RenSeq : méthodologie et application chez le Blé

Clémence Marchal

PhD Student

@ClemMarchal

Mapping-by-sequencing in wheat

Arabidopsis
0.12 b

Poplar
0.423 Gb

Wild grass
~7 Gb

Genes Gene family

Wheat
17 Gb
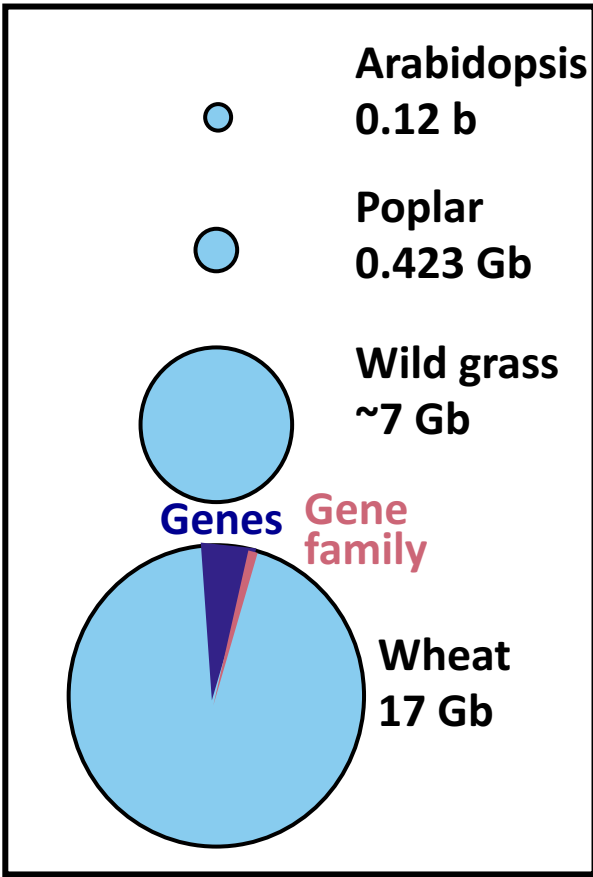
Whole-Genome-Sequencing based approaches

Schneeberger et al., 2009
Austin et al., 2011

Reduced representation sequencing approaches

RNA-sequencing

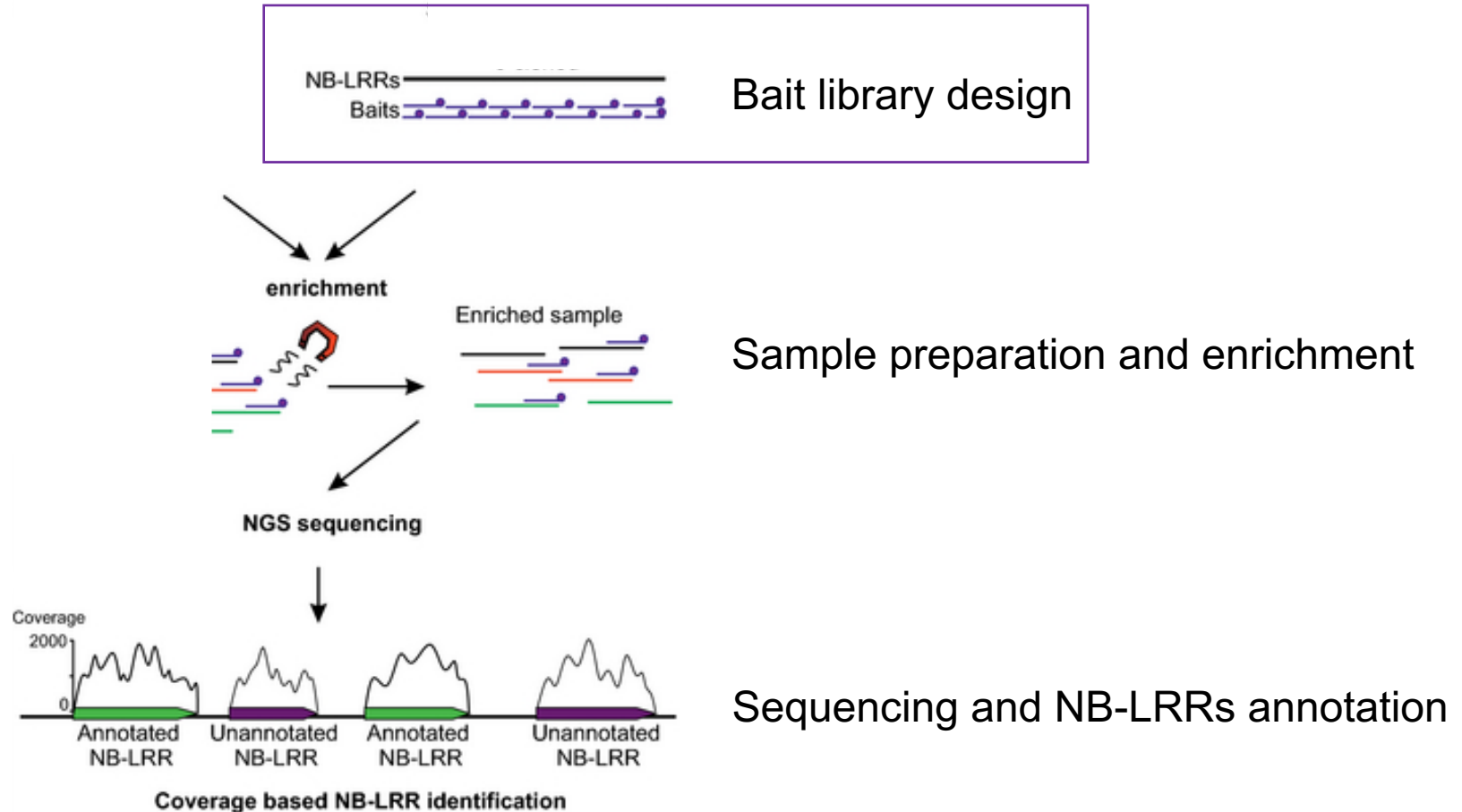*Ramirez-Gonzalez et al., 2015*

Exome capture and sequencing

King et al., 2015

Targeted sequencing

R-genes display a distinct pattern

Arabidopsis
0.12 Gb

Poplar
0.423 Gb

Wild grass
~7 Gb

Genes *R* **genes**

Wheat
17 Gb

N — TIR — NB-ARC — LRRs — C

N — CC — NB-ARC — LRRs — C

Reduced representation sequencing approaches

RNA-sequencing

Ramirez-Gonzalez et al., 2015

Exome capture and sequencing

King et al., 2015

Resistance gene enrichment Sequencing
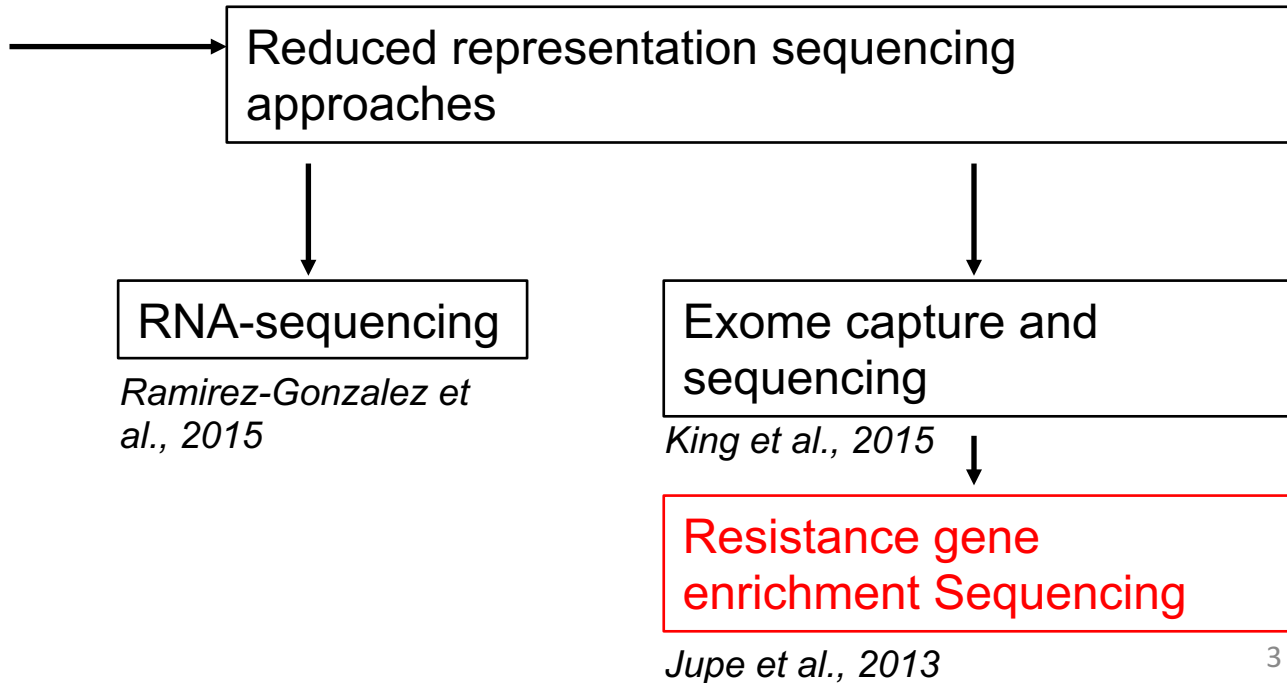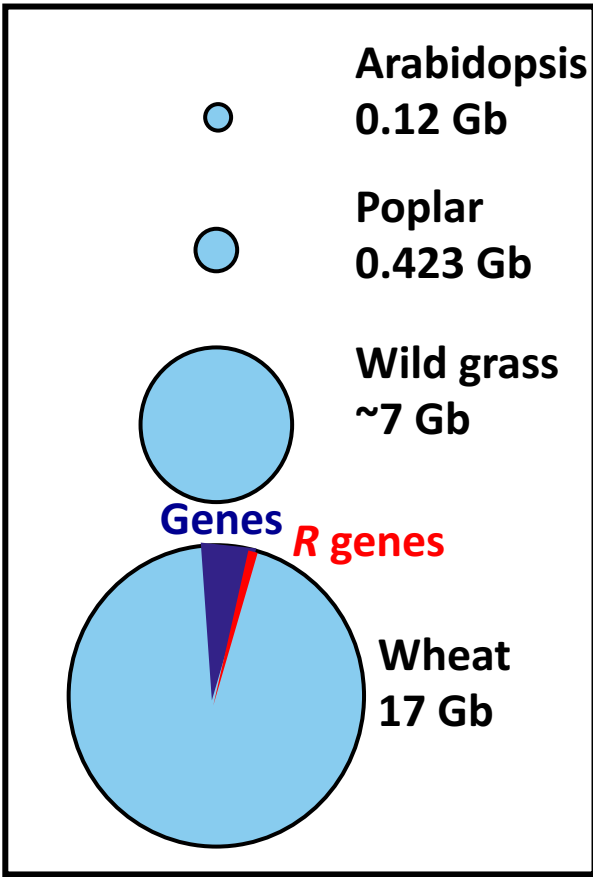
Jupe et al., 2013

RenSeq enables gene re-annotation and rapid mapping of resistance loci

Bait library design

Sample preparation and enrichment

Sequencing and NB-LRRs annotation

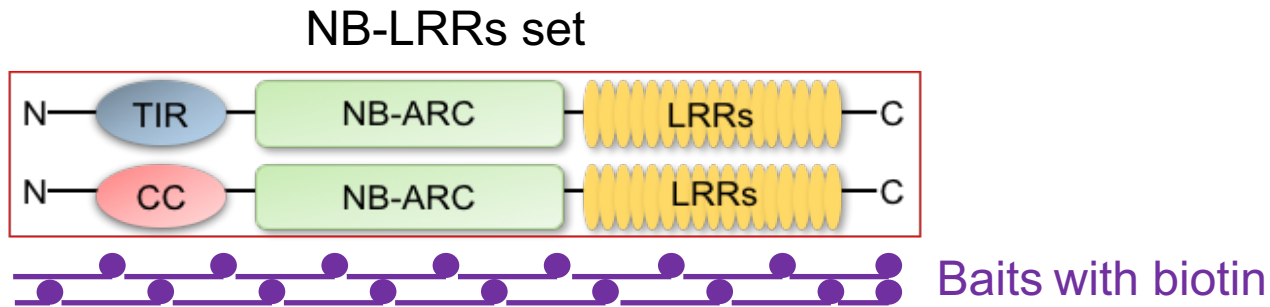*Adapted from Jupe et al., 2013*

Bait library design

➡ **Defining a NB-LRRs set in the targeted plant:**

- Previous annotation available

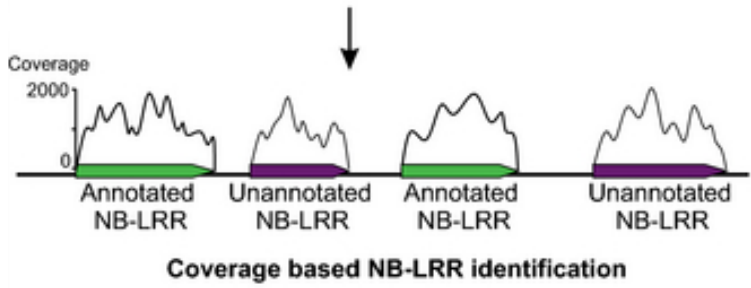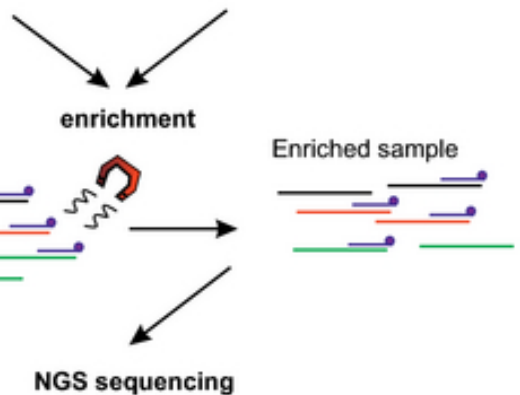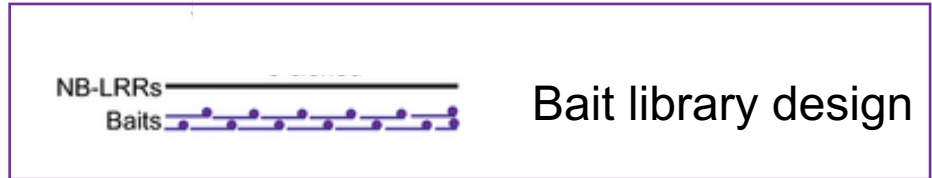- Pfam scan for NB-ARC domain

➡ **Bait design**

NB-LRRs set

Baits with biotin

Triticeae bait library available: *Steuernagel et al., 2016*

60,000 baits, each 120 bases ➡ MYcroarray
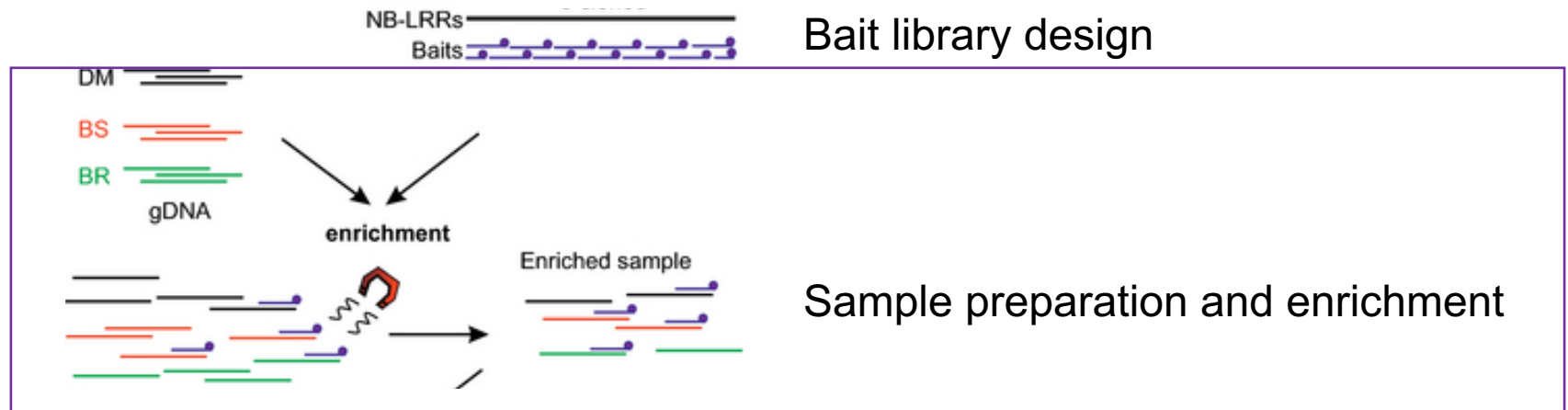
Bait library design

Bait library design

Sample preparation and enrichment

Sequencing and NB-LRRs annotation

Adapted from Jupe et al., 2013

Sample preparation and enrichment

Bait library design

Sample preparation and enrichment

Adapted from Jupe et al., 2013

Sample preparation and enrichment

1. gDNA sonication

Baits

BS

BR

gDNA

2. Hybridisation

3. Selection with streptavidin

Enriched sample
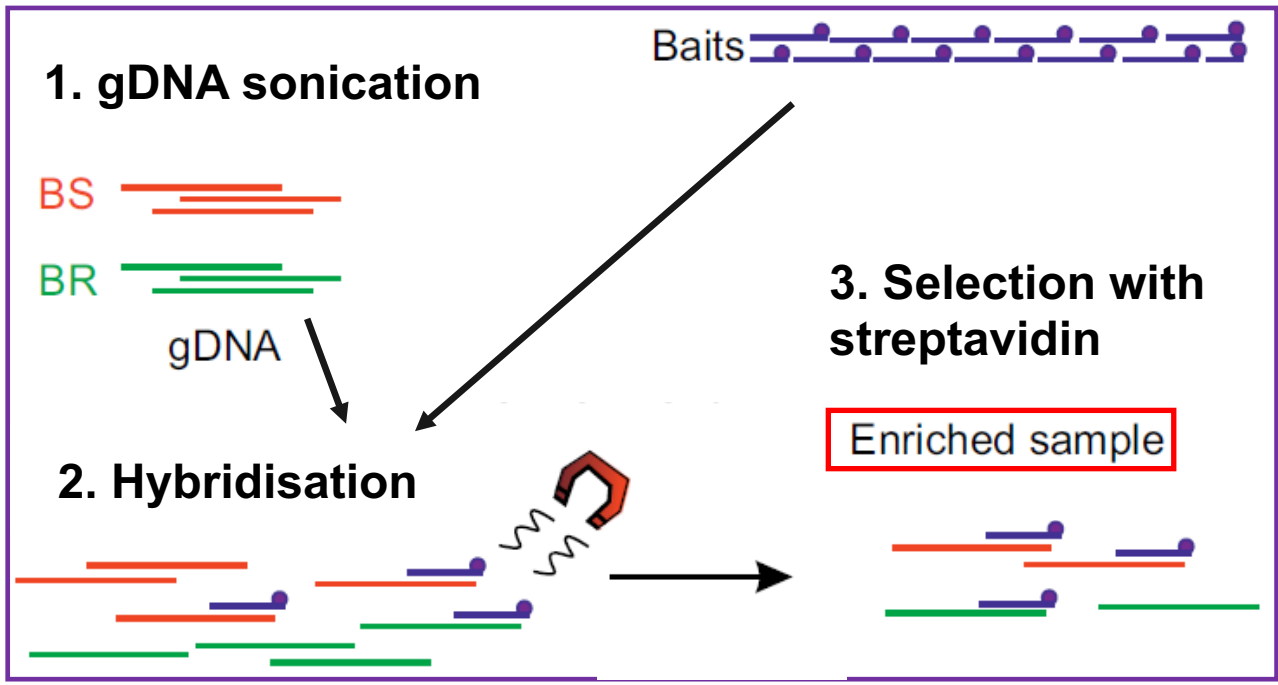
qPCR check for enrichment

After capture
Mean diff=9 cycles
$2^9 = 572x$
enrichment

Before capture

Sample preparation and enrichment

Bait library design

Sample preparation and enrichment

Adapted from Jupe et al., 2013

Sequencing and NB-LRR annotation

Bait library design

Sample preparation and enrichment

Sequencing and NB-LRRs annotation
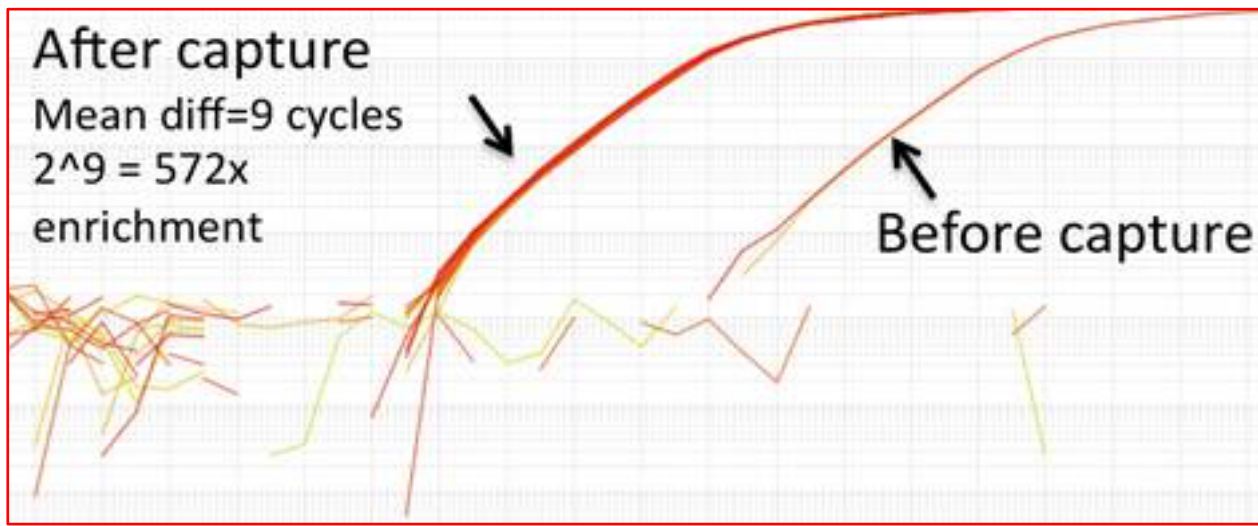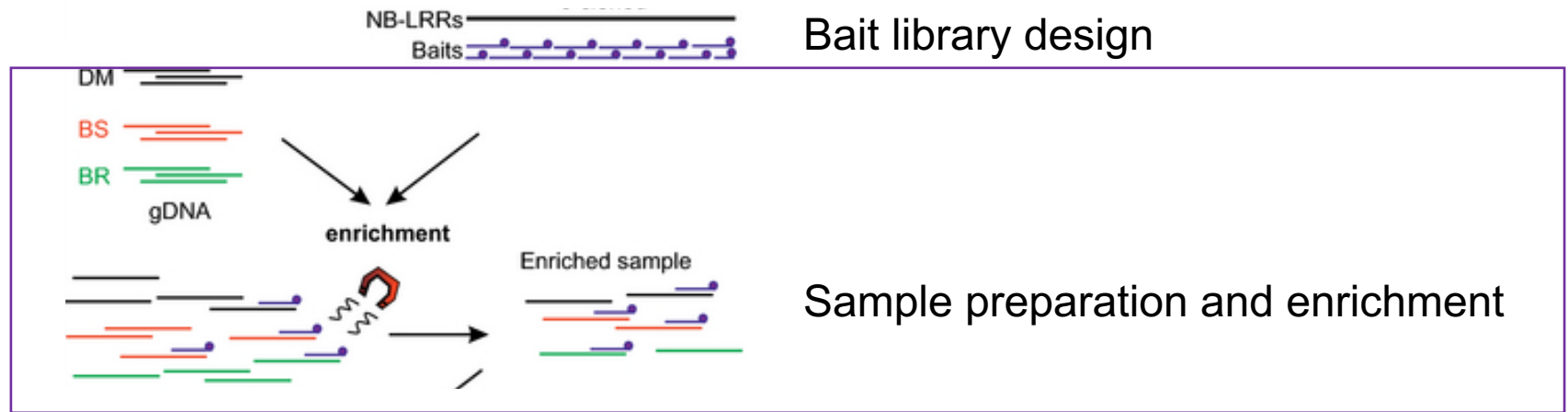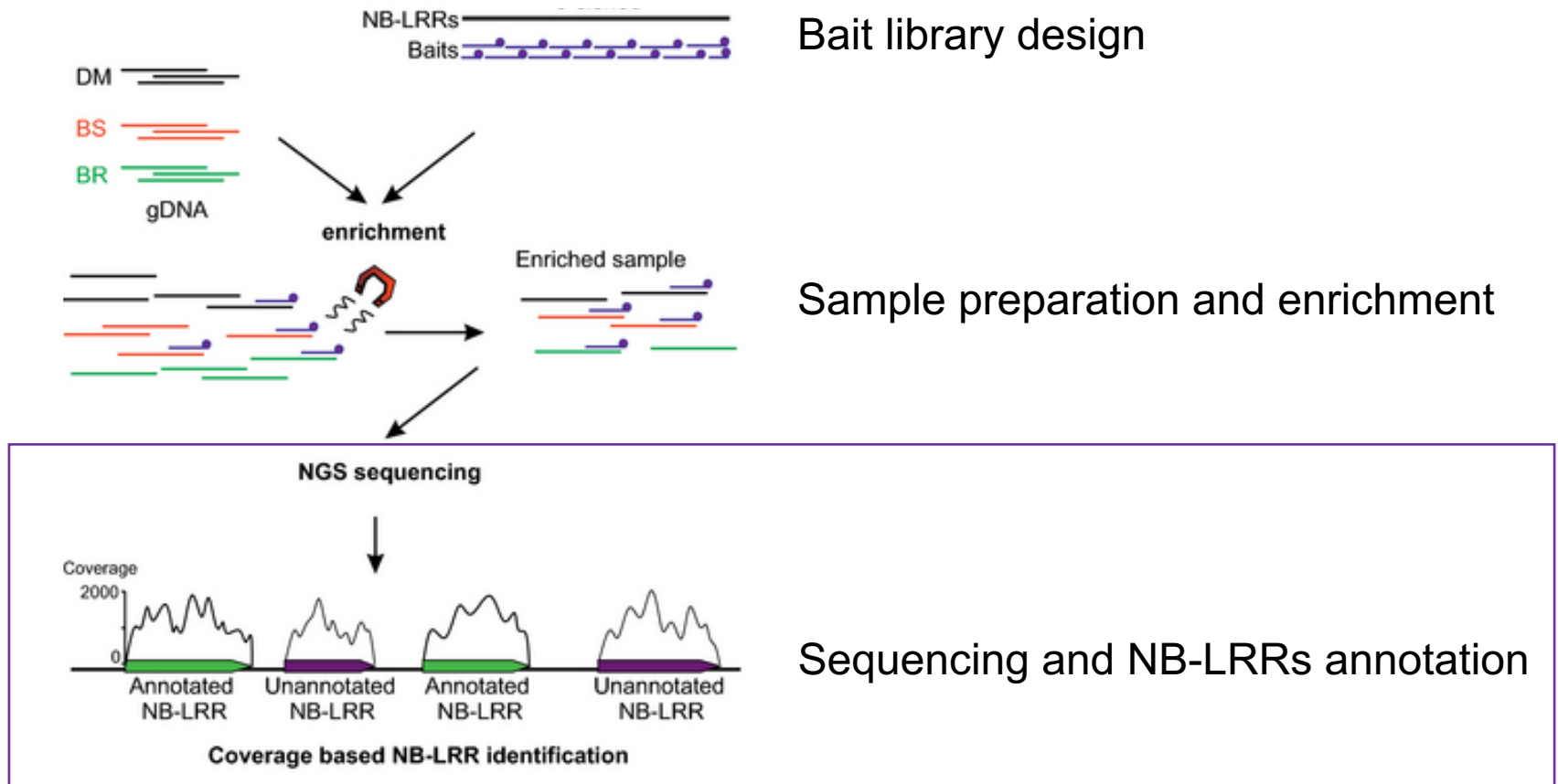
Adapted from Jupe et al., 2013

Sequencing and NB-LRR annotation

- MiSeq PE250 sufficient to allow re annotation and *de novo* annotation of NB-LRRs genes
- Higher coverage and better *de novo* assembly achieved with Illumina HiSeq 2500 PE250

Sequencing and NB-LRR annotation

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De novo assembly (masurca)

- #contigs: 69878

- #contigs > 1000bp: 8969

- 5573 Total NB-LRRs

- 432 Complete NB-LRRs

NLR-Parser
(*Steuernagel et al., 2015*)

Sequencing and NB-LRR annotation

- MiSeq PE250 sufficient to allow re annotation and *de novo* annotation of NB-LRRs genes
- Higher coverage and better *de novo* assembly achieved with Illumina HiSeq 2500 PE250

	Wild-Type	Mutant#1	Mutant#2	Mutant#3
Contigs covered (n=69878)	68664	44989	43647	44989
Average coverage	14.4	28.8	19	24.9
NB-LRRs covered (n=5573)	5562	5531	5524	5531
Average coverage NB-LRRs	66.2	170.4	113.3	151.2

MiSeq   HiSeq

Application

➡ **Assessing NB-LRR variation between resistant and susceptible varieties**

Bulk-Segregant Analysis:
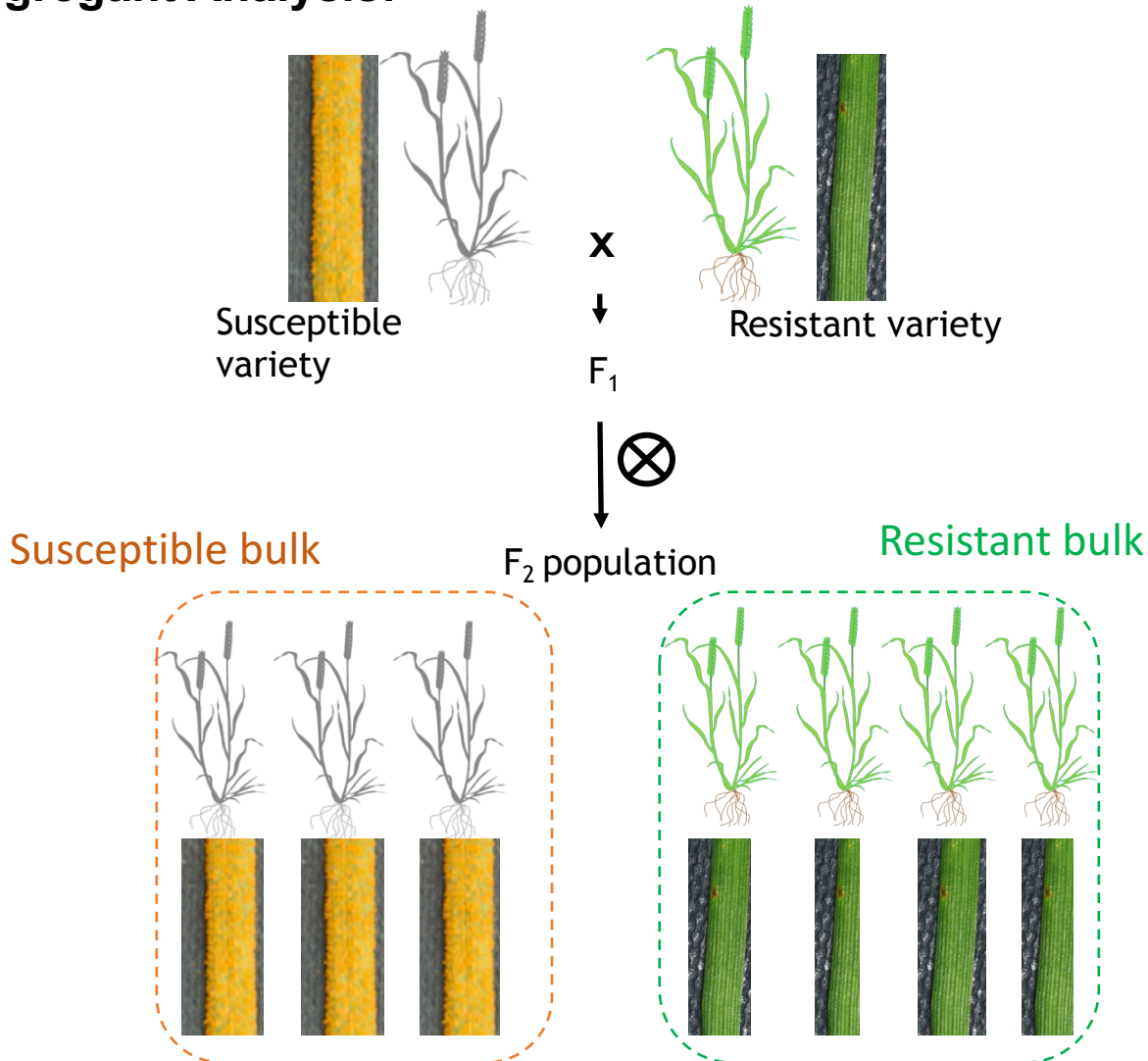

Susceptible variety X Resistant variety

F_1

$\otimes$

F_2 population

Application

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Bulk-Segregant Analysis:

Susceptible variety **X** Resistant variety

F_1

$\otimes$

F_2 population
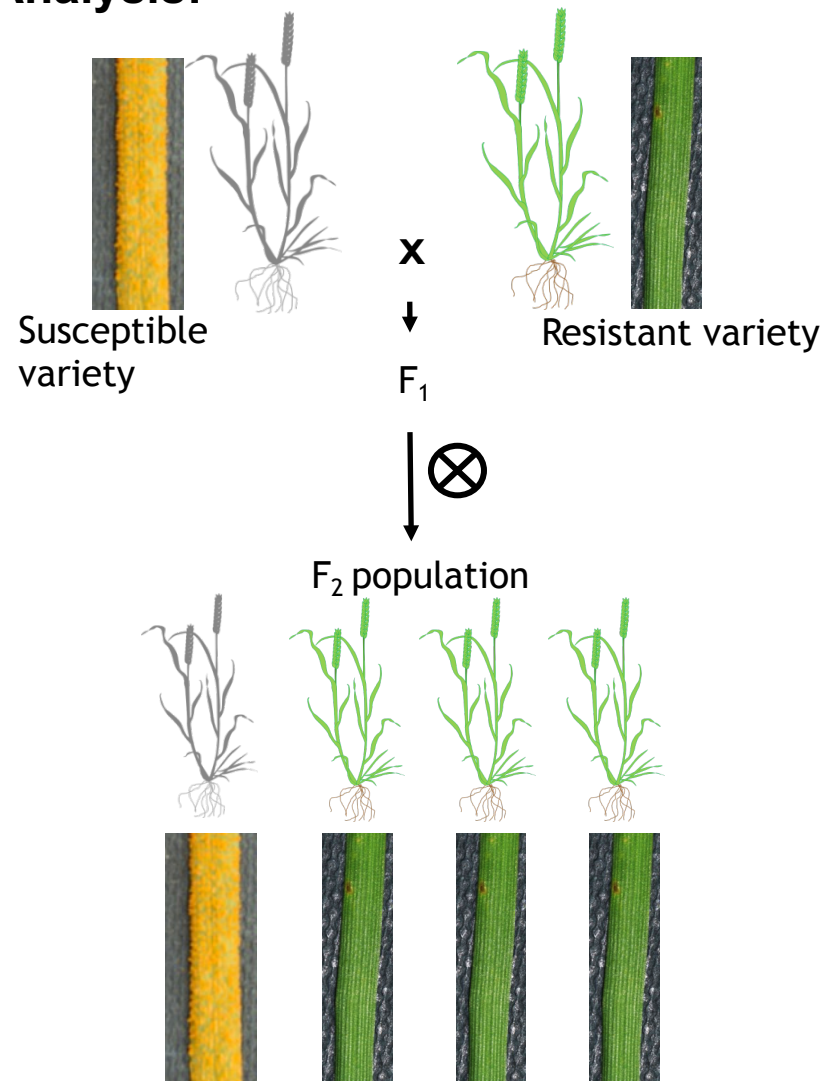
Susceptible bulk

Resistant bulk
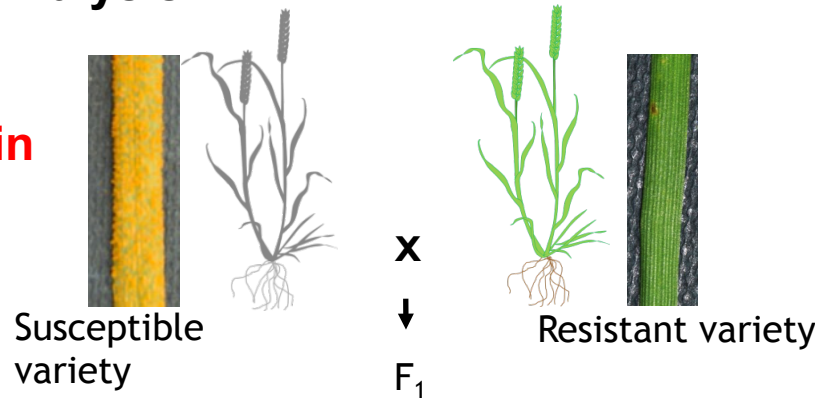
Application

➡ **Assessing NB-LRR variation between resistant and susceptible varieties**

Bulk-Segregant Analysis:
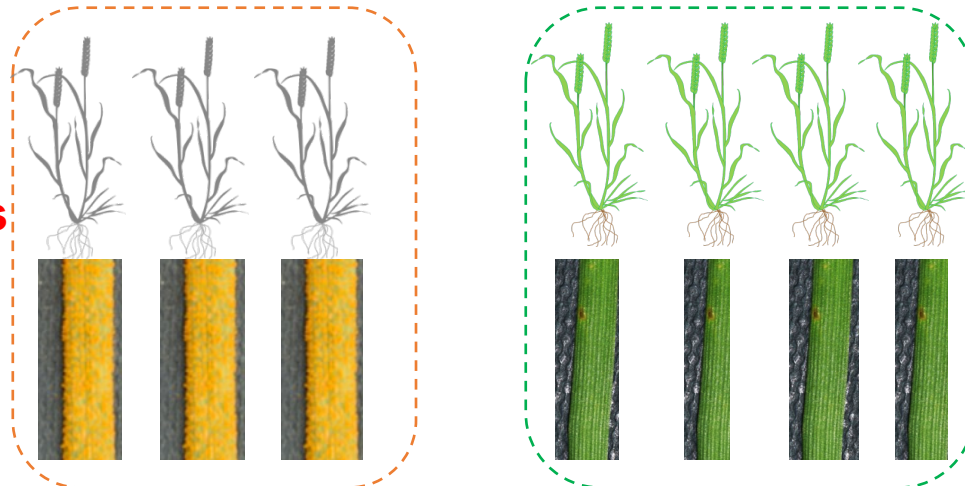
1. **Identify SNPs in parents**

Susceptible variety

X

Resistant variety

F$_1$

⊗

F$_2$ population

Susceptible bulk

Resistant bulk

2. **Determine allele frequencies within the bulks**

Application

➡️ **Assessing NB-LRR variation between resistant and susceptible varieties**
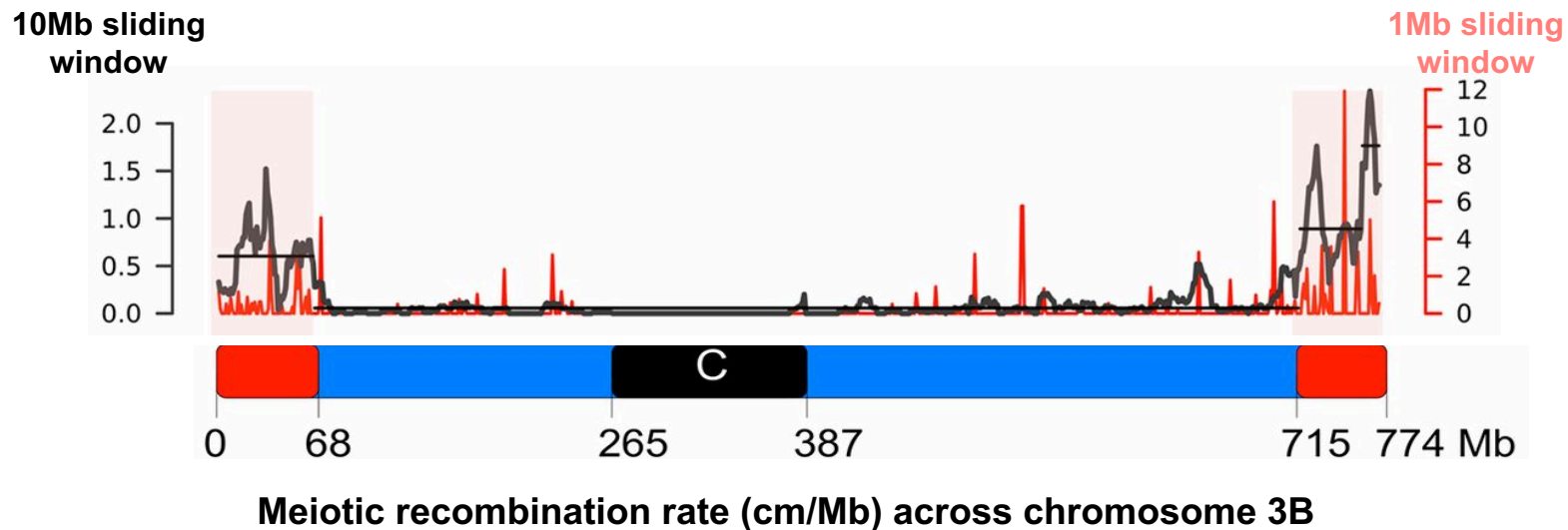
Bulk-Segregant Analysis - Limitations

Application

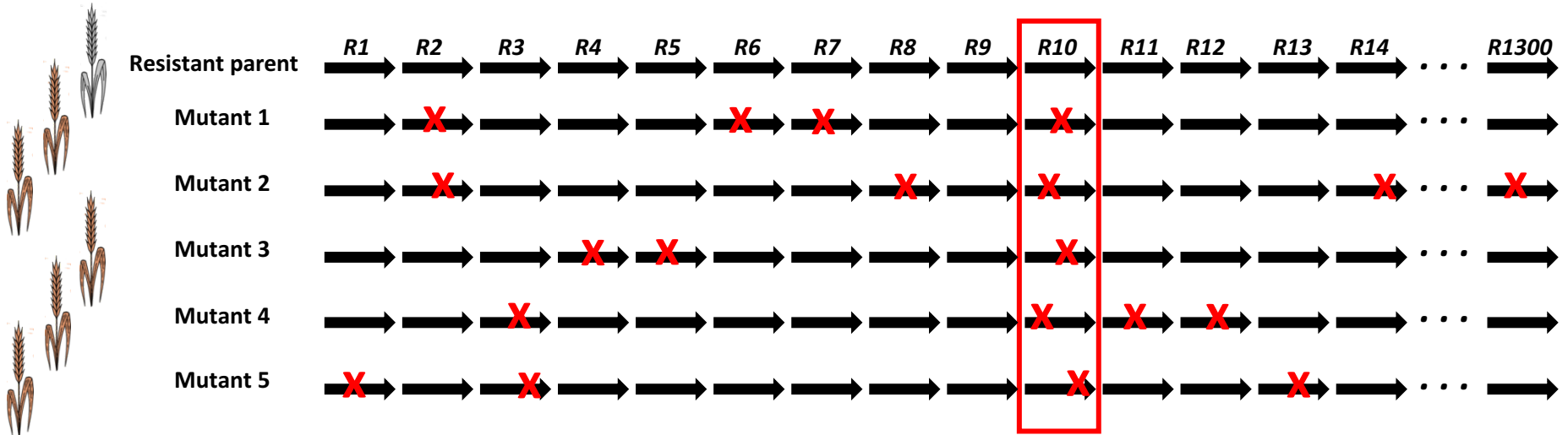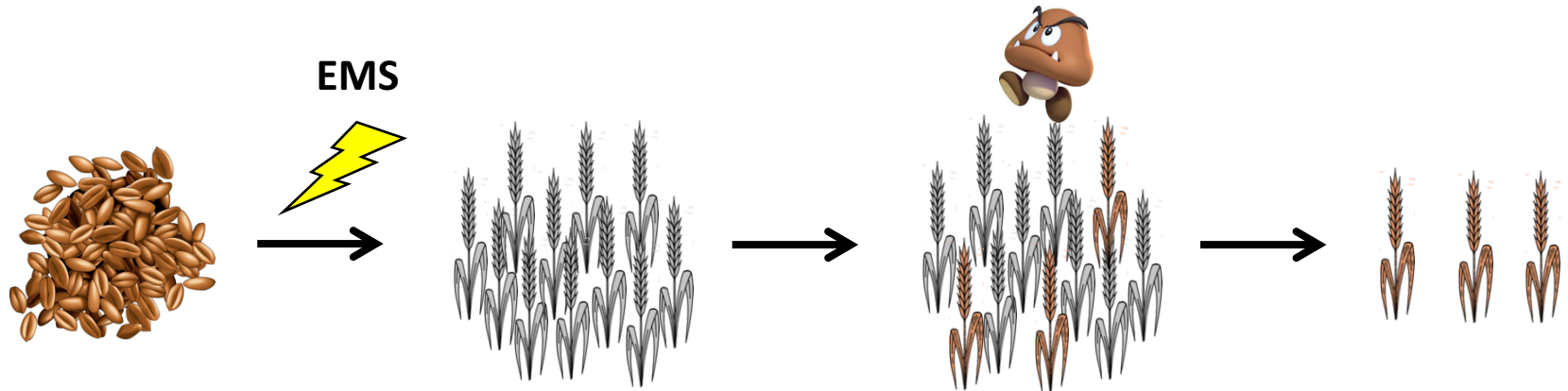**Assessing NB-LRR variation between resistant and susceptible varieties**

Bulk-Segregant Analysis - Limitations

- **Reduced recombination rate in the centromeric region**

10Mb sliding window

1Mb sliding window

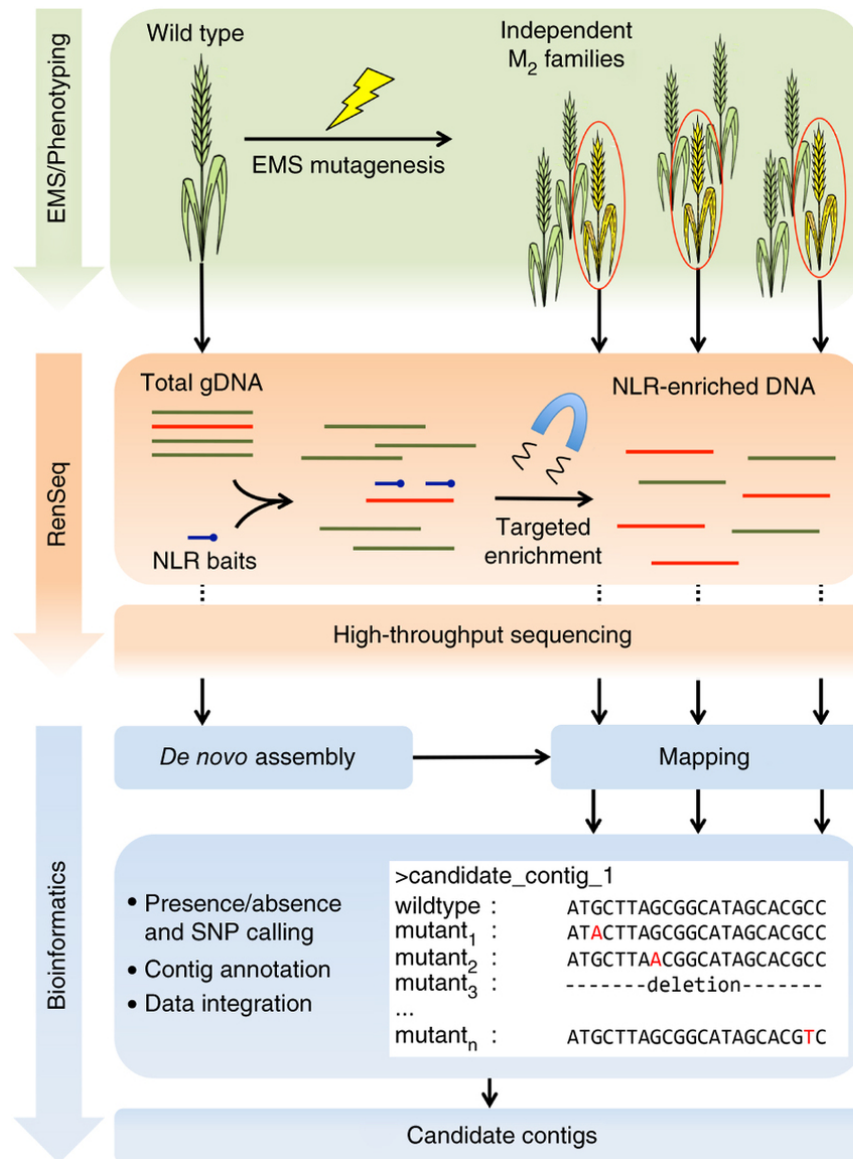Meiotic recombination rate (cm/Mb) across chromosome 3B
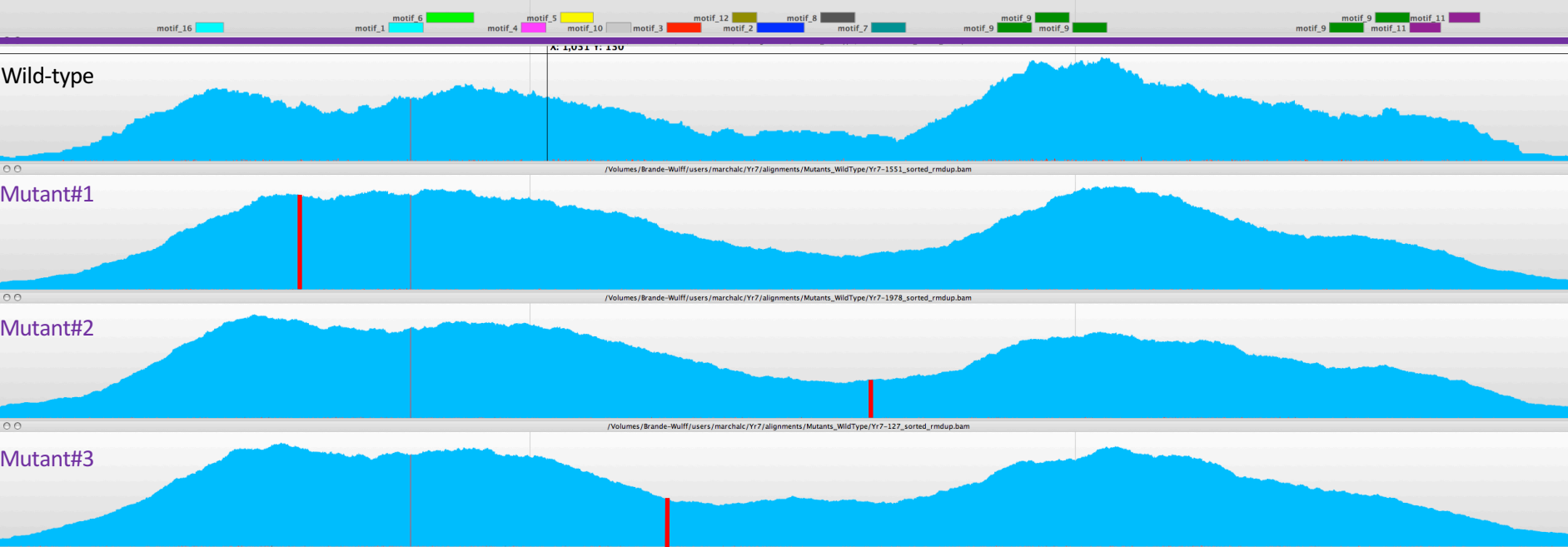
Adapted from Choulet et al., 2014

Mutational genomics
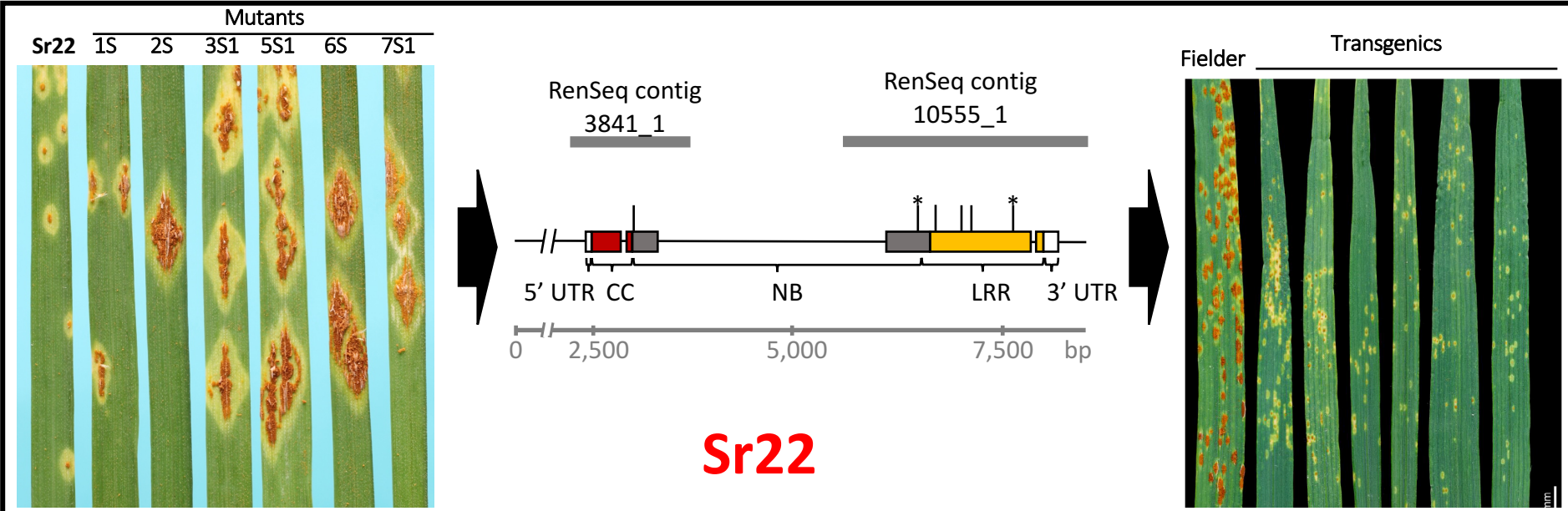
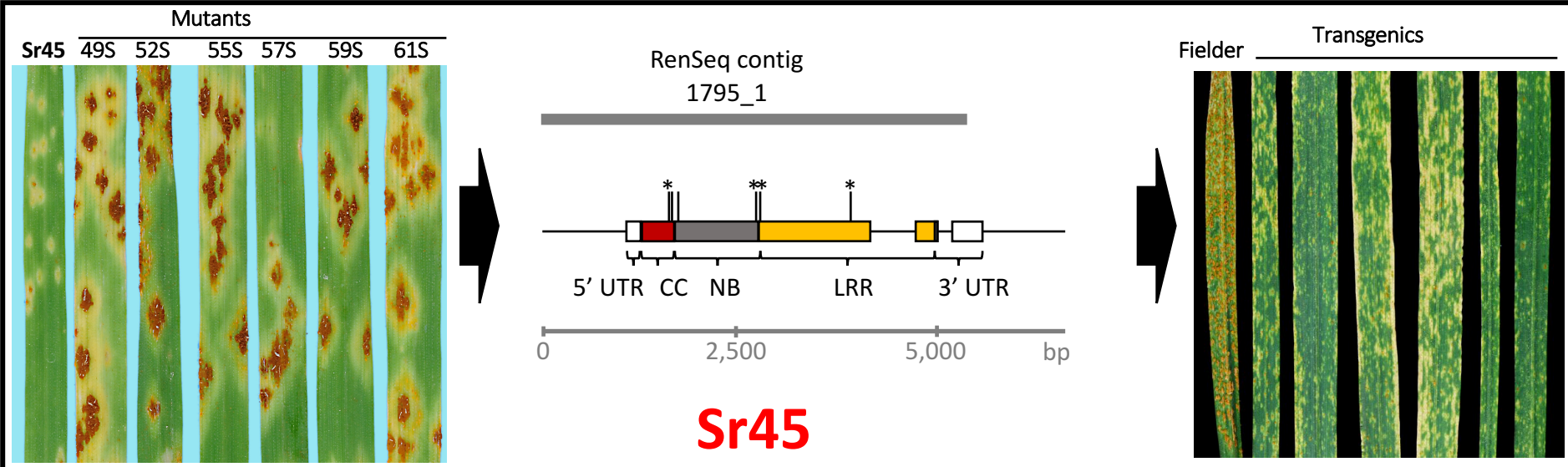# Mutational genomics + RenSeq = MutRenSeq

From Steuernagel et al., 2016

MutRenSeq - Candidate contig

Sr22

Sr45

Steuernagel et al., 2016
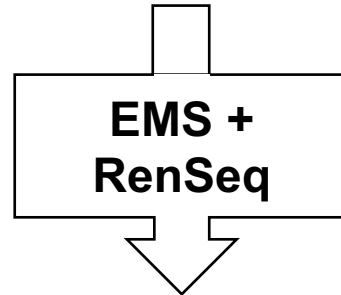
```
┌─────────────────────────────────────┐
│          21 Chromosomes             │
│        17 Billion base-pairs        │
│          124,201 genes              │
└─────────────────────────────────────┘
                  │
              EMS +
              RenSeq
                  ▼
┌─────────────────────────────────────┐
│                                     │
│         few candidate genes         │
│                                     │
└─────────────────────────────────────┘
```

**21 Chromosomes
17 Billion base-pairs
124,201 genes**

**EMS +
RenSeq**
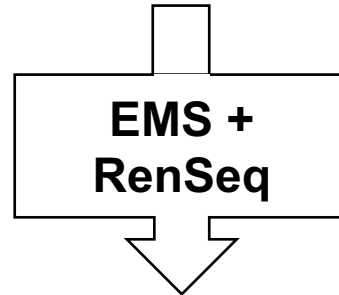
few candidate genes *

*Phenotype

Phenotype

Phenotype

Phenotype

…

Acknowledgments

Brande Wulff group

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Sanu Arora

Ngoni Kangara

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Guotai Yu

Yajuan Yue

Cristobal Uauy group

Clare Lewis

Philippa Borrill

Nikolai Adamski

Abdul Kader Alabdullah

Jemima Brinton

James Connorton

Karunesh Kumar

Ricardo Ramirez Gonzalez

Oluwaseyi Shorinola

Sophie Harrington

James Simmonds

Simon Berry

Paul Fenwick

John Innes Centre

Limagrain
from earth to life

Thank you for your attention!