

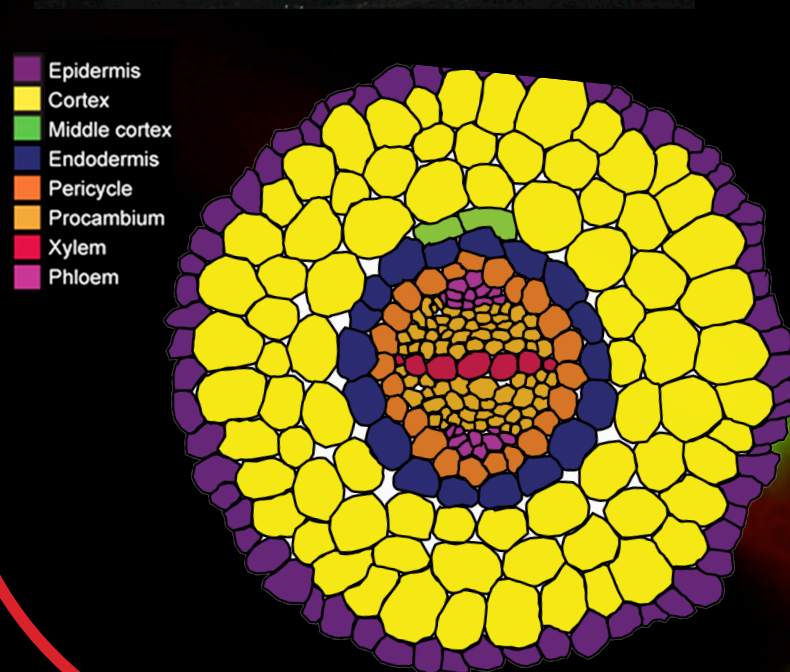
A RAPID METHOD FOR TRANSLATING MOLECULAR TOOLS TO CROPS



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1. FUNCTIONAL GENOMICS IN TOMATO



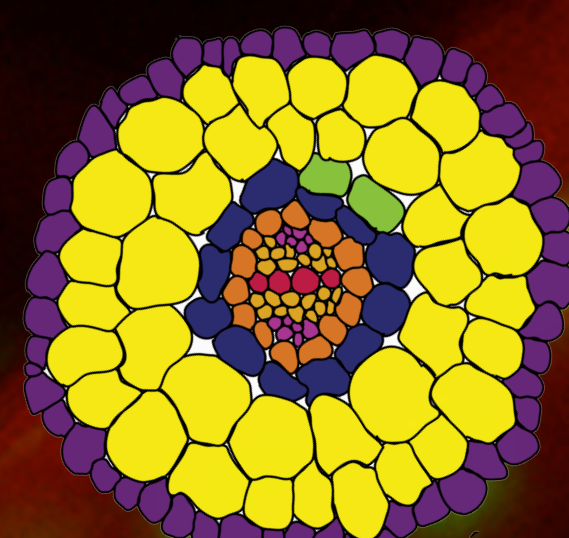
TOMATO HAS

- SEQUENCED GENOME
- TRANSCRIPTOME ATLASES
- GENETIC RESOURCES
- DROUGHT-TOLERANT CLOSE RELATIVES

WE PRESENT MOLECULAR TOOLS FOR

- KNOCKING OUT CANDIDATE GENES
- CELL-TYPE SPECIFIC MULTI-LAYER PROFILING OF GENE EXPRESSION

THAT WILL ALLOW US TO STUDY ROOT DEVELOPMENT AND RESPONSES TO WATER STRESSES.

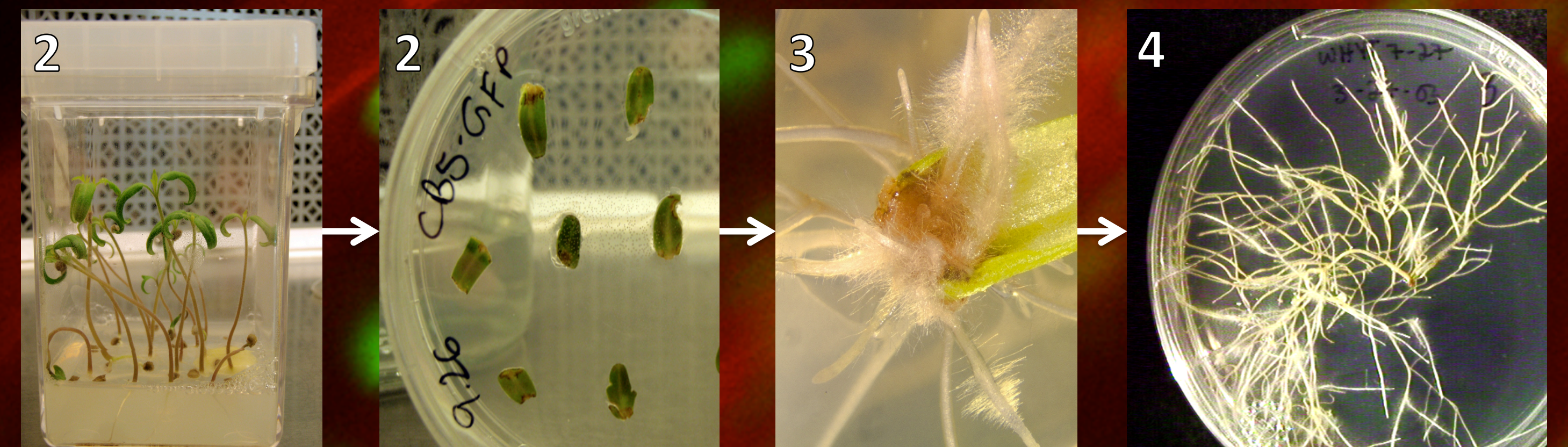


Ron et al, Plant Physiol, 2013

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!

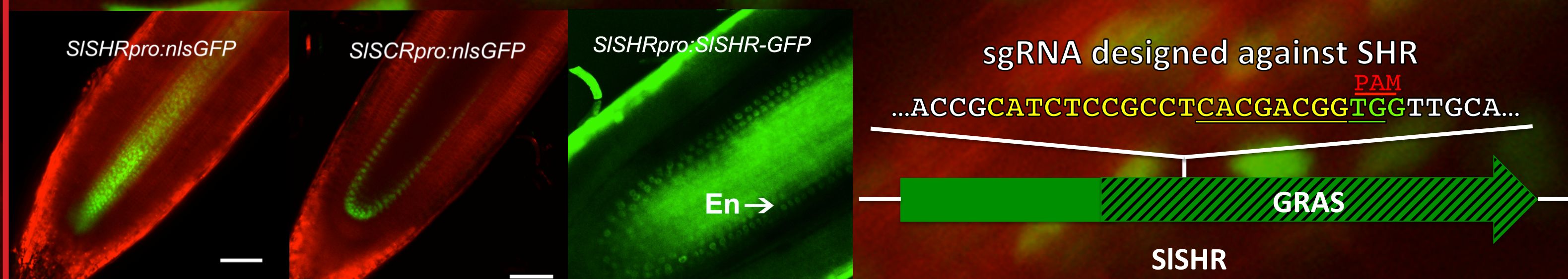
3. FUNCTIONAL STUDY USING CRISPR

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.

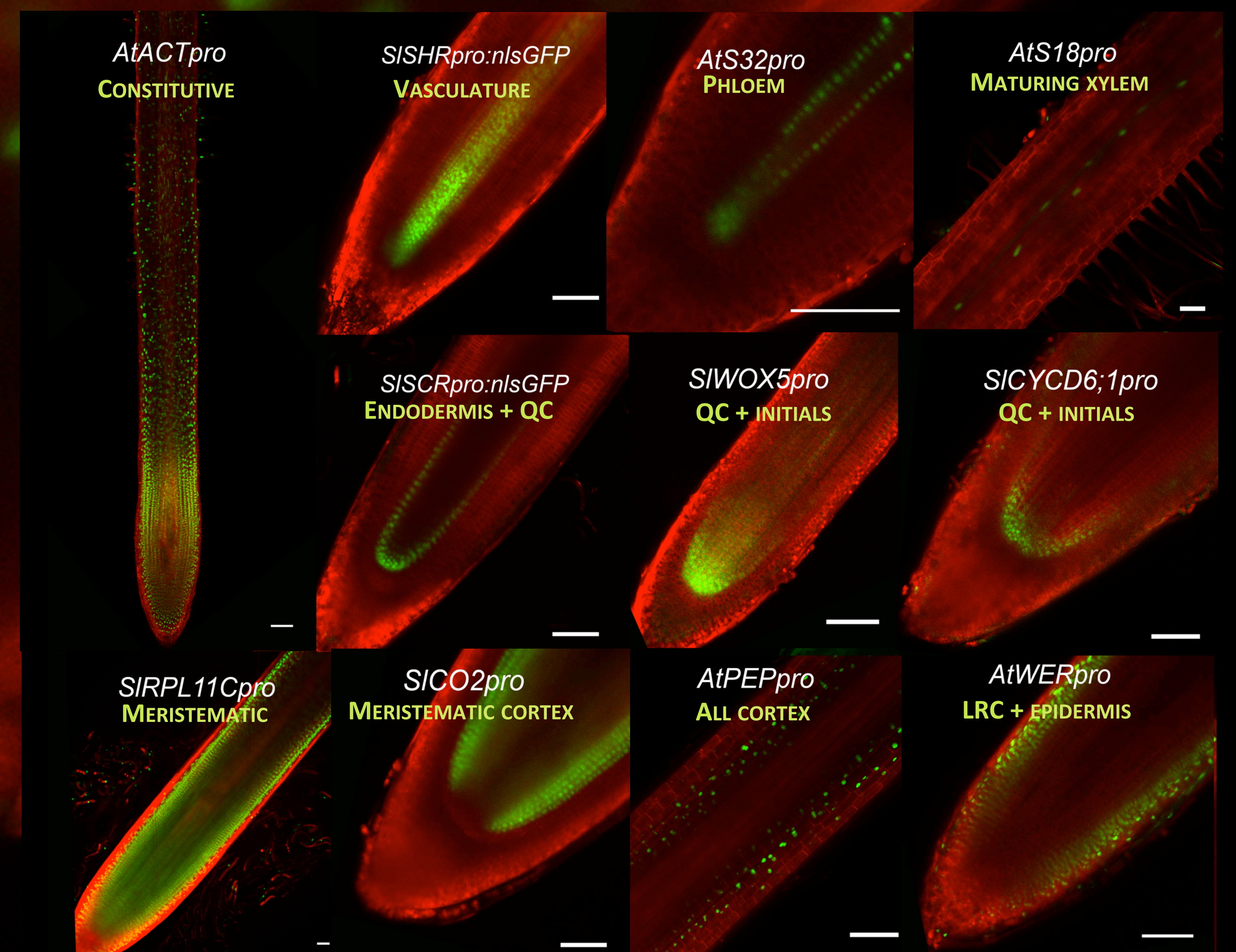


ROOTS EXPRESSING SCRPRO:GFP WERE TRANSFORMED WITH CRISPR/CAS9 CONSTRUCT TARGETING THE SHR GENE. ROOTS THAT LOST GFP EXPRESSION EXHIBIT A SHORT ROOT PHENOTYPE AND HAD A MUTATED SHR GENE.

ACACCGCATCTCCGCTCAGCA--CGGTTTGCAGCT WT
ACACCGCATCTCCGCTCAGC--CGGTTTGCAGCT -1
ACACCGCATCTCCGCTCAGC-A-CGGTTTGCAGCT -1
ACACCGCATCTCCGCTCAGC---CGGTTTGCAGCT -2
ACACCGCATCTCCGCTCAGC-----GGTTTGCAGCT -7
ACACCGCATCTCCGCTCAGCATCGGTTTGCAGCT +1



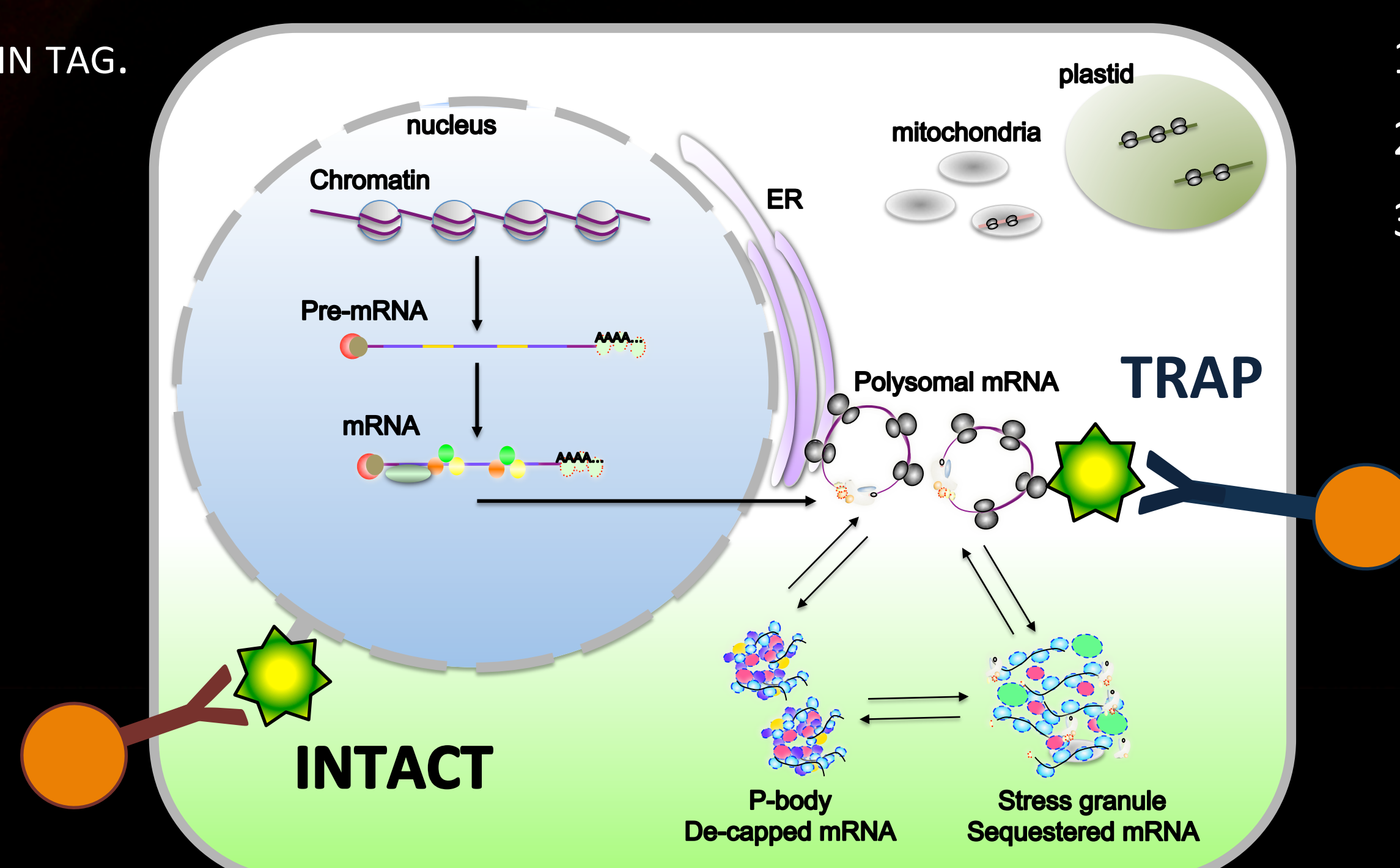
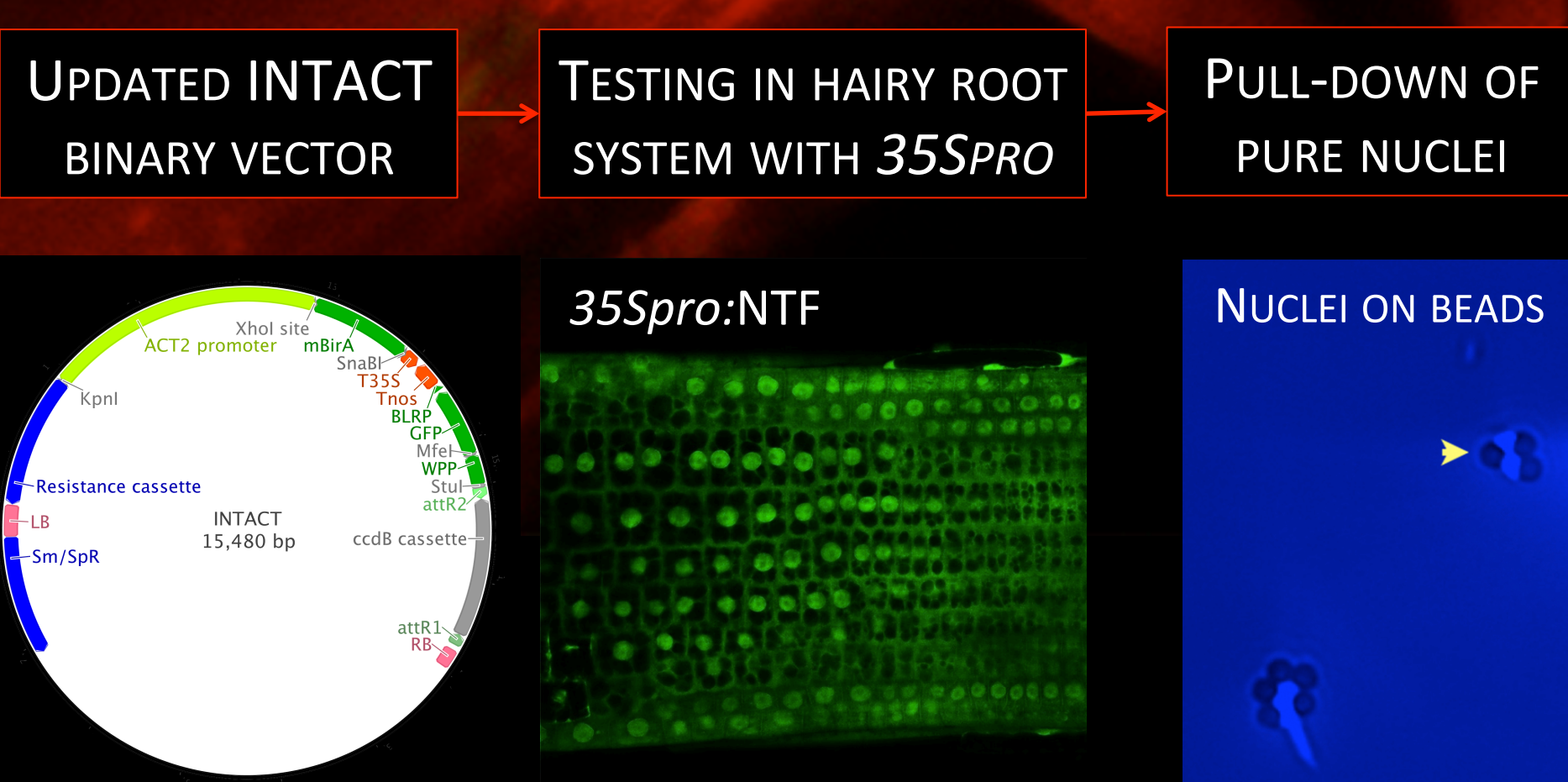
4. CELL-TYPE SPECIFIC PROMOTERS



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 α -FLAG BEADS.
- 3) SUBSEQUENT ANALYSES: RNA-SEQ, RIBOSOME FOOTPRINTING.

