

The UC Davis Genome Center, Next-Generation Sequencing And Plant Breeding

Richard Michelmore The Genome Center, UC Davis

http://genomecenter.ucdavis.edu http://michelmorelab.ucdavis.edu Genome Center

> A global approach to biology



Wednesday, November 9th

Time (Pacific time) Event

12:00 - 1:00 PM

Location: Plant & Environmental Sciences Building (Room 3001)

Click here

to register



Bonus presentation

Speaker:

Dr. Kent Bradford Director, Seed Biotechnology Center (SBC), UC Davis

Dr. Bradford will report on the Gene Flow and Co-existence workshop held in Washington, DC on September 7 and 8, 2011. He will summarize discussions among industry and academia experts on current and emerging strategies to minimize gene flow and maintain seed purity across agricultural communities.

2:00 - 3:45 PM Seed Central SCIENCE

Speakers:

Research & Technology presentations

Location: Plant & Environmental Sciences Building (Room 3001)

Click here

to register

Dr. Richard Michelmore Director The Genome Center, UC Davis

Topic: Strategies for using modern genomics in plant breeding



Dr. Allen van Devnze Director of Research Seed Biotechnology Center (SBC) UC Davis

Dr. Michelmore and Dr. van Deynze will share the Genome Center's and the SBC's strategies for integrating modern genomic-assisted approaches to plant breeding practices.

4:00 - 4:45 PM

Environmental

(Room 3001)

Seed Central BRAINSTORMING Brainstorming session

Location: Plant & Topic: The potential for establishing on the UC Davis campus collaborative, pre-competitive research facilities focused on research Sciences Building and technologies for using modern genomics in plant breeding.

5:00 - 7:30 PM

Seed Central FORUM

Networking event with featured speaker

Location: Buehler Alumni and Visitors Center

> Click here to register

Featured speaker (6:00 - 6:45):

Dr. Jorge Dubcovsky UC Davis Professor and HHMI-GBMF researcher

Dr. Dubcovsky's research illustrates the power of modern biotechnology approaches in delivering new tools to breeders. In his keynote address, he will share the successes of the USDA-NIFA

Sponsored by





THE CENTRAL RESEARCH DOGMA

Basic research Translational research DATA → KNOWLEDGE → SOCIETAL BENEFIT

Transitioning from a data poor to data (over-)rich reality Need to adjust research, teaching, and application strategies Access to large amounts data is no longer limiting; People's (researcher's) time and attention are. Need to be more concerned with false positives and data quality than with false negatives

The Transition from a Data-Poor to a Data-Rich Reality

GENOTYPE + ENVIRONMENT => PHENOTYPE

Controlled input

Variable component

New measurement opportunities:

High-Throughput Sequencing & Genotyping Detailed metadata collection technologies GIS What to measure? Consequential output multiple aspects & levels

RNA: arrays, -> sequencing Proteins: MS -> arrays? ~500 -> ~200,000⁺ proteins Metabolites: MS profiling, -> ID endogenous & exogenous cpds Live imaging: Whole organism -> single cells

The UC Davis Genome Center: UCD's Response to the Opportunities and Challenges of the Genomics Revolution

Biological paradigms being worked out in model species Need to use genomic information for medical and agricultural benefits Comparative functional genomics increasingly informative Big strength at UC Davis = diversity of organisms studied UC Davis = one of largest & most diverse biology campuses in the world

Rapidly changing technologies Biology becoming more computational, data poor to data rich Genetic components of biological/medical studies essential

New genomics technologies expensive and optimal ones uncertain Centralized access to enabling technologies on as-needed, at-cost basis Ability to generate and manipulate very large datasets Can spend money much faster, even if cost per data point very low

http://genomecenter.ucdavis.edu

Senome center

Objectives:

To ensure diverse research campuswide remains current.

To house ~8 bioinformatics & 8 wet lab, technology-driven faculty.

To provide genomic technologies atcost, as-needed through service cores:



DNA Technologies, Expression Analysis, Proteomics, Metabolomics, Bioinformatics

Rationale:

No longer possible for a single investigator to do everything Need multi-disciplinary capabilities at-cost, as-needed. Not "Big Biology". Extension of individual labs. Enable innovation at individual level. Good ideas should not be technology limited

> Technology antenna for genomics on campus http://www.genomecenter.ucdavis.edu

The Transition from a Data-Poor to a Data-Rich Reality

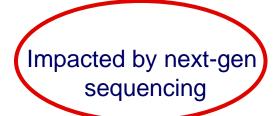
GENOTYPE + ENVIRONMENT => PHENOTYPE

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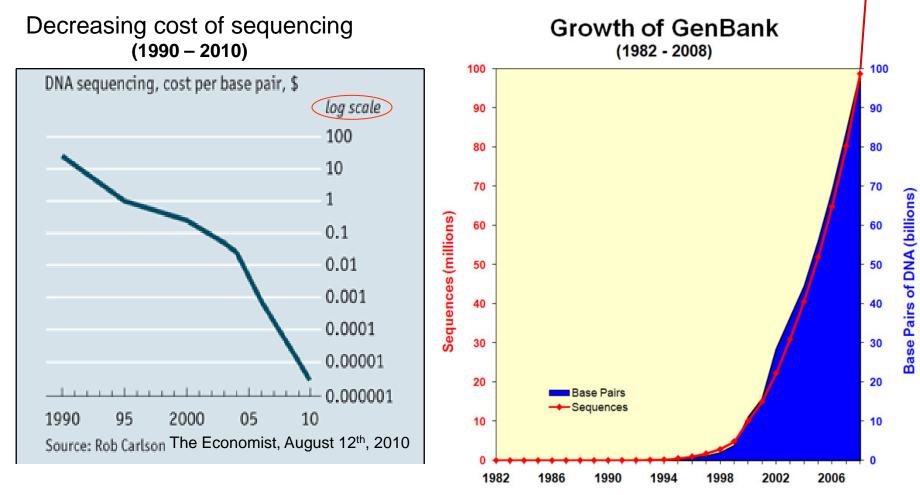
Consequential output multiple aspects & levels

New measurement opportunities: High-Throughput Detailed Sequencing metado & Genotyping collection



Detailed metadata collection technologies GIS What to measure?

RNA: arrays, -> RNAseq Proteins: Mass Spec -> arrays? ~500 -> ~200,000⁺ proteins Metabolites: MS profiling, -> ID endogenous & exogenous cpds Live imaging: Whole organism -> single cells DNA sequence becoming an inexpensive commodity. New paradigms as to how DNA sequence is generated, handled and valued.



Gary Shroth (Illumina): "A single lab with one HiSeq can generate as much sequence as was in GenBank in 2009, every four days".

Modes of Interaction & Recharge Categories

- Recharges per sample for standard analyses. Results ~guaranteed. GC assumes risk. Actual costs of consumables, labor, & service contracts. Minimal equipment and administrative overhead.
- 2) Research/exploratory mode.
 Recharges for units of project time, at cost.
 Results not guaranteed: only 'will do best'..
 Researcher assumes risk.
- 3) Annual training fee for 24/7 access to nonproduction machines for high-volume users.
- 4) Seed/pilot projects (\$2,000). Calls every ~6 months. Preliminary data for grant proposals

Priority: Campus > Off-campus > Commercial Clientele (cost + OH) (cost + 53% OH) (cost + 80% OH)

LATEST GENERATION SEQUENCING AND GENOTYPING AVAILABLE IN DNA TECHNOLOGY AND EXPRESSION CORES





Massively parallel DNA sequencing

- 2 Illumina Genome Analyzers
- 1 Hiseq 2000, 1 Miseq
- 1 Roche 454 Junior
- 1 Pacific Biosystems RS

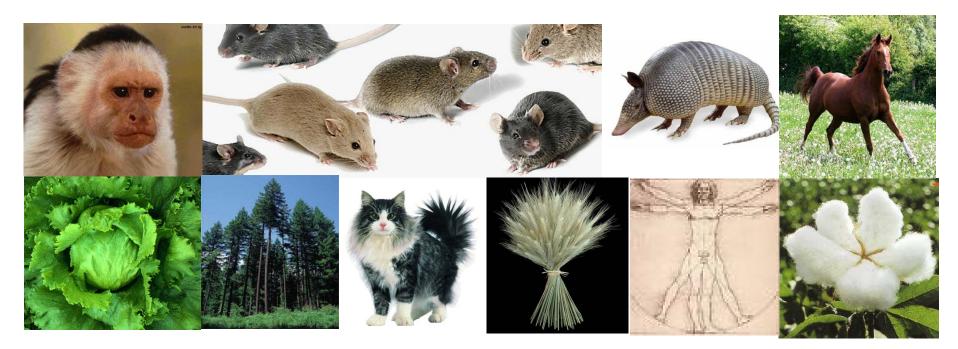
Uses

Transcriptome: Gene discovery Resequencing: Diversity analysis Highly efficient SNP discovery Expression analysis (RNAseq) Whole genome sequencing Metagenomics Segregation analysis: assembly validation

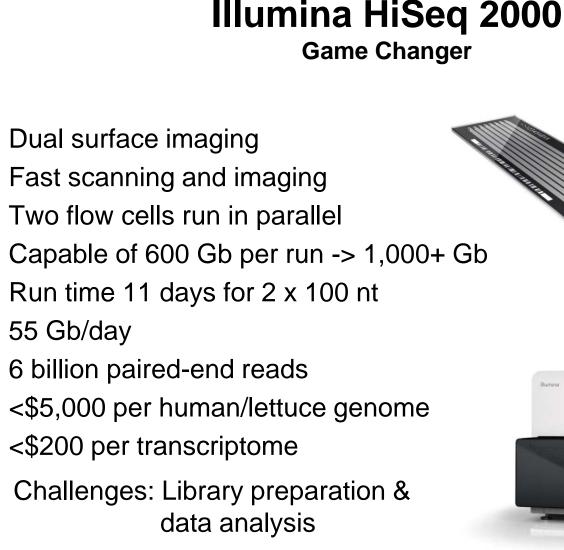
GoldenGate SNP genotyping iScan, BeadArray & BeadExpress

Ryan Kim, Core Manager

Examples of Organisms Genotyped in the DNA Technologies Core using Illumina GoldenGate SNP Assay



Example Project: David Neale, Plant Sciences, CA&ES. High Throughput Genetic Analysis of Trees. USDA CAP \$5.9M, \$1M to GC DNA Tech. 10,000 pine trees = ~all potential trees used for breeding in USA being scored for 7,600 genetic markers (often in candidate genes) per individual. Scored for plant characteristics e.g. growth and wood quality throughout US. Analyzed for metabolic characteristics by Metabolomics Core.



Gary Shroth (Illumina): "A single lab with one HiSeq is able to generate more sequence as was in GenBank in 2009, every four days".

THIRD GENERATION SEQUENCING



http://pacificbiosciences.com



Single Molecule Real Time (SMRT[™]) sequencing

Recording natural DNA synthesis by DNA polymerase as it occurs

Single molecule resolution

Simple amplification-free sample prep

Long reads, average read over 1kb, Poisson distribution to 3 – 4+ kb

Fast, 1 to 3 bases incorporated per second

Sample prep to data analysis in less than a day

Low overall costs

160,000 Zero Mode Waveguides (ZMWs) per SMRT cell

~33% of ZMWs have only one polymerase

15% error rate (indels)

Complementary to Illumina

Not for counting large numbers of tags or sequencing large genomes

UCDAVIS UNIVERSITY OF CALIFORNIA



The BGI@UC Davis Partnership:

Transforming Data into Knowledge for Societal Benefit

West Coast sequencing facility in UCD School of Medicine. Immediately: 3 + 2 CLIA Hiseq2000s. 2012: 10 to 20 Hiseqs.

Research collaborations: human & animal health, global food security, bioenergy, biodiversity, and environmental sustainability. Educational exchanges.

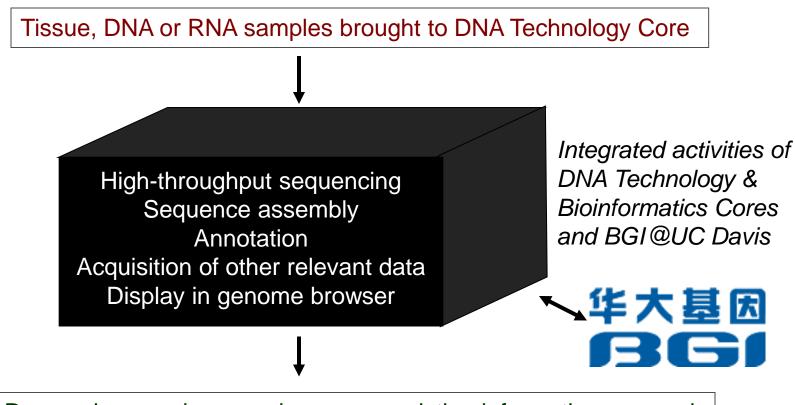
Access to sequencing and computational resources:

5 Tb/day = 1,600 human 1x, 312 wheat cultivars, 1 M bacteria

500 Tb in 2010 (= 10x NCBI over past 20 years), 100 Pb in 2011

Portal for other institutions.

UC Davis Sequencing & Gene Expression Service Cores



Researcher queries samples versus existing information over web

In near future: DNA sequence = an inexpensive commodity generated on a variety of platforms

\$1,000 (\$100?) human genome coming =>
 \$1,000 genome for many animals and plants
 \$100 genome for fungi
 \$10 genome for bacteria en masse

Metagenomics: sequencing of communities biomes (humans = 100x more bacteria) novel & unculturable organisms characterization of diversity & unique genes Not just genomic DNA sequence: DNA modifications epigenomics & copy number variation (CNV) expression analysis (RNAseq not arrays) Enormous amounts of sequence data Need for major data handling capabilities Vital role for bioinformatics

The Challenge and Opportunity: How to utilize the deluge of sequence data?

SEQUENCING APPLICATIONS: REVOLUTIONIZING BIOLOGY Genomic sequencing

De novo

Microbial, animal and plant diversity Novel & unculturable organisms Biomes (bacteria = 100x human)

Novel genespace

Re-sequencing

SNP and CNV discovery, TILLING Gene cloning, novel allelic diversity Genome Wide Association Studies (GWAS) High resolution population genetics Mapping, genotyping by sequencing BSAseq

Gene regulation

Transcriptome sequencing for gene models and splicing RNAseq for expression analysis Small and non-coding RNAs Ribosome profiling CHIPseq for DNA binding sites DNA modifications and epigenomics

Gene discovery & genotyping by sequencing of nonmodel organisms e.g. plant pathogens

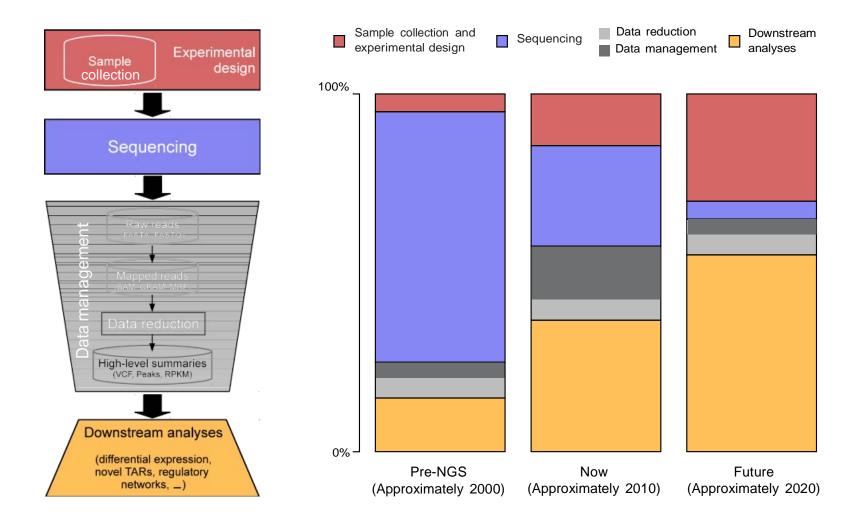
E.g. Cloning of 10 genes from wheat rust, *Puccinia striiformis* f.sp. *tritici* (collaboration with J. Dubcovsky)

Whole genome sequencing (<100Mb genome) Two lanes Illumina GA to ~60x. Quicker, cheaper, more informative than gene-by-gene. Permanent resource for other studies. Genotyping by low-coverage sequencing possible.



Cantu et al., 2011. Plos One

The real cost of sequencing: higher than you think! Sboner *et al. Genome Biology* 2011, **12**:125



COMPUTATIONAL CHALLENGES FOR CAMPUS (& ELSEWHERE): Need to greatly increase computational resources. Need local CPU and GPU compute clusters housed in

a dedicated, efficient facility with critical mass of diverse staff. Need to promote synergy between big data users on campus. Support for efficient use of resources, e.g. adaptations to GPUs. Need massive amounts of storage with reliable back-up. Need access to off-campus cloud and super-computing facilities. Need efficient methods of moving large amounts of data to

off-campus resources.



terabyte 10^{12} petabyte 10^{15} exabyte 10^{18} zettabyte 10^{21}



Petabytes in 2008... exabytes in 2011...?? in 2014...?? in 2017?

We need the proper equipment and we had better get it right.

BIOINFORMATICS SERVICE CORE

DATA ACQUISITION, CURATION, ANALYSIS, & DISTRIBUTION SUPPORT FOR TECHNOLOGY SERVICE CORES & INDEPENDENT BIOINFORMATICS PROJECTS STAFF SPECIALIZING IN DATABASES, SEQUENCE ASSEMBLY, ARRAY ANALYSIS, SYSTEM ADMINISTRATION, WEBSITES, et al. COMPUTE CLUSTERS (444 processors) & FAT NODES (1 @ 512 Gb RAM) **CPU & GPU NODES** SCALABLE STORAGE ARRAYS (300 Tb, Petabytes in future) **17 RACKS IN HIGH QUALITY MACHINE ROOM** ENABLE ACCESS TO EXTERNAL CLOUD COMPUTING

INTERFACE WITH BIOINFORMATICS AT BGI@UC DAVIS

Dawei Lin, Core Manager

Data-Poor to Data-Rich Environment

Biology becoming increasingly computational

Vast data sets from sequencing, genotyping and phenotyping Acquisition, curation, interrogation, integration, distribution Connectivity, data structures, computing speed and architecture, storage (archiving and retrieval)

Challenges: Data -> Knowledge

Identification of signal from noise

Relevant from irrelevant data: search tools

Good vs. poor relevant data: metadata and curation

Evaluation of significance of good, relevant data:

Query and statistical tools

Educated professionals and recipients

Utilization of knowledge

Integration with existing work flows

Development of novel products and workflows

Dealing with the implications of (uncertain) inferences

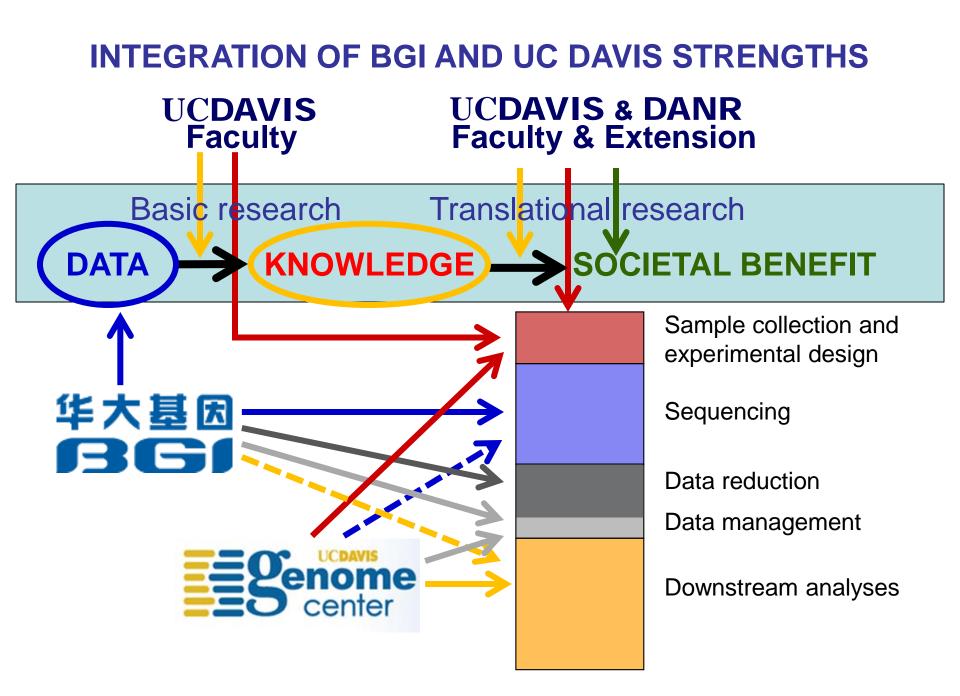
Implications of the Genomics Revolution

Very large amounts of sequence information for many organisms Genome sequences of individuals will become increasingly available Genetic predispositions for many normal and pathological traits The molecular basis of numerous normal

and pathological states will be understood Intervention strategies will lag behind knowledge of genetic predisposition and molecular understanding High resolution and very large datasets on genotypes and phenotypes at multiple levels: protein, metabolite, whole organism. Need to consider phenomena at the systems level.

Challenges:

Including a genetic component into many types of studies Sorting the signal from the noise Converting data to knowledge Staying current, training & sustaining sufficiently informed practitioners Conveying the useful information to the patient/public Dealing with the implications of (uncertain) inferences [when intervention strategies not available (yet)] Societal/ethical/legal issues of confidentiality and (mis)use



GENOMIC OPPORTUNITIES FOR BREEDING COMPANIES

Basic research Translational research DATA KNOWLEDGE SOCIETAL BENEFIT Pre-competitive research Commercial application

Collaborative, pre-competitive, knowledge-generating projects.

Development of ultra-dense, gene-based genetic maps with agriculturally phenotypes. Genome sequencing.

Analysis of crop diversity.

Identification of candidate genes for breeding targets.

Marker-assisted selection.

Monitoring pathogen populations to direct resistance gene deployment.

Genetic Mapping & Marker Development

Old paradigm (slow and inflexible): One-by-one marker development. Utilization of core set of reference markers.

Current paradigm (faster but specific to populations): Sequence transcriptome of parents to identify 10,000s of SNPs. Develop informative SNP panels for specific sets of crosses. Run SNPs on segregating individuals.

Latest paradigm (fast, flexible, & highly informative): Sequence segregating individuals.

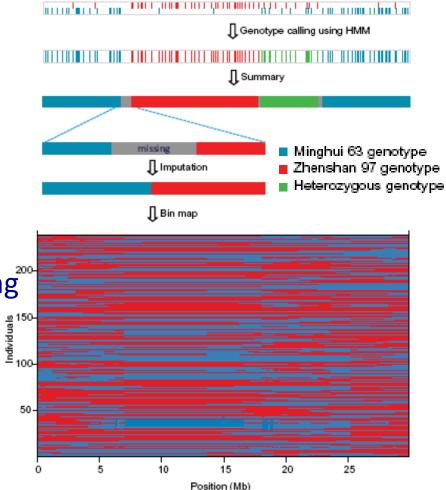
Rate limiting steps: Informative populations. Accurate phenotyping. Library preparation. Sequencing not limiting. Data analysis.

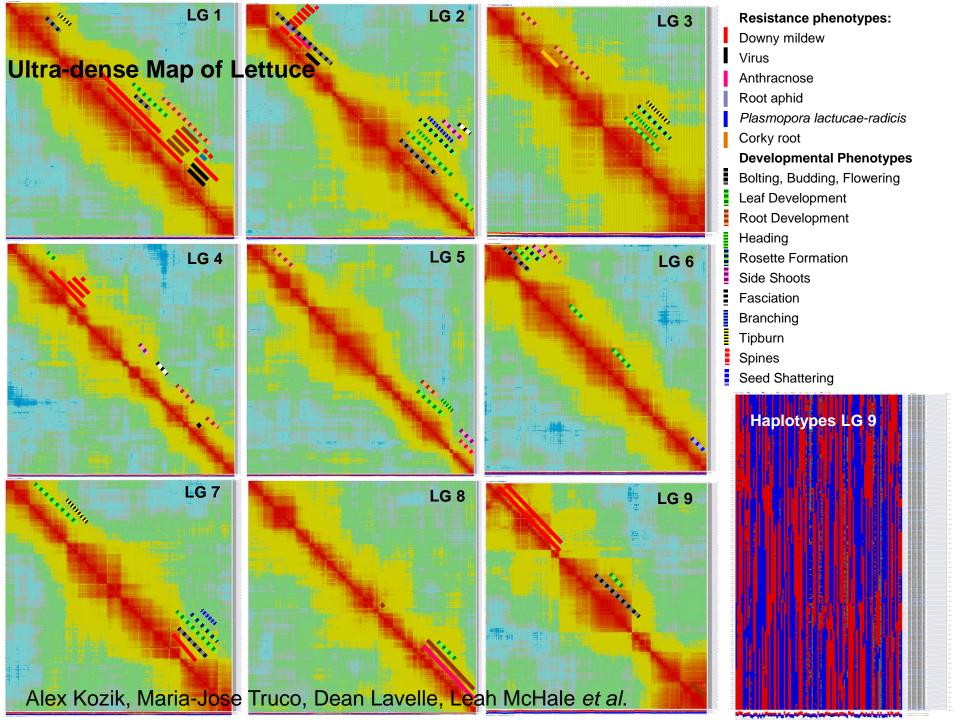
Parent-independent genotyping for constructing an ultrahigh-density linkage map based on population sequencing. *Xie et al., 2010.* PNAS 2010.

238 rice RILs each sequenced
to 0.055x, 13x in aggregate.
Barcoded and multiplexed.
2x 36 nt paired-end reads,
20.6 Mb total single run.

Genotypes inferred from RILs using maximum parsimony of recombination & HMM.

New capabilities => any species tractable in a single run.



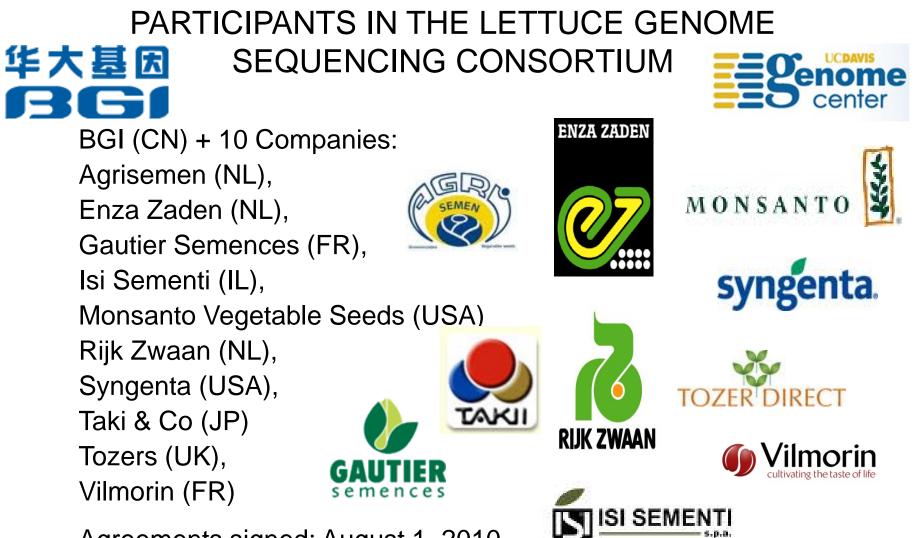


GBrowse Display of Lettuce PseudoSequence

🔮 LettuceAlpha Database: 20 kbp from LG1:11,625,21411,645,213 - Mozilla Firefox	x
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Image: State in the map calculation	
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13,788 mapped ESTs separated by 'N's scaled to map distance for 9 chromosomes

http://gviewer.gc.ucdavis.edu/cgi-bin/gbrowse/lettucePublic/ Sebastian Reyes Chin-Wo



Agreements signed: August 1, 2010

First draft to participants: January, 2010. Annotated draft: March 2011. Final report to participants: August 1, 2011.

Public data release: 12 months after release to participants.





Lettuce Genome Sequencing

Assembled into 13,352 scaffolds > 2 kb, 552,061 > 100 bp, N50 = 460 kb. 2.5 Gb of total 2.7 Gb genome = 93% assembled.

Genomic sequence data integrated with transcriptome, gene-space, to generate annotated draft genome sequence.
73% repeats, mostly retrotransposons and unknown repeats.
44,229 gene models, 79% genes functionally annotated.

Displayed using Gbrowse.

Assemblies validated using genetic data from ultra-dense map. ordered into chromosomal linkage groups. integrated with phenotypic and syntenic information.

Annotation and gene models refined at jamboree.

Zhiwen Wang, Song Chi, Xu Xun BCI & Sebastian Reves Chin-Wo et al.

Lettuce Genome Annotation Training and Jamboree

September 28th to October 19th, 2011

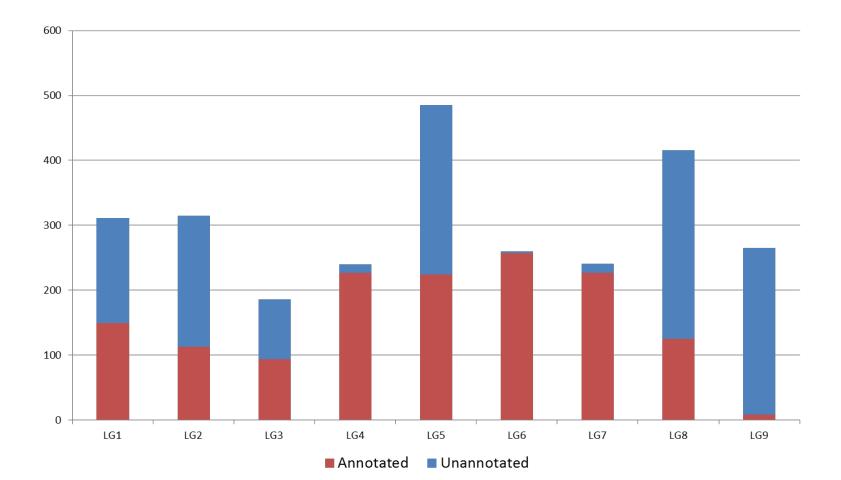


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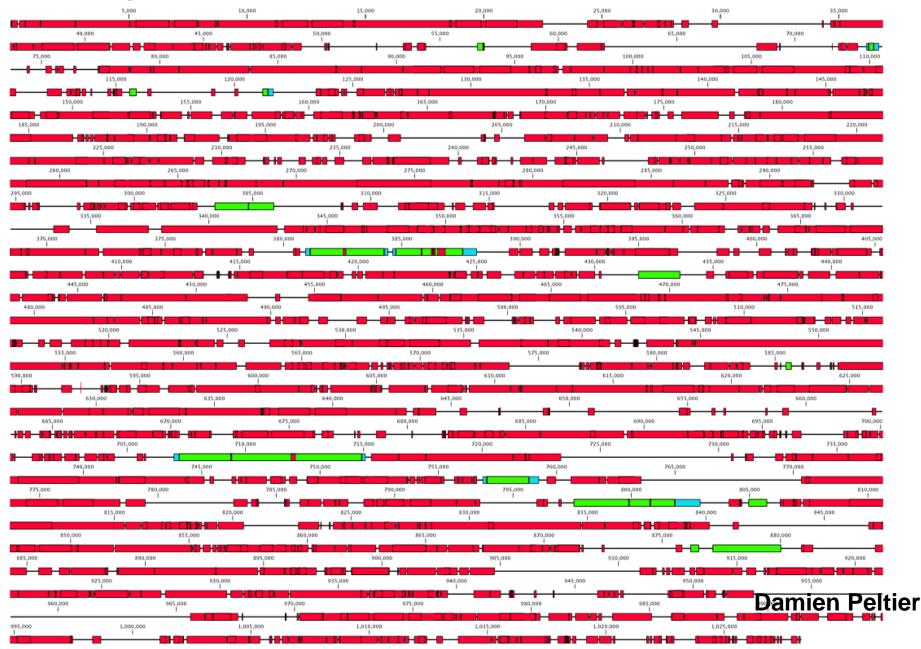
Participants Breeding companies: 11 BG1: 2 Michelmore lab: 12 Non-UCD academics: 9 Non-UCD students: 7 Countries: 7 Compositae species: 10+

JAMBOREE ANNOTATIONS

Eight teams of 3 to 4 people, each assigned a chromosome 8,333 gene annotations (~18% of 45,000 total) ~1,600 scaffolds (52% of mapped scaffolds, ~10% of total)



Lettuce genes are located in a "sea" of repeated sequences



Validation of Whole Genome Assemblies: Cosegregation of Unigenes in Scaffolds

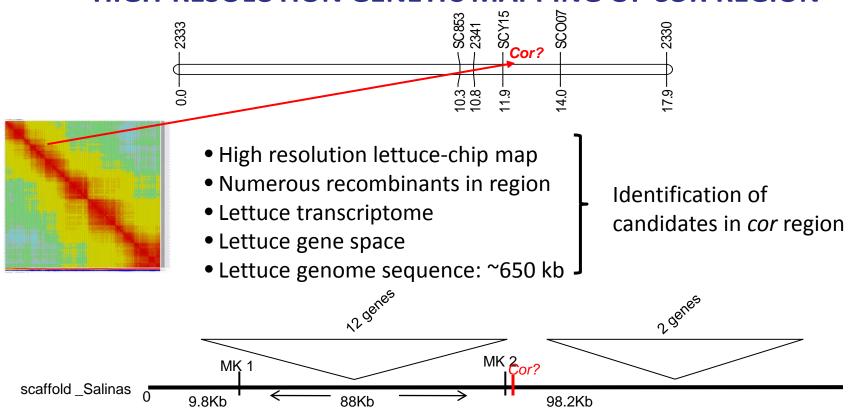
- 11,279 mapped unigenes align with 3,201 scaffolds
- Average 3 unigenes per scaffold (1 to 30)
- 3,023 (94.5%,) all unigenes co-segregate = correct assembly
- 87 (2.7%) unigenes map to 2 cosegregating groups = simple chimeras
- 79 (2.5%) unigenes map to 1 cosegregating group + single anomaly
- 9 (0.3%) unigenes map to 3 or more linkage groups = mis-assembly
- ~60% assembly (~55% total) in chromosomal linkage groups

Sebastian Reyes Chin-Wo, UCD

Genetic Coherence of Scaffolds on part of Chromosome 2

Zhiwen Wang, BGI

HIGH-RESOLUTION GENETIC MAPPING OF COR REGION



Disease screens of F₅ families



Future directions:

- Fine map relative to candidate gene(s) recombinants & BSAseq
- Assess allelic variation
- Functional analysis: RNAi
- Complementation analysis

Manju Govindarajulu, Oswaldo Ochoa

COMPONENTS OF A GENERIC PLANT GENOMICS PROJECT

- Generation of a high-quality, annotated reference genome sequence. Single genotype, draft assembly and automated annotation. Then manual annotation and finishing. Continual long-term process.
- 2. Development of ultra-high density genetic maps.

Genespace or WGS of ~100 descendants from one or more crosses. Genotyping by sequencing. Chromosomal ordering of assemblies. Candidate genes for traits.

3. Characterization of allelic variation.

Genespace or WGS of 500 to 1,000 diverse (randomly-mated) individuals. Genome-wide association studies (GWAS).

Fine mapping of traits to candidate genes.

4. Development of a gene expression atlas.

RNAseq from multiple tissues, developmental stages and conditions. Functional inferences on candidate genes.

5. Phenotyping and validation of marker associations and candidate genes.

Multi-locational testing of key genotypes and populations.

Mutant and transgenic analysis.

Dissection of complex traits. Systems biology.

6. Epigenetic analysis.

Requires technology development for routine global analysis of crop species.

- 7. Development and application of new technologies and analytical tools.
- 8. Database for curation, analysis and distribution.

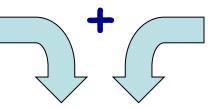
IMPACT OF HIGH THROUGHPUT SEQUENCING & MASSIVELY PARALLEL GENOTYPING ON RESISTANCE STRATEGIES

- Global rather then gene-by-gene analysis
- Saturation of identification of candidate genes Recognition, signal transduction, response SNPs in causal genes
- Characterization of germplasm
 Full genome resequencing of 10 100 genotypes
 Gobal genotyping of 1,000 10,000 genotypes
 Natural variation in 1°, 2°, & 3° genepools
 Vast numbers of resistance genes available
- Characterization of pathogens
 - (A)virulence factors
 - Pathogen variability
- Gene deployment
 - Marker assisted selection of causal genes Pyramids of multiple genes, conventional & transgenic Heterogeneity between genotypes in space & time Fragment selection pressure on pathogen populations Manage pathogen evolution

PATHOGEN POPULATION GENETICS SHOULD DRIVE DEPLOYMENT OF RESISTANCE GENES

Continual sampling of pathogen Virulence phenotyping Gene-space sequencing (10s) SNP genotyping (1000s)

Resistance gene discovery pipeline Germplasm screens Mapping, molecular markers Molecular characterization



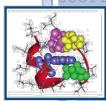
Deployment of effective resistance genes Pyramiding, MAS or effector-driven selection Allo- and sympatric diversity Temporal adjustment of resistance genes deployed Transgenic approaches for novel resistance strategies

Genome Center Courses Fall, 2011



Genome Center

A global approach to biology



Monday, August 29 – Friday, September 2 Proteomics and Metabolomics (Brett Phinney and Oliver Fiehn)

Tuesday, September 6 – Friday, September 9 Sequencing Library Preparation. (Henny O'Geen and Ryan Kim)

> Monday, September 12 – Friday, September 16 Next-Gen Sequence Analysis (Dawei Lin)

Monday, September 19 – Tuesday, September 20 Cloud Computing for Bioinformatics (Dawei Lin)

Wednesday, September 21 – Friday, September 23 PERL for Bioinformatics (Ian Korf)

Monday, September 26 – Tuesday, September 27 Data Analysis and Visualization Using R (Dawei Lin)

Wednesday, September 28 – Friday, September 30 Genome Annotation (Barry Moore and Ian Korf)

Details available at <u>http://courses.genomecenter.ucdavis.edu</u>

GENOME CENTER CONTACTS FOR ASSISTANCE



Dr. Ryan Kim. Director, DNA Technologies & Expression Cores. <u>rwkim@ucdavis.edu</u>



Dr. Dawei Lin. Director, Bioinformatics Core. <u>Ihslin@ucdavis.edu</u>



Dr. Brett Phinney. Director, Proteomics Core. <u>bsphinney@ucdavis.edu</u>



Professor Oliver Fiehn, Faculty lead, Metabolomics Core. <u>ofiehn@ucdavis.edu</u>

http://genomecenter.ucdavis.edu

Current RWM Lab Members

María José Truco Oswaldo Ochoa Tadeusz Wroblewski Lutz Froenicke Katie Caldwell Dean Lavelle Joan Wong Marilena Christopoulou Keri Cavanaugh Manjula Govindarajulu Juliana Gil Miguel Macias Gonzales **Pauline Sanders** Funding Natalie Pelter

Collaborators

BGI: Zhiwen Wang, Song Chi, Xun Xu Bicheng Yang, Sanwen Huang, GCP: Loren Rieseberg, Steve Knapp, Kent Bradford, Rick Kesseli, John Burke, David Still, Zhao Lai Allen van Deyzne & the LetChip team Ian Korf Ryan Hayes et al. (USDA Salinas) Jean Greenberg & lab (U. Chicago) Managers & staff of the GC Cores Many others for specific materials & populations

Databases & Bioinformatics

Alex Kozik Huagin Xu **Bertrand Perroud** Sebastian Reyes Chin-Wo Christopher Beitel **Damien Peltier** Davide Scaglione Belinda Martineau

NSF Plant Genome & 2010 UDSA NRI, AFRI, SCRI Seed Company Gifts UC Biostar Program & Bioseeds Co.s Novozymes Inc. Endowed Chair Ca. Leafy Greens Research Board The Lettuce Genome Sequencing Consortium http://michelmorelab.ucdavis.edu