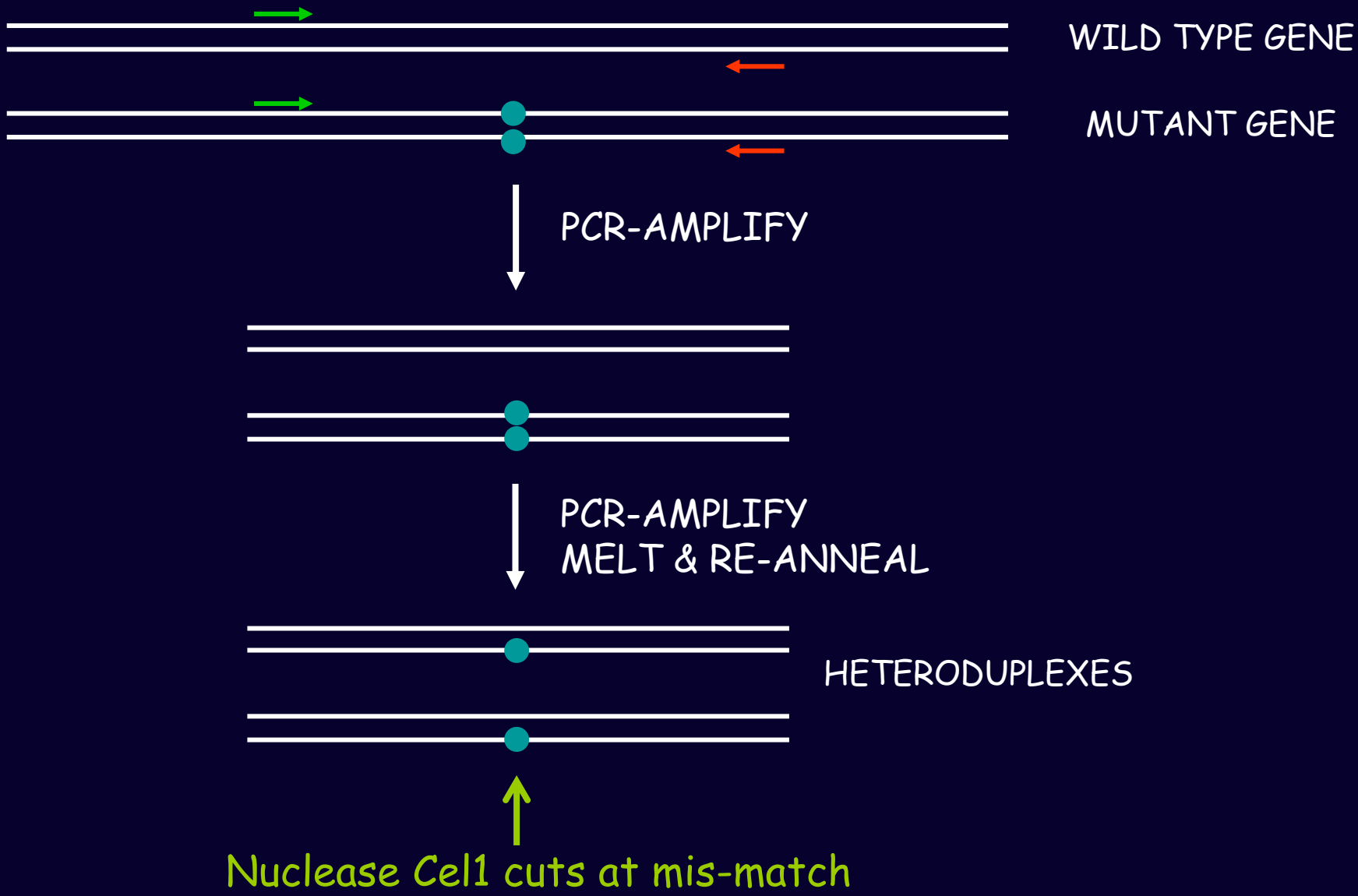
WGIN Programme: TILLING in hexaploid wheat

Year 1 Report

What is TILLING?

- Targeting Induced Local Lesions in Genomes
- PCR-based reverse genetics screen for polymorphisms in genes controlling key traits
- Non-GM method of targeted gene modification in mutagenised populations
- Identifies natural variation in genes of elite varieties, historic lines and landraces.

TILLING
(Targeting Induced Local Lesions in Genomes)



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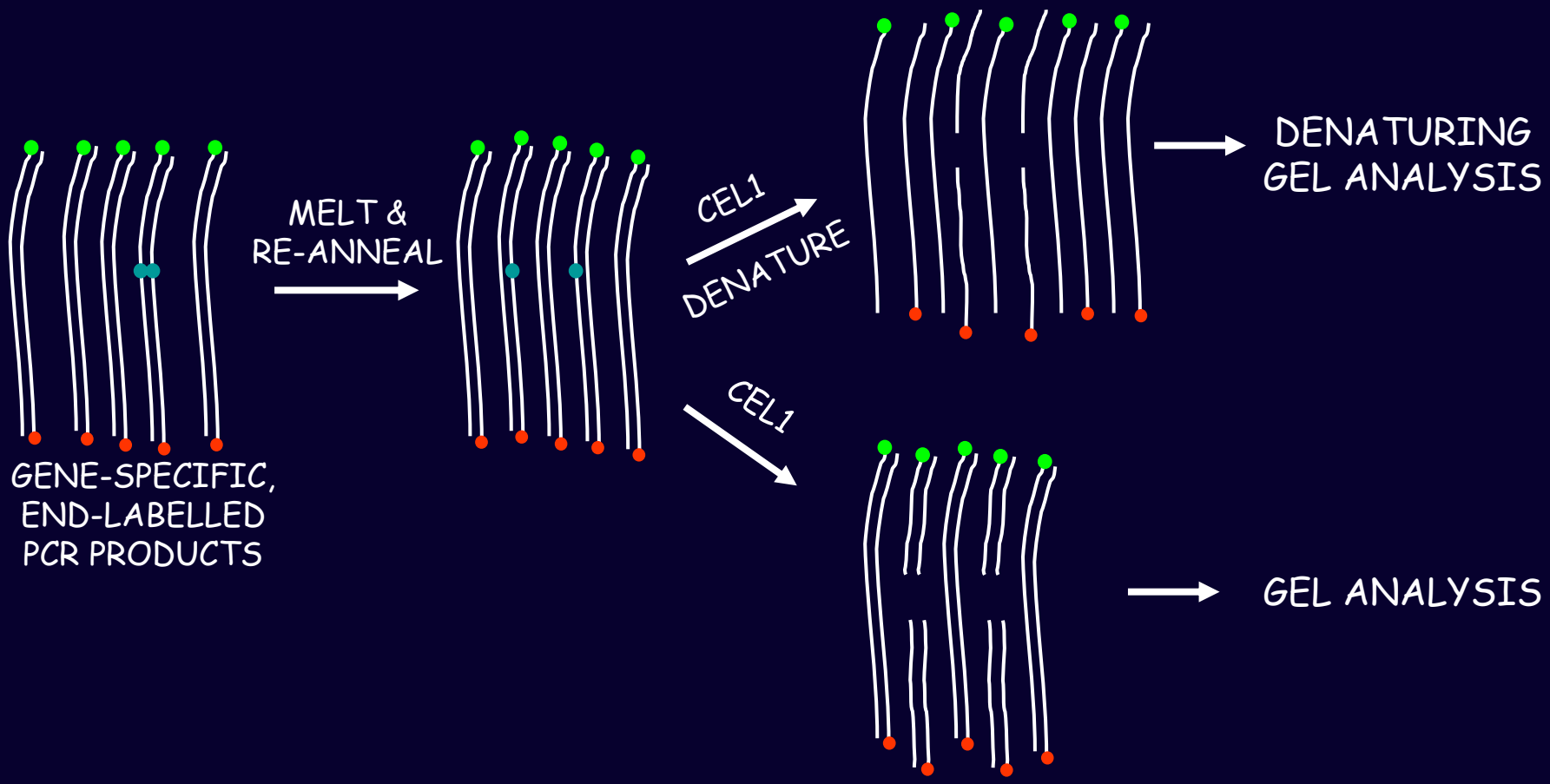
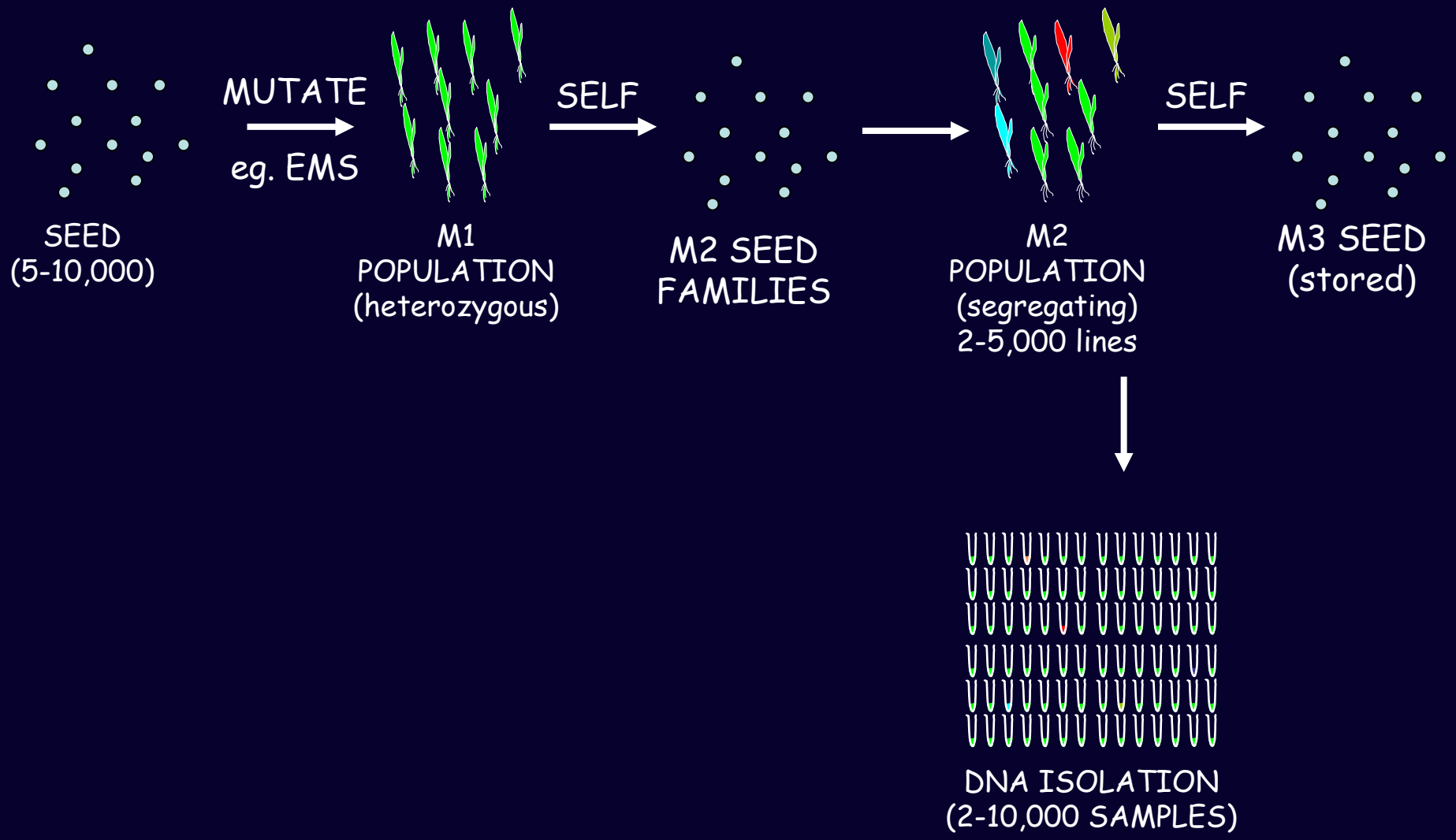


PRODUCTION OF MUTAGENIZED POPULATION



HIGH-THROUGHPUT TILLING

eg. 3,000 M_2 LINES

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3,000 DNA SAMPLES

↓

375 @ 8-FOLD POOLS

↓

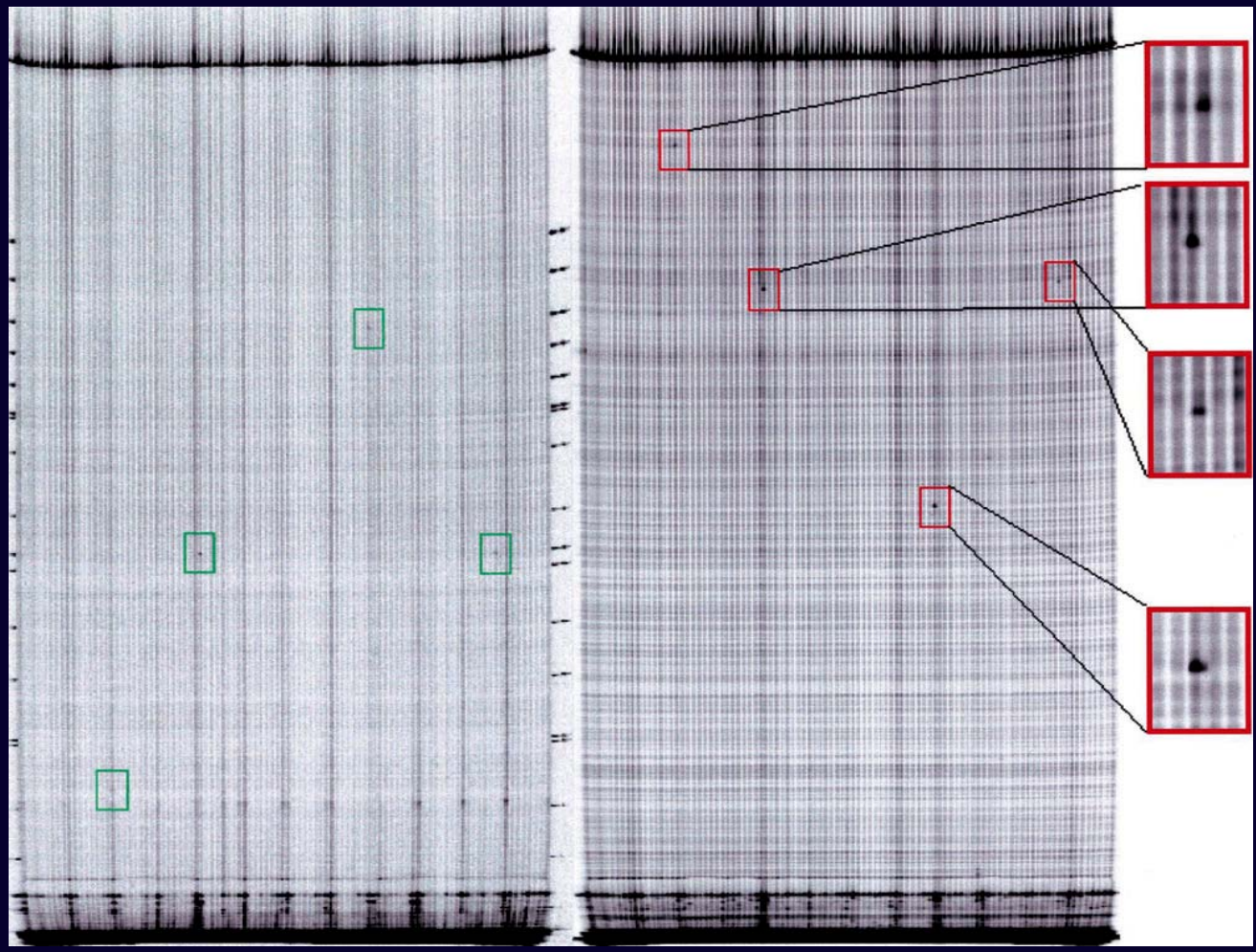
GENE-SPECIFIC PCR,
DENATURATION,
CEL1-CLEAVAGE

↓

4 @ 96-LANE GEL RUNS

TILLING EXAMPLE

ARABIDOPSIS DNA, 8-FOLD POOLING



LEFT PRIMER

RIGHT PRIMER

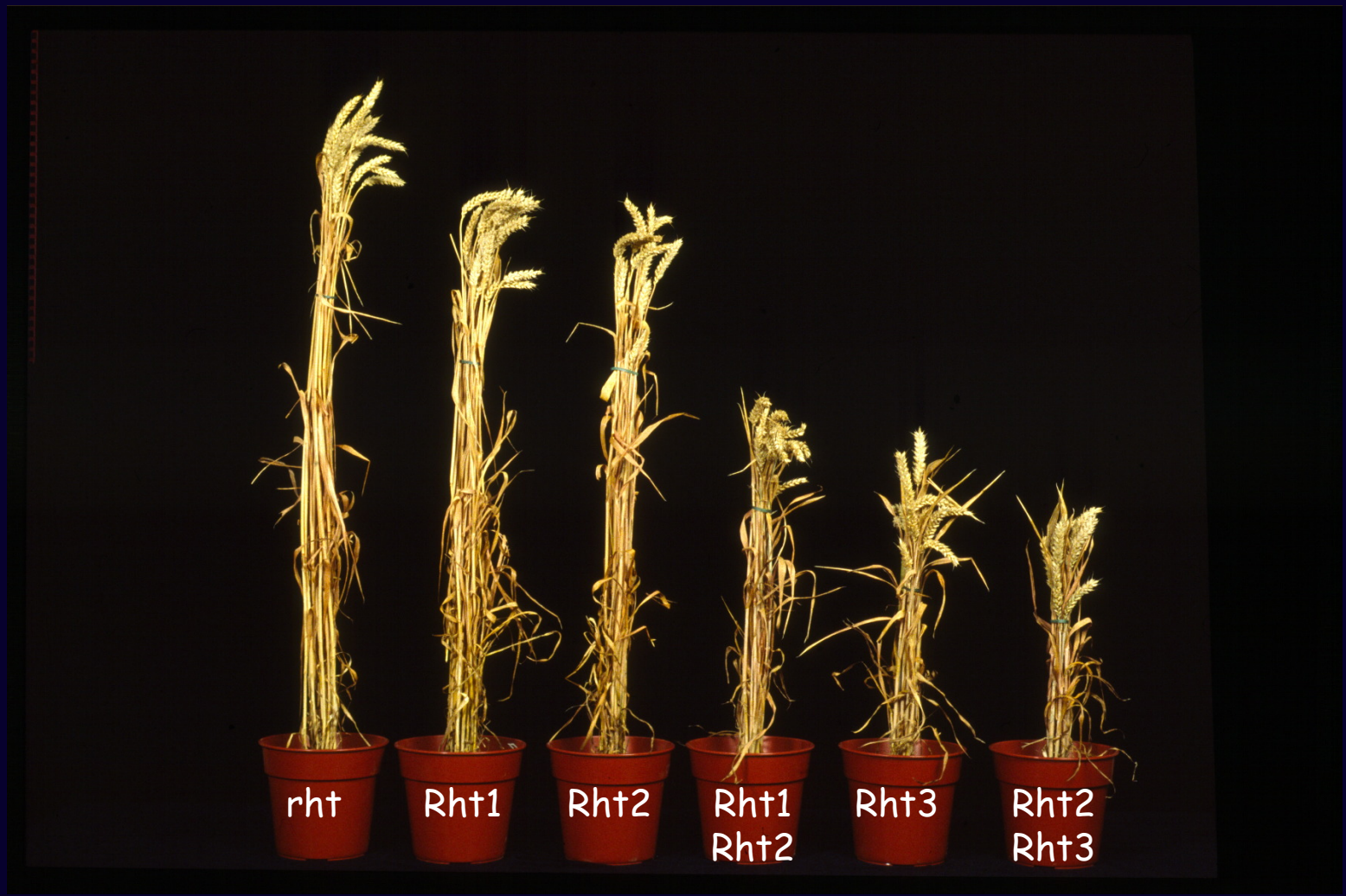
From Colbert T, *et al* (2001) *Plant Physiol* 126:480-4.

TILLING FOR WHEAT IMPROVEMENT

- Non-GM approach
- Can screen mutagenized lines or natural variation
- Provides a series of alleles of varying strength
- Generates perfect genetic markers for breeding
- Possible to target complex multigenic traits
- Targeting individual homoeologues of wheat uncovers traits not detectable by normal screens
- But – do need specific genes as targets – dependent on good basic knowledge of gene function
- And – need to develop homoeologue-specific PCR amplification at each target locus (rate-limiting)
- However - high ploidy level of wheat allows high levels of mutagenesis

INITIAL WGIN TILLING TARGETS

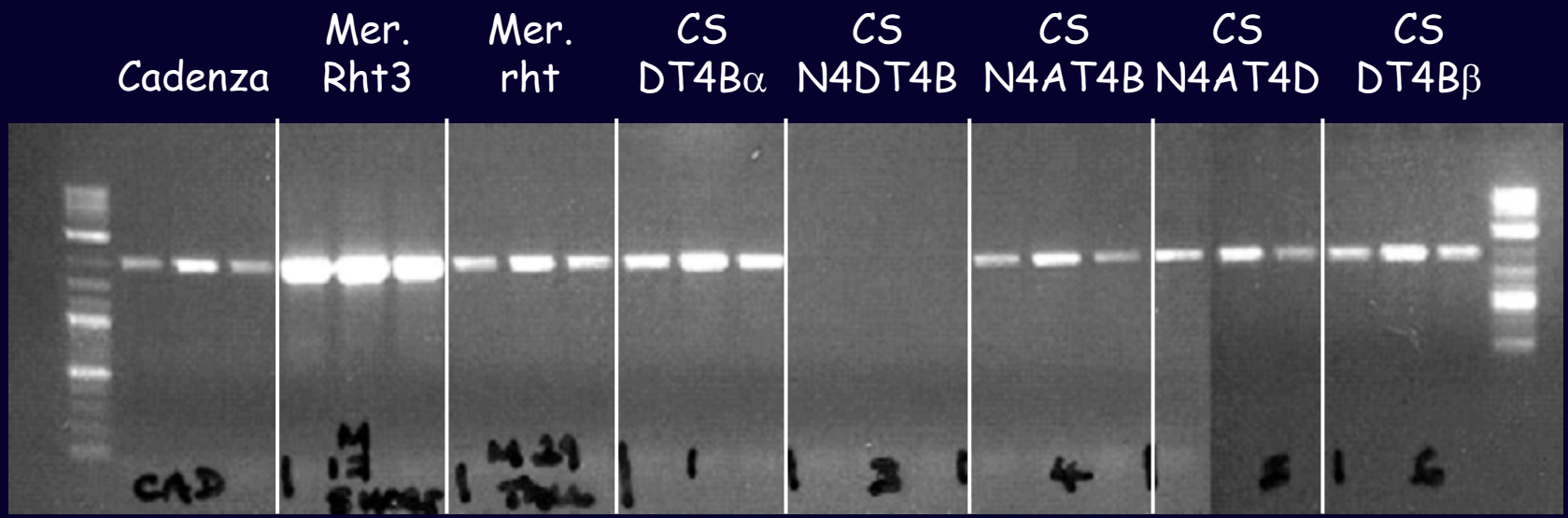
NEW ALLELES OF RHT AS PROOF-OF-CONCEPT



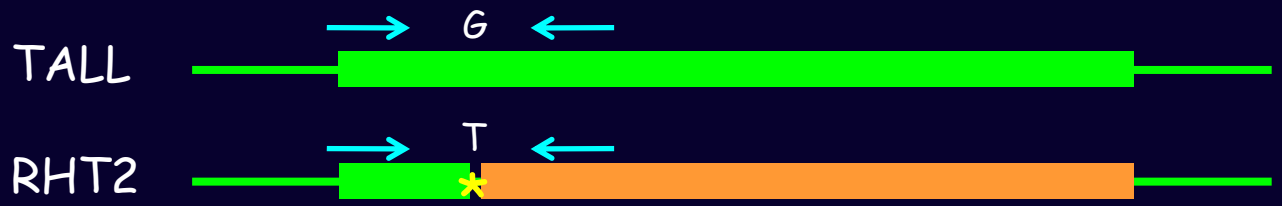
PCR TILLING IN RHT

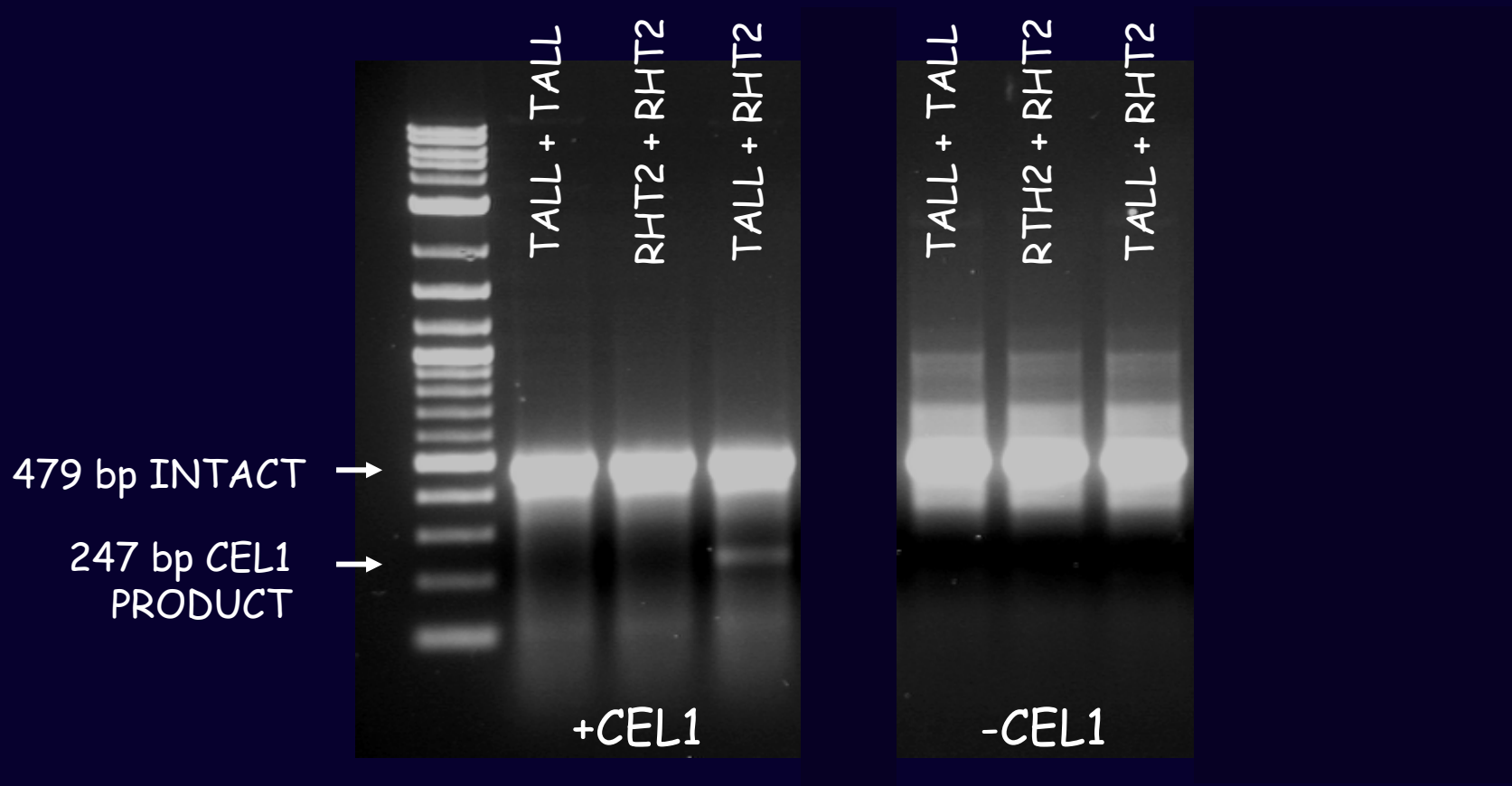
1. Designed and tested homeologue-specific primers for Rht-B1 (Rht1, Rht3) and Rht-D1 (Rht2).

Eg. Rht-D1-specific primers



TESTING PCR TILLING WITH RHT

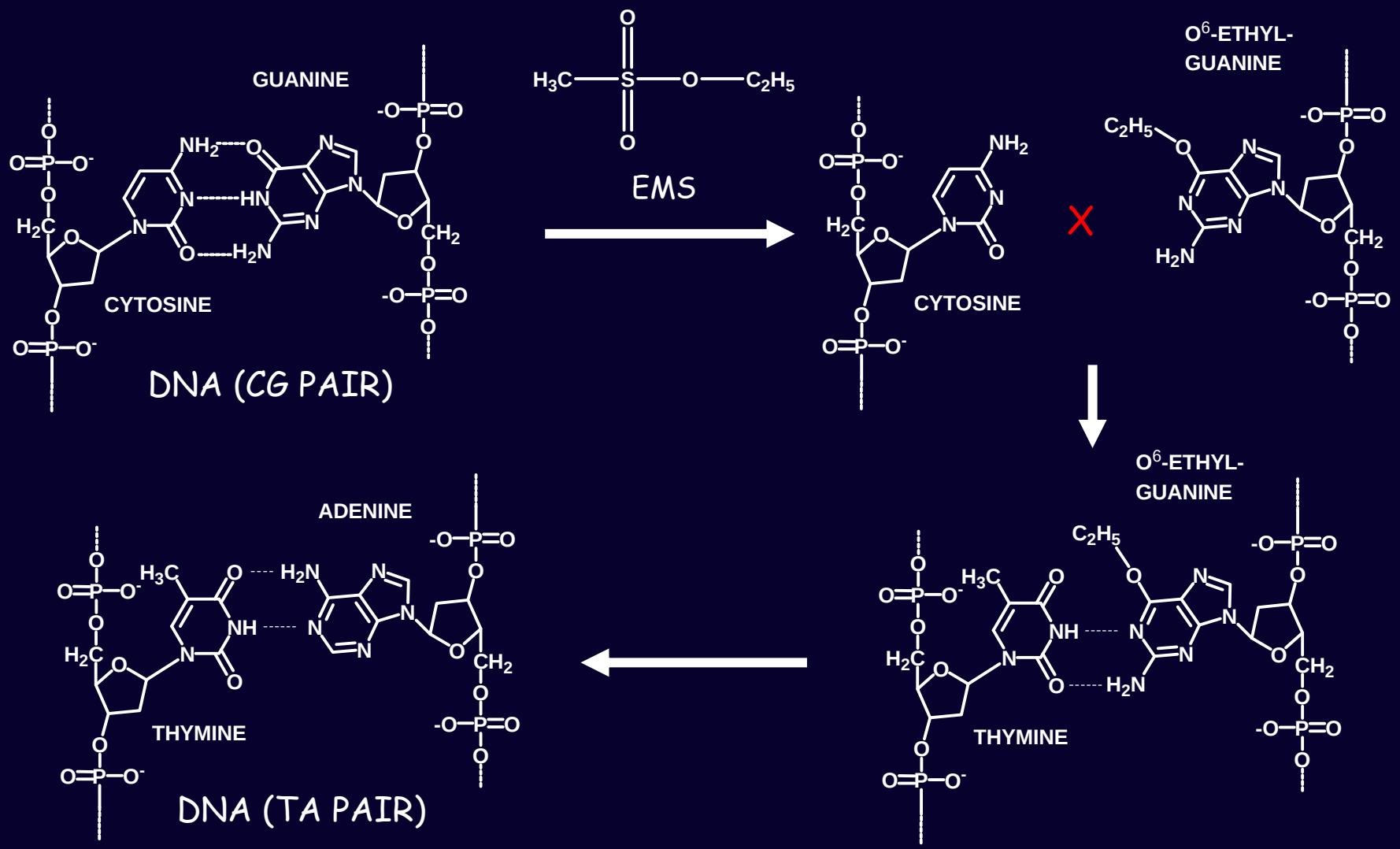




TILLING POPULATIONS FOR WGIN

1. ~10,000 Cadenza seeds treated with 0.3%, 0.6% & 0.9% ethylmethane sulphonate for 16h, field-sown 2004.
2. Ears from ~3000 M1 individuals harvested Sept 2004. Now replanting for M2 production.
3. ~2x 4000 Cadenza seeds treated with 0.6% and 0.9% for 16h, sown under glass as for SSD.
4. Harvested leaf material for DNA from 7,000 Paragon M_2, produced by Rob Koebner, JIC.

CHEMICAL MUTAGENESIS



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Cadenza M1 #2

ESTABLISHMENT OF A TILLING FACILITY

SUMMARY OF PROGRESS

- Visited TILLING labs at SCRI Dundee, U. Washington and JIC Norwich for advice & training
- Set up EMS-treated populations of hexaploid wheat (Cadenza) and collected from JIC M2 lines (Paragon)
- Imported high-throughput DNA extraction method from RAGT (Peter Jack)
- Established homoeologue-specific PCR for primary targets
- Purified Cel1 from celery
- Shown Cel1 cleavage of heteroduplex PCR products
- Won BBSRC grant for TILLING equipment:
  - Liquid handling robot
  - Licor sequence analysis system

RRes WGIN Programme Team

Kim Hammond-Kosack (PPI) – Cereal pathogenesis & diploid TILLING

Andy Phillips (CPI) – Gibberellin signalling and hexaploid TILLING

Hai-Chun Jing (PPI) – Pathogenesis and diploid TILLING

Katie Tearall (CPI) – Hexaploid Tilling

Sam Irving – WGIN administration