



Building Excellence in Genomics and Computational Bioscience

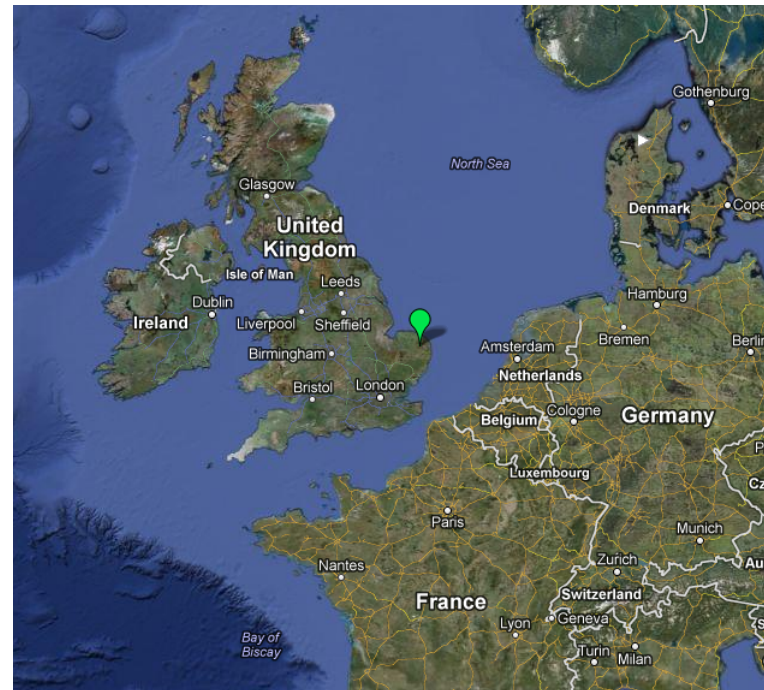
TILLING @ TGAC

Sarah Ayling
Crop Genomics and Diversity
sarah.ayling@tgac.ac.uk





Building Excellence in Genomics and Computational Bioscience



Forward genetics is the approach of determining the **genetic** basis responsible for a phenotype – classical genetics

Reverse genetics is an approach to discover the function of a gene by analyzing the phenotypic effects of specific engineered gene sequences.

TILLING: Targeting Induced Local Lesions in Genomes

Source: Wikipedia

TILLING by sequencing



Induce
genetic
variation

Base changes:
C > T; G > A



GCGATGAGCTCGAAGACGCA



GCGAT**A**AGCTCGAAGAT**T**GCA

Detect base
changes in
genes



Phenotype
plants with
changes in
your gene of
interest



Sequence once, database for everyone...

TILLING projects:

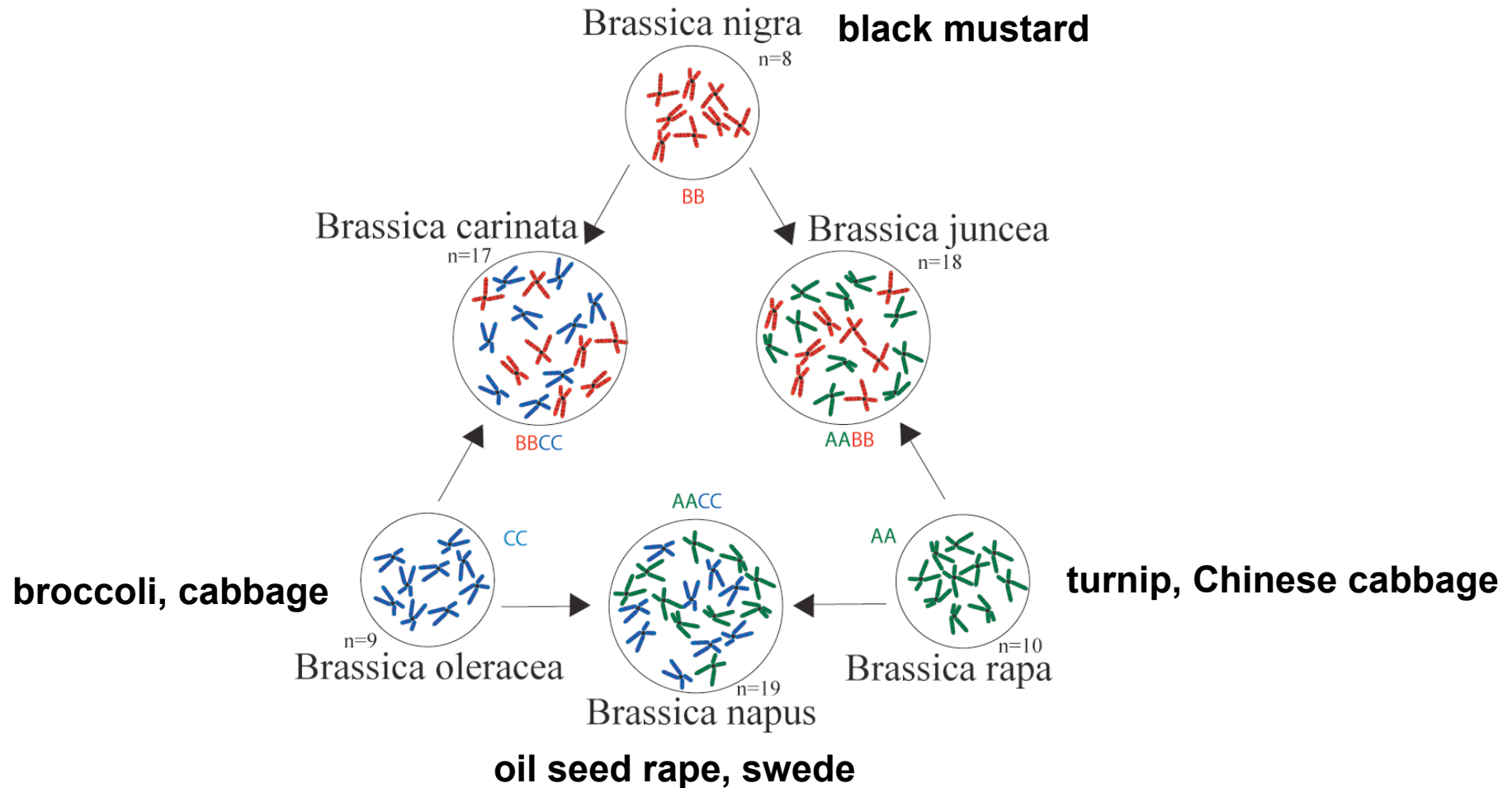


Brassica rapa



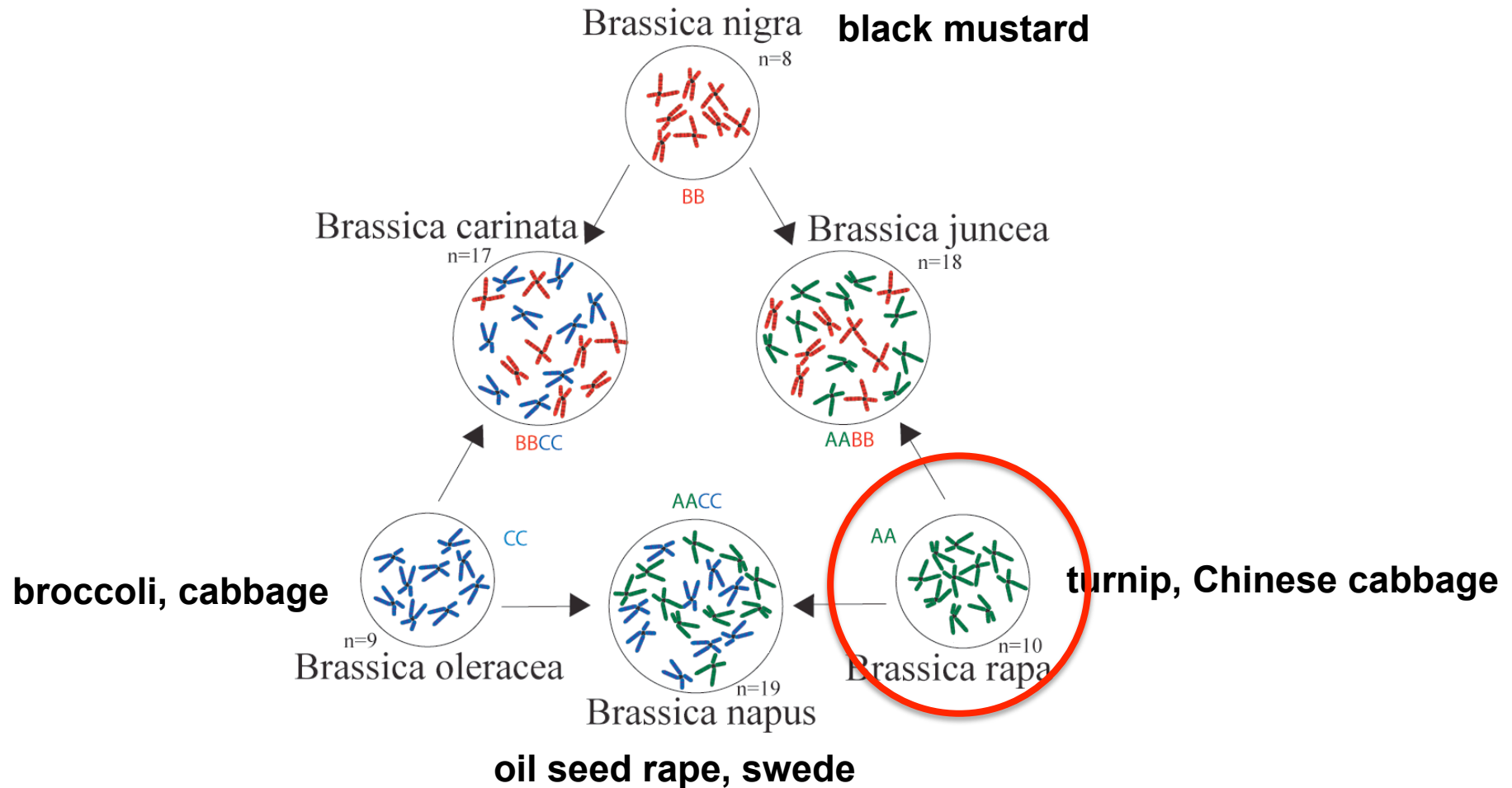
Triticum aestivum

Triangle of U



Wikipedia (U, Jpn J Bot
7:389452, 1935)

Triangle of U



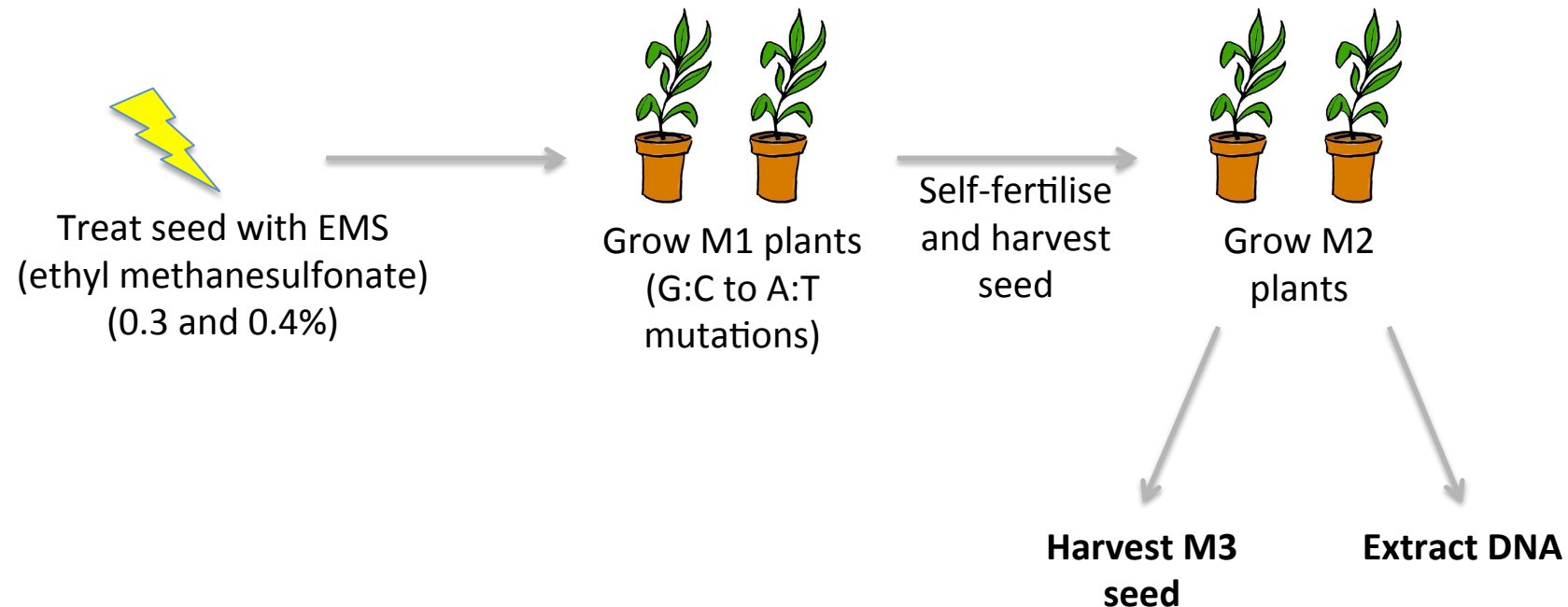
Wikipedia (U, Jpn J Bot
7:389452, 1935)

Genotype: R-o-18

- Inbred line of *B. rapa* subsp. *Trilocularis* (Yellow Sarson)
- Closely related to *B. rapa* oilseed crops grown in Pakistan

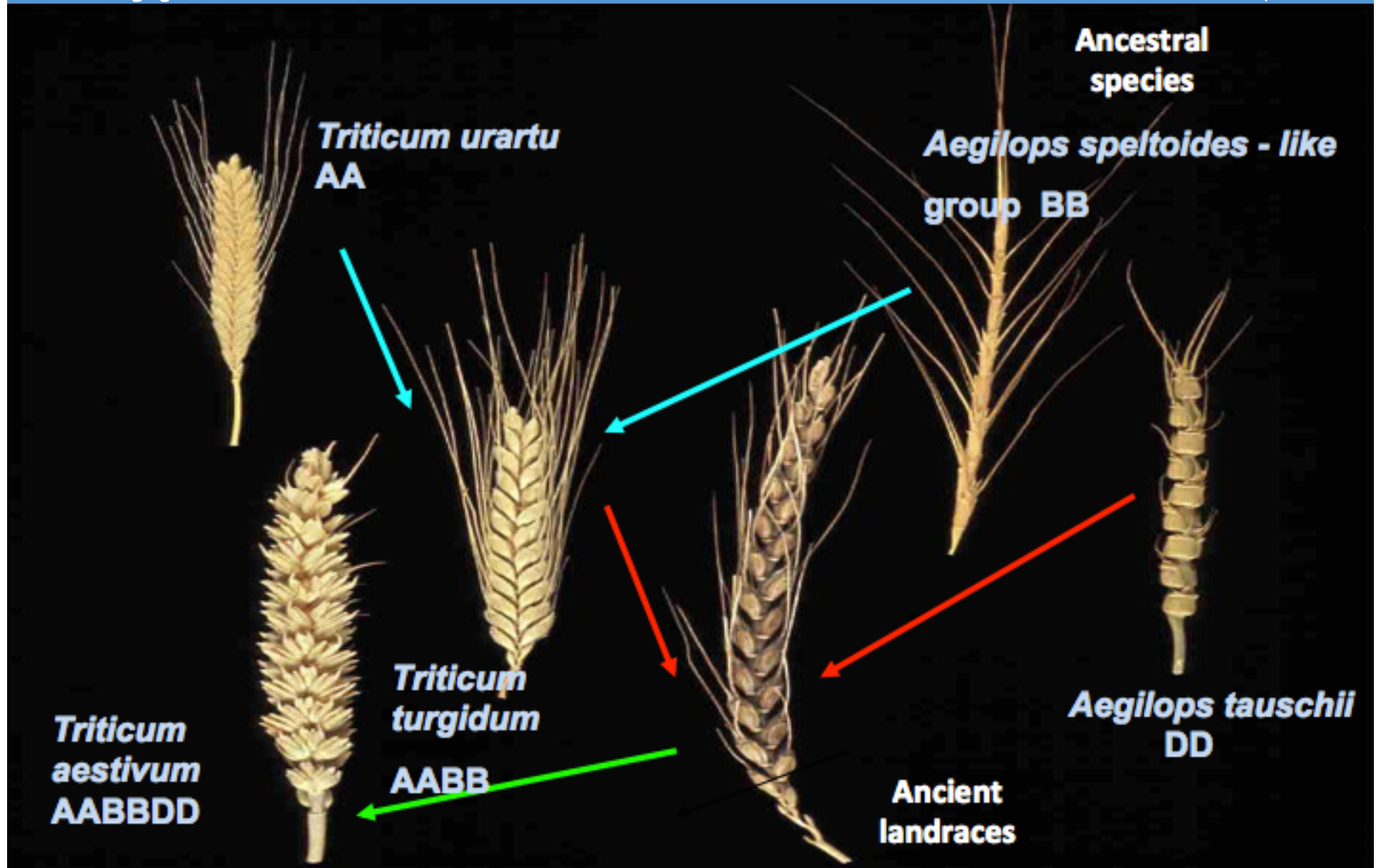


EMS population developed in Lars Østergaard group, >1000 lines

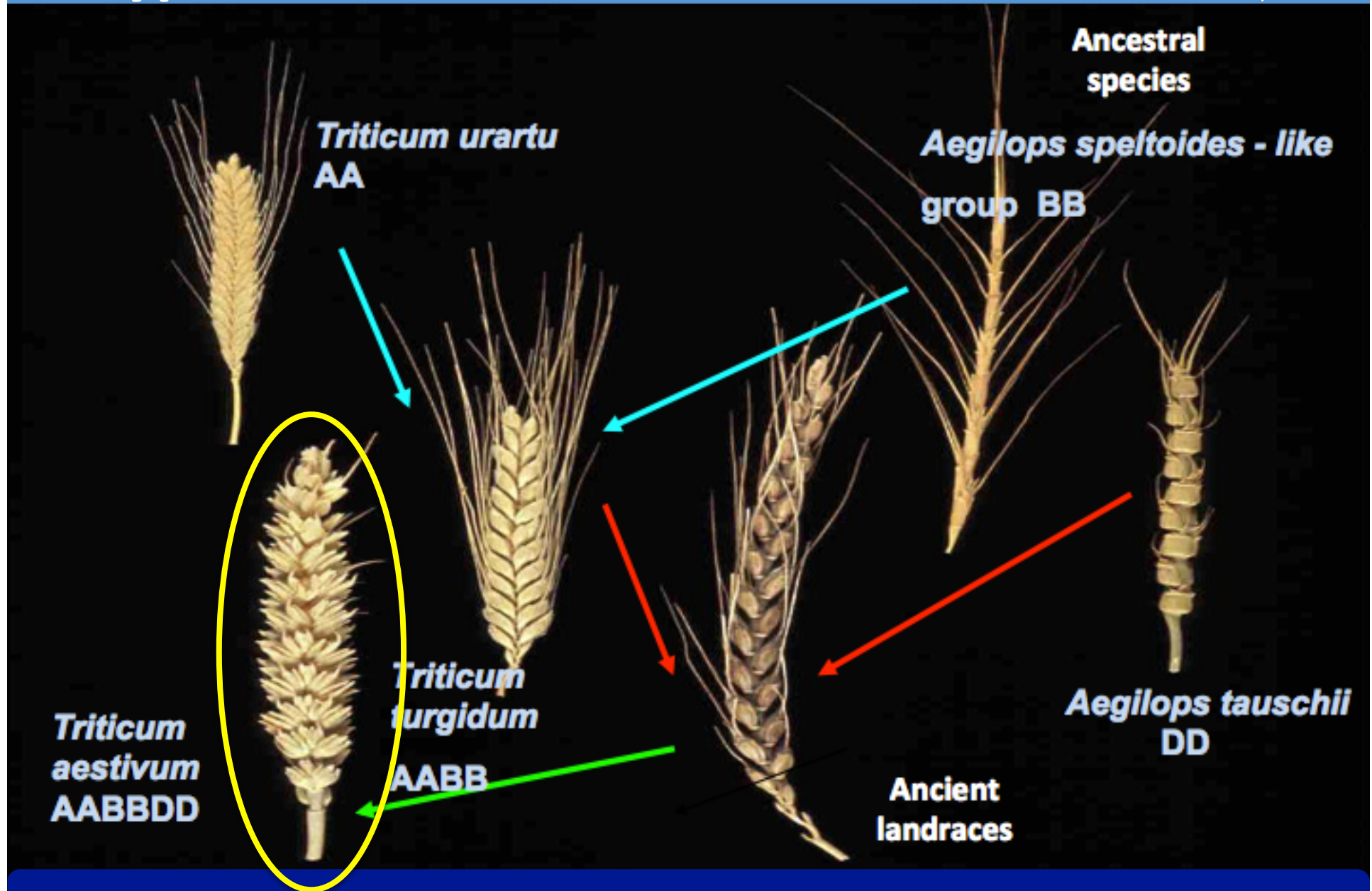


Stephenson *et al.*, BMC Plant Biology 2010

Polyploid wheat



Polyploid wheat

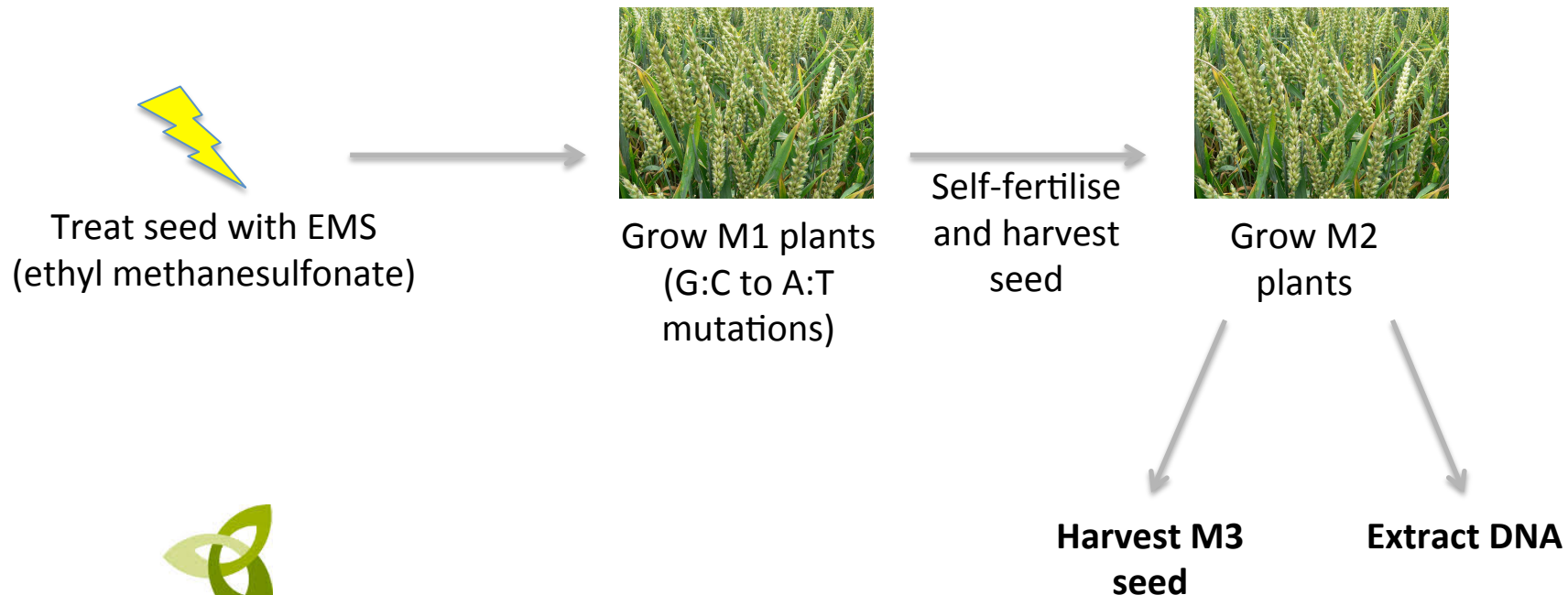


Mutant population - wheat

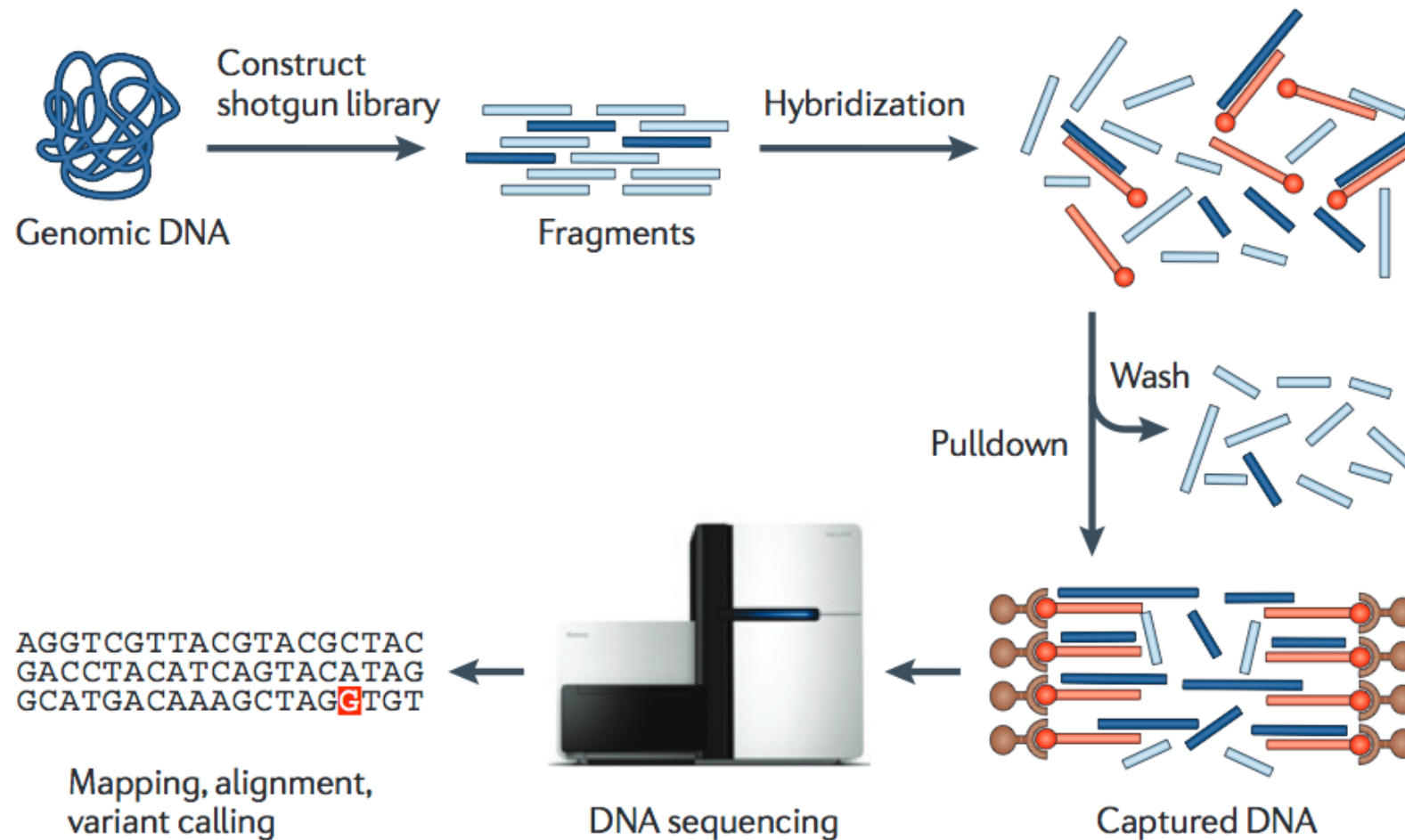
Genotype:

Triticum aestivum cv Cadenza (Bread wheat)

EMS population developed in Andy Philips group,
>1500 lines as part of WGIN1



Exome capture



Bamshad *et al.*, Nature Reviews Genetics, 2011

 **EnsemblPlants** ▾ BLAST | BioMart |

Brassica rapa ▾

 **Brassica rapa**
Brassica rapa subsp. *pekinensis*
Data Source [VFCAAS](#) | Taxonomy ID [51351](#)

Gene counts

<u>Coding genes:</u>	41,018
<u>Non coding genes:</u>	1,011
<u>Small non coding genes:</u>	1,009
<u>Long non coding genes:</u>	1
<u>Misc non coding genes:</u>	1
<u>Pseudogenes:</u>	817
<u>Gene transcripts:</u>	42,853

- Ian Bancroft and Martin Trick at the John Innes Centre for providing the gene annotation.

B. rapa genome

<http://brassicadb.org>, v1.2

41,019 protein-coding genes, **48 Mb** total sequence



48 Mb



30 Mb

CODDLE-like: Comai lab

(Codons Optimized to Detect Deleterious Lesions)

 **NimbleGen**
SeqCap EZ Library

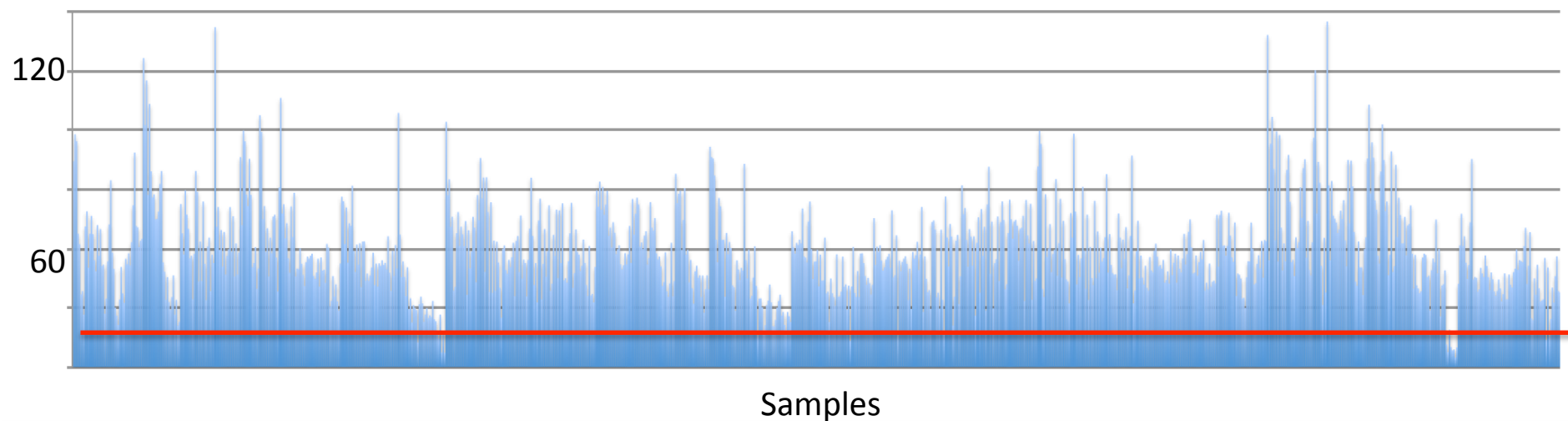


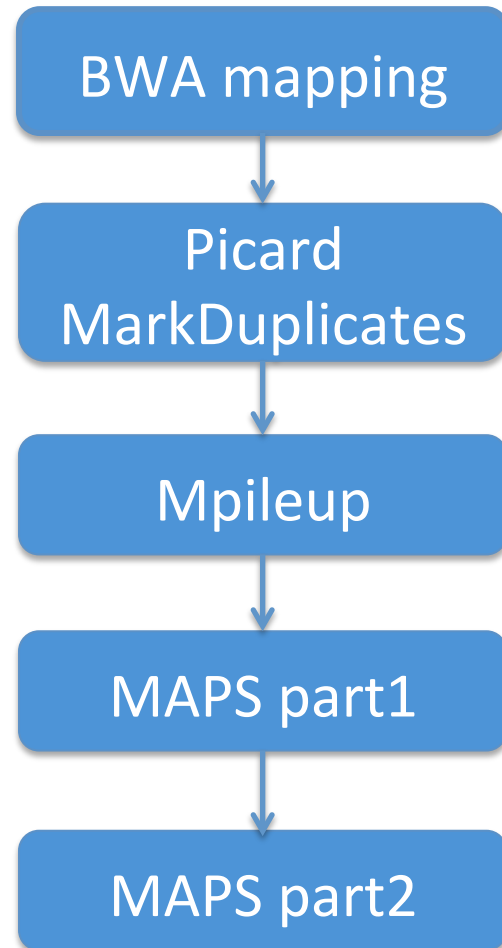
- Paired-end Illumina
- Fragment size: 300bp
- Read lengths: 2x150bp
- 24-plex run over 2 lanes
 - = 12-plex



Number of samples processed	998
Mean number of reads per sample	25,892,695
Minimum number of reads for a sample	3,755,166
Mean target coverage	38.5
Mean percentage of targets with <2x coverage	5.1
Mean percentage of target bases with $\geq 10x$ coverage	86.3

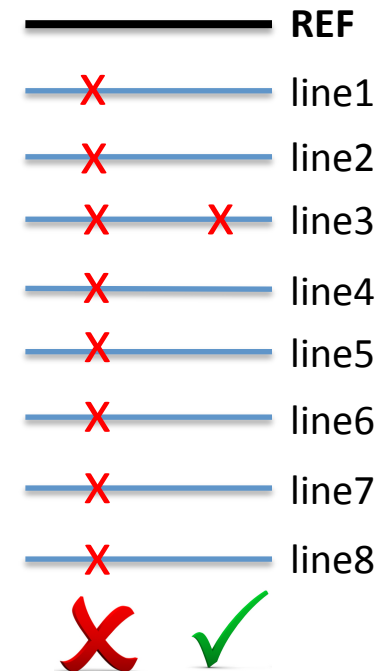
Target coverage variability between samples





MAPS part1:
(whole batch)
minLib=n-2
minCov=10
maxCov=1000
hetOneMinPer=20

MAPS part2:
(per sample)
minLib=n-2
minCov=10
hetMinPer=30
homMinCov=3
hetMinCov=6



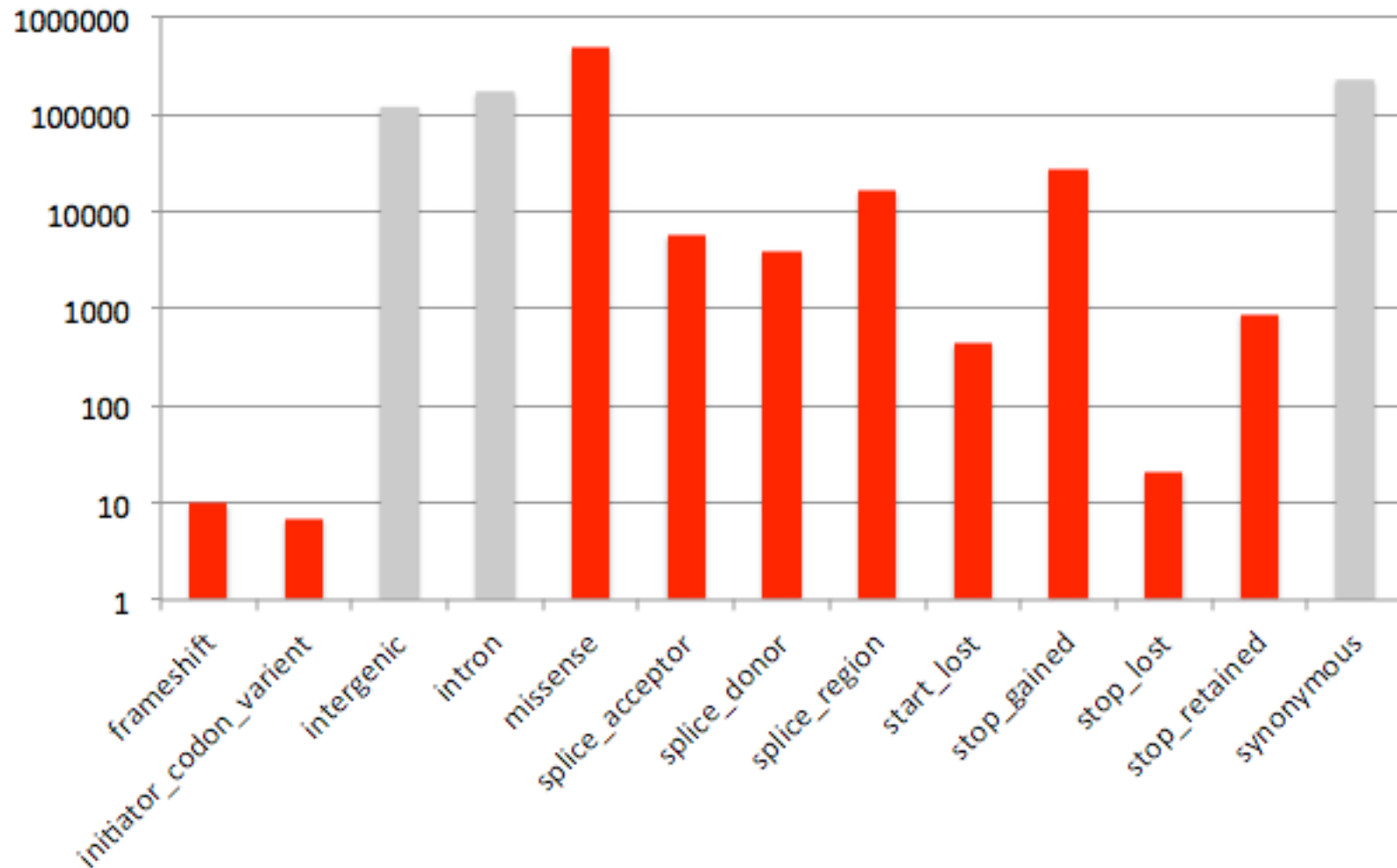
Mean number of mutations per sample	1188.7
Mean mutation density	1 per 49.9 kb
% Homozygous	10
% Heterozygous	90
Mean % EMS mutations (C:G->A:T)	99.1



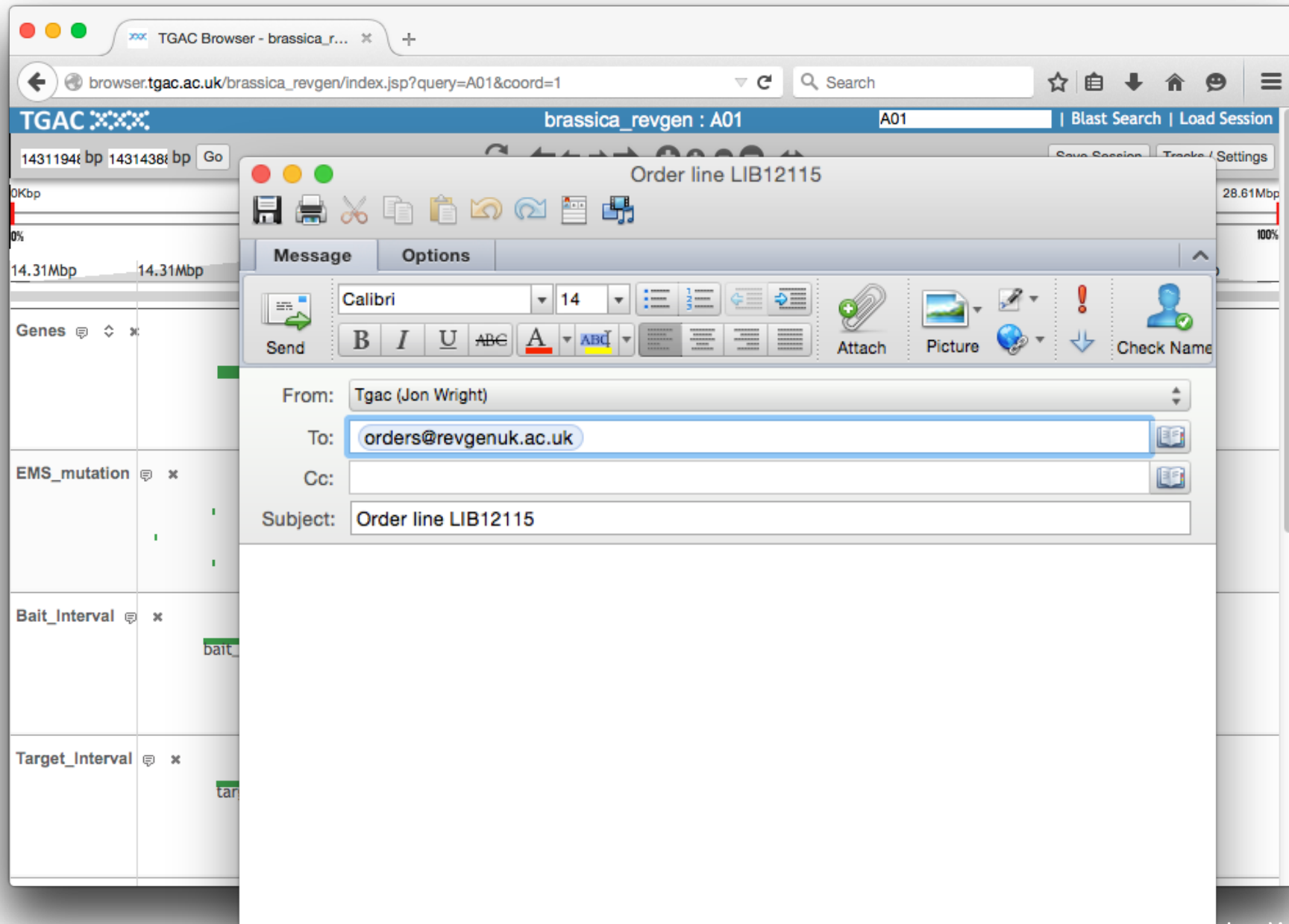
SnpEff

Genetic variant annotation and effect prediction toolbox.

92.2% of *B. rapa* genes contain non-synonymous mutations



Accessing the data



Accessing the data

The screenshot shows a web browser window with the address bar displaying `browser.tgac.ac.uk/brassica_revgen/index.jsp?query=A01&coord=1`. The page title is "TGAC Browser - brassica_r...". The main header area shows "brassica_revgen : bra033779" and a search bar with "bra033779" entered. There are links for "Blast Search" and "Load Session".

Results for searched query

(Limited to first 100 match)
Search Result

Genes Transcripts GO

Track	Gene	Reference Name	Reference Coord Sys	Position	Link
Gene_genes	Bra033779	A01	1	14312347-14314113	

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Accessing the data

Browser window: TGAC Browser - brassica_r...
 URL: browser.tgac.ac.uk/brassica_revgen/blast.jsp

brassica_revgen : A01 | Blast Search | Load Session

Parameter: Gap Costs Existence: 5 Extension: 2

Word Size: 16

Short Queries: ☐ Automatically adjust parameters for short input sequences.

BLAST History 1
 BLAST job v9GyXPJf

Query	Subject	% identity	alignment length	mismatch	gap opening	q.start	q.end	s.start	s.end	e-value	bit score	Subject db	Download Sequence
my_gene	A01	99.60	1767	0	3	1	1760	14312347	14314113	0.0	3328	Brapa_sequence_v1	↓
my_gene	A01	88.64	449	30	3	573	1020	2514568	2514140	7e-152	540	Brapa_sequence_v1	↓
my_gene	A01	87.80	451	39	5	573	1022	11459833	11460268	3e-140	502	Brapa_sequence_v1	↓
my_gene	A01	87.90	463	39	7	572	1020	9234522	9234063	1e-139	500	Brapa_sequence_v1	↓
my_gene	A01	94.80	269	13	1	756	1024	15447658	15447925	1e-117	427	Brapa_sequence_v1	↓
my_gene	A01	85.24	454	41	7	572	1023	9198217	9198646	2e-115	419	Brapa_sequence_v1	↓
my_gene	A01	94.01	267	14	2	754	1019	8599362	8599097	3e-110	402	Brapa_sequence_v1	↓
my_gene	A01	92.62	271	18	2	754	1023	13990327	13990058	1e-105	387	Brapa_sequence_v1	↓
my_gene	A01	93.23	266	15	3	757	1020	16040003	16039739	3e-103	379	Brapa_sequence_v1	↓
my_gene	A01	92.54	268	17	3	754	1020	15428525	15428790	6e-101	371	Brapa_sequence_v1	↓
my_gene	A01	92.54	268	16	3	754	1019	21296996	21297261	6e-101	371	Brapa_sequence_v1	↓

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RevGenUK

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TILLING is sweet: see Lin et al. Nature

Welcome to RevGenUK

The John Innes Centre (JIC) is at the forefront of reverse genetics through its establishment of TILLING and de-TILLING technologies in model legumes. Reverse genetics involves making a change to a specific gene, seeing what effect this has, and so working out the gene's normal function. We aim to develop these into a biological and bioinformatics resource for the wider plant scientific community via the RevGenUK project, providing an integrated reverse genetics platform for model plants.

RevGenUK aims to be "A 'single-stop' shop for use in functional genomics research consisting of an efficient, self-sustaining, extendable service for reverse genetics."



We should be very grateful if you would take a few minutes to fill in our [questionnaire](#). Your answers and opinions will help us to shape and improve the RevGenUK service in the future.



- Order your seeds (multiple mutants per gene)
- Grow your plants
 - Check phenotypes
- Cross/self to obtain homozygous alleles
 - Check with KASP markers
- Grow your plants
 - Check phenotypes
- Publish 😊

Acknowledgements

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Brassica:

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Lars Østergaard

Saleha Bakht



John Innes Centre

Cristobal Uauy

UCDAVIS
UNIVERSITY OF CALIFORNIA

Jorge Dubcovsky

Hans Vasquez-
Gross

Luca Comai

Meric Lieberman

Isabelle Henry



ROTHAMSTED
RESEARCH

Andy
Phillips

