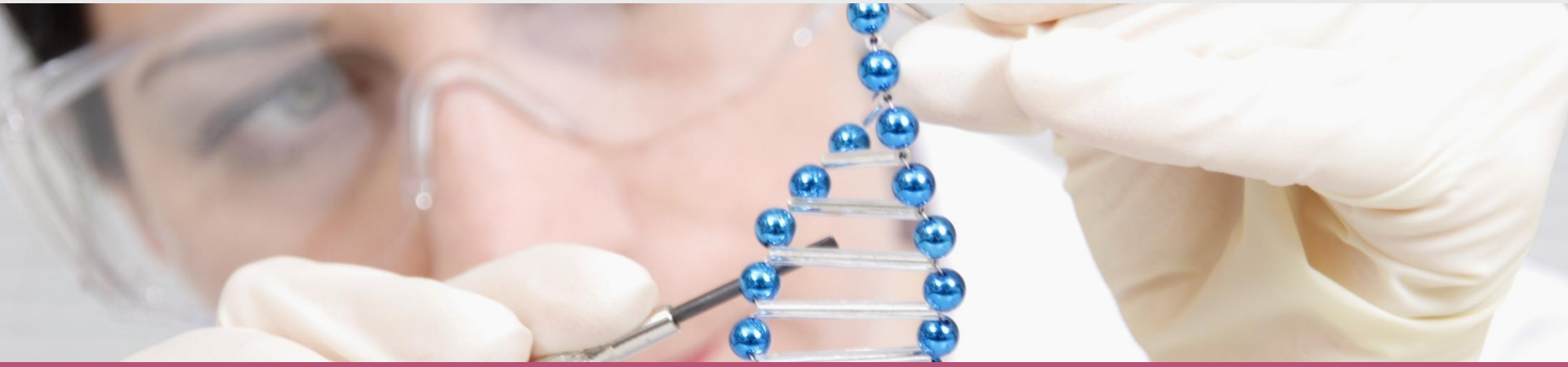
LGC Genomics

now incorporating KBioscience



Optimizing extraction technologies for efficiency in high throughput genotyping data generation

April, 17th, 2013

Spring 2013 AEIC Meeting, Madison (WI)

Dr. Frank Schubert
Business Unit Manager Nucleic Acid Preparation (NAP)

Amy Pierce
US Lab Director

LGC Genomics

now incorporating KBioscience

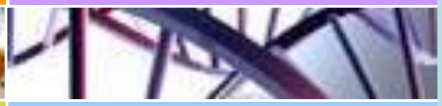
LGC Genomics – part of the LGC Group

- Established in 1842 as a scientific testing laboratory for the UK Government
  - Privatised in 1996
- Retains role as UK National Measurement Institute
  - Standard bearer for chemical & bio-analytical measurement
- Global presence
  - Over 30 locations
  - 1800+ staff
  - 3-fold growth since 2004

Five Operating Divisions

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

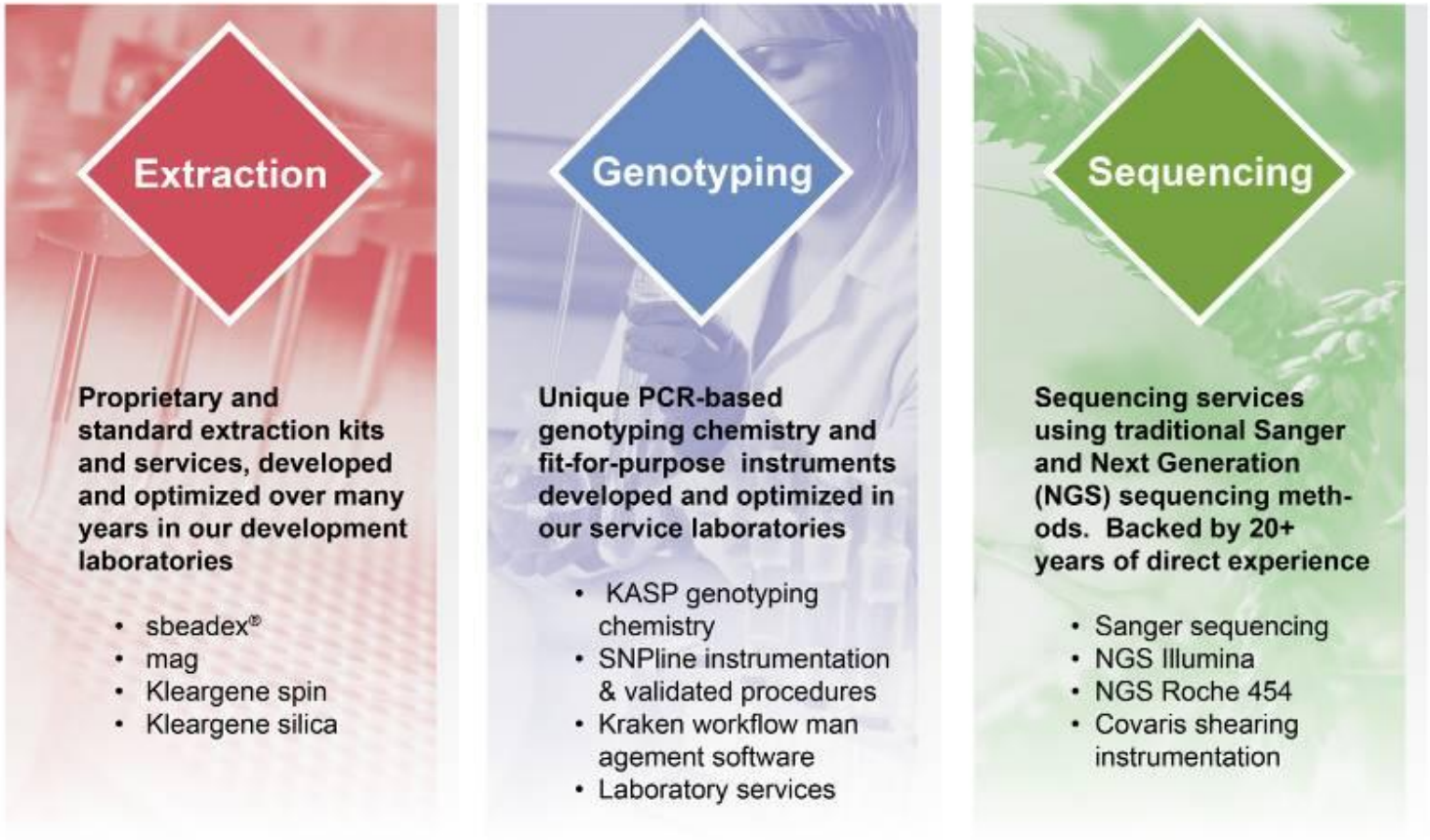
LGC Standards
Production and supply of chemical and biochemical reference materials

LGC Science & Technology
Home of the UK National Measurement Institute

LGC Genomics

now incorporating KBioscience

LGC Genomics – solutions for genomic research



Accurate, flexible, highly cost-effective solutions

LGC Genomics in summary

- Deliver accurate, reproducible analytical science solutions

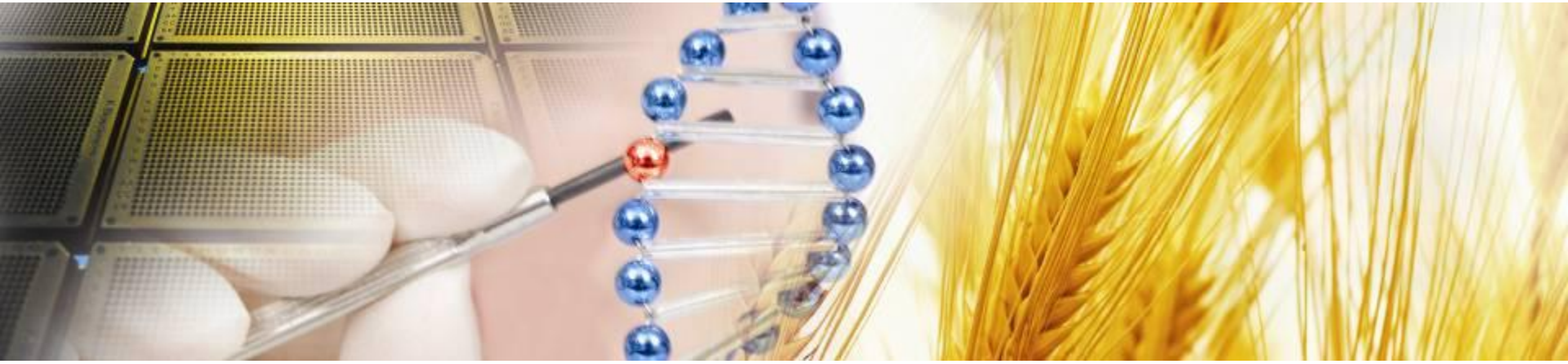
- **We use what we sell**

  and sell what we use

- We use the instruments and reagents that we sell to also drive a profitable service business

Extraction

Extraction Products and Services



- Patented sbeadex® technology
- Mag™

Optimised solutions for any application - Just a few examples

Plants

Example applications

- •Genetic analysis
- • Marker assisted breeding
- • Quality control

Forensic samples

Example applications

- •Genetic fingerprinting
- • Paternity testing
- • Trace analysis

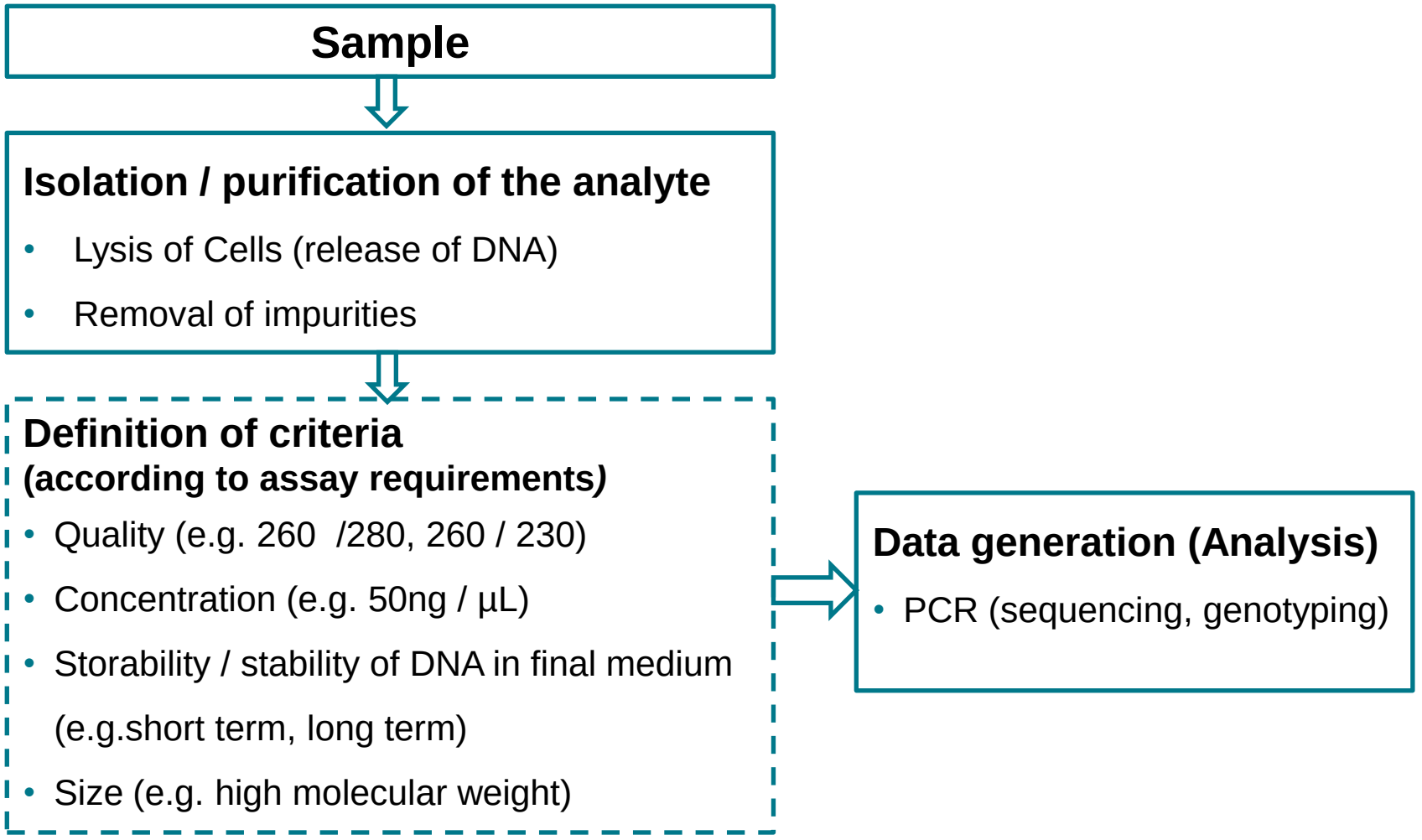
Blood & tissue

Example applications

- •HLA typing
- • SNP genotyping
- • Biobanking

Food, saliva, plasmids, prokaryotes etc.

Analytical pathway in DNA analysis



How to cope with m(illions of) any samples

- Parallel processing to increase throughput (MTPs)
- Decrease scale (Miniaturization)
- ***Automation***

Increase data output / time and money

- Throughput (samples per run, day etc.)
- Investment
- Running cost
- Reliability of chemistry and instrumentation
- Flexibility of protocols regarding sample material, scale

Portfolio – Nucleic Acid Preparation (NAP)

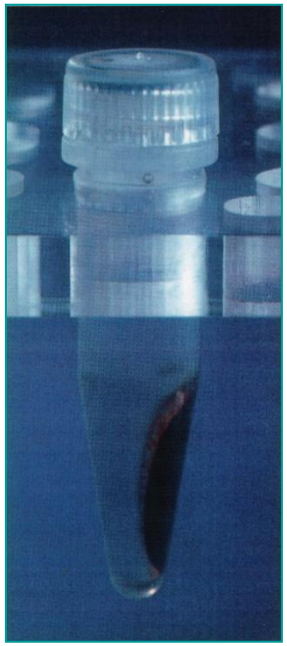
Kits for automated DNA extractions

- sbeadex® kits
- mag™ kits

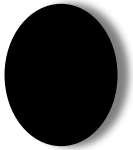
Instruments

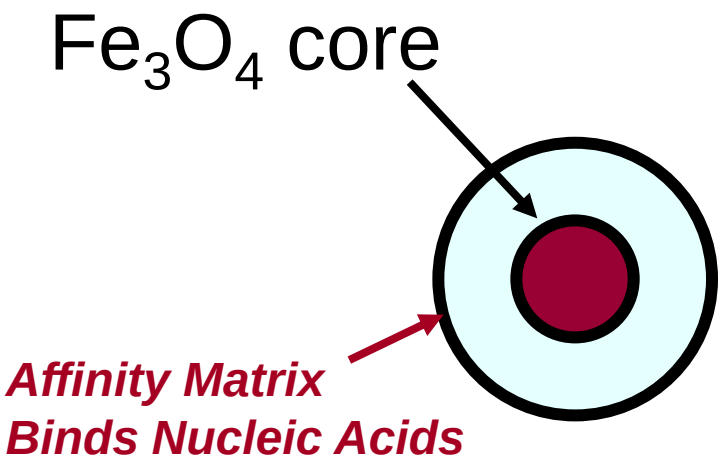
- Magnetic particle manipulators

Magnetic bead based SPE of DNA



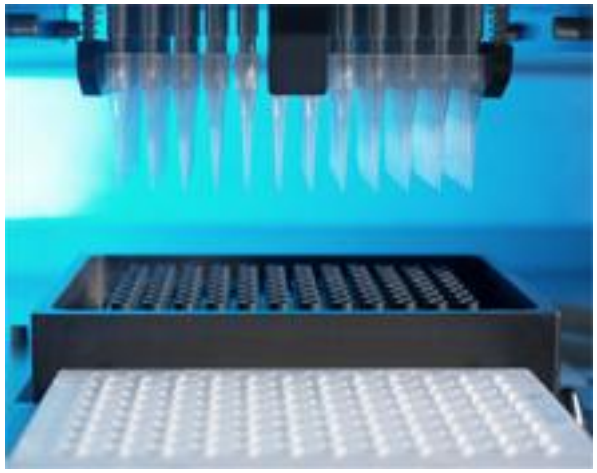
Magnet





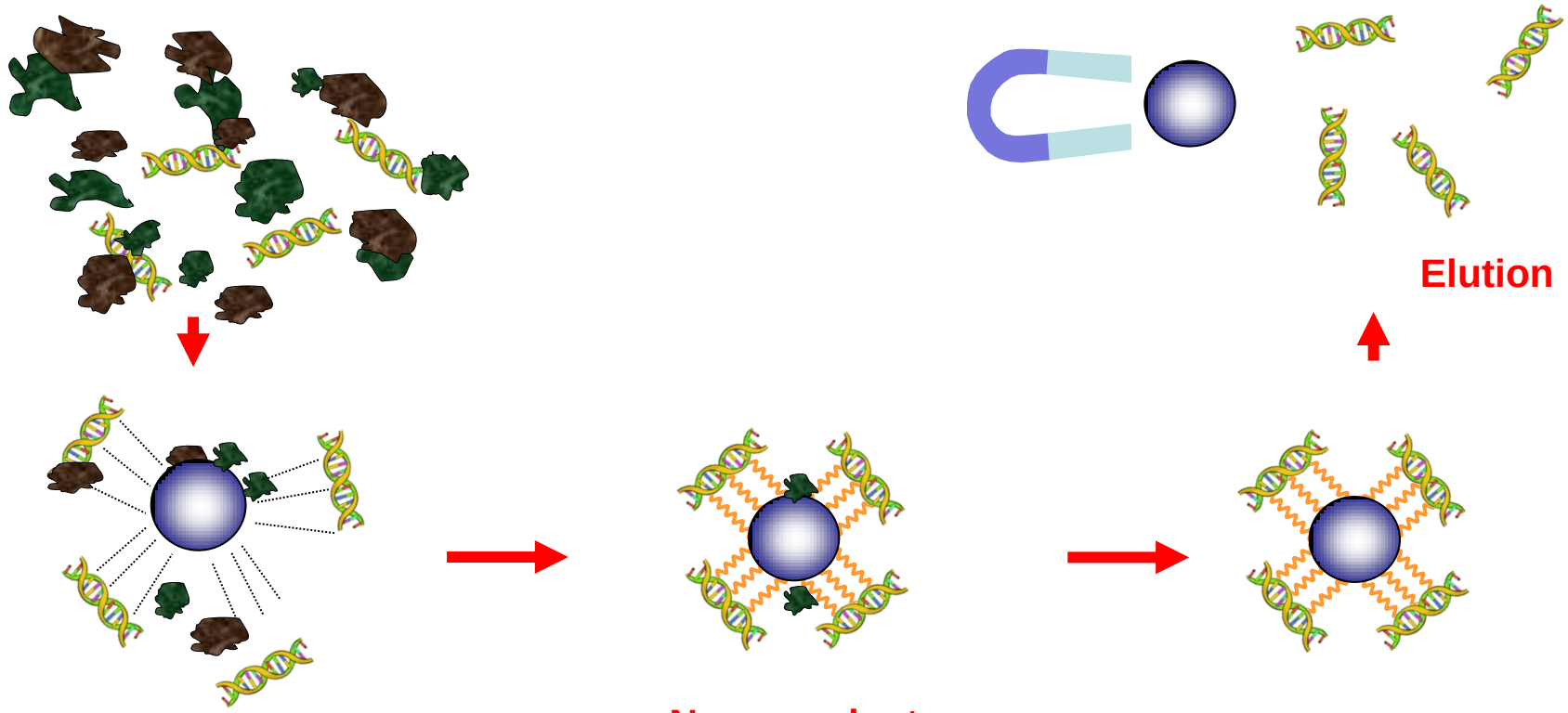
General benefits of magnetic beads technology:

- Reduced labor, ***cost effective*** and usually highly scalable
- Facilitate high-throughput extraction, miniturization & easy to automate
- Reduced set up costs
- Ongoing running costs are low



sbeadex® principle

- a change of binding mechanism during extraction -



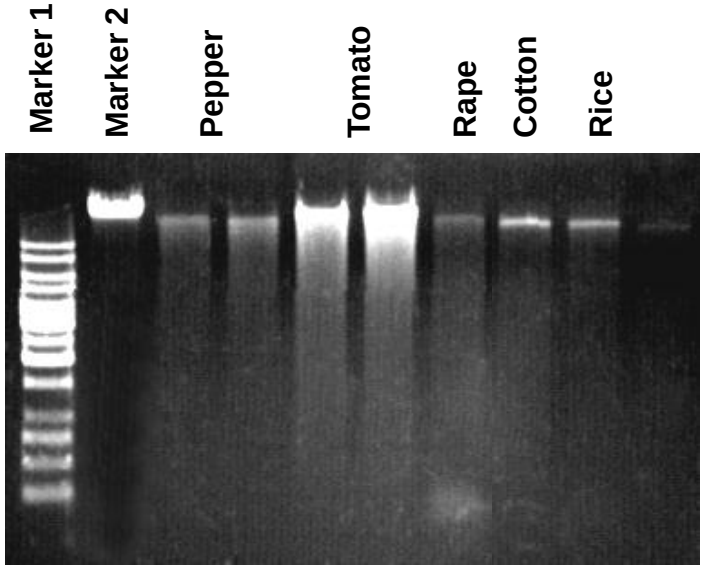
sbeadex ® - advantages / benefits

- No compromise in lysis conditions
- No organic solvents in wash buffers - Price, convenience
- No drying - Speed
- In principle, no chaotrophs for binding required
- Fast extractions

sbeadex® - plant kit

DNA extraction from different plant (leaf material)

Material	ID	ng/ µL	260/280	260/230	Total [µg]	RNase
Pepper plant[1)]	1/1	68.3	2.1	1.5	5.5	-
	1/2	66.0	2.1	1.7	5.3	-
Tomato[2)]	2/1	29.1	2.2	1.7	1.5	-
	2/2	26.7	2.0	1.6	1.3	-
Rape[1)]	3/1	39.9	1.9	0.8	3.6	+
	3/2	21.0	1.9	1.2	1.9	+
Cotton plant [1)]	4/1	84.3	1.8	1.2	7.6	+
	4/2	91.7	1.8	1.1	8.3	+
Rice[1)]	5/1	9.5	1.8	0.8	0.9	+
	5/2	9.1	1.5	0.7	0.8	+



sbeadex® - plant kit

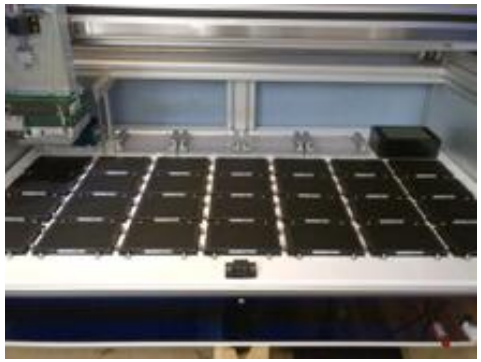
Examples of validated protocols

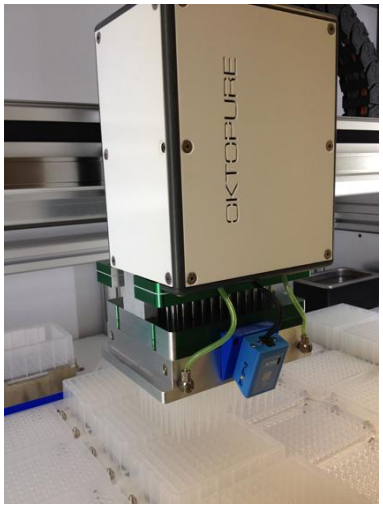
- Cotton
- Chicory
- Grapewine
- Hops
- Lettuce
- Maize
- Oak
- Onion
- Pepper
- Pine
- Rapeseed
- Rice
- Sunflower
- Tomato
- Wheat

No contractual / technical obligation!

Automated liquid handling

- oktopure™





Co-operations with manufacturers of magnetic bead handling systems

- TECAN
- Hamilton
- PerkinElmer
- King Fisher; Thermo Scientific
- Beckman

Applications & analysis

- **Molecular biology:**
  e.g. PCR, SNPs
- **Plant & animal breeding:**
  e.g. Genotyping & Sequencing
- **Diagnostics:**
  e.g. pathogens, viruses etc.

The sbeadex® toolbox

Standard ingredients

1. Lysis buffer
2. Binding buffer
3. sbeadex beads
4. Wash buffer 1
5. Wash buffer 2
6. Elution buffer

Individual adds on / Tailor made solutions

- Customized bulk kit for almost all robotic systems (home made included)
- Challenging samples / protocol development
- DNA concentration
- Total amount of DNA

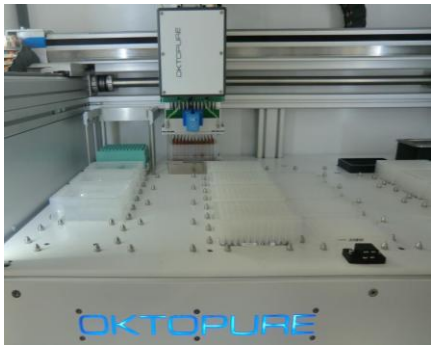
oKtopure™

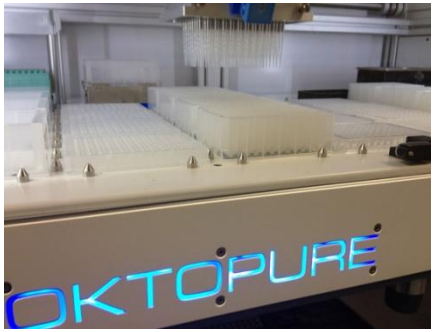
- 96 channel pipetting head
- Optimized for magnetic bead based extractions
- Max. 8 plates x 96-well platess per run

  = 768 samples (extraction, only)
- Run time

  mini scale 1h 10min

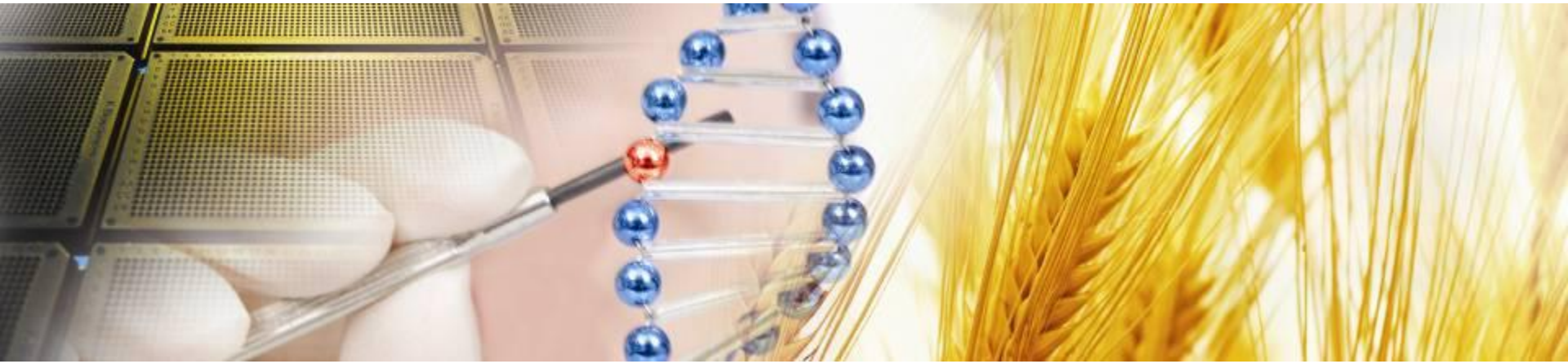
  maxi scale ca. 2h





Genotyping

Genotyping Products and Services

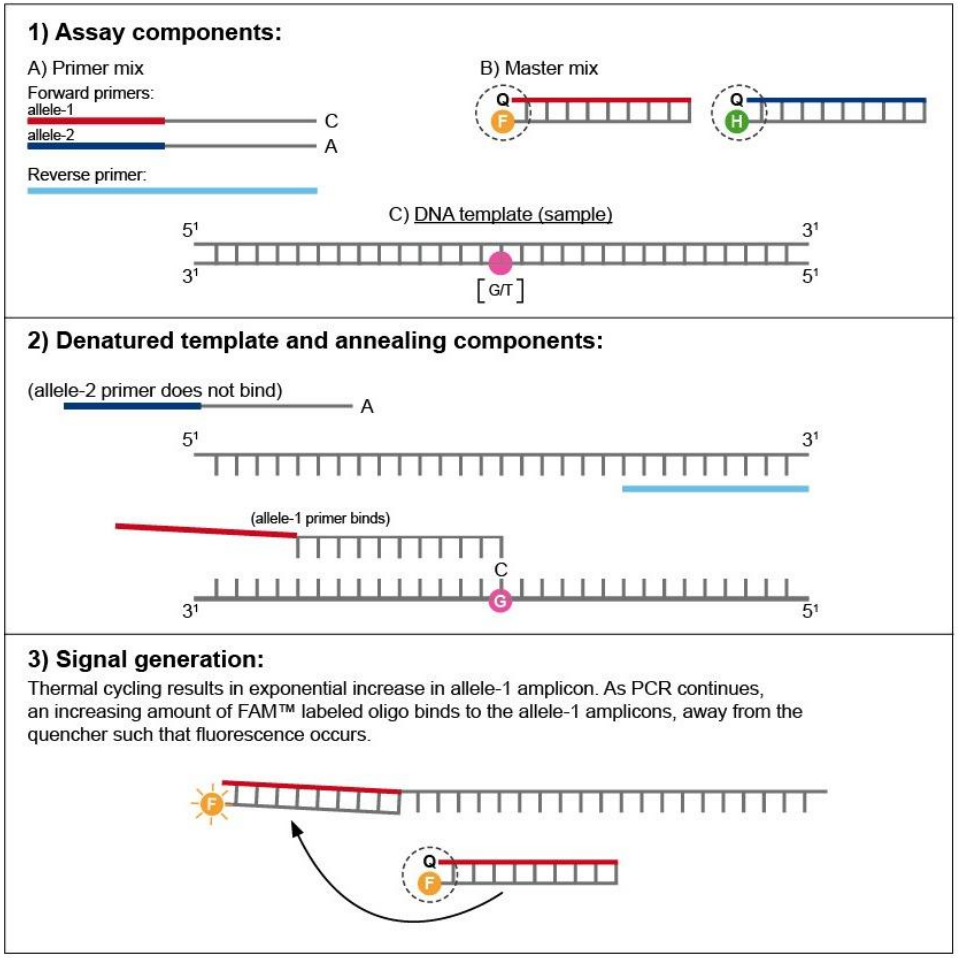


- KASP genotyping chemistry
- SNPline workflow

KASP™ Genotyping System

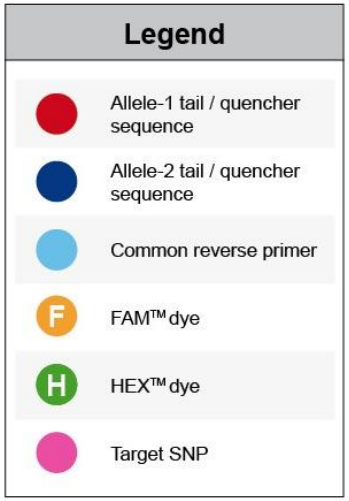
- Novel **K**ompetitive **A**llele-**S**pecific **P**CR
- Designed to overcome high cost of dual-labeled probes
- Homogeneous fluorescent assay - no separation steps
- Scalable single-plex PCR: 96/384/1536 plates
- Delivers accurate SNP and InDel genotyping data while providing maximum flexibility and cost savings.
  - Improved genotype clustering, especially with plant and polyploid organisms; fewer primer-dimer (PD) issues.
  - Run as few or as many assays as you like; repeat what you want
  - Price per data-point remains consistently low

KASP Chemistry

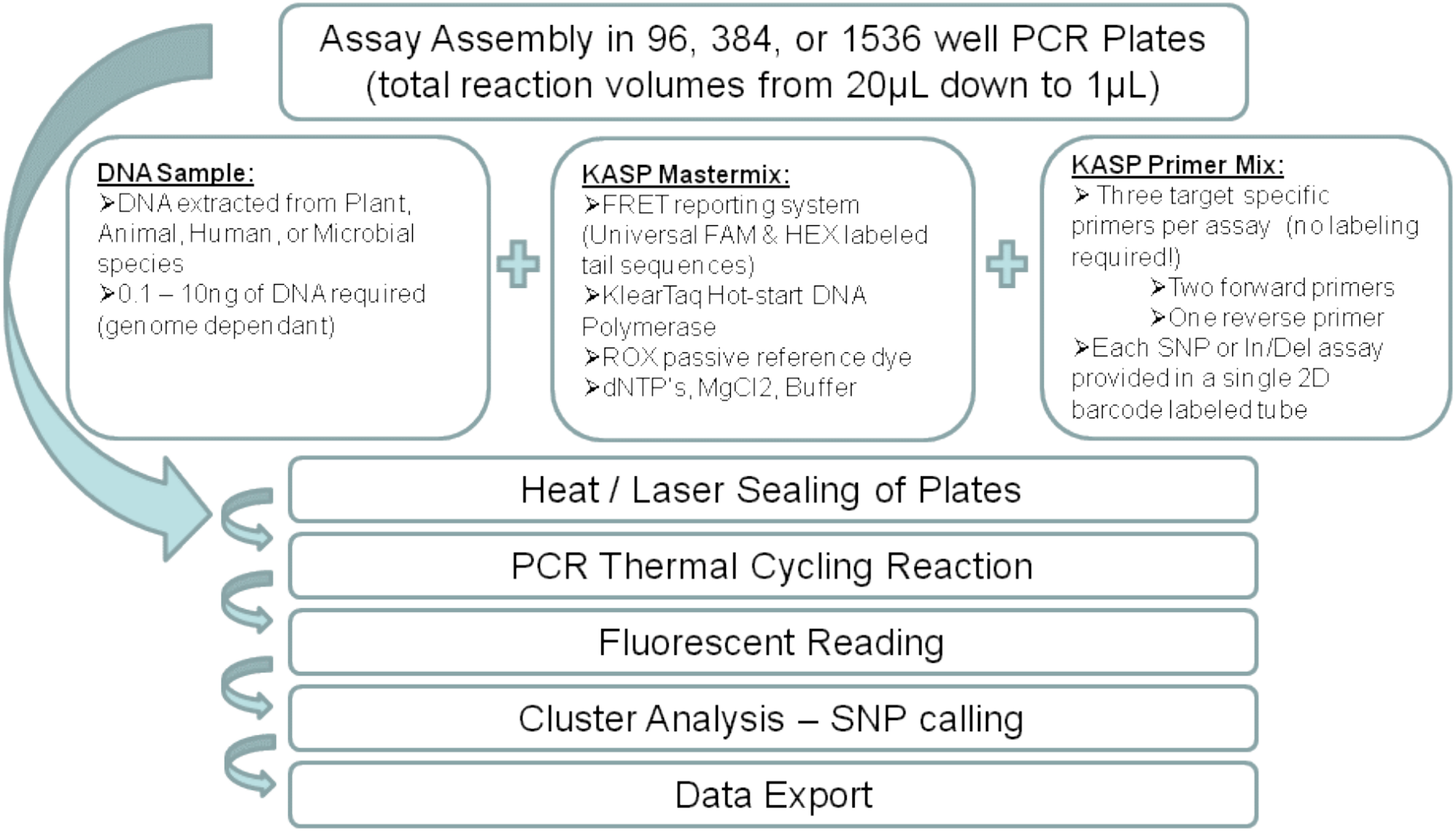


Allelic discrimination achieved through competitive annealing of two allele-specific forward primers, each containing a unique tail sequence that corresponds with a distinctly labeled FRET cassette in the master mix.

- Excellent data quality
- Maximum flexibility
- Cost-effective
- Minimal DNA material required
- Leading SNP to assay conversion rate (>90%)



KASP workflow



SNPline workflow – process overview

DNA Stamping; plate replication & reformatting

Assay Assembly; reagent dispensing

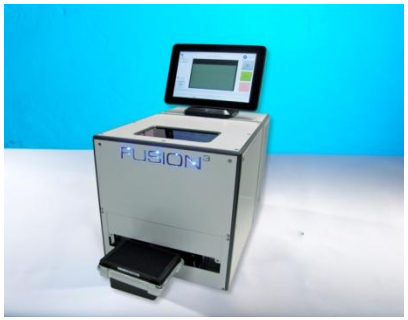
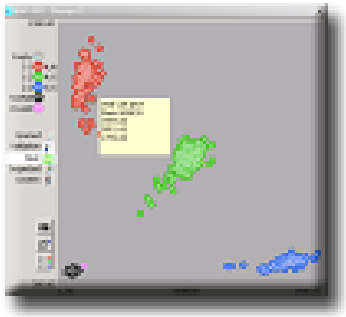


Plate Sealing



Kraken™ Workflow Manager; assay design, sample/ workflow mgmt., data analysis, etc.

Plate Reading; FRET based Fluorescence

High Throughput PCR; Waterbath thermal cycling

SNPline workflow:
DNA stamping - plate replication & reformatting

SNPline Lite: Kpette – manual

- Manually stamp DNA into 96/384/1536 plate formats
- Tip guides make for quick processing
- Optional tip regeneration station with optimized protocol can virtually eliminate tip costs

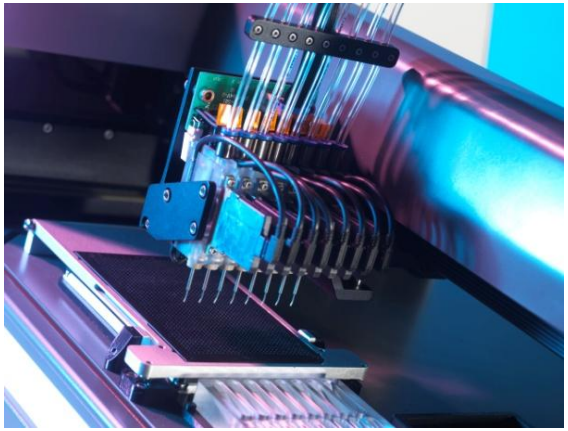
SNPline XL: repliKator – fully automated

- 24 x 1536 well plates in 15 minutes
- Onboard tip washing – eliminate tip costs
- 96/ 384/1536 – integrated into Kraken Software
- Integration friendly

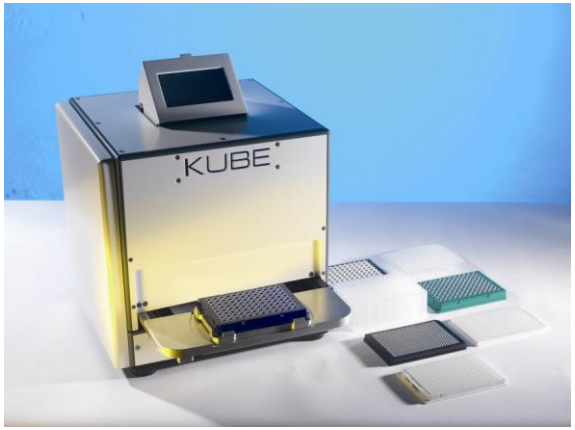
SNPline workflow
Assay assembly – reagent dispensing

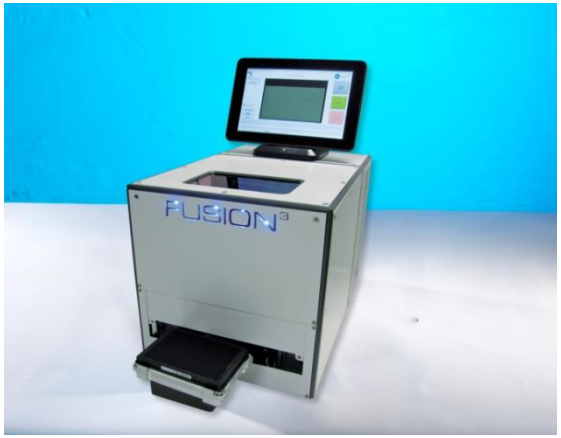
SNPline Lite & XL: Meridian Dispenser

- Single or 8 Channel configurations
- Dispense volume: 300nl to 100ul
- Unique aspirate function greatly reduces waste of expensive reagents
- Standalone PC control interface, and integrated control through Kraken software
- Angled tips to avoid cross contamination
- Peltier cooled plate locator
- Fill 24 x 1536 plates per hour



SNPline workflow: Plate Sealing





SNPline Lite: Kube Plate Sealer

- Automated heat sealer
- Hermetically seals 96 & 384 plates
  - Standard height and deep well
- Advanced onboard control interface
- Integration friendly

SNPline XL: Fusion Plate Sealer

- Automated laser sealer – patented
- Hermetically seals 96, 384, & 1536 plates
- Ability to re-seal up to 50 times!
- Intuitive software control interface
- Integration friendly

SNPline workflow:
High throughput PCR – water bath thermal cycling

SNPline Lite: Hydrocycler-4

- Process 4 x 96, 384, or 1536 plates at a time
- Up to 30% faster than Peltier based systems
  - Typical KASP PCR run = 1.15 hours
  - 6 full runs in an 8 hour day
- Automated, robust and consistent

SNPline XL – Hydrocycler-16, 32, or 64

- Process 16 to 64 x 384 PCR plates at a time; or 14 to 56 x 1536 PCR plates.
  - Up to 500,000 data points/day!
- Energy efficient; throws off less heat
- Eliminates need for special AC environment

SNPline workflow: Fluorescent Plate Reading

- Kraken interface for both models – automatic file generation, export, and scoring

- Optional 36-plate stacker available for both models.

SNPline Lite: BMG FLUOstar Omega

- Single excitation/emission
- Three passes, three reads
- Reads KASP assays in 96 & 384 plates
- Speed: 1.5 minutes/384 well plate

SNPline XL: BMG PHERAstar

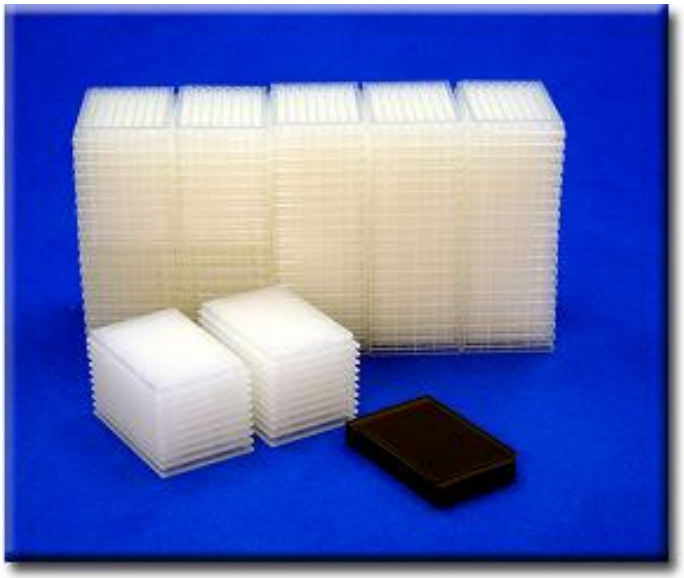
- Simultaneous dual-emission technology
- Two passes, four reads
  - Faster with greater sensitivity
- Reads KASP assays in 96, 384, & 1536 plates
- Speed: 1.2 minutes/1536 well plate

Scalability and Cost Savings

96 x 96 well plates

24 x 384 well plates

6 x 1536 well plates



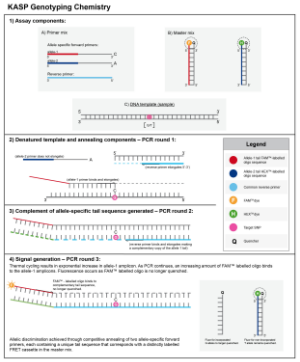
10µl PCR reaction

5µl PCR reaction

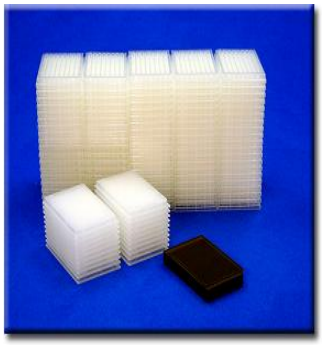
1µl PCR reaction

- Reagent consumption is the primary cost driver
  - Two-fold savings from 96 to 384, Ten-fold from 96 to 1536
- Next is equipment: cost, throughput, flexibility
  - Hydrocycler 64 will process 64 x 384, or 52 x 1536 plates in 1.2 hours!
- Plate and seal costs are a wash when analyzed per well

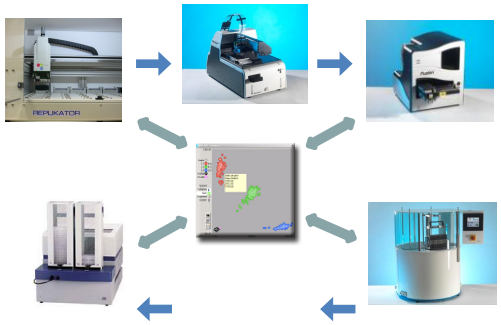
Driving scalability and cost savings



Novel primer approach with KASP



Minitaturisation to enable 1µL reaction volumes



The flexibility and scalability of SNPline

All this means we can drive cost per data point to as low as 1 or 2 cents per data point, which is back where we started and why we do all this, which is to enable our customers to do more with their research dollars

Thank You For Your Time