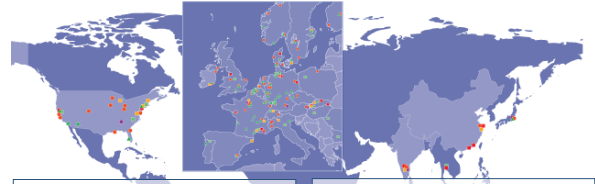**eurofins**
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The Eurofins Agrogenomics Initiative
-
Strategies for Molecular Breeding

Dr Georg Gradl

Takahiro Obana

Eurofins MWG Operon

Eurofins Global Map

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Eurofins is a leading provider of testing, analytical and support services for environmental, agriculture, food, pharmaceutical and consumer products, for industries, governments and scientific communities.

>**35** countries, >**14.000** employes, >**180** labs

> 1 Billion **€** revenue in **2012**

Eurofins is a leading provider of testing, analytical and support services for environmental, agriculture, food, pharmaceutical and consumer products, for industries, governments and scientific communities.

>32 countries, >12.000 employes, >170 labs

> 1 Billion $ revenue in 2011

Eurofins' leading market positions

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No 1
worldwide

No 1 to 3
worldwide

No 1
worldwide

Food & Feed Testing

Testing for Pharma/ Biotech

Environment Testing

Services to the biotechnology industry are within the 3 major business lines

No 1 in Europe
No 1 in Germany
No 1 in France
No 1 in Scandinavia
No 1 in Benelux
No 1 in the UK*
N 1 in Brazil

No 1 worldwide in pharma product testing
Among top 3 global providers of central laboratory, genomic and agrosciences services
No 1 or 2 in most segments/ countries in Europe

No 1 in Europe
No 1 in Germany
No 1 in France
No 1 in Scandinavia
No 1 in Benelux

To the best of Eurofins' knowledge, based on data available to the Group   * except routine Bacteriology - focus on high end analysis
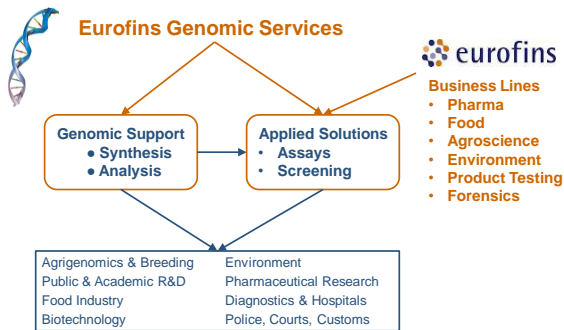
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Eurofins Genomics Services

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Eurofins Genomics is the genomic service provider of the Eurofins Group with 5 major production sites in USA, Europe and Asia, and a total of appr. 500 staff

Aarhus, Denmark

Munich/Ebersberg, Germany

Huntsville, AL

Bangalore, India

Tokyo, Japan

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How Eurofins Genomics works

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Eurofins Genomic Services

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Business Lines
• **Pharma**
• **Food**
• **Agroscience**
• **Environment**
• **Product Testing**
• **Forensics**

Genomic Support
● **Synthesis**
● **Analysis**

Applied Solutions
• **Assays**
• **Screening**

Agrigenomics & Breeding
Public & Academic R&D
Food Industry
Biotechnology

Environment
Pharmaceutical Research
Diagnostics & Hospitals
Police, Courts, Customs

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Genomics Services within the Eurofins Portfolio

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Environment Testing	Agro-Sciences	Food & Feed Testing	Genomics Services	Pharma Services	Product Testing

Eurofins Genomics Campus

•Pharmacogenetic and Pharmacogenomic Services, Diagnostics, Forensics, Applied Genetics: Regulatory Market
•Custom DNA Sequencing, Next Generation Sequencing, Gene synthesis, Oligonucleotide Synthesis: R&D Market

Eurofins AROS

•Affymetrix & Illumina Microarrays
•Illumina HiSeq Next Generation Sequencing
•High throughput qPCR (Fluidigm)
• Experience in Pharma R&D, Biotech and human research

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Platforms at Eurofins Genomics

- Roche Genome Sequencers Junior and FLX/FLX++
- Illumina MiSeq and HiSeq 2000/2500
- ABI 3730 XL 96/ 3100/3130 16 Capillary Sequencers
- ABI TaqMan 7500, 7900HT, Low density arrays Roche Lightcycler LC 480 II
- Fluidigm Biomark
- Sequenom MALDI-TOF
- Affymetrix 3000 G7 and GeneTitan
- Illumina BeadStation and iScan
- Beckman Pipetting Robots
- Hamilton Robotic Systems
- PerkinElmer Chemagen Chemagic MSMI
- Qiagen QIAsymphony, Universal Workstation, QIAcube

Food & Feed Testing within the Eurofins Portfolio

Environment Testing	Agro-Sciences	Food & Feed Testing	Genomics Services	Pharma Services	Product Testing

Eurofins Genomics Campus

- Leading Competence in DNA based tests

Yellow Fin Tuna
Thunnus albacares

Skipjack Tuna
Katsuwonus pelamis

Agro Sciences within the Eurofins Portfolio

Environment Testing	Agro-Sciences	Food & Feed Testing	Genomics Services	Pharma Services	Product Testing

The Eurofins Agrogenomics Initiative

- **Large, connected portfolio**
- **Services interfacing to reduce client workload**
- **Efficient and flexible**
- **Low risk for client**
- **Global presence and offering**
- **Willing and able to fill gaps in portfolio, capacity, expertise**
- **Pro-active and innovative**
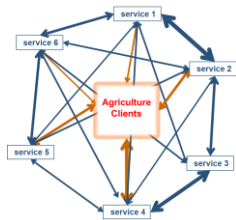
... be client-oriented, scalable and easy

About Molecular Breeding

- Early agriculture development started independent some 15.000 years ago in Near East, China, Africa and South America.
- After several thousand years of conventional breeding, modern agriculture started end of the nineteenth century with artifical fertilizer and early chemical pest control.

Marker assisted selection (MAS)

- The development of DNA (or molecular) markers has irreversibly changed the disciplines of plant genetics and plant breeding. While there are several applications of DNA markers in breeding, the most promising for cultivar development is called marker assisted selection (MAS).

- What are the advantages of MAS?
- Simpler compared to phenotypic screening
- Selection may be carried out at seedling stage
- Single plants may be selected with high reliability.
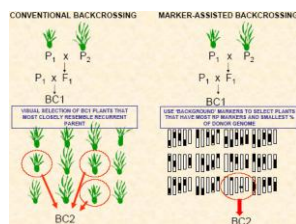
Introduction to RAD-Seq

What is RAD-Seq and why use it?

- **Restriction Site Associated DNA Sequencing (RAD-Seq)**
- **Genome "complexity reduction" technique**
- **Interrogates a small part instead of whole target genome**
- **Tags are screened for presence of genetic variation (e.g. SNPs)**

Advantages:

- **reduced sequencing cost vs. whole genome approaches**
- **high-multiplex sequencing**
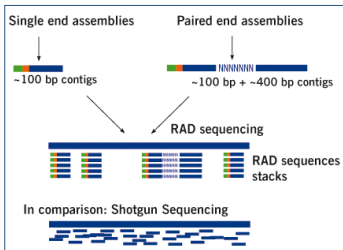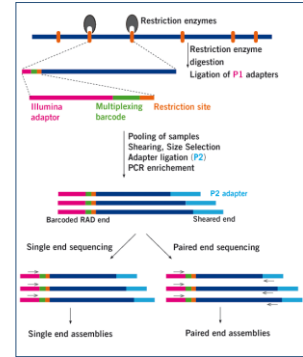- **more streamlined bioinformatic analysis**

About RAD-Seq

What types of scientific studies are possible with RAD-Seq?

RAD-Seq is a genetic variant discovery and genotyping system

- **Genetic Marker Discovery**
- Identify and catalog large numbers of genetic variants (e.g. microsatellites, SNPs and InDels)
- **Local Genome Assembly**
- De novo genome assembly for survey sequencing, comparative genomics and variant detection
- **Genotyping by Sequencing (GBS)**
- Develop genotype information for linkage maps association mapping or population genetic analysis

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From wet lab to sequencing

RAD-Seq protocol:

a. **DNA digestion**
b. **Adapter ligation (P1)**
c. **Pooling samples**
d. **Shearing**
e. **Size selection**
f. **Blunting / A-tailing**
g. **Adapter ligation (P2)**
h. **PCR enrichment**
i. **Size selection**

Bioinformatic basics

- DNA is digested with a specific set of restriction enzymes and two adaptors ligated to the fragments afterwards.
- Sequencing the fragments from either one side or both sides results in RAD sequences stacks.
- The stacks are the perfect starting point to identify and analyse genetic markers.

RAD Genotyping of *Helianthus annuus L.*

De novo sequencing of sunflower genome for SNP discovery using RAD (Restriction site Associated DNA) approach

Venkatramana Pegadaraju, Rick Nipper, Brent Hulke, Lili Qi and Quentin Schultz
BMC Genomics 2013, 14:556

- Sunflower is one of the leading oilseed and confectionery crops in North America,
- Annual crop mass of approx. 1 billion kilograms
- Economic value >720 million US$
- RAD-Seq (PstI) resulted in >15.1 Mb de novo sequence
- >94,000 putative SNPs identified in 6 sunflower lines
- 16,467 SNPs used for an Illumina Infinium Genotyping Array


RAD-Sequence Analysis

- 6 lines are RAD sequenced: RHA 464 and BDI lines 01 – 05
- RHA 464 was de-novo assembled
- All 5 lines are aligned against the contigs
- From 105,662 putative SNPs 16,464 SNPs meet the criteria for Array design

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RAD-Seq assembly results and repeat elements contribution
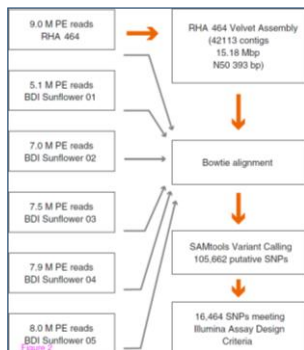
A. The length distribution of RAD-Seq contigs is plotted as a histogram.
B. The contribution of known repetitive elements in the *H. annuus* RAD sequence assemblies is shown. Results were obtained through RepeatMasker analysis using the Repbase Arabidopsis database.

1.75% of A) was classified as repetitive and is shown in B)

3
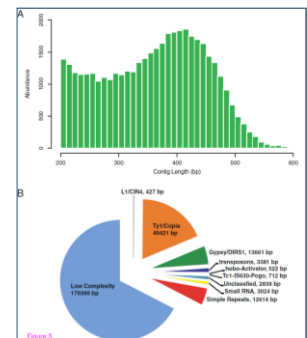
Helianthus SNP Discovery

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A. The number and ratio of SNP transitions and transversions observed in the Helianthus population is graphed.

B. The frequency of SNPs by position in each respective contig is plotted.

**C.** The number of sequence variations observed across each RAD-Seq contig is shown.
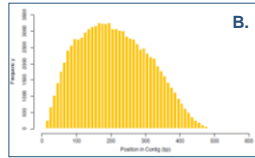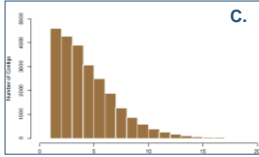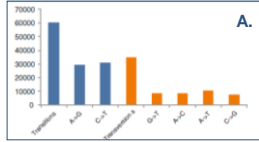
Summary: Marker assisted selection

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- **MAS is the perfection of 15,000 years of convential agriculture.**
- **With a growing world population and climate change , we need to speed up the production of volume and quality of food and feed.**

Can we even go a step further?

Some thoughts about „genetic engeneering"

## Genetically Modified Organisms (GMOs)

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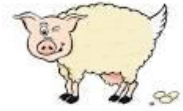
- Genetically modified organisms (GMOs) can be defined as organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally. The technology is often called "gene technology" or "genetic engineering". It allows selected individual genes to be transferred from one organism into another, also between non-related species.
- Such methods are used to create GM plants
- This results e.g. in increased tolerance towards herbicides or insects or optimisation of ingredients (golden rice).
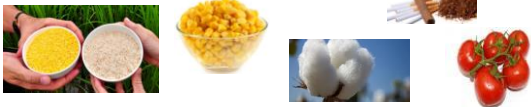
## The effect of genetic modification

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- Insect resistance is achieved by incorporating into the food plant the gene for toxin production from the bacterium *Bacillus thuringiensis* (BT).

- Herbicide tolerance is achieved through the introduction of a gene from a bacterium (glyphosat or bromoxynil).

- While theoretical discussions have covered a broad range of aspects, the three main issues debated are tendencies to provoke allergic reaction (allergenicity), gene transfer and outcrossing.

$$HOOC\text{-}CH_2\text{-}NH\text{-}CH_2PO_3H$$
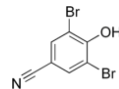
Detection of transgenic DNA in GMOs

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- **Detection of illegal GMOs**
- **Support in breading to get approval for registration**
- **Testing of identity of food ingredients with the wild type**

SCIENTISTS ARE HOPING TO ISOLATE A **BIG HAND GENE** TO GIVE PEOPLE THE POWER OF FLIGHT.
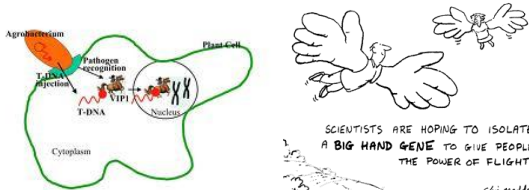
Targeted Resequencing

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- **Enrichment of selected genomic regions of interest by custom designed capture oligonucleotides**

Typical Applications:
- **Whole Exome Sequencing**
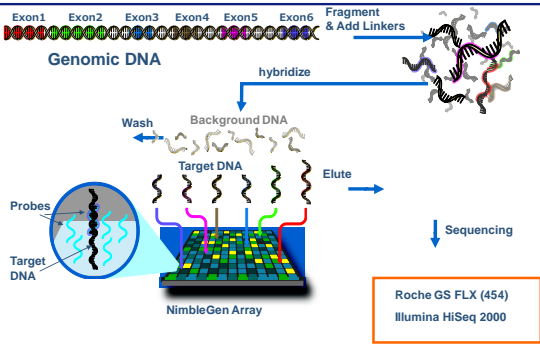- **Sequencing of selected genomic regions**

- **Special Applications:**
- **Chromosomal Breakpoint Analysis**
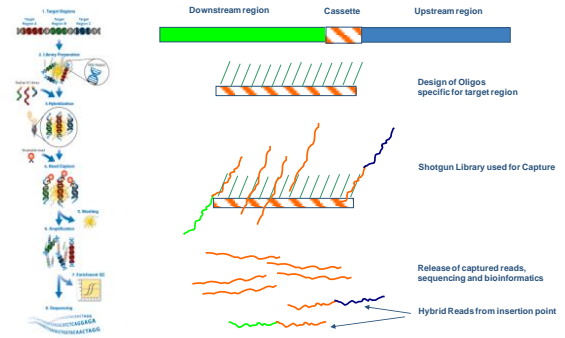- **Analysis of Cassettes and Insertion Points in GMOs**

Process Scheme

Exon1 Exon2 Exon3 Exon4 Exon5 Exon6

Genomic DNA

Fragment & Add Linkers

hybridize

Background DNA

Wash

Target DNA

Elute

Probes

Target DNA

NimbleGen Array

Sequencing

Roche GS FLX (454)
Illumina HiSeq 2000

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Why can we find Insertion Points?

Downstream region | Cassette | Upstream region

Design of Oligos specific for target region

Shotgun Library used for Capture

Release of captured reads, sequencing and bioinformatics

Hybrid Reads from insertion point

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Example of a transgene in a chromosome

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Targeted Locus Amplification (TLA)

- In combination with Next Generation Sequencing technologies, the TLA Technology enables the complete targeted sequencing of any genomic locus of interest. The TLA Technology is hypothesis neutral and will detect all mutations and structural variants.

- TLA is a proprietary technology of Cergentis B.V., Netherlands in early stage development.

- We are currently beta-testing TLA.

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Key features and principles of the Targeted Locus Amplification (TLA)

- The TLA Technology uses the physical proximity of nucleotides within a locus of interest as the basis of selection
- TLA uses a locus specific tag (depicted in yellow) for the targeted amplification and complete sequencing of any locus of interest (depicted in red).
- First, genomic DNA is crosslinked with a crosslinking agent
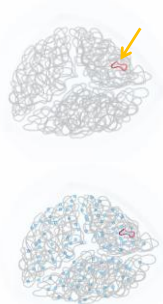
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TLA in Detail

- Following crosslinking sequences within any locus are physically linked.

- This cross-linked DNA is then cut with a restriction enzyme and in turn re-ligated with a ligase enzyme


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TLA in Detail
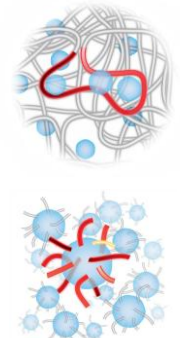
- This results in circular DNA fragments that consist of a number of restriction fragments that originated from the same locus.
- Stochastic variation in the folding and crosslinking of every individual copy of a genome results in a repertoire of ligated DNA circles that are composed of unique combinations of restriction fragments from that locus.
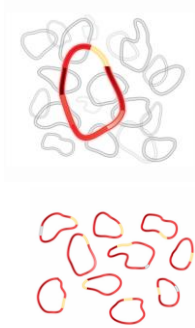

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- These circular DNA fragments are then amplified with inverse primers corresponding to the locus specific tag sequence.
- As a result, the complete region of interest is amplified and can be sequenced using Next Generation Sequencing technologies
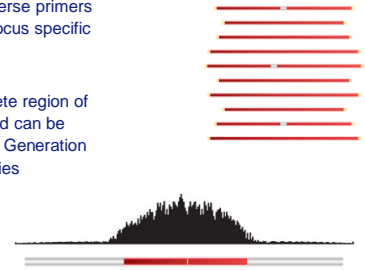
32

- TLA enables targeted hypothesis neutral sequencing. It detects all sequence and structural variants in loci of interest, also in heterogeneous samples such as tumors.
- TLA permits multiplexing. Loci can thus be analyzed using multiple locus specific tags and different loci of interest can be amplified and sequenced *in one analysis*.
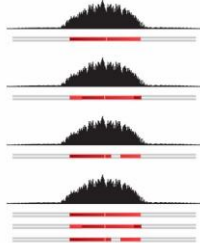
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Summary

- **Molecular breeding has the potential to revolutionize agriculture.**
- **Eurofins Genomics has the organization, know how and platforms to support plant and animal breeding organizations worldwide.**
- **Eurofins Scientific as an analytical testing supplier has also the power and tools to control such activities in order to ensure safety and health for consumer, environment and the future.**

We are happy to answer your questions now

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