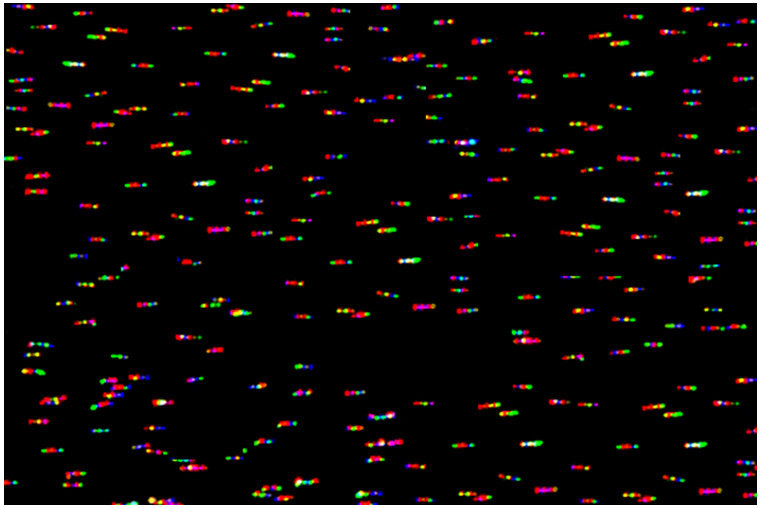


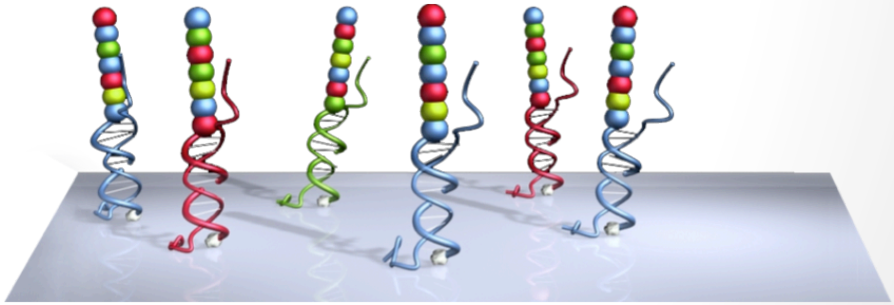
NanoString nCounter MAX

- Hybridization/quantification overview



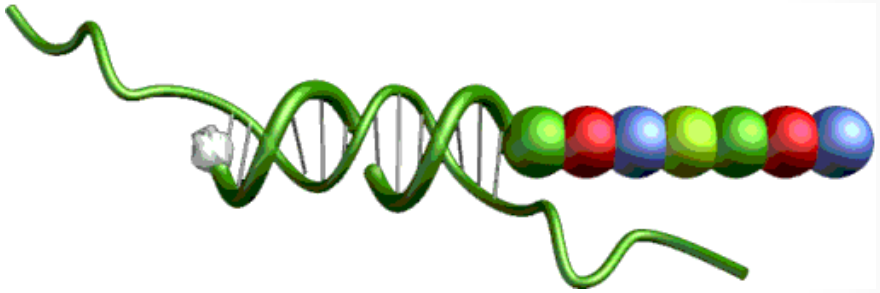






NanoString nCounter MAX

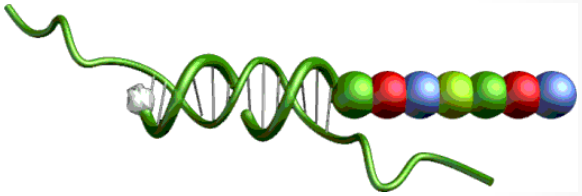
- Probe-based quantification
  - Capture probe
  - Reporter probe

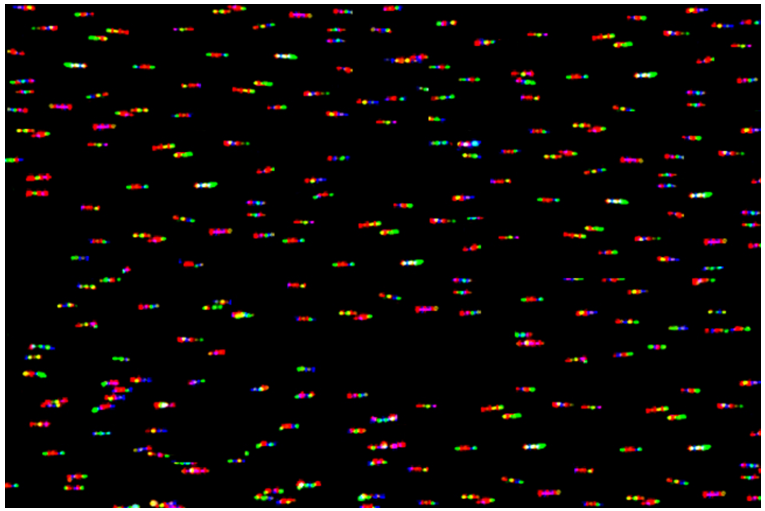


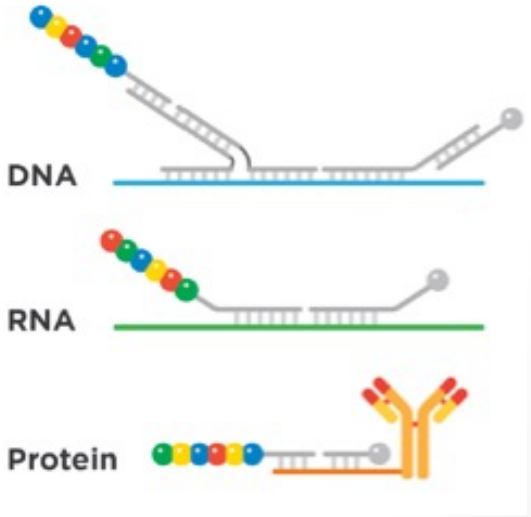
- Many stock panels
- Up to ~700 targets per panel
  - Human, rat, mouse
- Typical runs use 12 samples
- Custom probe sets possible
  - Multiplexing possible for 24/96 genes

NanoString nCounter MAX

- Quantifies mRNA, miRNA, DNA CNV, proteins
- Cost effective
  - 770 gene panel $275/sample
  - 200 gene panel $140/sample
- NanoString works closely with users

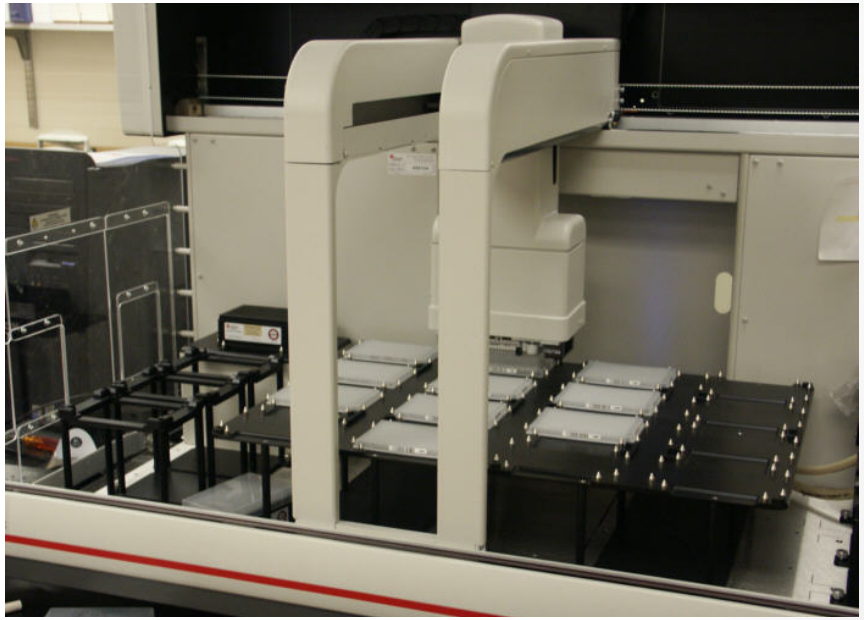


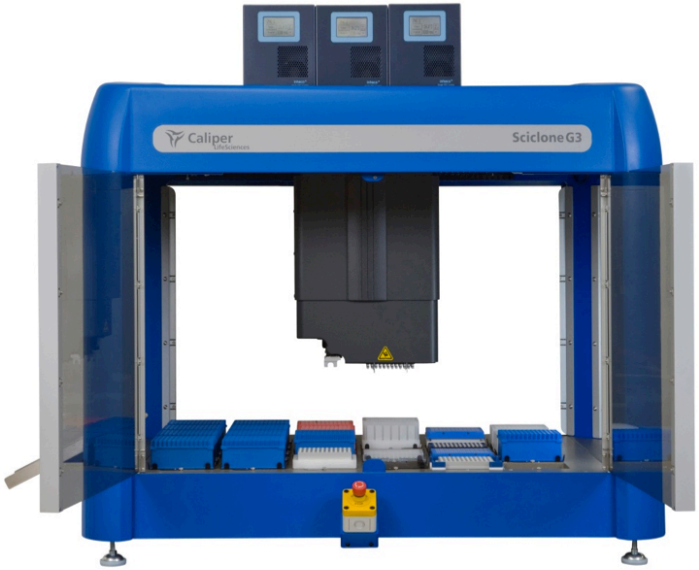




Automated Liquid Handling

- Biomek 3000 (8 channel)
- Biomek FX (96 channel)
- Sciclone G3





Miscellaneous Instruments

- Caliper LabChip GX
  - Multisample automated electrophoresis
- Blue Pippin
  - DNA size selection with pulse field electrophoresis

Self-Service Equipment

- Qubit fluorometer
  - Target specific fluorescence
- FLUOstar OPTIMA plate reader
  - Target specific fluorescence
- BioDrop spectrofluorometer
  - UV-absorbtion
- 2100 Bioanalyzer
  - Automated electrophoresis
- Covaris ultrasonic shearer

Where Is Your Data?

- FTP site
  - DNA-seq and RNA-seq
  - FastQC reports
  - Metagenomic/amplicon results
- Genomics Depot
  - Sanger results
  - RT-qPCR/marker analysis
  - nCounter results
- Instructions on our website
  - https://rtsf.natsci.msu.edu/genomics/

Consulting

Cost Recovery Pricing

- Pricing formula
  - Cost of reagents, disposables, labor, service contract
- Same prices for off-campus academic users

Long Read Sequencing

- PacBio Sequel
- Oxford Nanopore GridIon
- Coming soon…

Genomics Core Seminars

Sept 19 - Illumina RNA-seq libraries (Jeanne Geskes, Illumina)
Oct 3 - 10X Sequencing (Marie Adams, Van Andel Institute)
Oct 17 - Genome assembly strategies (Bob VanBuren)
Oct 31 - NanoString PlexSet (Peter Dornbos)
Nov 14 - Nanopore sequencing (Kevin Childs)
Dec 5 - SmartChip RT-qPCR/technical discussion (Maila Crist, Takara Bio)

12 PM to 1 PM
162-C Food Safety and Toxicology

You may sign up to our mailing list by sending the text "SUBSCRIBE GENOMICS-CORE" on a single line and as the only text in the body of an email to LISTSERV@LIST.MSU.EDU. Or write to Kevin at kchilds@msu.edu.