Next Generation Sequencing in Next Generation Agriculture

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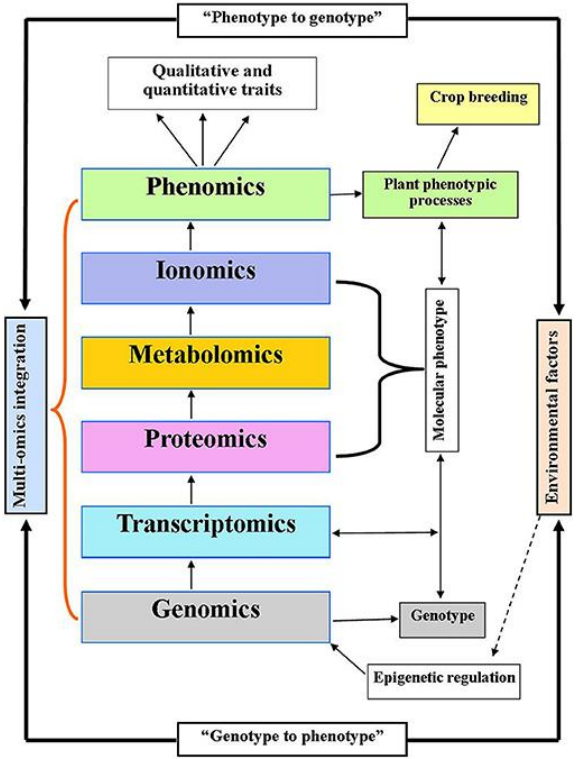
Next Generation Agriculture

Multi-omics technologies are being used today to move the crop breeding and management to the next generation agricultural era!

The combination of different omics analysis has helped scientist in identifying potential candidate genes and their pathways and created the path for MAS and genetic modifications via traditional breeding and genome editing approaches.



frontiers in Plant Science

REVIEW
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Applications of Multi-Omics Technologies for Crop Improvement

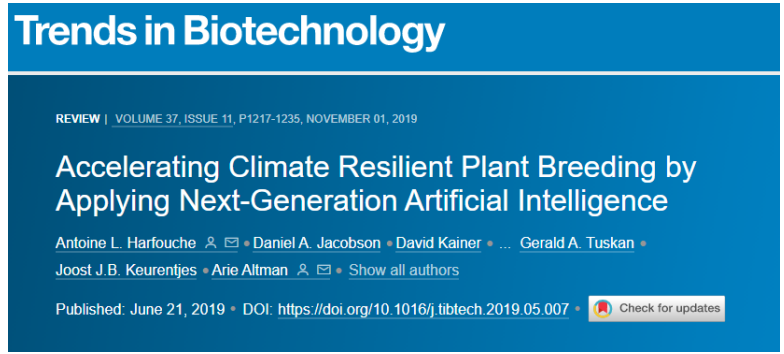
Yaodong Yang[1†], Mumtaz Ali Saand[1,2†], Liyun Huang[1], Walid Badawy Abdelaal[1], Jun Zhang[1], Yi Wu[1], Jing Li[1], Muzafar Hussain Sirohi[2] and Fuyou Wang[1]*

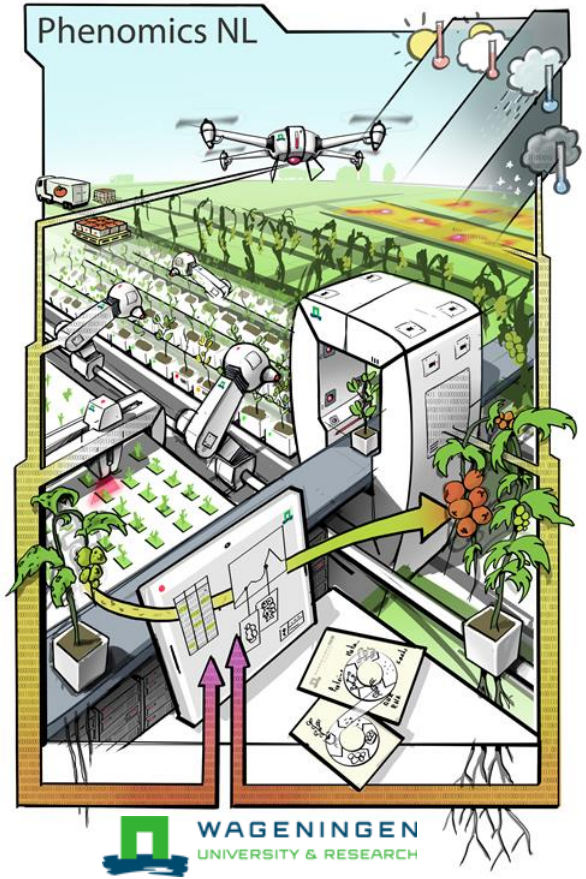
Next Generation Phenomics and AI

IPPN

International Plant Phenotyping Network

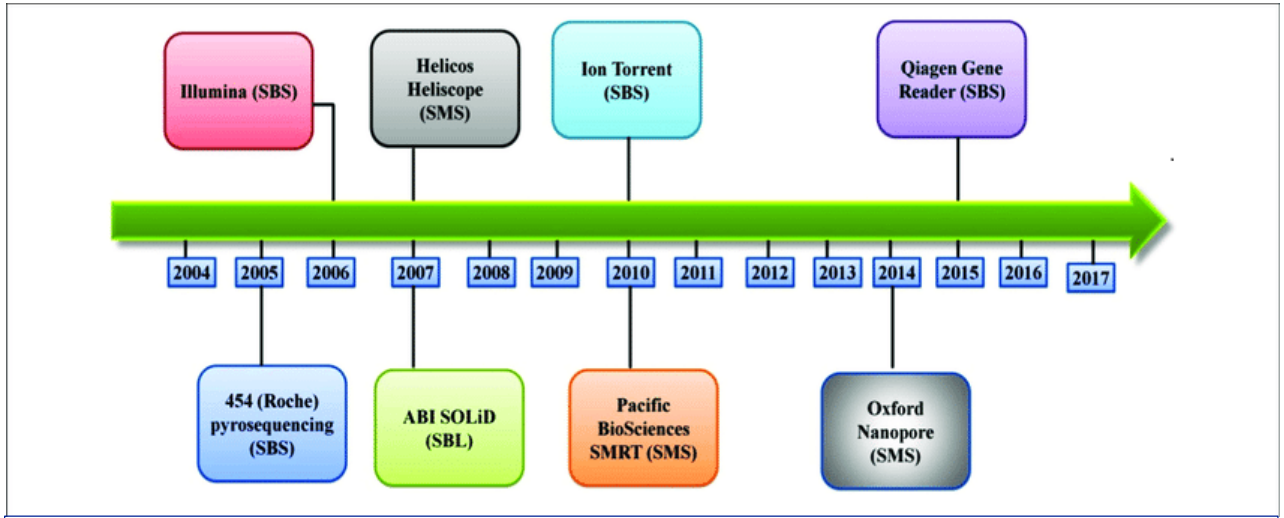
Mission: IPPN members recognize the need to integrate globally plant phenotyping approaches across all levels of plant systems, from molecular to field. IPPN members are also aware that developing and breeding new varieties of plants with improved performance is necessary for the future.





Next Generation Sequencing

Second generation sequencing or massively parallel sequencing emerged in 1998 and is commercially available since 2005 by the name of 454 sequencing using pyrosequencing technology. Illumina purchased the Solexa genome analyzer on 2006 and commercialized it in 2007 and then revolutionized the second generation sequencing by providing accurate and affordable sequencing.



Ahmad, Muneer et al. (2018). Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment. Frontiers in Immunology. 10.3389/fimmu.2018.02868.

Next Generation Sequencing

Third generation sequencing methods mostly focused on single molecule long reads or reducing the cost of sequencing. Pacific BioSciences introduced the PacBio RSII in 2010 to address the limitation of the short read sequencing effecting genome assemblies.

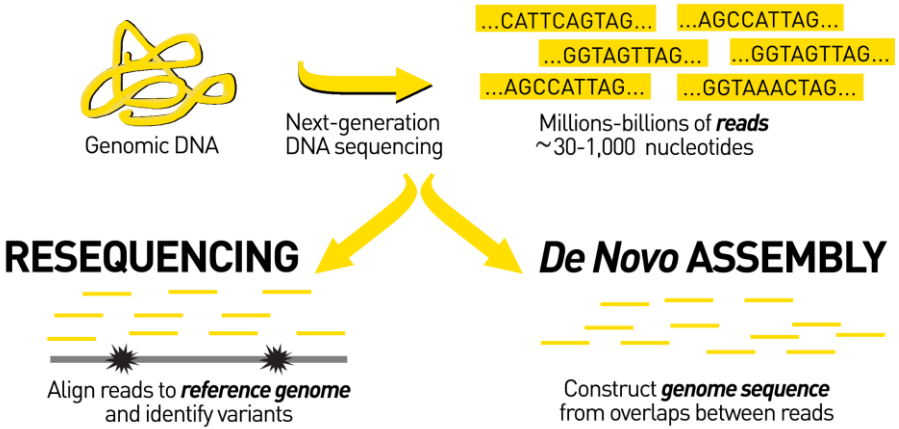
GS platforms/company/max output per run	Read length per run (bp)	No. reads per run	Time (h or days)	Cost per 10^6 bases	Raw error rate (%)	Platform cost (USD approx.)	Chemistry
First generation							
Sanger/Life Technologies/84 kb	800	1	2 h	2400	0.3	95,000	Dideoxy terminator
Second generation							
454 GS FLX+/Roche/0.7 Gb	700	1×10^6	24/48 h	10	1	500,000	Pyrosequencing
GS Junior/Roche/70 Mb	500	1×10^5	18 h	9		100,000	Pyrosequencing
HiSeq/Illumina/1500 Gb	2x150	5×10^9	27/240 h	0.1	0.8	750,000	Reversible terminators
MiSeq/Illumina/15 Gb	2x300	3×10^8	27 h	0.13	0.8	125,000	Reversible terminators
SOLiD/Life Technologies/120 Gb	50	1×10^9	14 days	0.13	0.01	350,000	Ligation
Retrovolocity/BGI/3000 Gb	50	1×10^9	14 days	0.01	0.01	12×106	Nanoball/ligation
Ion Proton/Life Technologies/100 Gb	200	6×10^7	2–5 h	1	1.7	215,000	Proton detection
Ion PGM/Life Technologies/2 Gb	200	5×10^6	2–5 h	1	1.7	80,000	Proton detection
Third generation							
SMRT/Pac Bio/1 Gb	>10,000	1×10^6	1–2 h	2	12.9	750,000	Real-time SMS
Heliscope/Helicos/25 Gb	35	7×10^9	8 days	0.01	0.2	1.35×10^6	Real-time SMS
Nanopore/Oxford Nanopore Technologies/1 Gb	>5000	6×10^4	48/72 h	<1	34	1000	Real-time SMS
Electron microscopy/ZS	7200		14 h	<0.01		1×10^6	Real-time SMS
Genia nanopore (http://www.geniachip.com)							Real-time SMS

Next-Generation Sequencing — An Overview of the History, Tools, and "Omic" Applications
By Jerzy K. Kulski (DOI: 10.5772/61964)

Applications of Next Generation Sequencing

Whole Genome Sequencing

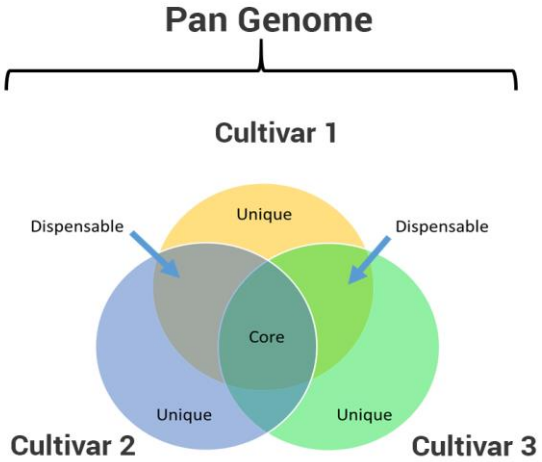
The whole genome sequencing is an essential step for finding the sequence variants (SVs) used for marker discovery, gene discovery and genome structural variations.



Della Coletta et al. Genome Biology (2021) 22:3
https://doi.org/10.1186/s13059-020-02224-8
Genome Biology

How the pan-genome is changing crop genomics and improvement

Rafael Della Coletta[1], Yinjie Qiu[1], Shujun Ou[2], Matthew B. Hufford[2*] and Candice N. Hirsch[1*]



Transcriptome Sequencing

Transcriptome analysis using SAGE, Microarrays, DD AFLPs and Northern blots is now expired! And RNA Seq is the new tool!

Published in final edited form as:
Wiley Interdiscip Rev RNA. 2017 January ; 8(1): . doi:10.1002/wrna.1364.

RNA-Seq methods for transcriptome analysis

Radmila Hrdlickova[1], **Masoud Toloue**[1,*], and **Bin Tian**[2,*]

[1]Bioo Scientific Inc., Austin, TX, USA

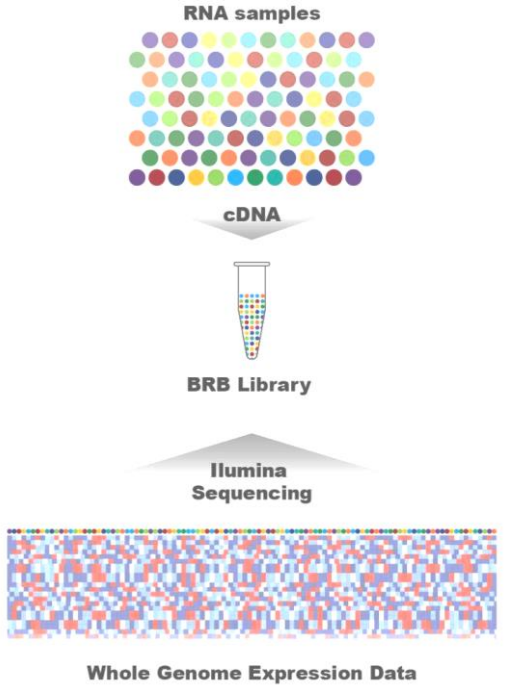
[2]Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers New Jersey Medical School, Newark, NJ, USA

Alpern *et al. Genome Biology* (2019) 20:71
https://doi.org/10.1186/s13059-019-1671-x

Genome Biology

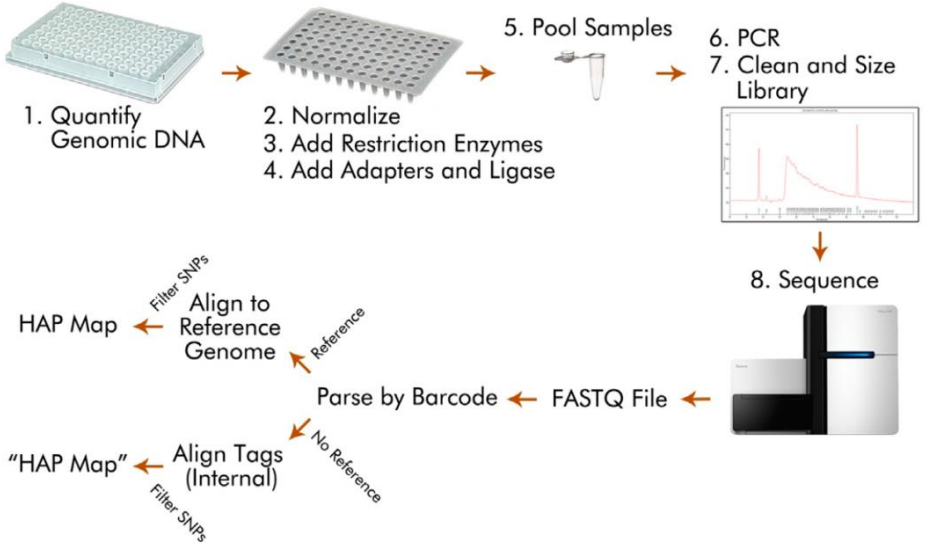
BRB-seq: ultra-affordable high-throughput transcriptomics enabled by bulk RNA barcoding and sequencing

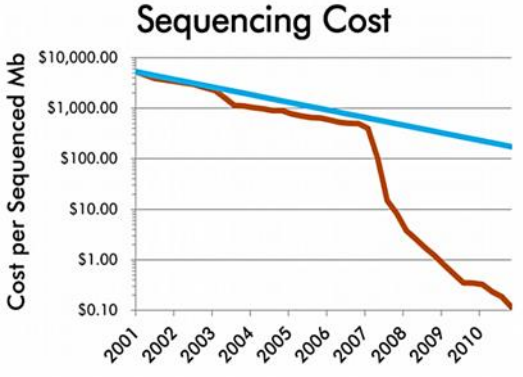
Daniel Alpern[1,2†], Vincent Gardeux[1,2†], Julie Russeil[1], Bastien Mangeat[3], Antonio C. A. Meireles-Filho[1,2], Romane Breysse[1], David Hacker[4] and Bart Deplancke[1,2*]



Genotyping by Sequencing

1) Restriction enzyme mediated genotyping by sequencing
2) Hybridization based targeted sequencing
3) Amplicon based targeted sequencing
4) Skim sequencing (re-sequencing by low coverage)



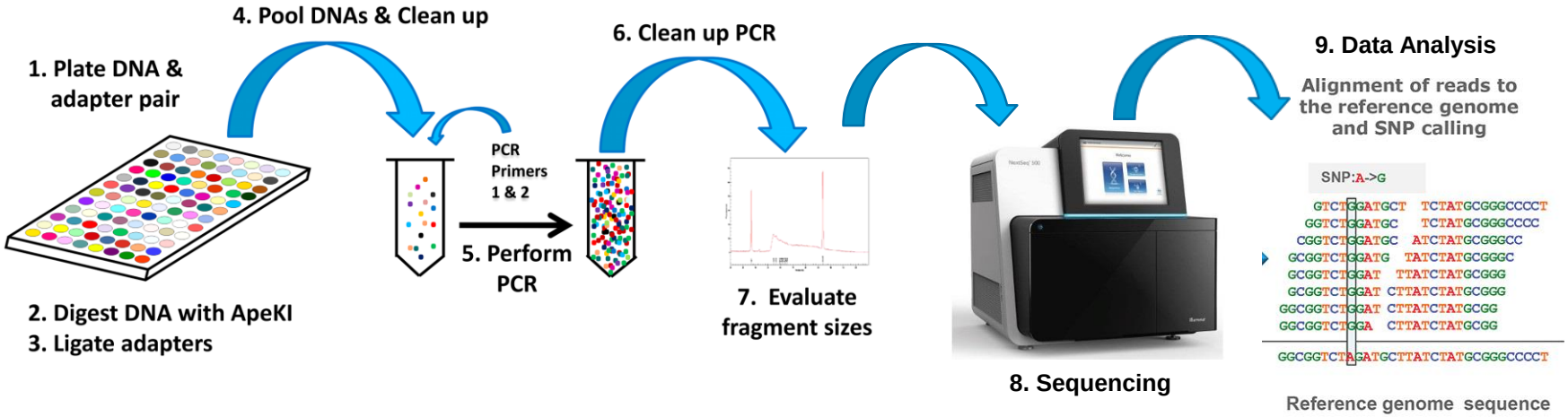


Genotyping-by-Sequencing for Plant Breeding and Genetics

Jesse A. Poland* and Trevor W. Rife

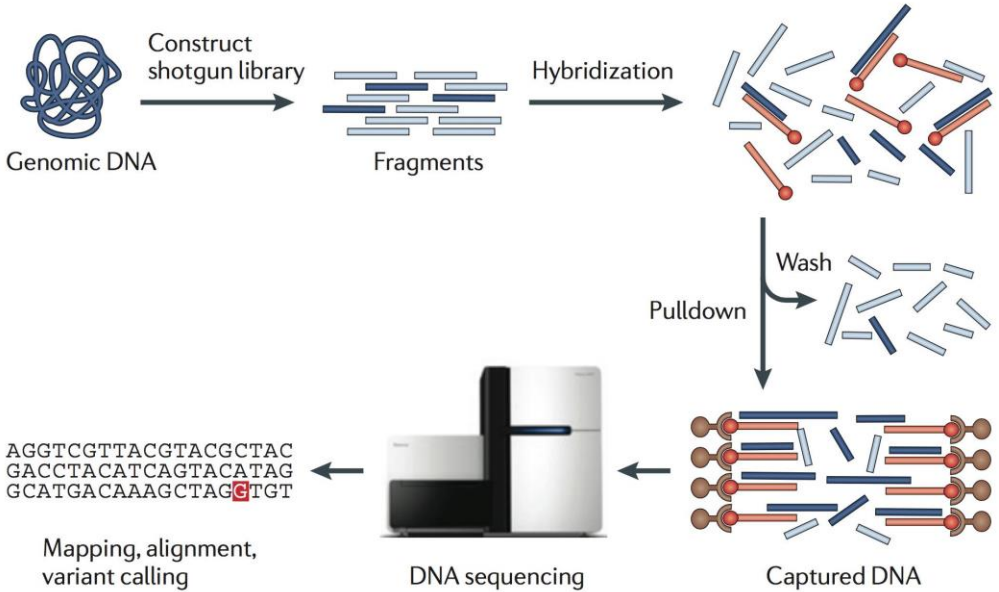
Restriction Enzyme Mediated GBS

GBS via restriction enzyme: using different restriction enzymes, the genome will be digested and only the fragments with certain sizes will be amplified and sequenced.



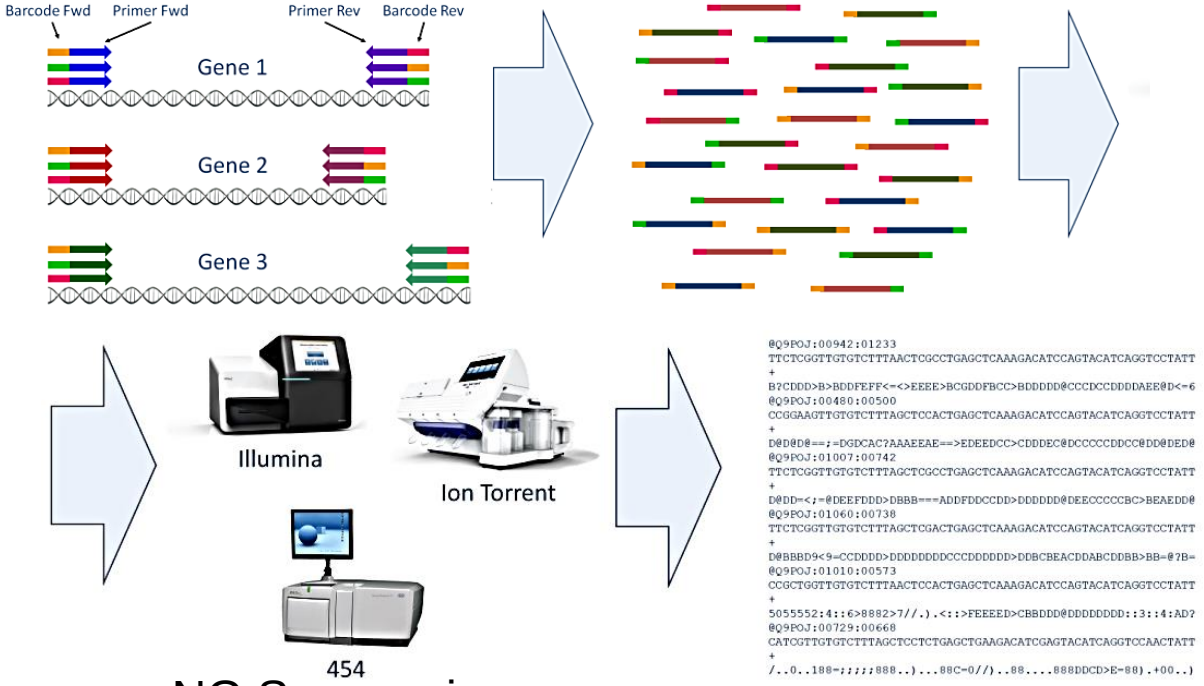
Hybridization Based GBS (Sequence Capture)

The genome will be physically or enzymatically fragmented to small fragments. Then the targets will be captured by probes attached to beads or arrays and will be sequenced.



Amplicon Based Targeted Sequencing

Hundreds of regions in the genome will be amplified using multiplex PCR approach. Then the PCR fragments will be sequenced.



NG Sequencing

Data Analysis

Amplicon Based Targeted Sequencing

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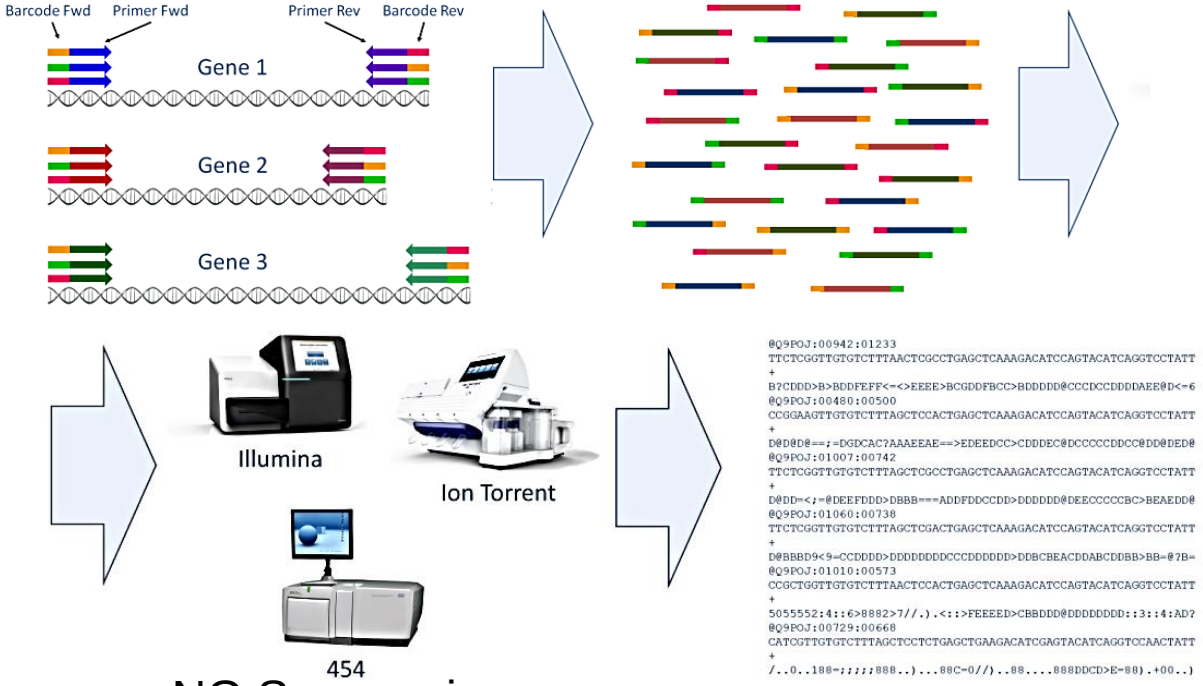
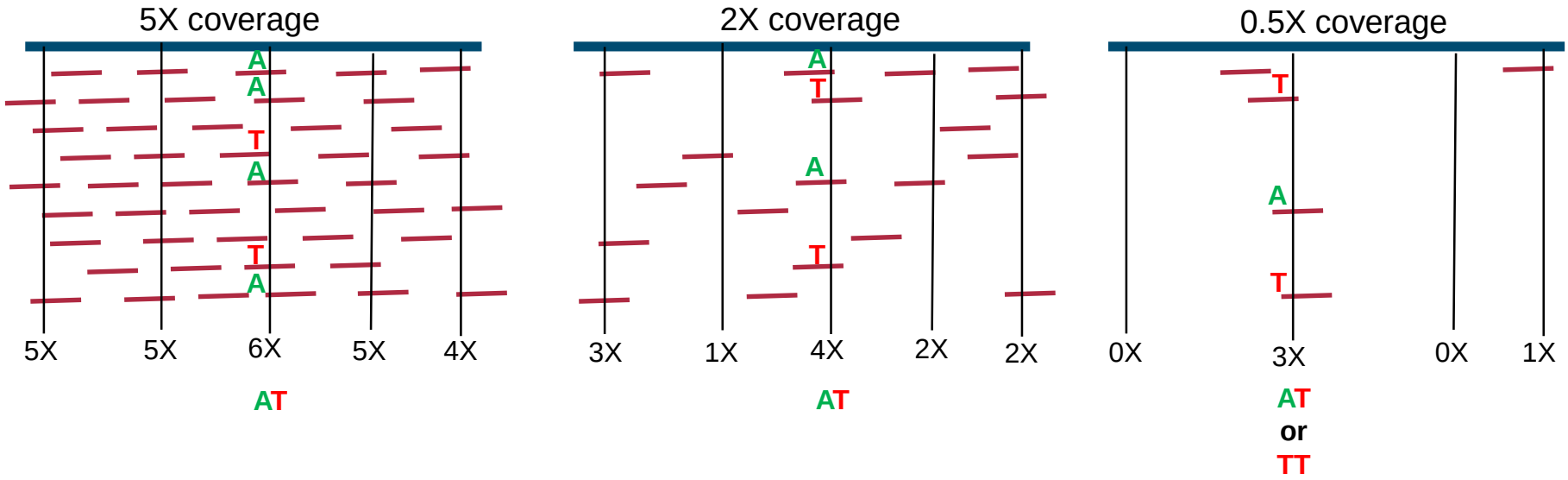


NG Sequencing

Data Analysis

Skim Sequencing (Shallow Sequencing)

Substantial reduction in sequencing cost has made low depth whole genome resequencing a novel strategy for genotyping. Shallow sequencing by a coverage less than 1X (0.25X, 0.5X, 1X) have been used successfully in genotyping plant and animals.



Skim Sequencing (Shallow Sequencing)

Skim sequencing is a great alternative for array based genotyping when >50,000 markers is needed. However having a deep sequencing of the parental lines or a well defined pan genome information of the species of interest and a great imputation strategy is essential for this method.

frontiers in Plant Science

ORIGINAL RESEARCH
published: 07 December 2018
doi: 10.3389/fpls.2018.01809

Evaluation and Recommendations for Routine Genotyping Using Skim Whole Genome Re-sequencing in Canola

*M. Michelle Malmberg[1,2], Denise M. Barbulescu[3], Michelle C. Drayton[1], Maiko Shinozuka[1], Preeti Thakur[1], Yvonne O. Ogaji[1], German C. Spangenberg[1,2], Hans D. Daetwyler[1,2] and Noel O. I. Cogan[1,2]**

PLOS ONE

Evaluating Imputation Algorithms for Low-Depth Genotyping-By-Sequencing (GBS) Data

Ariel W. Chan[1]*, Martha T. Hamblin[2], Jean-Luc Jannink[1,3]

SNP-skimming: a fast approach to map loci generating quantitative variation in natural populations

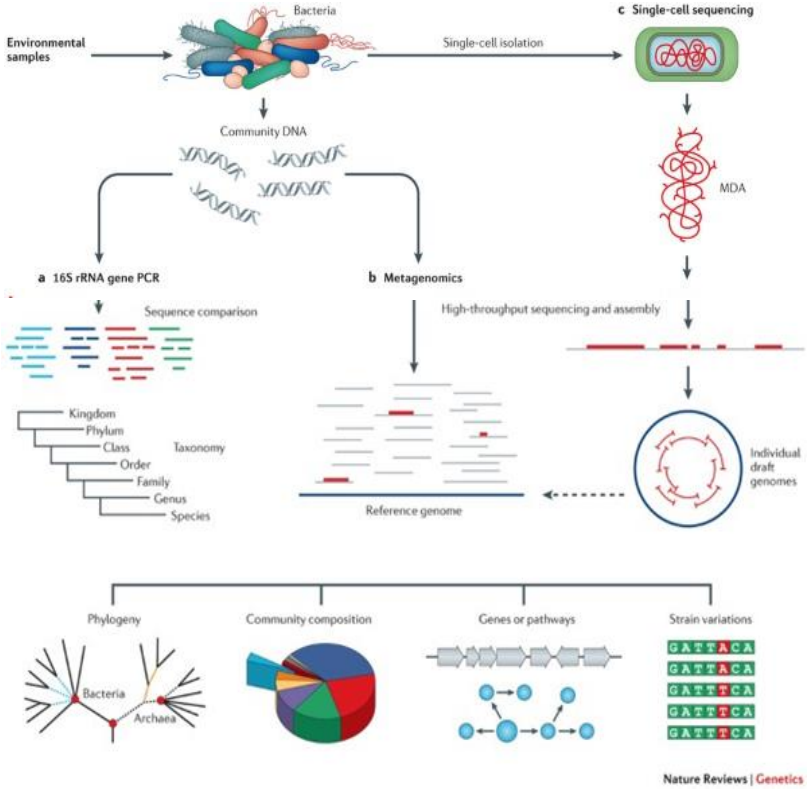
Carolyn A. Wessinger[1,*], John K. Kelly[1], Peng Jiang[2], Mark D. Rausher[2], and Lena C. Hileman[1]

[1]Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS

[2]Department of Biology, Duke University, Durham, NC

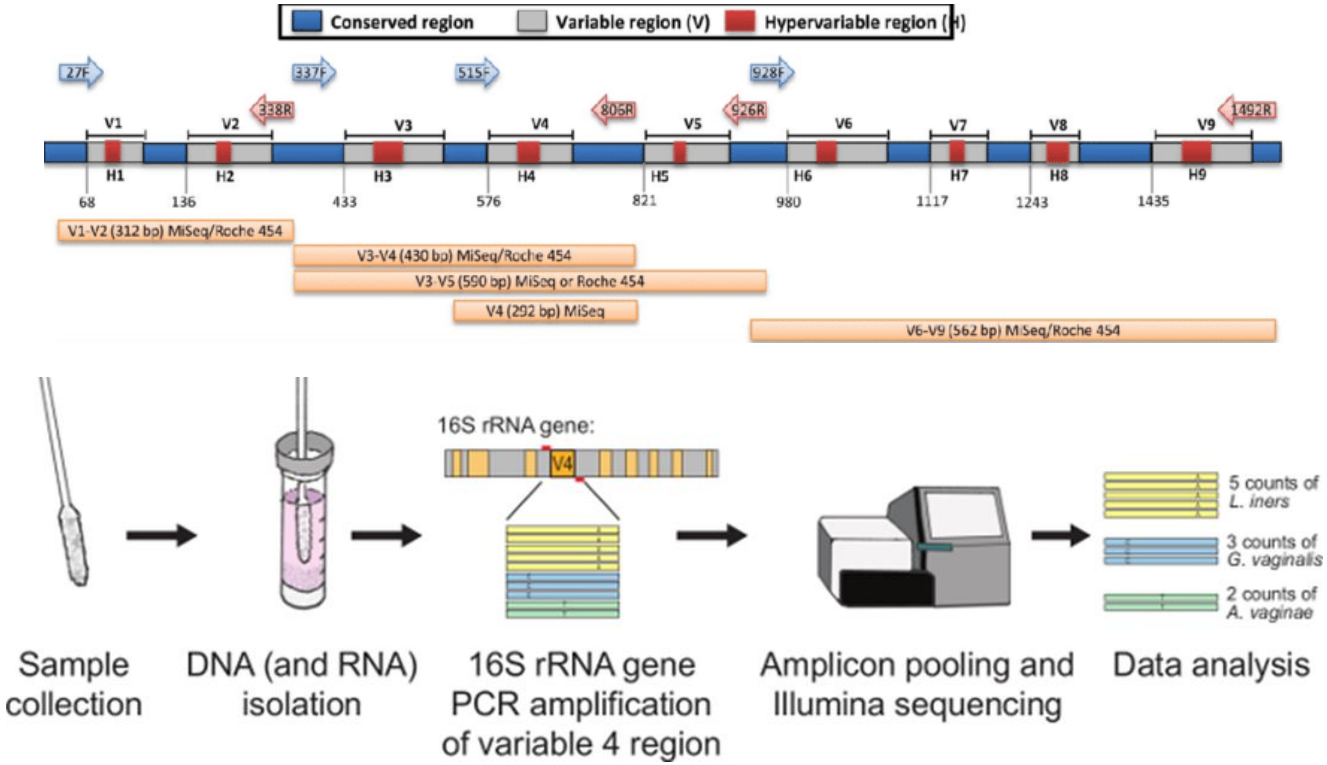
Microbiome Detection Methods

- Next Generation Sequencing
  - Whole genome Sequencing
  - 16S/18S/ITS Sequencing
  - Metagenomics (Shotgun Sequencing)
  - Single cell sequencing



Nat Rev Genet 15, 577–584 (2014). https://doi.org/10.1038/nrg3785

Targeted 16S, 18S, ITS Sequencing



Microbiome in Plants

Bacterial Microbiota

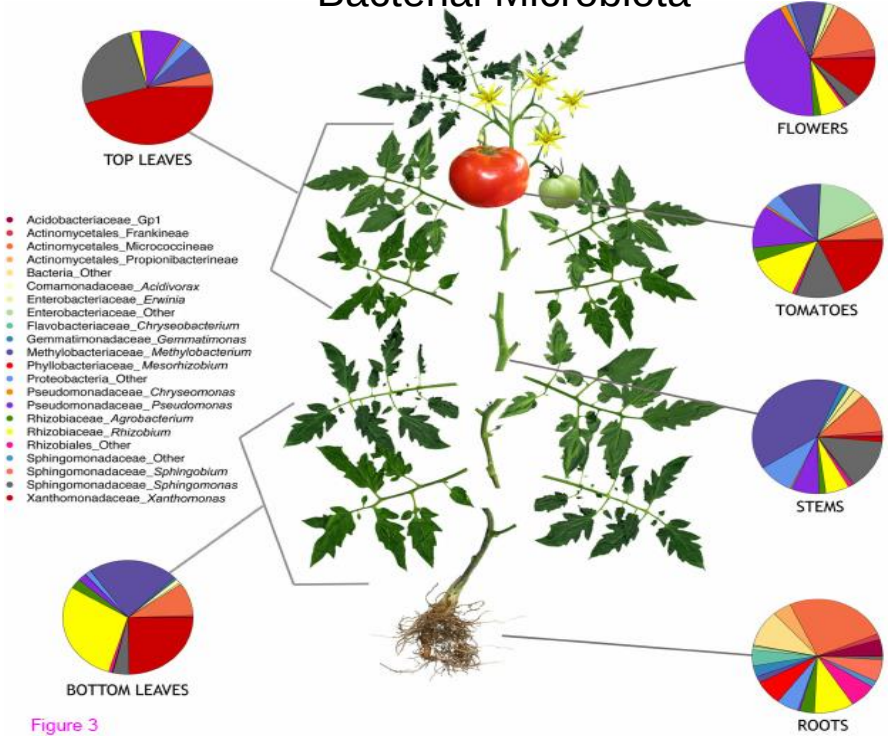
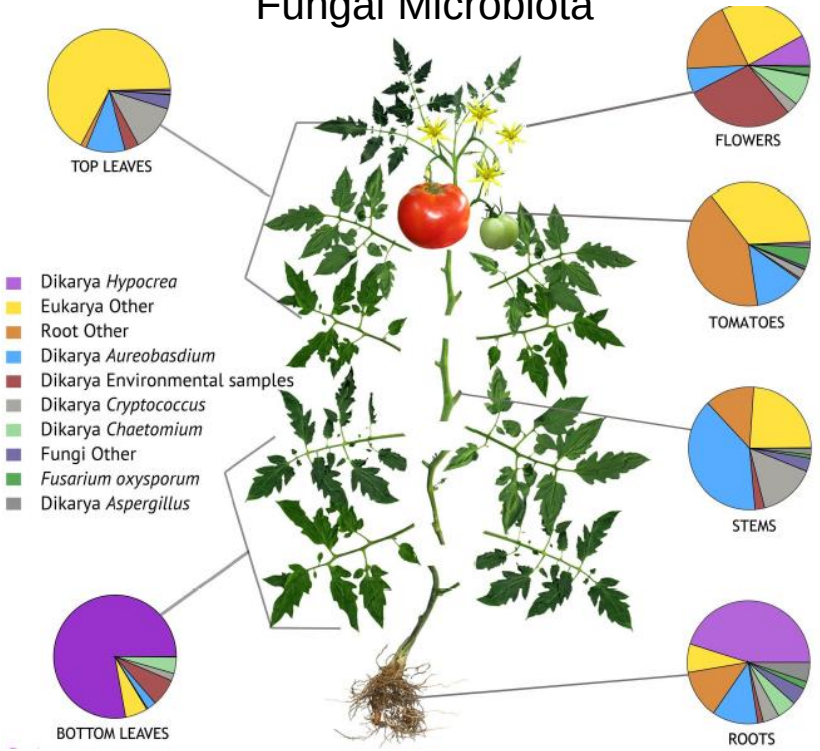


Figure 3

Fungal Microbiota

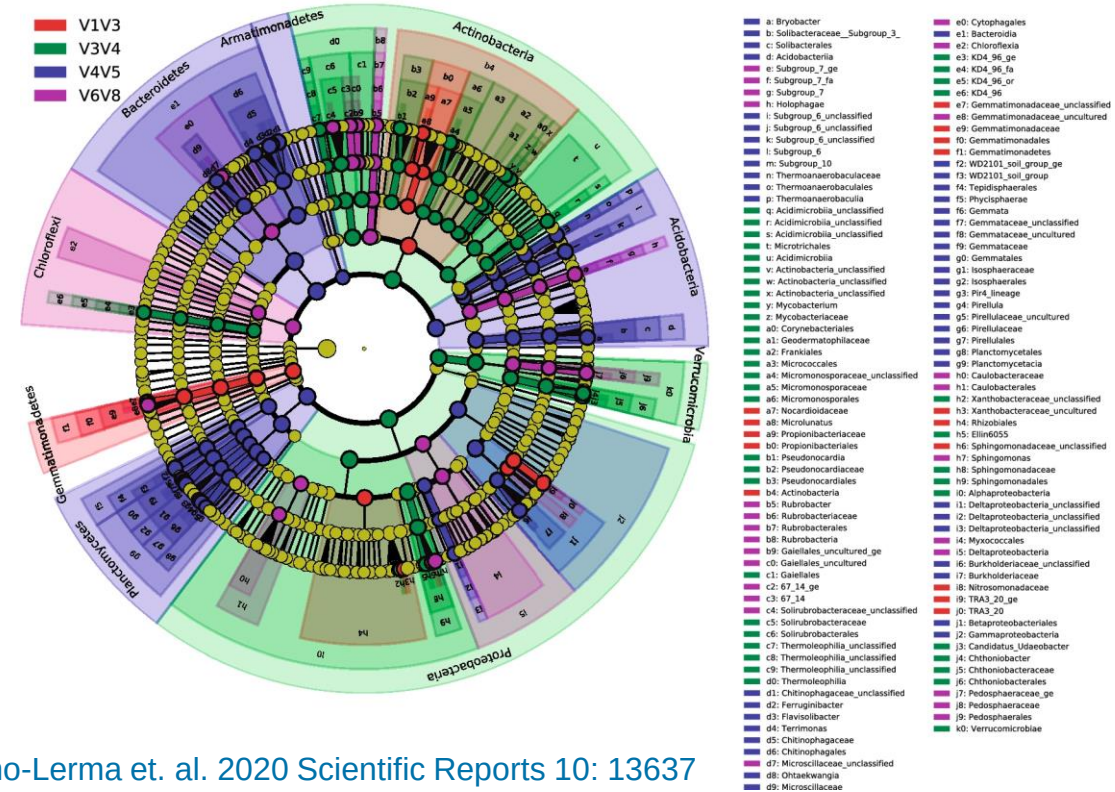


Ottesen et al BMC MICROBIOL 2013

Soil Microbiome

16S targeted Microbiome analysis can detect the bacteria to their genera and their abundance ratio in the soil.
In soil samples, the taxa detected by V1V3 and V3V4 regions were similar, in order of abundance: *Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Planctomycetes, Gemmatimonadetes*, *Chloroflexi* and *Verrucomicrobia*. But V4V5 and V6V8 produced a slighty different patterns.

Cladogram for 16S rRNA regions in soil samples



Soriano-Lerma et. al. 2020 Scientific Reports 10: 13637

Conclusion

- The advent of next generation sequencing methods have changed the landscape of agricultural studies.

- The ongoing cost reduction in next generation sequencing made it the proper tool for different applications like genotyping, microbiome analysis, differential gene expression analysis, epigenomics studies and numerous other applications when nucleic acids are involved.

- Genotyping by sequencing is now the main tool for ultra high throughput screening of >50 markers when lots of samples needs to be genotyped.

- Microbiome studies using whole genome or targeted sequencing shed light into a new definition of plant/animal and human health management and host environment interactions.

Thank you !

Questions?

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