

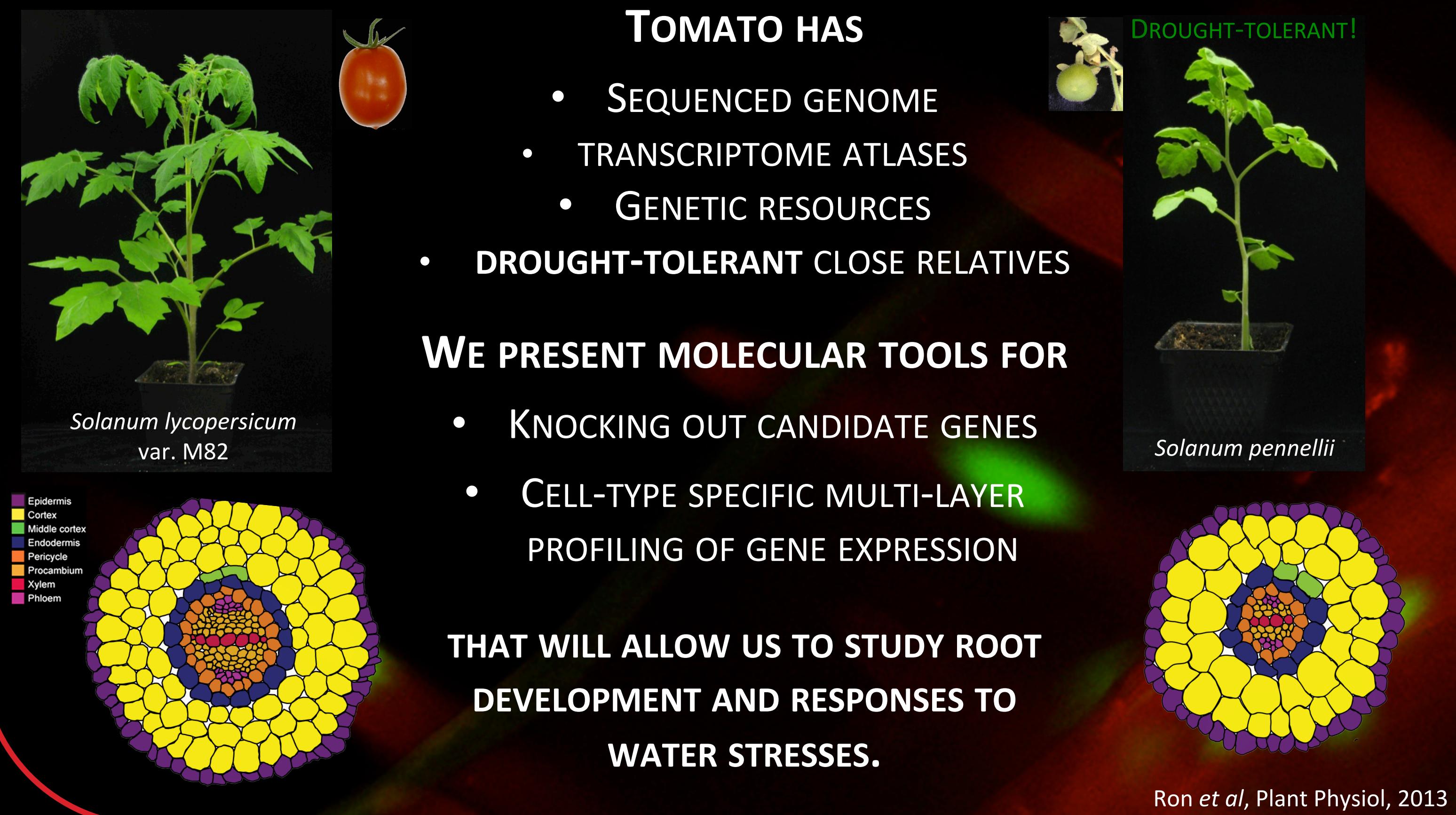
# A RAPID METHOD FOR TRANSLATING MOLECULAR TOOLS TO CROPS



MILY RON<sup>1,2</sup>, KAISA KAJALA<sup>1,2</sup>, GERMAIN PAULUZZI<sup>3</sup>, DONGXUE WANG<sup>4</sup>, MAURICIO A. REYNOSO<sup>3</sup>, KRISTINA ZUMSTEIN<sup>1</sup>, SONJA WINTE<sup>1,2</sup>, JASMINE GARGHA<sup>1,2</sup>, HELEN MASSON<sup>1,2</sup>, SOICHI INAGAKI<sup>2,5</sup>, FERNÁN FEDERICI<sup>6,7</sup>, NEELIMA SINHA<sup>1</sup>, ROGER DEAL<sup>4</sup>, JULIA BAILEY-SERRES<sup>3</sup>, SIOBHAN BRADY<sup>1,2</sup>

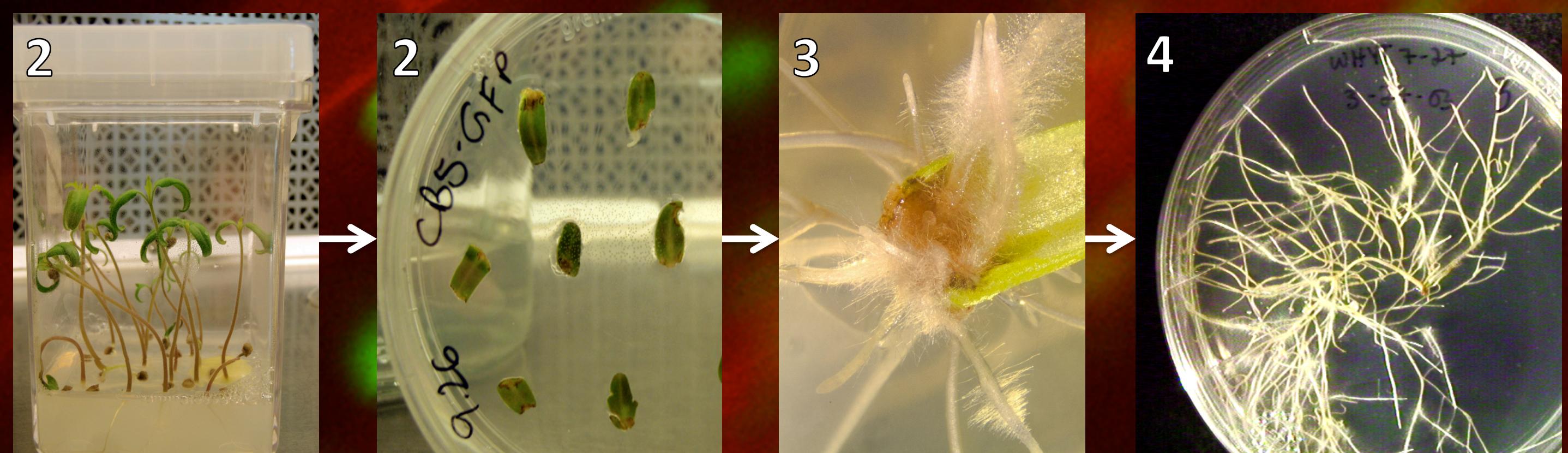
<sup>1</sup> DEPARTMENT OF PLANT BIOLOGY, UC DAVIS, USA. <sup>2</sup> GENOME CENTER, UC DAVIS, USA. <sup>3</sup> CENTER FOR PLANT CELL BIOLOGY, BOTANY AND PLANT SCIENCES DEPARTMENT, UC RIVERSIDE, USA. <sup>4</sup> DEPARTMENT OF BIOLOGY, EMORY UNIVERSITY, USA. <sup>5</sup> DEPARTMENT OF INTEGRATED GENETICS, NATIONAL INSTITUTE OF GENETICS, MISHIMA JAPAN. <sup>6</sup> DEPARTMENT OF PLANT SCIENCES, UNIVERSITY OF CAMBRIDGE, UK. <sup>7</sup> DEPARTAMENTO DE GENÉTICA MOLECULAR Y MICROBIOLOGÍA, PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE, CHILE.

## 1. FUNCTIONAL GENOMICS IN TOMATO



## 2. TRANSGENIC ROOTS IN THREE WEEKS!

HAIRY ROOT INDUCTION WITH *AGROBACTERIUM RHIZOGENES*  
(CAN BE DONE IN MANY DIFFERENT PLANT SPECIES)



- 1) BINARY VECTOR ELECTROPORATED INTO VIRULENT *A. RHIZOGENES*.
- 2) TOMATO COTYLEDONS ARE CO-CULTIVATED WITH *A. RHIZOGENES*
- 3) T-DNA CONTAINING THE RI-LOCUS INDUCES HAIRY ROOT GROWTH.
- 4) ROOTS CAN BE SUBCLONED AND CULTURED FOR YEARS!

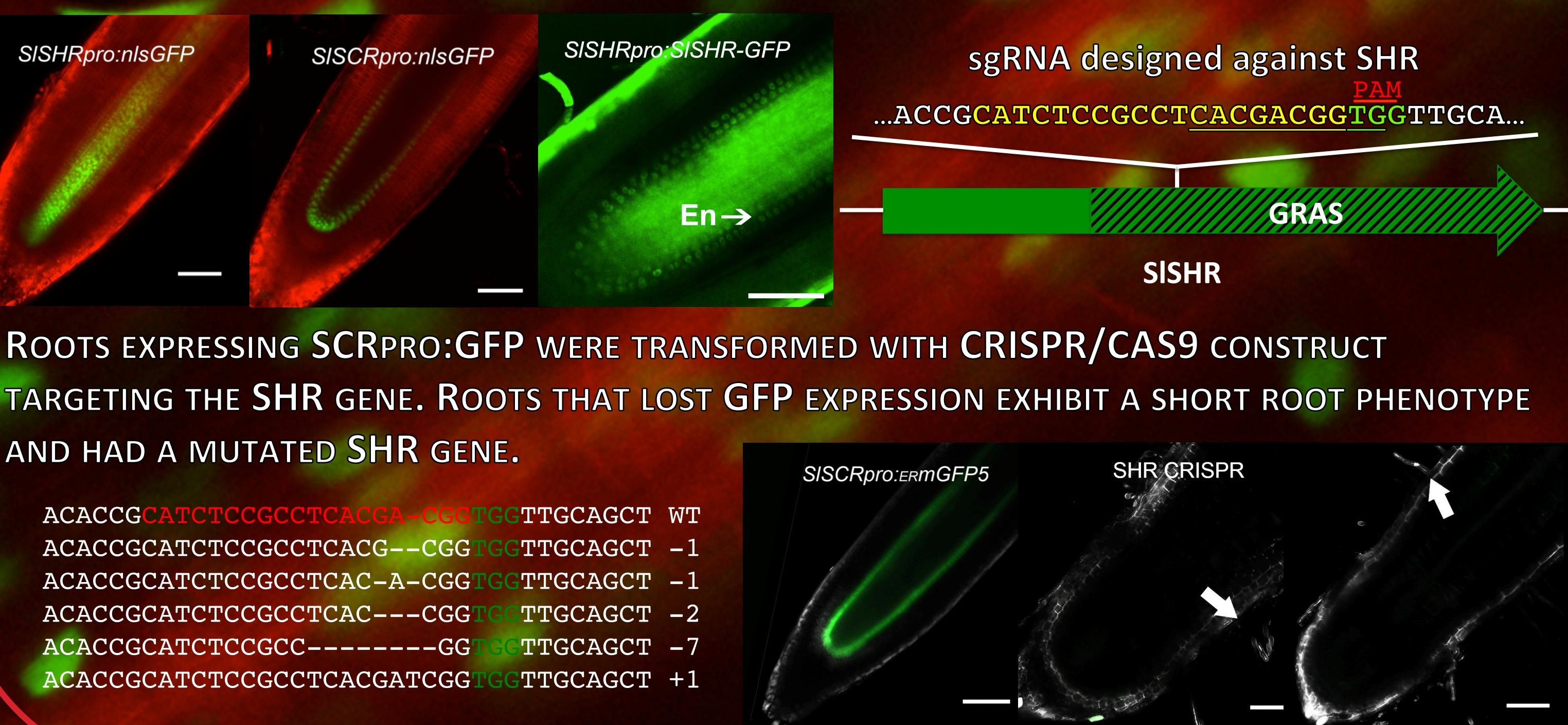
## 3. FUNCTIONAL STUDY USING CRISPR

### SHORT-ROOT IN ARABIDOPSIS:

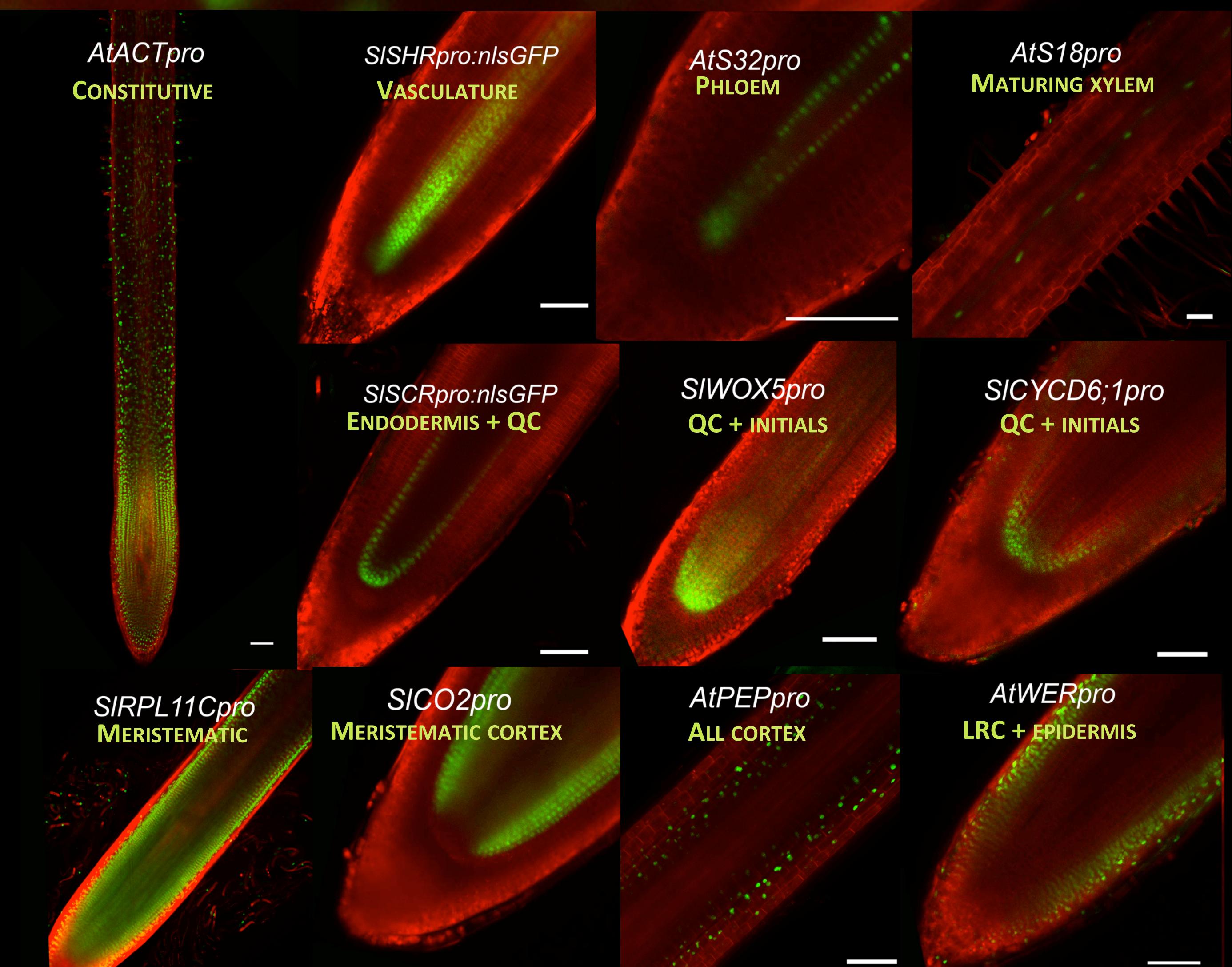
SHR IS A NON-CELL-AUTONOMOUS TRANSCRIPTION FACTOR EXPRESSED IN THE STELE.  
THE PROTEIN MOVES TO THE ENDODERMIS AND ACTIVATES SCARECROW (SCR) EXPRESSION.  
*shr* MUTANTS HAVE SHORT MERISTEMS RESULTING IN SHORT ROOTS.

### SHORT-ROOT IN TOMATO:

SHR IS EXPRESSED IN THE STELE AND SCR IS EXPRESSED IN THE ENDODERMIS.  
THE SHR PROTEIN MOVES TO THE ENDODERMIS.



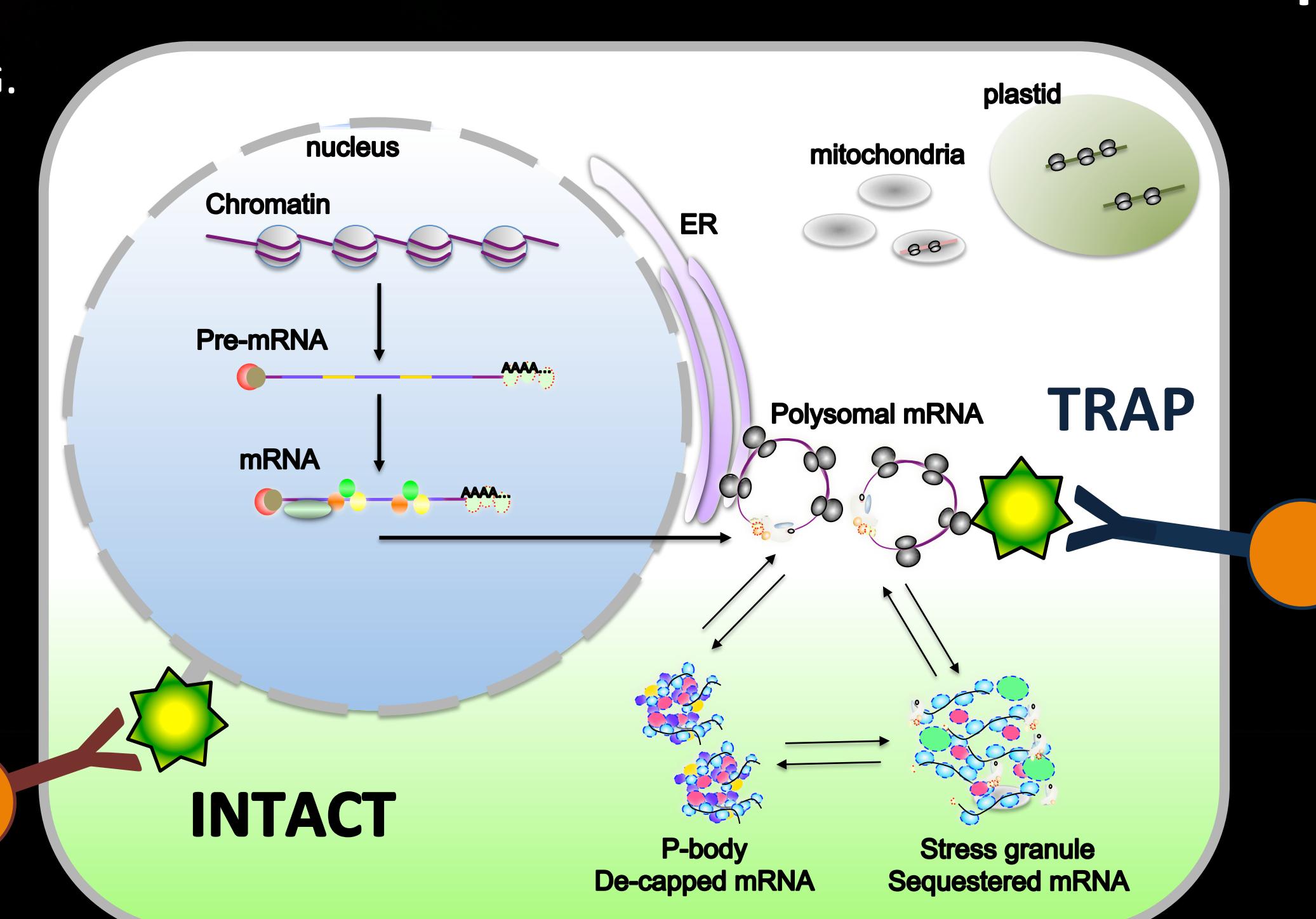
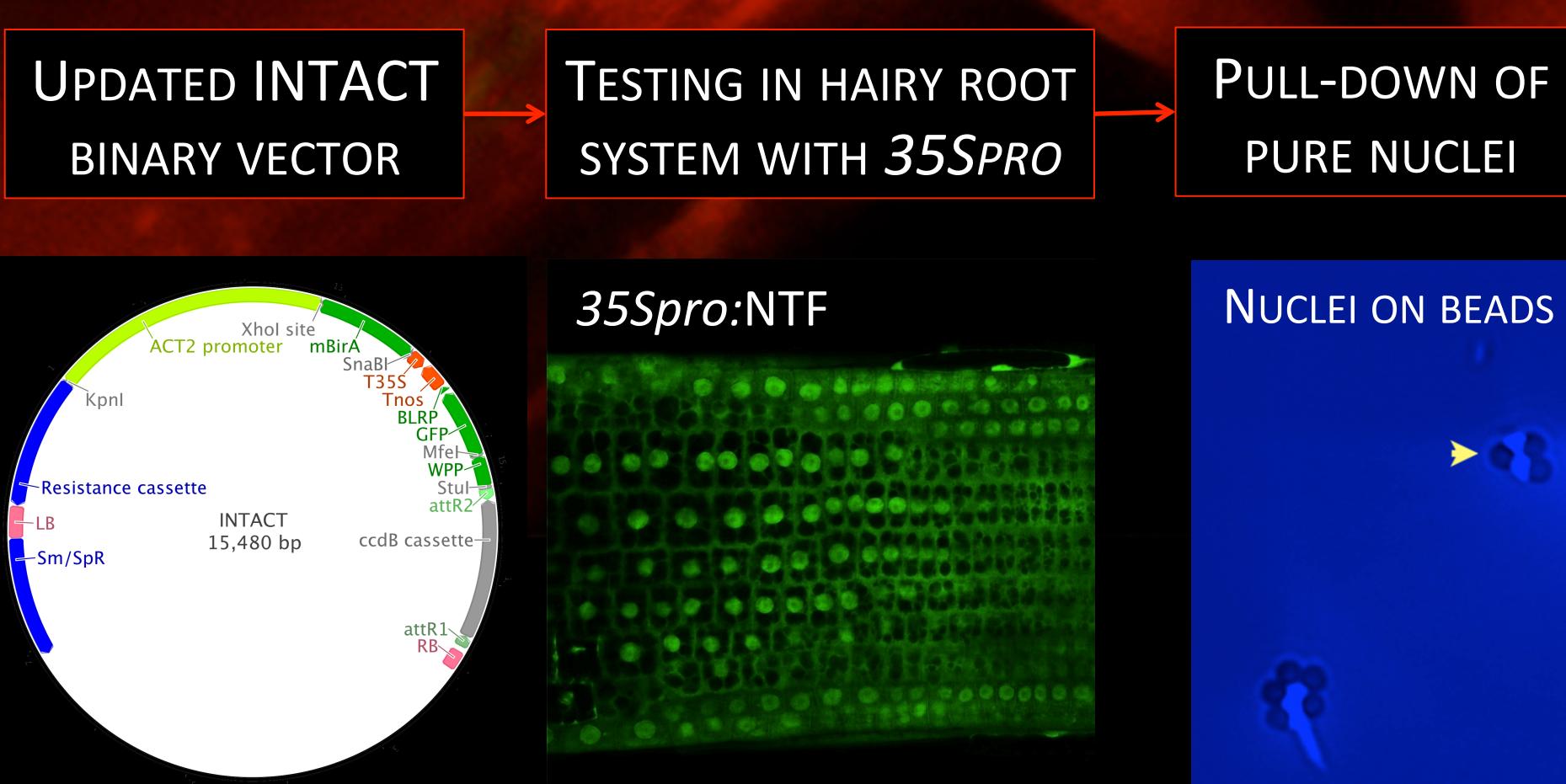
## 4. CELL-TYPE SPECIFIC PROMOTERS



## 5. TOOLS FOR ISOLATING NUCLEI AND RIBOSOMES FROM SPECIFIC CELL-TYPES

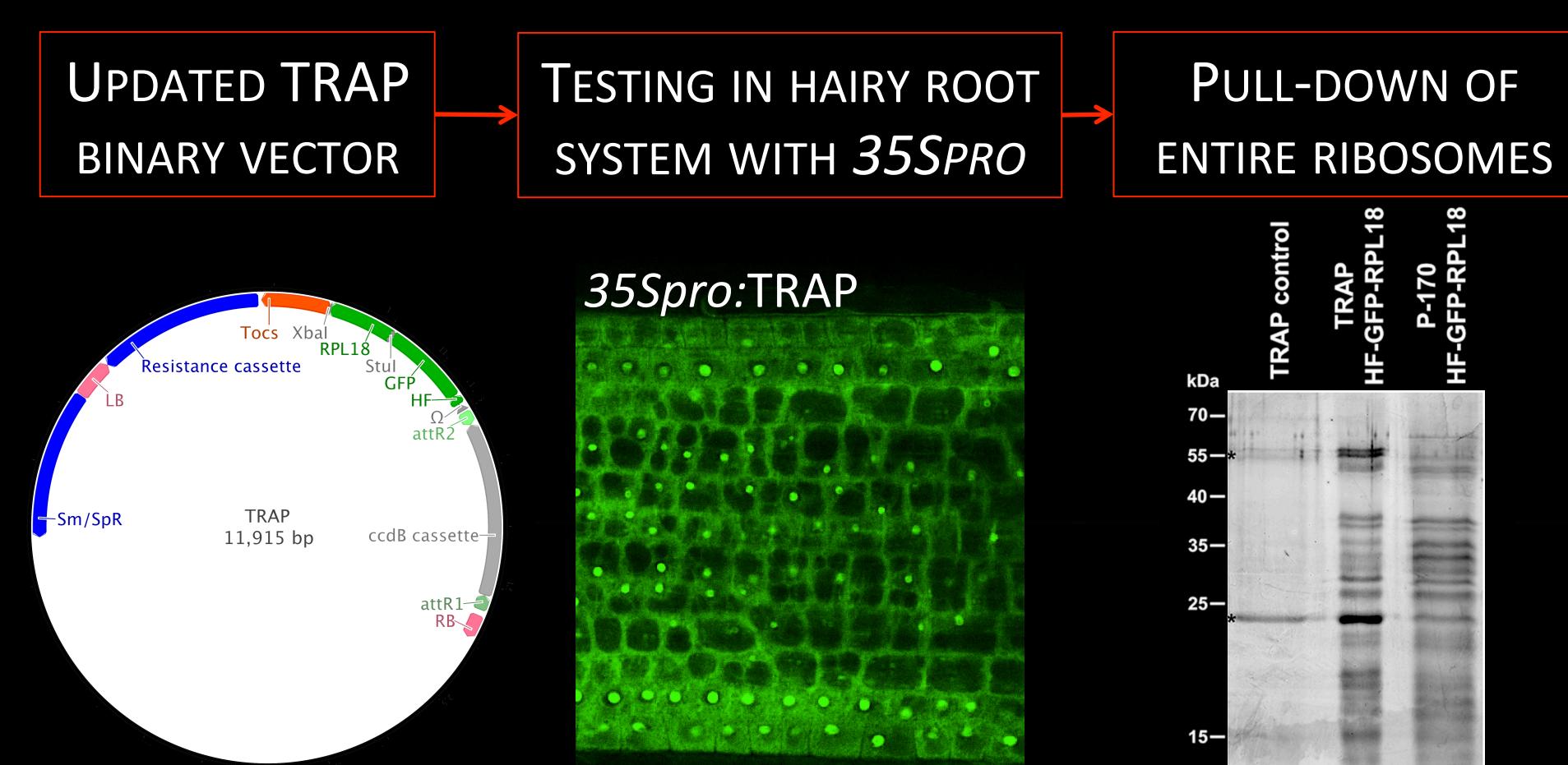
### INTACT: ISOLATION OF NUCLEI TAGGED IN CELL TYPES

- 1) USE YOUR FAVORITE PROMOTER TO EXPRESS A NUCLEAR BIOTIN TAG.
- 2) PULL DOWN THE NUCLEI WITH STREPTAVIDIN BEADS.
- 3) SUBSEQUENT ANALYSES: CHIP-SEQ, RNA-SEQ, ETC.



### TRAP: TRANSLATING RIBOSOME AFFINITY PURIFICATION

- 1) USE YOUR FAVORITE PROMOTER TO EXPRESS A RIBOSOME FLAG TAG.
- 2) PULL DOWN THE RIBOSOMES WITH  $\alpha$ -FLAG BEADS.
- 3) SUBSEQUENT ANALYSES: RNA-SEQ, RIBOSOME FOOTPRINTING.



Bailey-Serres 2013; Deal and Henikoff 2010; Zanetti et al., 2005; Mustroph et al., 2009.

UCDAVIS



USE QR READER TO GET THE PAPER AND COMPLETE PROTOCOL ☺

