Accelerating marker-assisted selection - from dirt to data

Dr. Jonathan Curry – Senior Scientist - Genomics

Introduction

- Who are LGC?
- Where do SNPs grow?
- How might they be harvested for breeding?
- You reap what you sow – applying technology

LGC Genomics is a part of the LGC Group

- LGC established in 1842 as a scientific testing laboratory for the UK government
  - Privatised in 1996
- Retains its role as UK National Measurement Institute
  - Standard bearer for chemical and bio-analytical measurement
- Global presence
  - 33 locations
  - 2,000+ staff
  - >3-fold growth since 2004

LGC Health Sciences
Leader in analytical testing services for food, drug, and consumer products

LGC Genomics
Genomic solutions for DNA/RNA extraction, genotyping, and sequencing

LGC Forensics
The world's largest supplier of products and services for forensic analysis

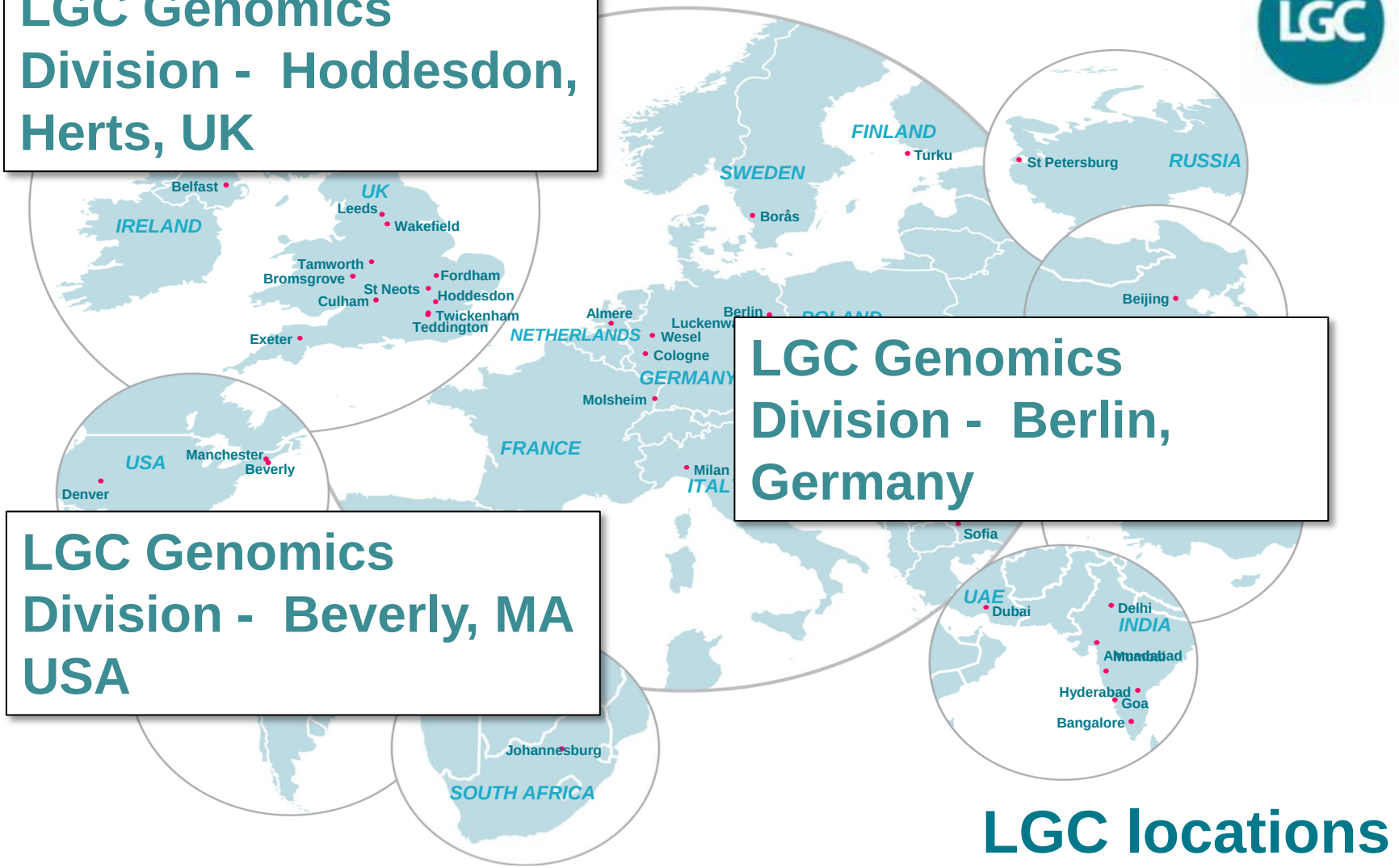
LGC Standards
Production and supply of chemical and biochemical reference materials

LGC Science & Technology
Home of the UK National Measurement Institute

LGC Genomics Division - Hoddesdon, Herts, UK

LGC Genomics Division - Berlin, Germany

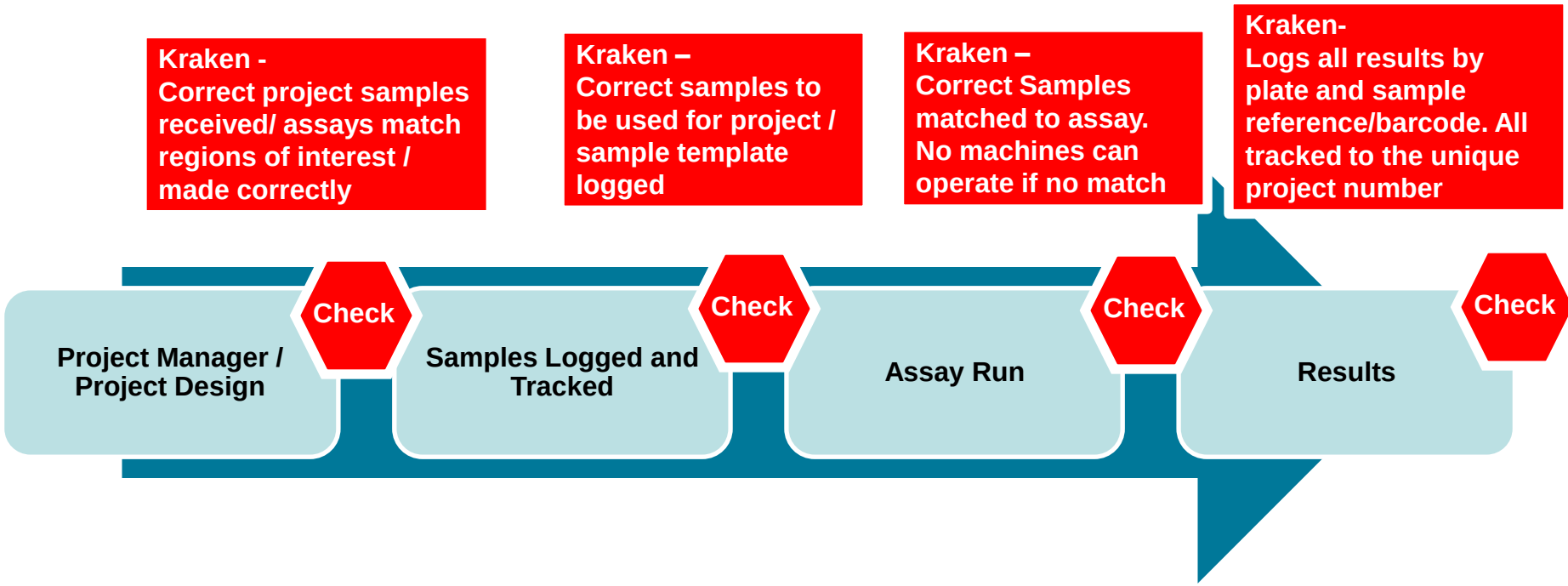
LGC Genomics Division - Beverly, MA USA



LGC locations

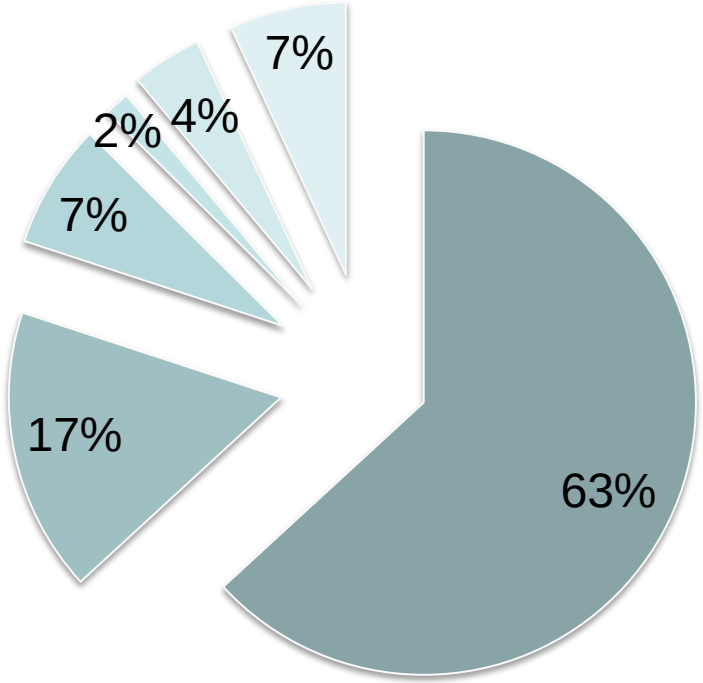
LGC Genomics Labs - Hoddesdon

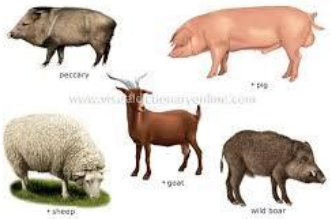
SNP genotyping project process – Kraken™ integration check points



Kraken™ integrated SNPline checks at vital points of the process ensure the correct sample and project are always tied.

Distribution of Life in Kraken





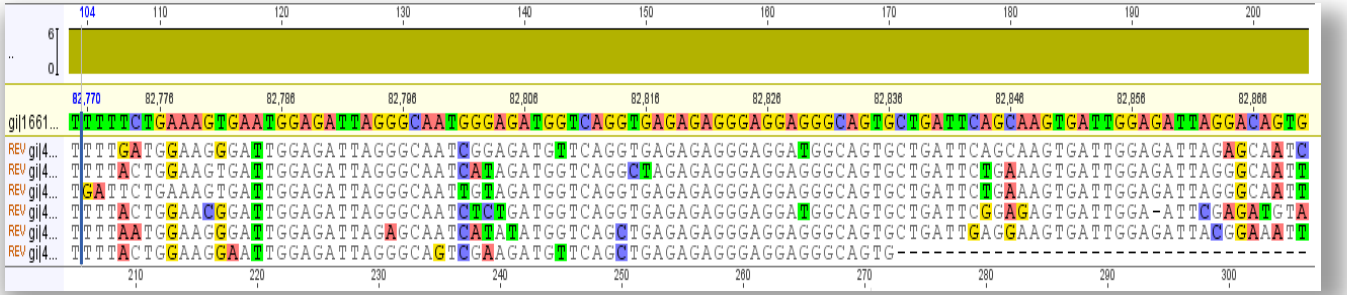
Plant Animal Fish Insect Bacteria / Yeast / Protozoan Human

Where do SNPs grow?

High-Throughput Marker Discovery

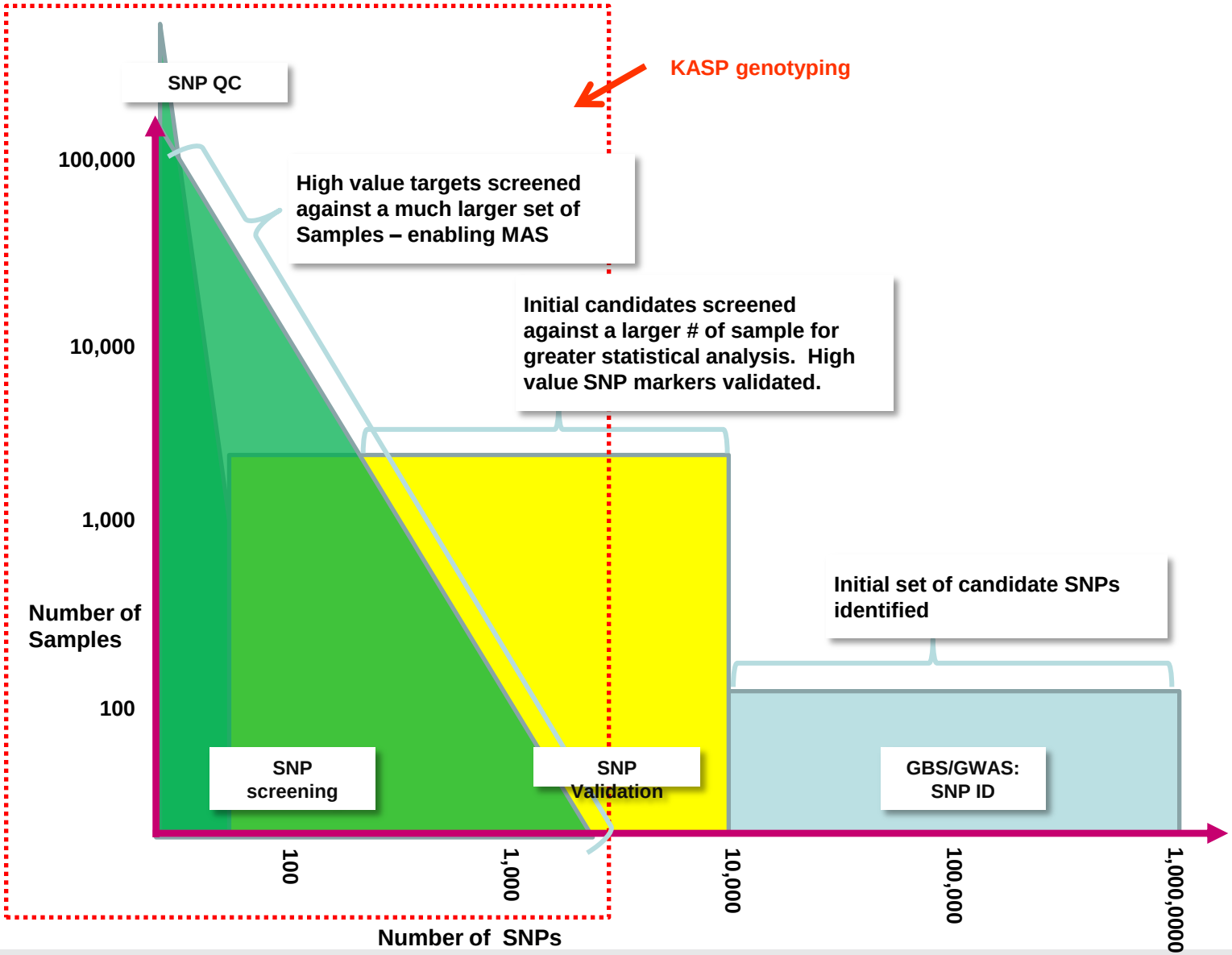
Discovery of valuable traits using Next-generation sequencing

- **Using High-throughput sequencing to survey genomes in bulk**
- **Markers are used to identify differences between genomes.**



- **Use markers when cross breading varieties.**
- **Once markers are selected they can be run over hundreds / thousands / millions of samples.**
- **Reduction of time to market – reduces cost / increases profitability**

KASP genotyping landscape

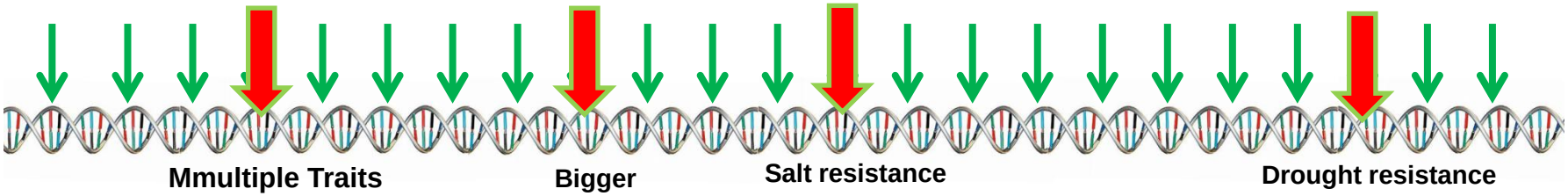


Trait Identification

The reduction in cost of NextGen sequencing has meant a dramatic increase in possible loci to target

Position of desired trait marker

Fine mapping with KASP genotyping

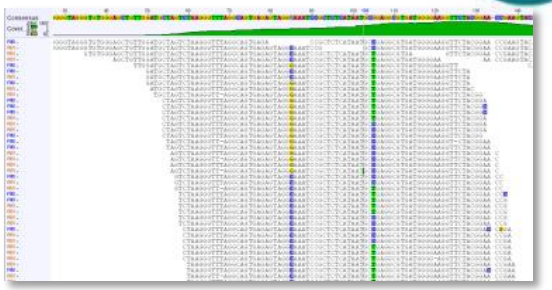


Design flexibility allows more markers to be targeted to saturate regions

By using more SNP markers (higher resolution mapping) you will find desired trait faster

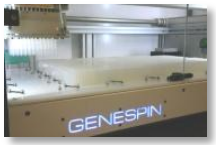
KASP assays allow higher resolution, flexible mapping of organisms

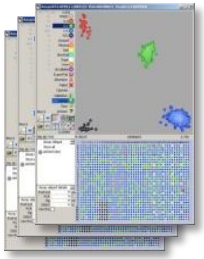
High Throughput Marker Assisted Selection



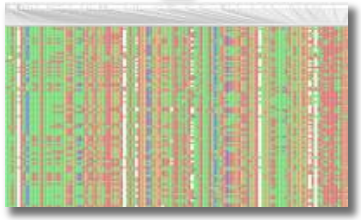
Sequencing and informatics
Marker Identification

KASP





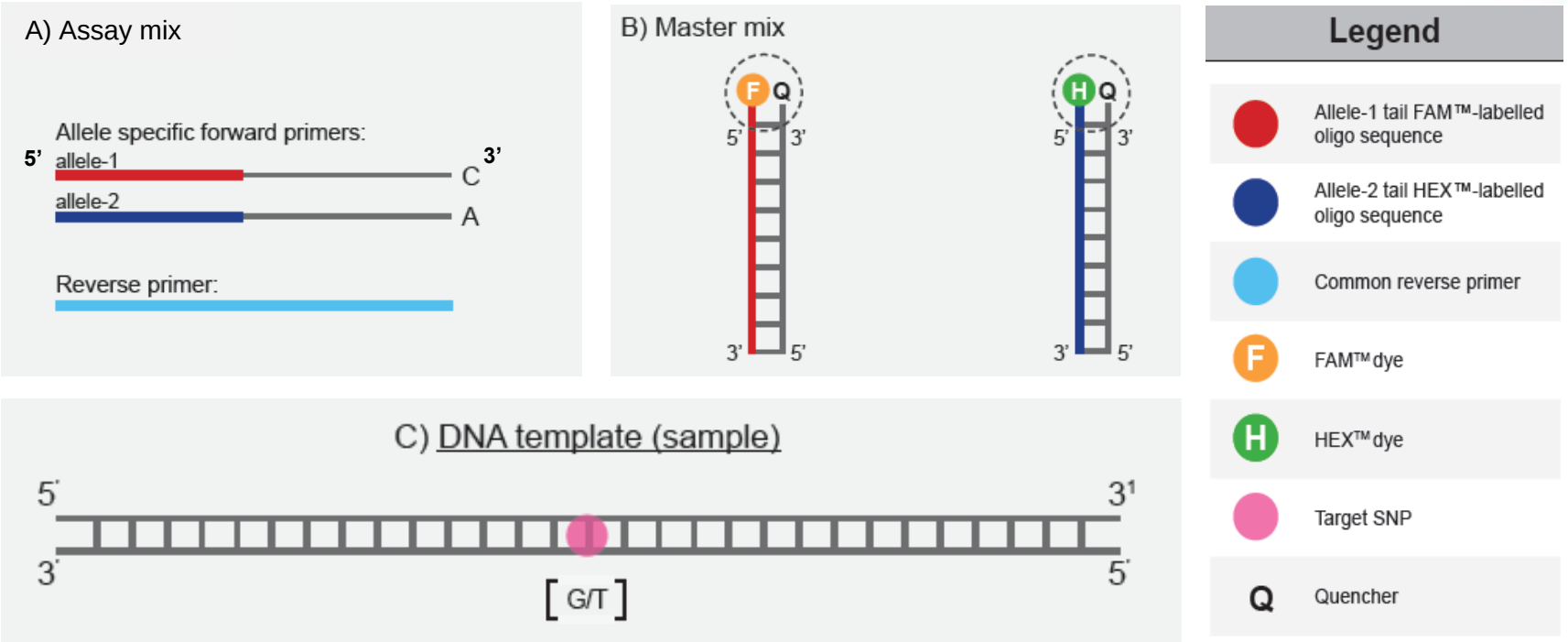
Development



Evaluate different generations in High - Throughput.

How might they be harvested for breeding?

KASP Components



- Assay mix - contains two allele specific primers and common reverse primer(s). This is often referred to as a KASP Assay.
- Universal Master mix - contains fluorophores, quenchers and *Taq* polymerase.

KASP – flexible for all platforms

KASP is very simple and scalable…

- Anyone who has a pipette, PCR machine and a way of reading FRET can run KASP!!
- System agnostic
- LGC Genomics service labs can run your project for you.
- Bi-allelic SNP data is general purpose marker currency – easy to exchange and work with.

Flexible design

- KASP is PCR
  - Can be designed as such
  - Can be optimised as such (Temp / Betaine / DMSO/ Mg^{2+})
  - 90% assay conversion
- Choose loci with a fair chance of designing primers.
- i.e. not:
  - Non-unique and highly spread throughout genome (although this can be solved)
  - Long homo-polymer repeats i.e. AAAAAA
  - VNTRs / micro-satellites – although Hybeacons can be used here.
  - Copy number

Solutions for homology and polyploidy

Want to save time and money on breeding / mapping projects with KASP?

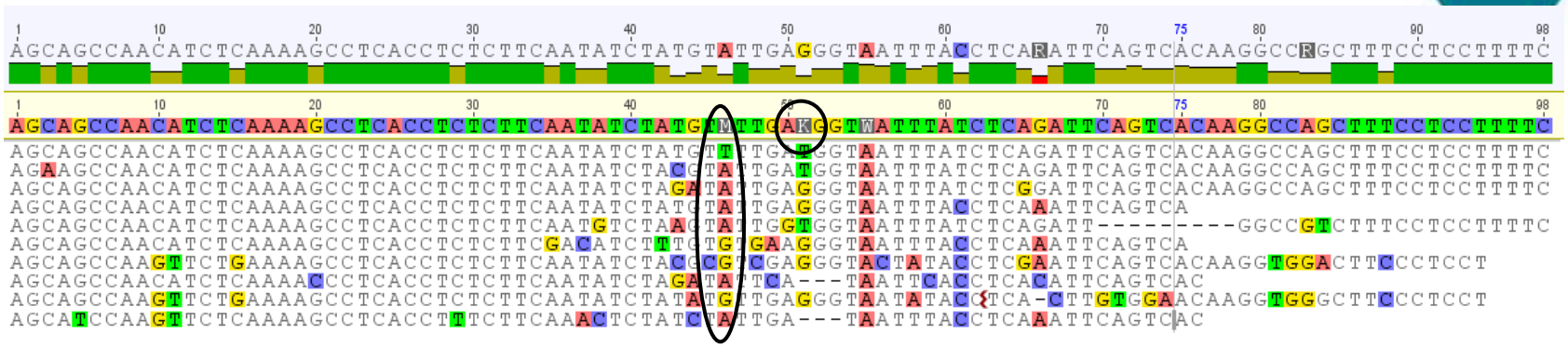
- Answer?
  - BLAST, BLAST and BLAST some more!!!!!!!

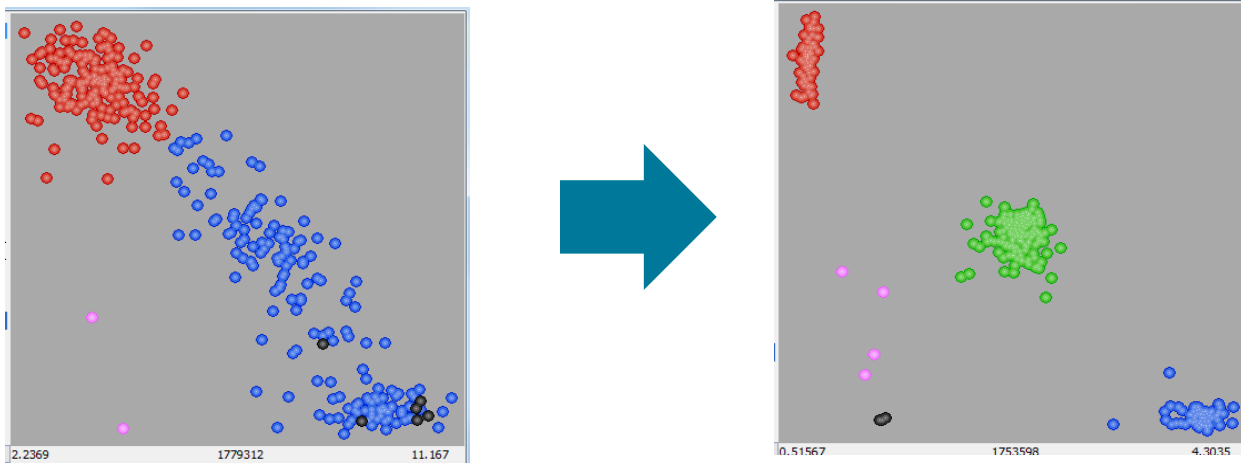
- Very simple to do with available databases for many common crops.

- Turn off complexity filters and run Mega-BLAST then BLASTN.

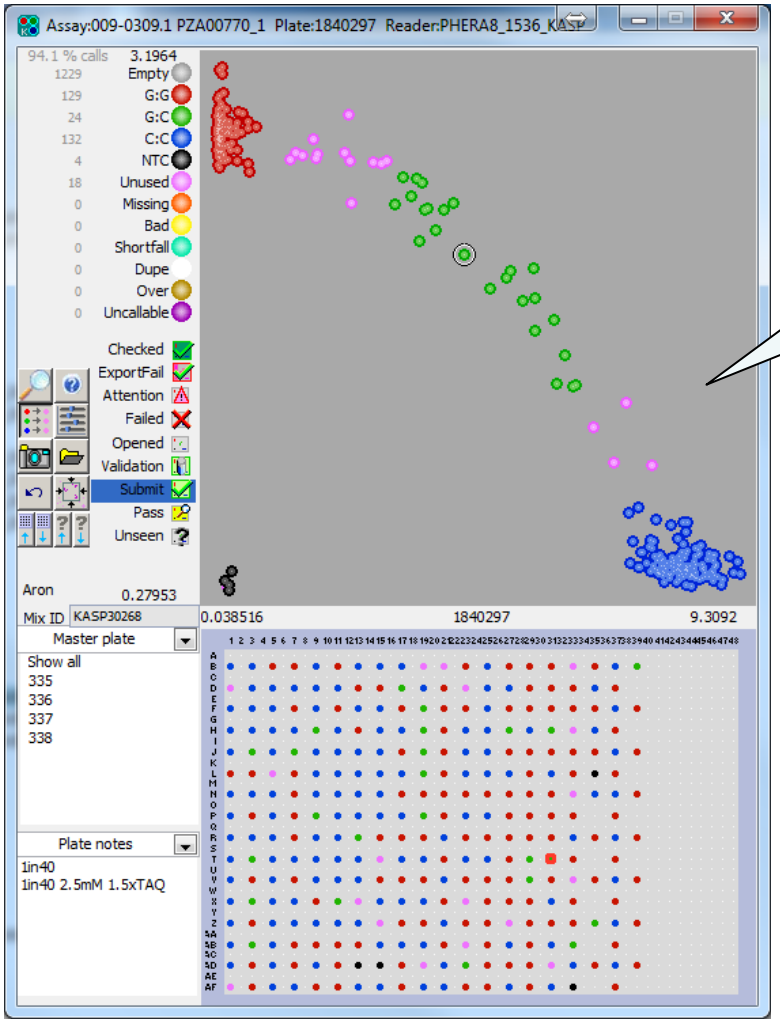
- Even partially sequenced genomes can give some information about choice of markers to design.

Solutions for homology and polyploidy





Polyploid data – allele dosage



Heterozygotes.
In a tetraploid organism these samples could have any of the following genotypes:
1:1:1:2
1:1:2:2
1:2:2:2

- **The central set of data points are from known heterozygotes.**
- **These are wheat crosses with clear allele dosage effect seen centrally.**
- **If using this kind of experiment then well normalised DNA is crucial.**

You reap what you sow – applying technology


USDA
United States Department of Agriculture
NATIONAL INSTITUTES OF HEALTH
EMBL
Institut Pasteur de Lille
Massachusetts Institute of Technology
TEXAS A&M UNIVERSITY
LGC
Queen's University Belfast
INRA
dkfz.
DEUTSCHES KREBSFORSCHUNGSZENTRUM
IN DER HELMHOLTZ-GEMEINSCHAFT
THE UNIVERSITY OF CHICAGO
UNIVERSITÄT LEIPZIG
SALK INSTITUTE
Where cures begin.
FRED HUTCHINSON CANCER RESEARCH CENTER
A LIFE OF SCIENCE
MEDIZINISCHE UNIVERSITÄT INNSBRUCK
Karolinska Institutet
deCODE genetics
UNIVERSITY OF COPENHAGEN
LUNDS UNIVERSITET
Lunds Tekniska Högskola
Deutsches Institut für Ernährungsforschung Potsdam - Rehbrücke
Medizinische Hochschule Hannover
POLYTECH NICE-SOPHIA
Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI
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香港中文大學
The Chinese University of Hong Kong
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UNIVERSITY OF TARTU
UNIVERSITY OF MANITOBA
institut Curie
Maastricht University
Universidad del Tolima
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Institut d'Oncologia
ULB
UNIVERSITÉ LIBRE DE BRUXELLES
AARHUS UNIVERSITY
Universidad Nacional Autónoma de México
UNAM
UCDAVIS
UNIVERSITY OF CALIFORNIA
SRI RAMACHANDRA UNIVERSITY
UNIVERSITY of the WESTERN CAPE
UAB
Universitat Autònoma de Barcelona
TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE
THE UNIVERSITY OF DUBLIN
NUS
National University of Singapore
RGCB
Rajiv Gandhi Centre for Biotechnology
KATHOLIEKE UNIVERSITEIT LEUVEN
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Universiteit Antwerpen
INCA
INSTITUTO NACIONAL DE CÁNCER
UNIVERSITEIT STELLENBOSCH UNIVERSITY
WAGENINGEN UR
For quality of life
UNIVERSITÀ DEGLI STUDI DI MILANO
SRC
SASKATCHEWAN RESEARCH COUNCIL
UNN
WE CARE – AND SHARE RESPONSIBILITY TOGETHER

KASP research with academics and industry

Plant Biotechnology Journal

Plant Biotechnology Journal (2012), pp. 1–17 doi: 10.1111/pbi.12009

Discovery and development of exome-based, co-dominant single nucleotide polymorphism markers in hexaploid wheat (*Triticum aestivum* L.)

Alexandra M. Allen[1,*], Gary L. A. Barker[1], Paul Wilkinson[1], Amanda Burridge[1], Mark Winfield[1], Jane Coghill[1], Cristobal Uauy[2], Simon Griffiths[2], Peter Jack[3], Simon Berry[4], Peter Werner[5], James P. E. Melichar[6], Jane McDougall[7], Rhian Gwilliam[7], Phil Robinson[7] and Keith J. Edwards[1]

[1]*School of Biological Sciences, University of Bristol, Bristol, UK*
[2]*John Innes Centre, Norwich, UK*
[3]*RAGT, Ickleton, Essex, UK*
[4]*Limagrain, Woolpit, Suffolk, UK*
[5]*KWS, Thriplow, Hertfordshire, UK*
[6]*Syngenta Seeds Ltd, Whittlesford, Cambridge, UK*
[7]*KBioscience Unit 7, Hertfordshire, UK*

Wheat SNP validation – public projects

Bristol/JIC KASP assays

Cross	Mapped Markers	
Avalon v Cadenza	3,028	1,122 A genome
		1,520 B genome
		386 D genome
Savannah x Rialto	1,543	790 A genome
		594 B genome
		159 D genome
Synthetic x Opata	201	70 A genome
		73 B genome
		49 D genome

- First report of a public linkage map for hexaploid wheat based on KASP to genotype wheat varieties and generate a linkage map.
- 67% polymorphic in varietal screen.
- 4% monomorphic in hexaploid wheat, but polymorphic compared to diploid/tetraploid varieties.

GCP KASP assays

GCP originator	Validated KASP Assays
Dr Susanne Dreisigacker (CIMMYT)	1,864

- Validation on wheat cultivars originating from Australia, China, India and Mexico.
- Included high anther culture ability and disease resistant varieties.

Generation Challenge Programme
Partnerships in modern crop breeding for food security

Feed the world

CGIAR Global Rice Science Partnership

CIFOR

BILL & MELINDA GATES foundation

Integrated Breeding Platform
Today's tools for tomorrow's crops

FAO FIAT PANIS

LGC

CIMMYT

CIAT
International Center for Tropical Agriculture
Since 1967 / Science to cultivate change

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

ICRISAT
Science with a human face

World Agroforestry Centre

AOCC

IRRI
INTERNATIONAL RICE RESEARCH INSTITUTE

INRA
Institut National de la Recherche Agronomique

ICARDA

Generation

World Agroforestry Centre
TRANSFORMING LIVES AND LANDSCAPES

IRD

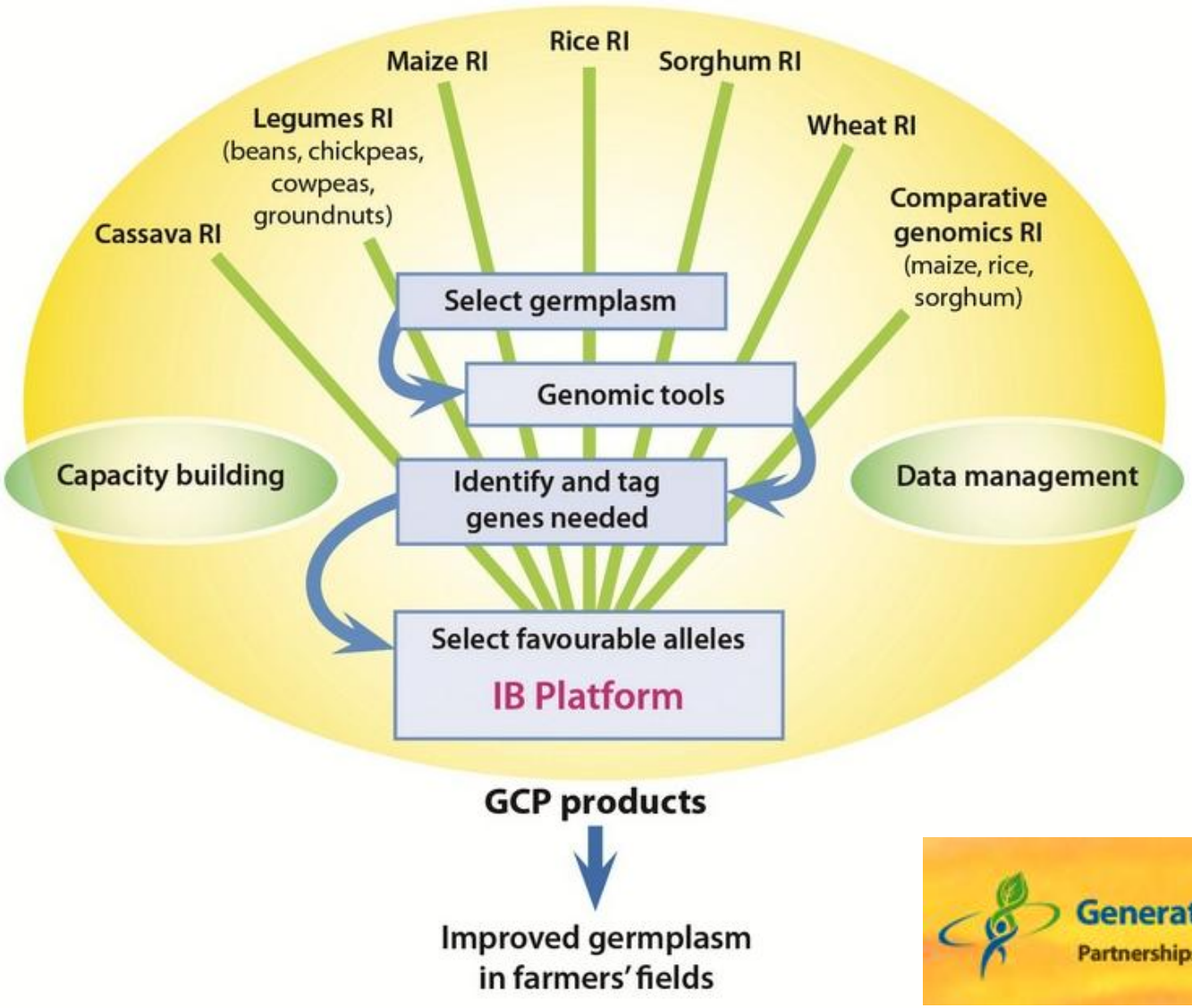
CGIAR

AfricaRice

cirad

JIRCAS
JAPAN

Generation Challenge Program (GCP) panels



Generation Challenge Programme
Partnerships in modern crop breeding for food security

Feed the world

African Orphan Crops Consortium
Genomics Laboratory

- Aim is to reduce stunting by increasing the nutritional value and yields of 100 local African crops by training the best African breeders to use the best tools in the world

- LGC are primary partner of the program and took part in training the 1st 25 breeders in Nairobi 2014

Plant sampling essentials

AOCC Nairobi 2014

Outline protocol

LGC leaf sampling kit

Kit contents:

- 1 x 96-well tube storage rack with lid
- 12 x perforated strip caps
- 1 x 50g desiccant sachet (in a bag)
- 1 x larger (labelled) sealable bag
- 1 x cutting tool & 1 x cutting mat

If multiple kits have been requested, only 1 x cutting tool and 1 x cutting mat will be sent to you.

We may be able to handle projects outside the specifications given in this document; if this is the case please contact us directly to discuss your requirements:

+44 (0)1992 470757 info.uk@lgcgenomics.com

Collecting leaf samples

Repeat this process for each plant you want to sample, using a new tube each time.

Cut leaf discs

- Place the leaf to be sampled on the cutting mat.
- Uncap the cutting tool.
- Cut a disc out of the leaf by pushing the cutting tool into the leaf; twist the tool as you push to make the tool pick the disc up.

Fill tubes with samples

- Insert the end of the tool into one of the racked tubes, and depress the plunger to dispense the disc.
- *The tubes are in strips of 12, and can be removed from the rack if needed at this step.*
- *Add 1-12 discs per tube, depending on the project (see 'How many disks are required?')*

Wash cutting tool

- Place the end of the cutting tool in some clean water and depress the plunger a few times.
- Flick the tool until completely dry.

Please ensure the rack is labelled appropriately.

Preparing samples for transport

Seal tubes

- Place the perforated Strip caps on top of the tubes.
- Press firmly to ensure caps are secured.
- **Remove the desiccant from its small sealed bag.**

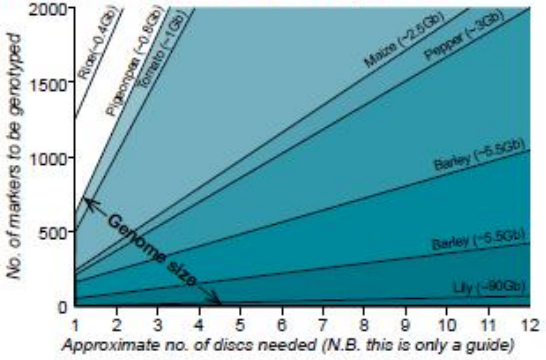
Add dessicant

- **Place the dessicant sachet on top of the rack of tubes and replace lid.**
- Place completed rack inside the larger (labelled) bag.
- **Force the air out of the bag and then seal it.**

Prepare for shipping

- Place the sealed bag in a suitable container.
- Our address: **LGC, Extractions department, Units 1 & 2 Trident Industrial Estate, Pindar Road, Hoddesdon, Hertfordshire, UK, EN11 0WZ.**
- *Provide a description of the contents for customs.*

How many disks are required?



CS0614

Agricultural crop improvement case study: The story of the Pigeon pea revolution

- KASP Genotyping assay run by LGC genotyping service lab
- Marker-assisted breeding for improved crops

Pigeon pea circa. 1992

- Slow growing
- Low yield
- No commercial value
- Neglected by crop breeders
- Un-developed potential

- **Breeding program sponsored**
- **Accelerated MAS using SNPs**
- **SNPs analysed with KASP**

Pigeon pea circa. 2013

- Drought & disease resistant
- Excellent food supply
- Better market value
- Established life changing market value chain for growers in Tanzania

Colorado State University
Wheat Breeding and Genetics Program

- Scott Healy's group use KASP to identify and monitor traits for Colorado's harsh climate

- They also use KASP to find traits for improving human health such as starch quality and antioxidants.

Development of gene specific KASP markers: a toolbox for marker-assisted selection in wheat.

Gina Brown-Guedira [1], Neelam Kumari [2], Susan Dreisigacker [3], Peter Sharp [4], Catherine Ravel [5], and Cristobal Uauy [6].

[1] USDA–ARS Plant Science Research, North Carolina State University, Raleigh, NC, USA; [2] Department of Crop Science, North Carolina State University, Raleigh, NC, USA; [3] International Maize and Wheat Improvement Center (CIMMYT), Mexico; [4] Plant Breeding Institute, University of Sydney, Narellan NSW, Australia; [5] INRA, Université Blaise Pascal, Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand, France; and [6] Department of Crop Genetics, John Innes Centre, Colney, Norwich, United Kingdom.

Whole-genome, SNP detection technologies now available in
struction of linkage maps, genome-wide association mapping
these technologies are not suited for marker-assisted breeding
the same polymorphism on tens of thousands of plants in the
tive markers amenable to high-throughput genotyping are nee
and in combination with other marker-assisted breeding appro
of an international collaboration to develop and make publicly
wheat. Stream-lined homogeneous assays were developed usi
assay design, making it well-suited for use in polyploid wheat
methods. Reported sequence variation (SNPs and indels) were
the reduced height, vernalization, and photoperiod-response g
for cloned disease resistance and end-use quality genes. In so
the causal gene sequence. Also, a number of genes in wheat a
assays for these, associated SSR and STS markers were evalu
Wheat Infinium Assay. SNP in linkage disequilibrium (r^2 >0.9
approach, markers were developed that are highly predictive
the *Sr36* and *Sbm1* resistance genes, and alleles at the *Glu-D1*
association mapping experiments, coupled with SNP genotyping and current sequencing projects in wheat, will result in identification of numerous sequence targets for development of new predictive, homogeneous assays for important genes. We encourage researchers to contribute sequences for development of additional publicly available KASPar assays for use in wheat improvement programs world-wide.

"KASP provides flexibility in assay design, making it well-suited for use in polyploid wheat, and is low cost"

"We encourage researchers to contribute sequences for development of additional publicly available KASPar assays for use in wheat improvement programs world-wide"

Plant assay search tool

Marker: 3-hydroxy-3-methylglutaryl-coenzyme A reductase 1 **Chromosome:** 1 **Position:** 32 **Trait:** Ggc grey ground colour (seed coat colour)	add to cart
Marker: 30S ribosomal protein S31, chloroplastic **Chromosome:** 1 **Position:** 32 **Trait:** Yc cotyledon colour	add to cart
Marker: 40S ribosomal protein S15-4 **Chromosome:** 2 **Position:** 32 **Trait:** Ggc grey ground colour (seed coat colour)	add to cart
Marker: 40S ribosomal protein S15a-1 **Chromosome:** 2 **Position:** 32 **Trait:** Tgc tan ground colour (seed coat colour)	add to cart
Marker: 40S ribosomal protein S18 **Chromosome:** 2 **Position:** 32 **Trait:** Tgc tan ground colour (seed coat colour)	add to cart
Marker: 40S ribosomal protein S18 **Chromosome:** 3 **Position:** 32 **Trait:** Tgc tan ground colour (seed coat colour)	add to cart
Marker: 50S ribosomal protein L31, chloroplastic **Chromosome:** 3 **Position:** 32 **Trait:** scp seed coat pattern (not close though - 7cM)	add to cart
Marker: 60S acidic ribosomal protein P1-2 **Chromosome:** 3 **Position:** 32 **Trait:** Seed diameter, plumpness	add to cart
Marker: 60S ribosomal protein L27a-2 **Chromosome:** 3 **Position:** 42 **Trait:** Seed diameter, plumpness	add to cart
Marker: ABSCISIC ACID-INSENSITIVE 5-like protein 5 **Chromosome:** 4 **Position:** 42 **Trait:** Seed thickness	add to cart
Marker: ABSCISIC ACID-INSENSITIVE 5-like protein 5 **Chromosome:** 4 **Position:** 42 **Trait:** Seed thickness	add to cart

Reset search

Lentil

Marker Name:

Genetic

Chromosome: cM

Physical

Mapping Min:

Mapping Max:

Distance/Mapping Interval: cM

Trait...

Plant Assay Cart

Hide cart

Your cart is empty.

Web assay search tool

- A web based tool for locating functionally validated SNPs for crops.
- Started out for human validated SNP (more than 100 k!)
- We thought that it might be useful for breeding communities.
- Not there to compete but compliment projects
- We aim for it to:
  - Deposit markers for everyone to use – Can be quickly (minutes) with new markers for sharing.
  - Links to original material
  - Gives basic information about the marker (i.e. sequence or map position (cM))
  - An easy way to order assays

Assay Search Tool – Submitting

- You can add information linking to reference
- Point me towards where the information is and I'll do the rest.
  - OR
- Provide either map units (cM – reference map) or nucleotide positions (provide the reference genome name).
- Provide marker reference name / sequence
- I'll do the rest

What will be available?

- Know Pulse – Lentil – core 154 initial assays but more to come
- Sol Cap - All Tomato validated 384 assays and 7k *in silico* designed
- Wheat – cerealsDB assays – around 9000 validated
- More to come!!

Any Questions?