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La transition fruit immature/fruit mature: analyse globale des profils transcriptomiques

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UMR 990 INRA/INP-ENSAT Génomique et Biotechnologie des Fruits

Maturation des fruits

<u>Changement physiologiques et biochimiques profonds:</u>

* Activité respiratoire :
  - augmentation subite et intense chez les fruits climactériques
  - pas de changement d'intensité chez les fruits non climactériques

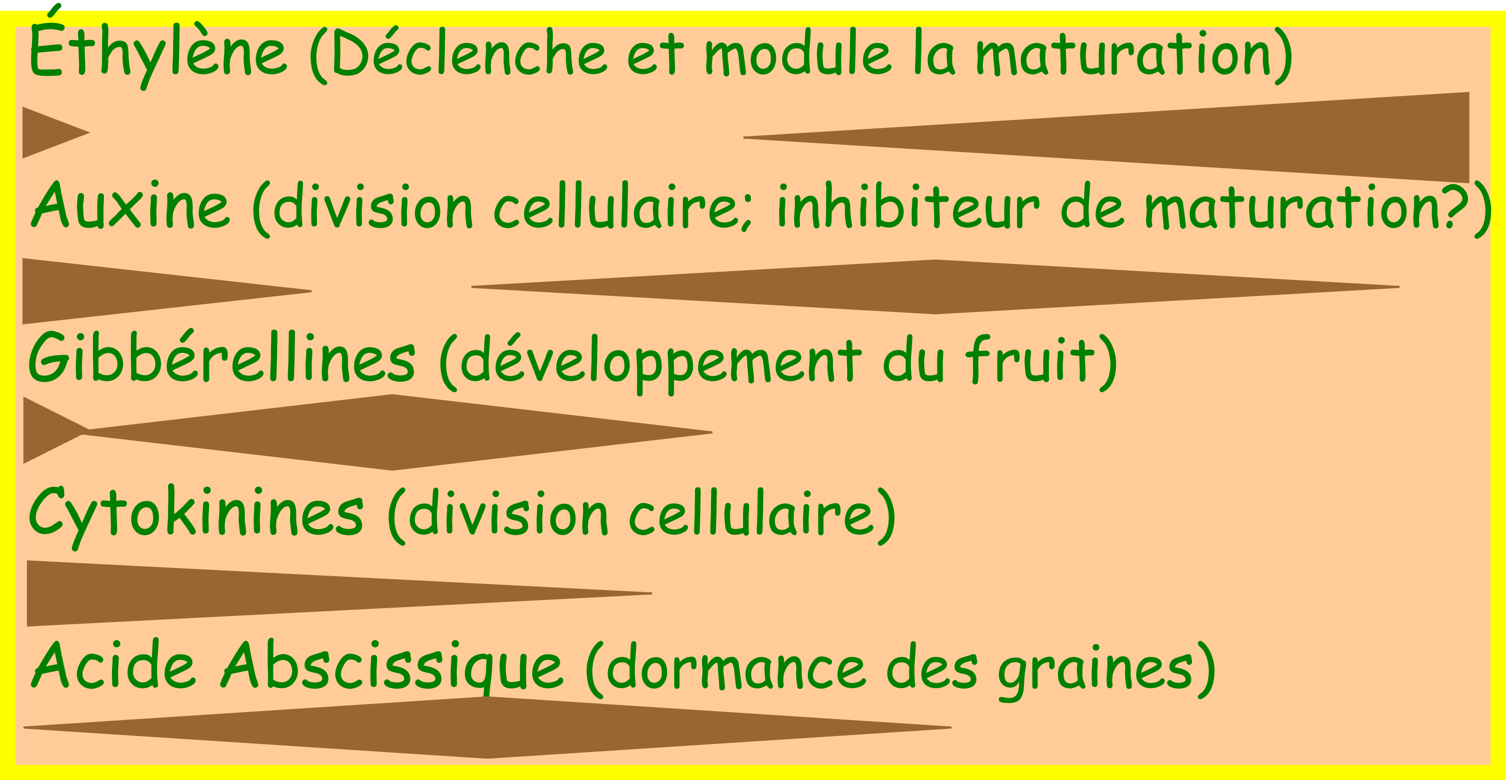
* Diminution de la fermeté

* Formation de pigments

* Évolution des sucres et des acides

* Biosynthèse d’arômes

Régulation du développement des fruits par les hormones



Éthylène (Déclenche et module la maturation)

Auxine (division cellulaire; inhibiteur de maturation?)

Gibbérellines (développement du fruit)

Cytokinines (division cellulaire)

Acide Abscissique (dormance des graines)

Régulation de la Maturation des fruits climactériques

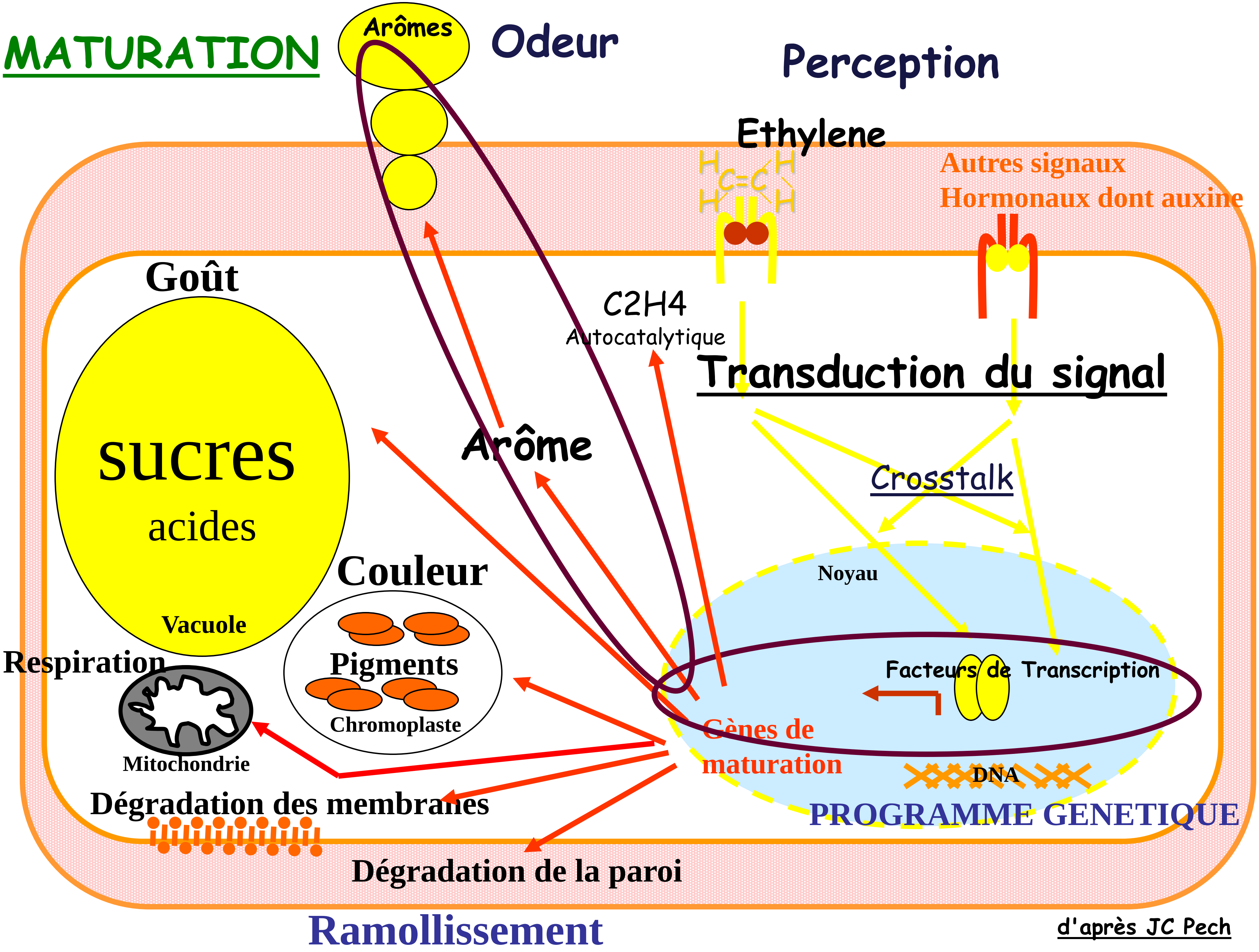
Éthylène

- Production autocatylique de l'éthylène
- Autonomie de maturation

Quelques fruits climactériques:

abricot, avocat, banane, kiwi, melon, pêche, poire, pomme, tomate …

MATURATION



Arômes

Odeur

Perception

Ethylene

H H
C=C
H H

Autres signaux
Hormonaux dont auxine

Goût

sucres

acides

Vacuole

C2H4
Autocatalytique

Transduction du signal

Arôme

Crosstalk

Couleur

Noyau

Pigments

Chromoplaste

Facteurs de Transcription

Respiration

Mitochondrie

Gènes de maturation

DNA

Dégradation des membranes

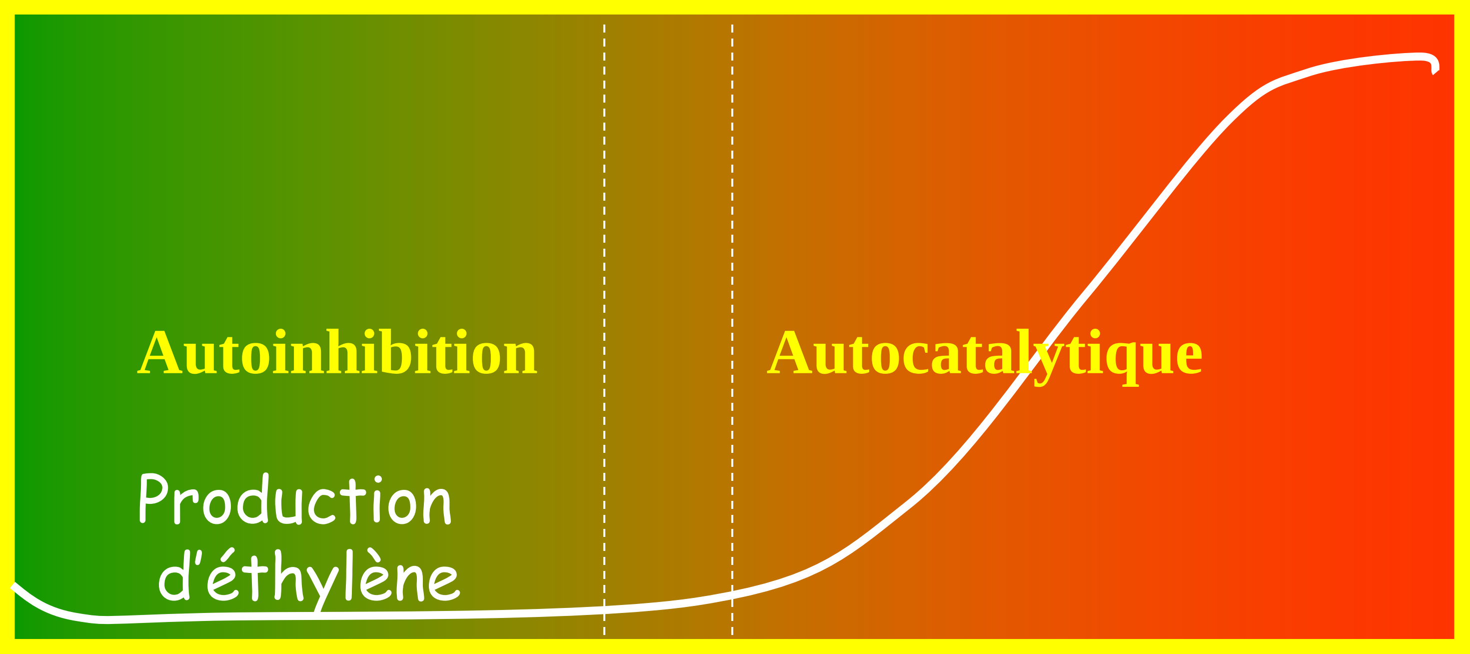
PROGRAMME GENETIQUE

Dégradation de la paroi

Ramollissement

d'après JC Pech

Maturation du fruit de Tomate

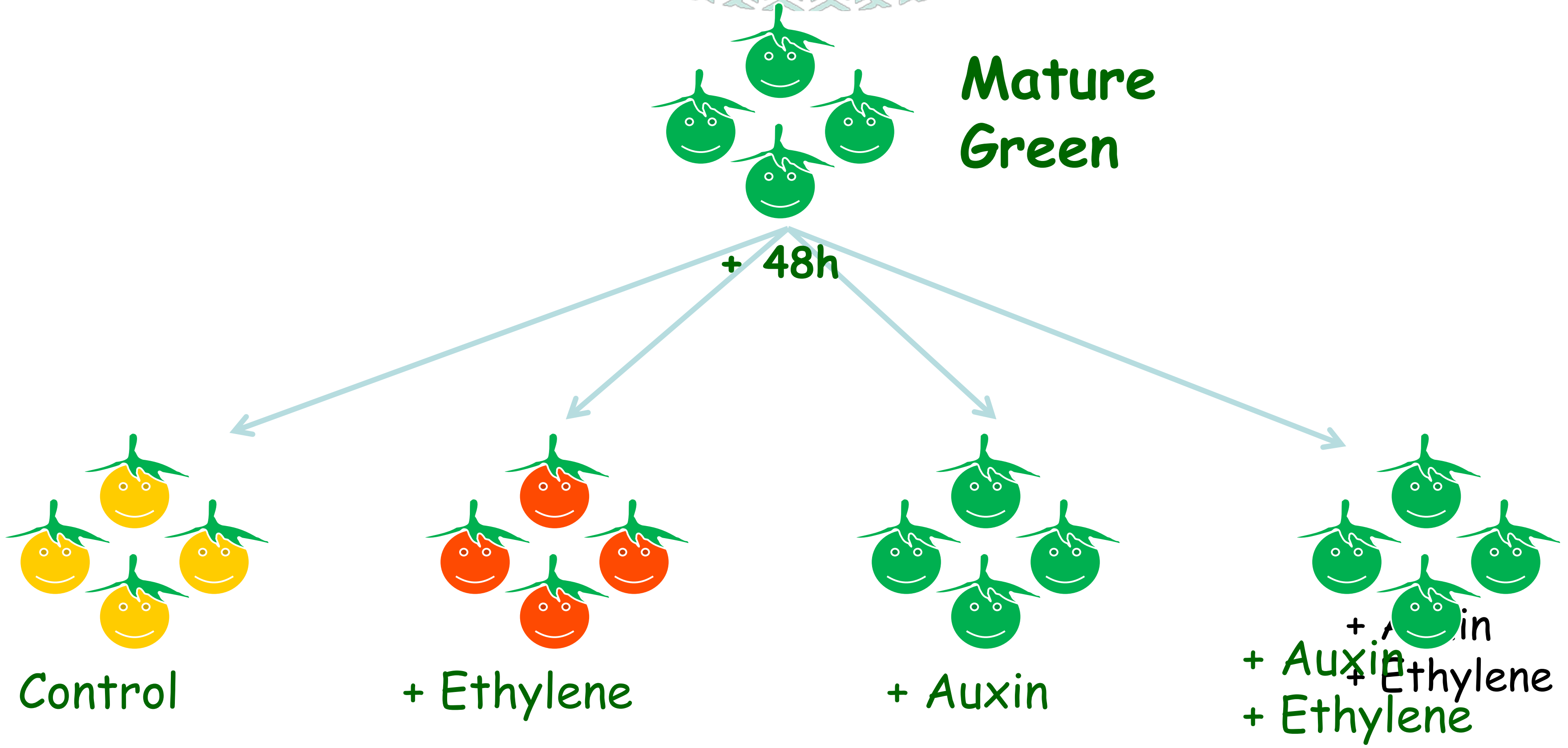


Transition fruit immature/fruit mature
ou
Comment le fruit acquière sa capacité à murir ?

Influence croisée de l'éthylène et de l'auxine
au moment de la transition fruit immature/fruit mature
(stade de développement vert mature :mature green)

Analyse du transcriptome de tomates traitées avec de l'éthylène ou de l'auxine par RNASeq : séquençage quantitatif des ARN messagers

Approche d'étude et Plan d'experience



+ A in
+ Ethylene

RNAseq

RNAseq

- RNASeq – technology based on the second generation of sequencing machines (NGS) to catalog full collection of RNA in the cell (a.k.a. transcriptome)

- Using this technology we are able to look at the transcriptome snapshot inferring all possible variations in transcription and quantify levels of expression

RNAseq experiments: workflow

1. Sampling, RNA extraction

5 treatments
X 3 biological replicates

2. Sequencing

Illumina Hiseq2000,
PLAGe plateforme

genotoul bioinfo

3. Bioinformatics
data processing
in-silico discovery

4. In-vitro &
In-vivo
validation

qRT-PCR,
analyzes

Transcripts capture with RNAseq,

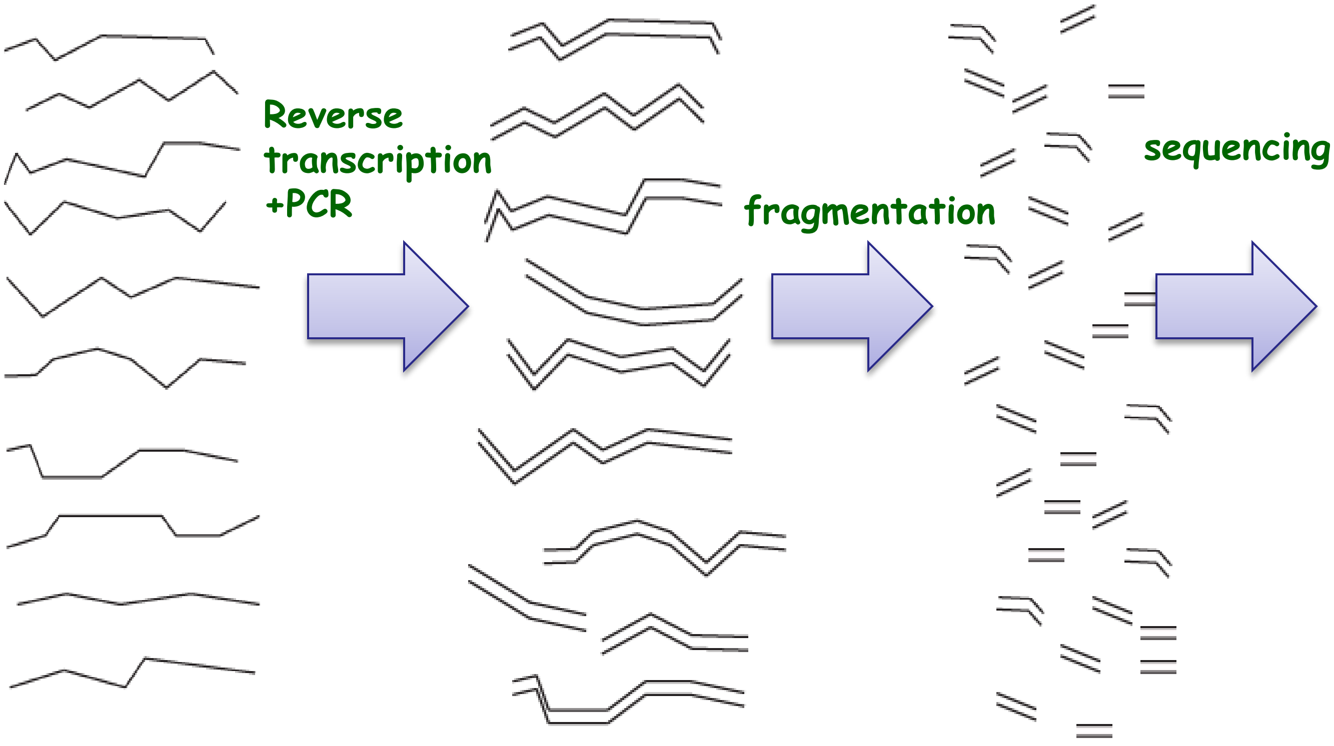
sampling, sequencing

Sample RNA

Amplified cDNA

cDNA fragments

Millions of short reads per sample



Reverse transcription +PCR

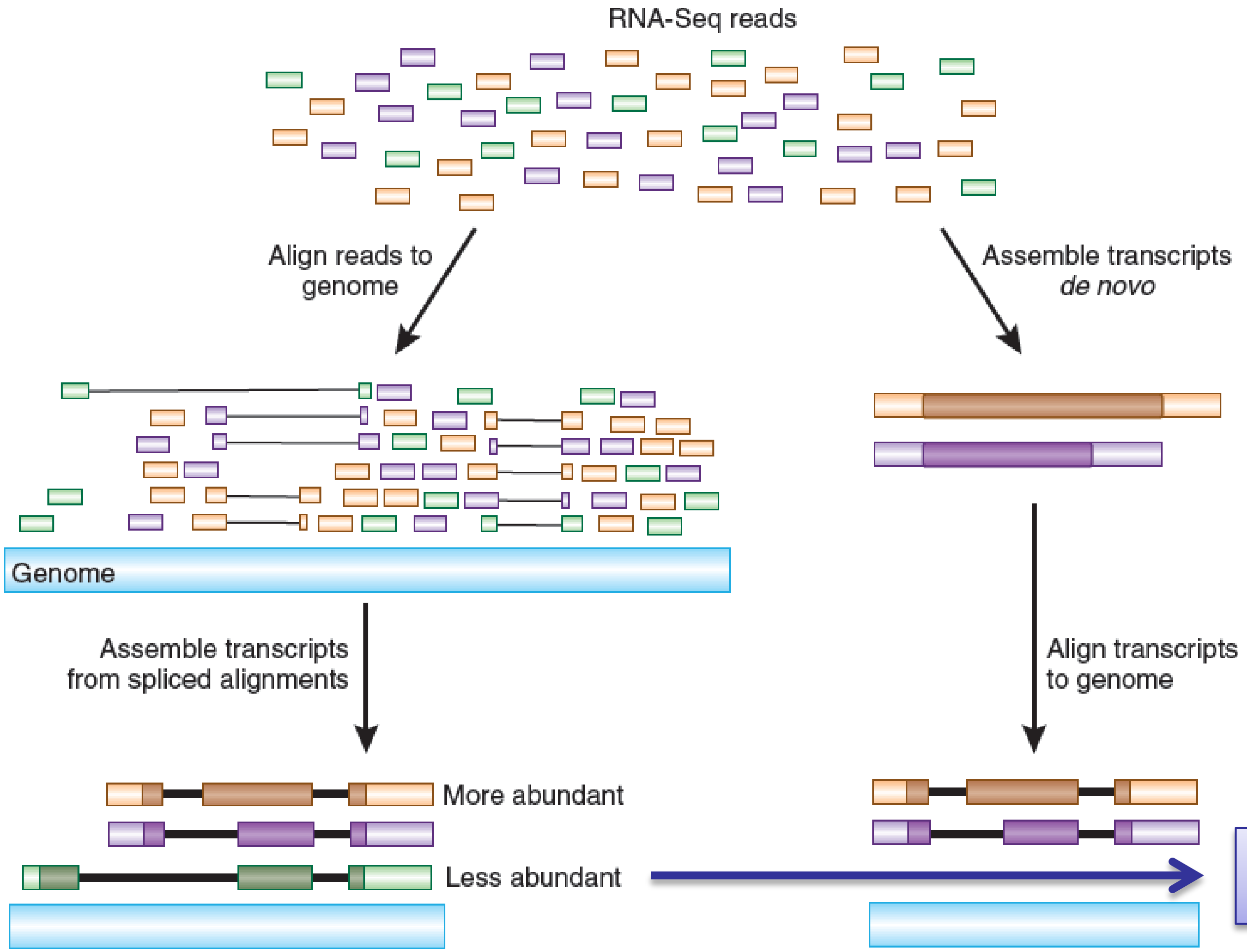
fragmentation

sequencing

```
@HWI-ST314_0085:8:1101:10000:
CGAGTGTATAGTGAATCCCCTTTTGA
+
HHHHHHHHHHHHHHHHEG########
@HWI-ST314_0085:8:1101:10000:
CTTGCATCTATCTTCAAACCAATAAAT
+
HHHHHHHHHHHHHHHHHHHHHHHHHH
+
@HWI-ST314_0085:8:1101:10003:
TGGAAGGAGAGAGGCCTGAGAAAC
+
HHHHHGHHHFHGHHHHHHHHHHHH
@HWI-ST314_0085:8:1101:10004:
TCCGGTTACATTCTTAAACCCAACCG
+
@CC@CDEDECDFEFEE8DBEFBCFD8C
@HWI-ST314_0085:8:1101:10005:
CCAAAATAAAGAAAACATTCAACTCA
+
GD>FFD:5869%<2'++,:9.C<;@;<<A:-
@HWI-ST314_0085:8:1101:10008:
CTGCAAGATAGAATTCCTTACATTCTT
+
HHHHHHHFHHHHHHFHHHHHHHHHHH
@HWI-ST314_0085:8:1101:10008:
CTGGGTTTCTTTGTTTCCGGAGTTTTTC
```

We sequenced 450M reads in total: 30M x 5 samples x 3 replicates

Strategies for reconstructing transcripts from RNA-Seq reads, bionformatics



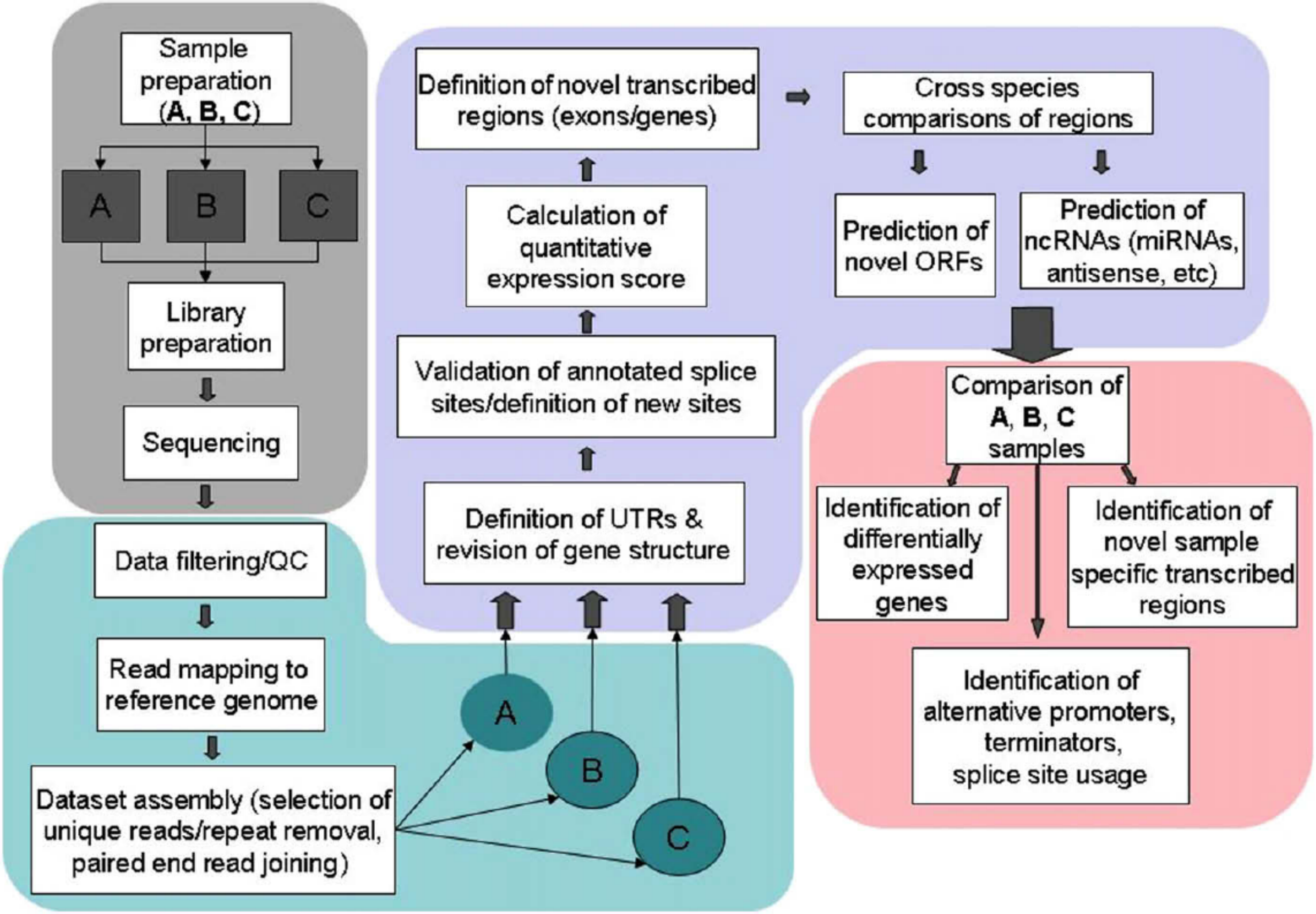
Not assembled = not quantified

Align-then-assemble: Cufflinks, Scripture

Assemble-then-align: ABySS

Advancing RNA-Seq analysis
Brian J Haas & Michael C Zody

Bioinformatics workflow diagram



Sample preparation (**A, B, C**)

A

B

C

Library preparation

Sequencing

Data filtering/QC

Read mapping to reference genome

Dataset assembly (selection of unique reads/repeat removal, paired end read joining)

A

B

C

Definition of UTRs & revision of gene structure

Validation of annotated splice sites/definition of new sites

Calculation of quantitative expression score

Definition of novel transcribed regions (exons/genes)

Cross species comparisons of regions

Prediction of novel ORFs

Prediction of ncRNAs (miRNAs, antisense, etc)

Comparison of **A**, **B**, **C** samples

Identification of differentially expressed genes

Identification of novel sample specific transcribed regions

Identification of alternative promoters, terminators, splice site usage

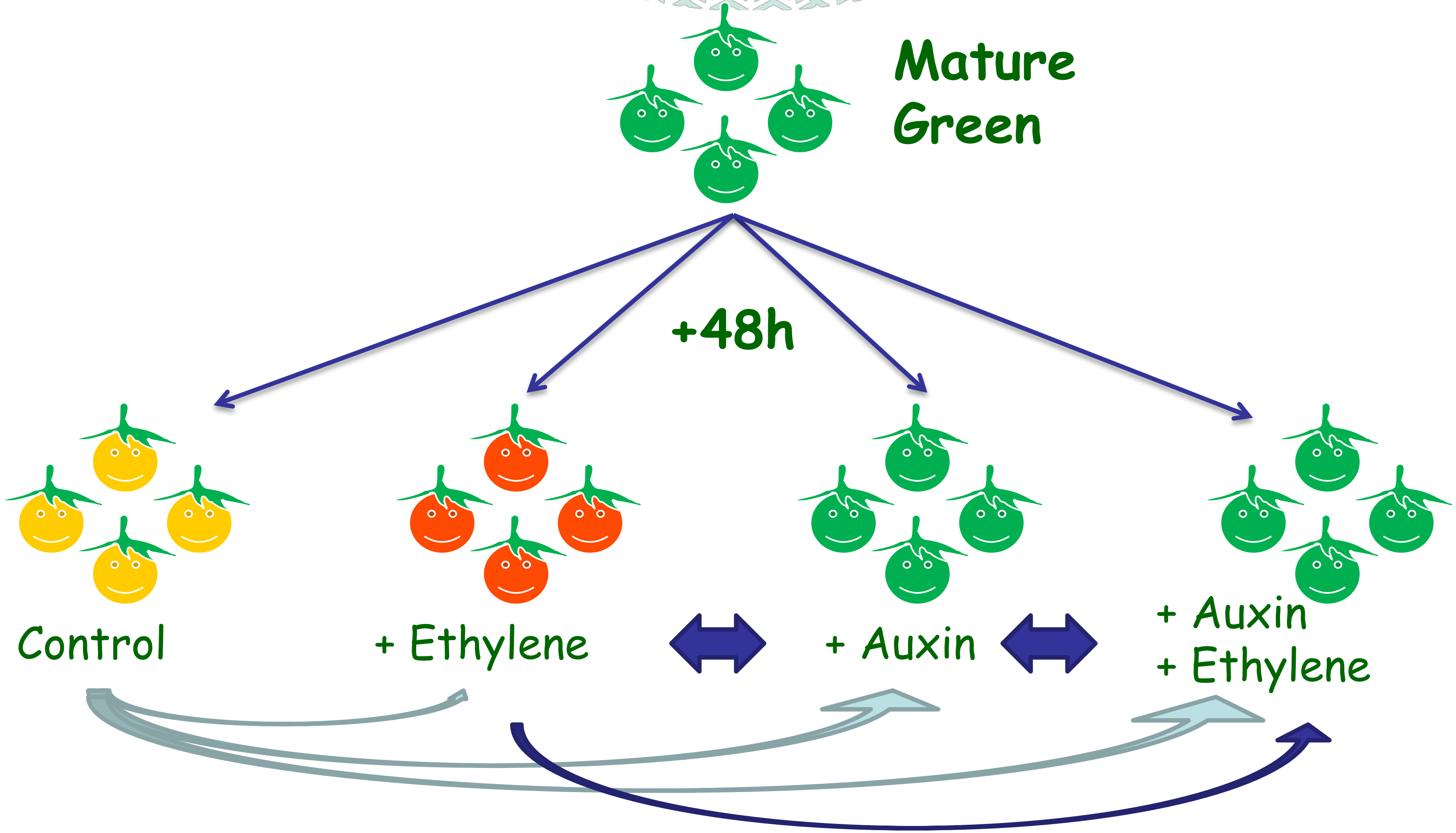
RNA-Seq—quantitative measurement of expression through massively parallel RNA-sequencing, Brian T. Wilhelm a,b,*, Josette-Renée Landry

Biostatistics: data normalization and significance test

- **Normalization by account for technical errors/bias:**
  - different library sizes: different numbers of reads
  - different gene lengths; limited read capacity: highly-expressed genes "steal" more of reads
  - sequencing biases

- **DE analysis: is a gene significantly differentially expressed under two conditions?**
  - we are working on accurate stochastic data model design
  - we use Binomial, Poisson and Negative Binomial distributions
  - employing Fisher Exact Test, Mann-Whitney U test, MARS.

Expression levels comparison and Differentially expressed genes identification



Mature Green

+48h

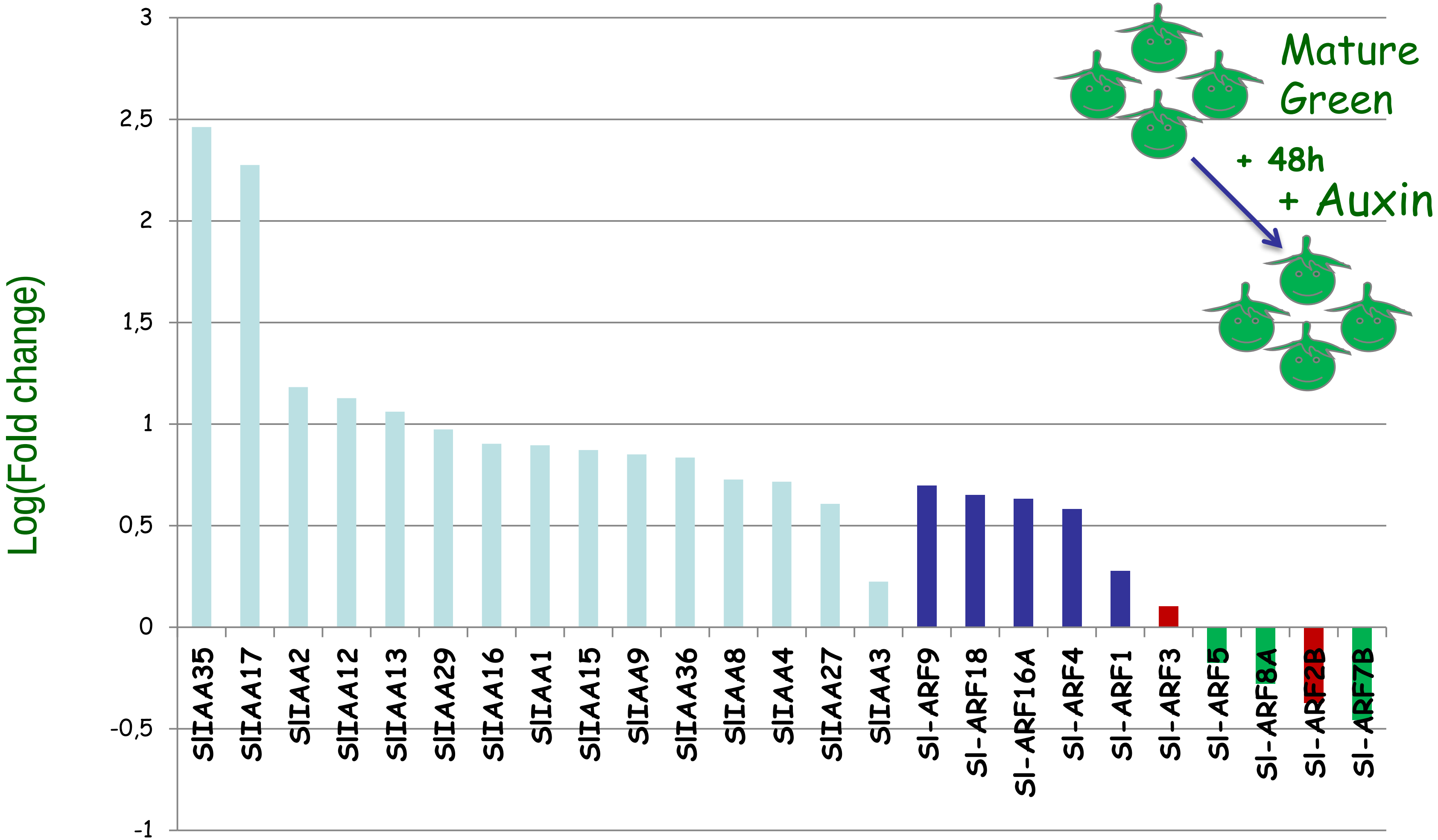
Control

+ Ethylene

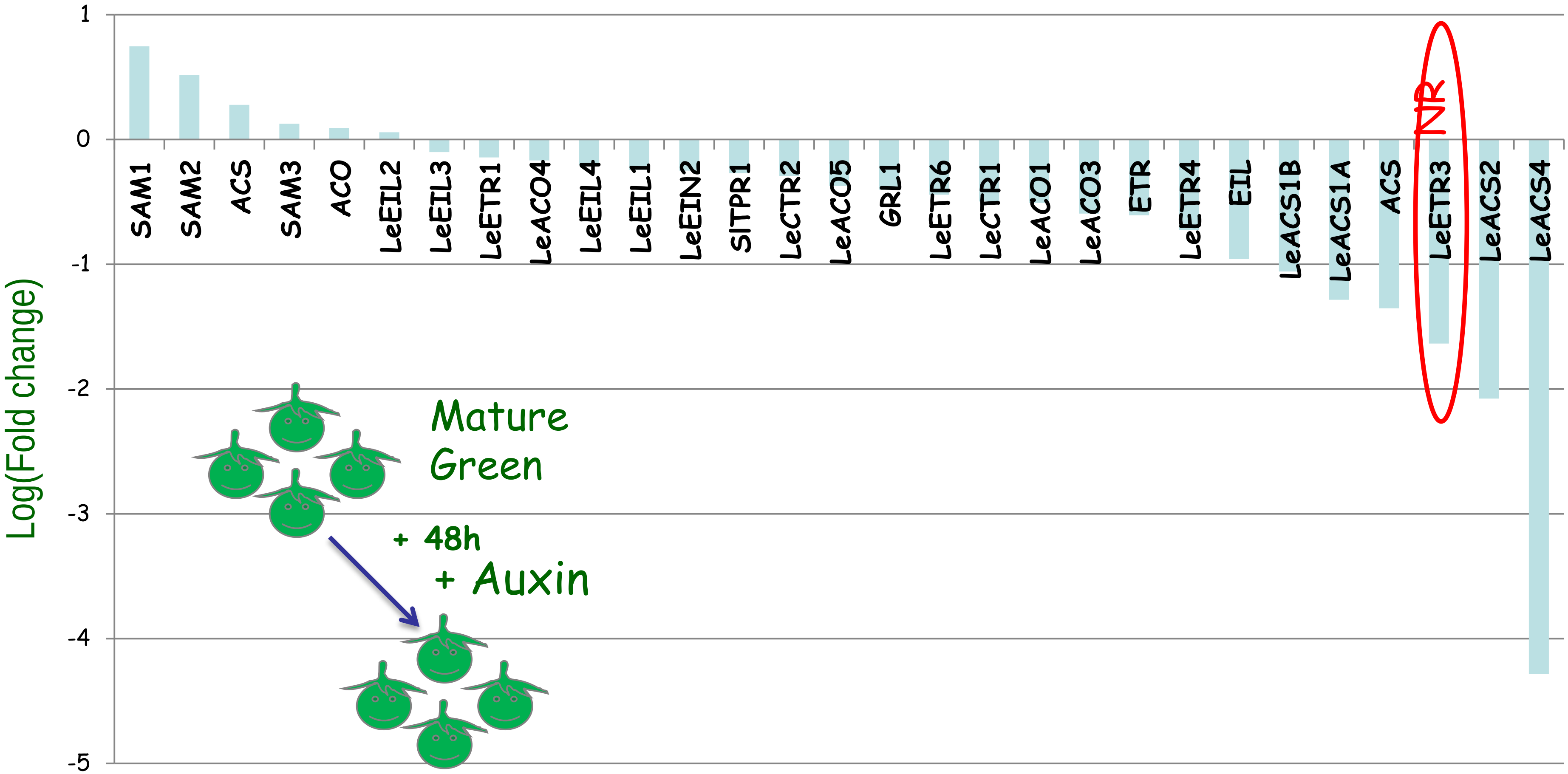
+ Auxin

+ Auxin
+ Ethylene

Induction of Auxin related genes by auxin treatment



Suppression of Ethylene related genes by auxin treatment



Conclusion and perspectives

- RNASeq allowed us to measure the mRNA expression levels in tomato fruits in the presence of external signals (hormone treatment)

- Analysis of this data identified a number of genes actively responding to treatment, thus shedding light on the process of the fruit ripening

- Our RNASeq experiment revealed hundreds of new transcripts (genes and RNA) in tomato which were previously un-annotated

- We identified a need and started a development of robust statistical methods for DE genes identification

Contributeurs

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  - Biologie:
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- Plateforme Plage
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- Plateforme Bioinfo-genotoul
  - C. Klopp
  - D. Laborie