

# LGC Biosearch Technologies 基因分型解决 方案及应用



## Agenda

- KASP/BHQPlus 介绍
- BHQPlex Coprimer介绍
- 单位点检测优势
- Troubleshooting
- 应用案例

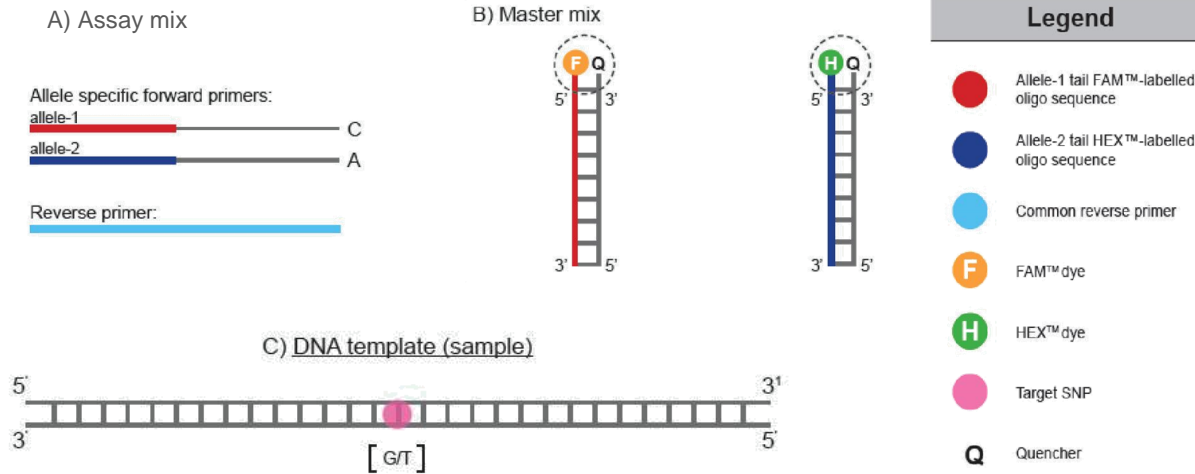
# KASP/BHQPlus/BHQPlex Introduction

# Biosearch PCR Oligo & Assay Portfolio

	BHQ Probes™				Dual-labeled and LNA Probes	KASP™ Genotyping Chemistry	Molecular Beacons
	BHQplus®	BHQnova™	BHQ® Dual-labeled Probes	BHQplex™ CoPrimers™			
qPCR & Multiplex qPCR	X	X	X	X	X		
SNP Genotyping / Allelic Discrimination	X			X	X	X	
Multiplex SNP Genotyping with Assay Interchangeability				X			
Gene expression analysis	X	X	X	X	X		X
Presence/Absence	X	X	X	X	X	X	
Insertions/Deletions	X		X	X	X	X	
Pathogen Detection / Viral Load Quantification	X	X	X	X	X		X
AT rich templates	X	X		X		X	
Copy Number Variation	X	X	X	X	X		
Polyploid genotyping, plants				X		X	
Valumix™ Assays - Single tube, pre-mixed SNP genotyping assays	X						
Valumix™ Assays - Single tube, pre-mixed gene expression assays			X				
Long probe sequence		X					
In Silico & Functionally Validated Genotyping Assay options						X	
Black Hole Quencher® (BHQ)dye Technology	X	X	X	X			

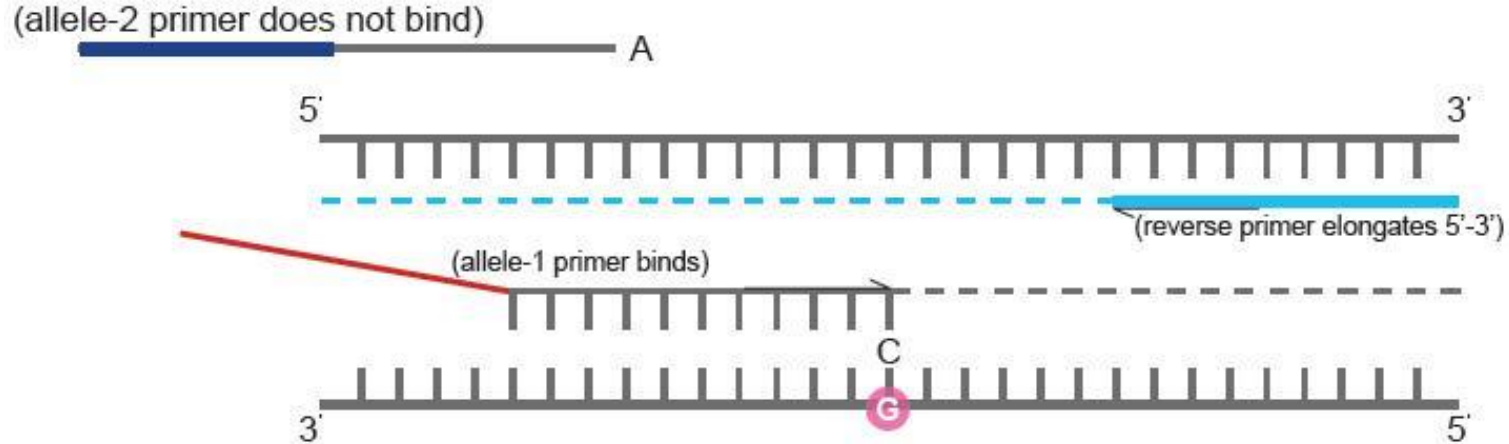


# KASP Components

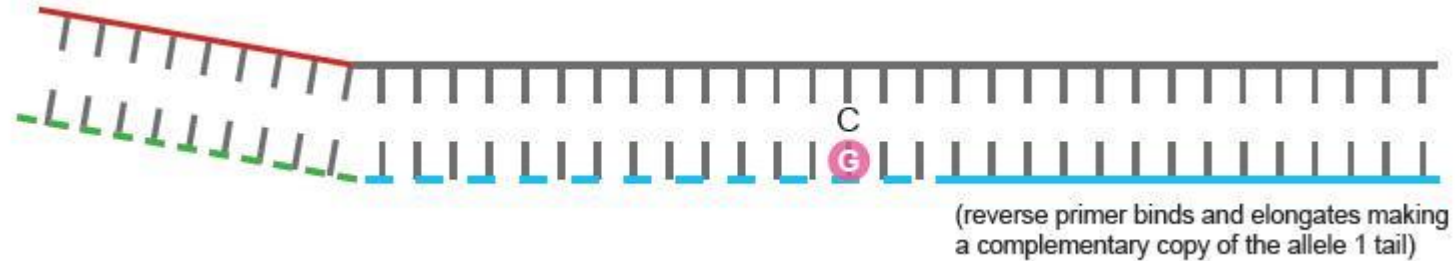


- Assay mix - contains two allele specific primers and common reverse primer(s). This is often referred to as a KASP Assay.
- Universal Master mix - contains fluorophores, quenchers and *Taq* polymerase.

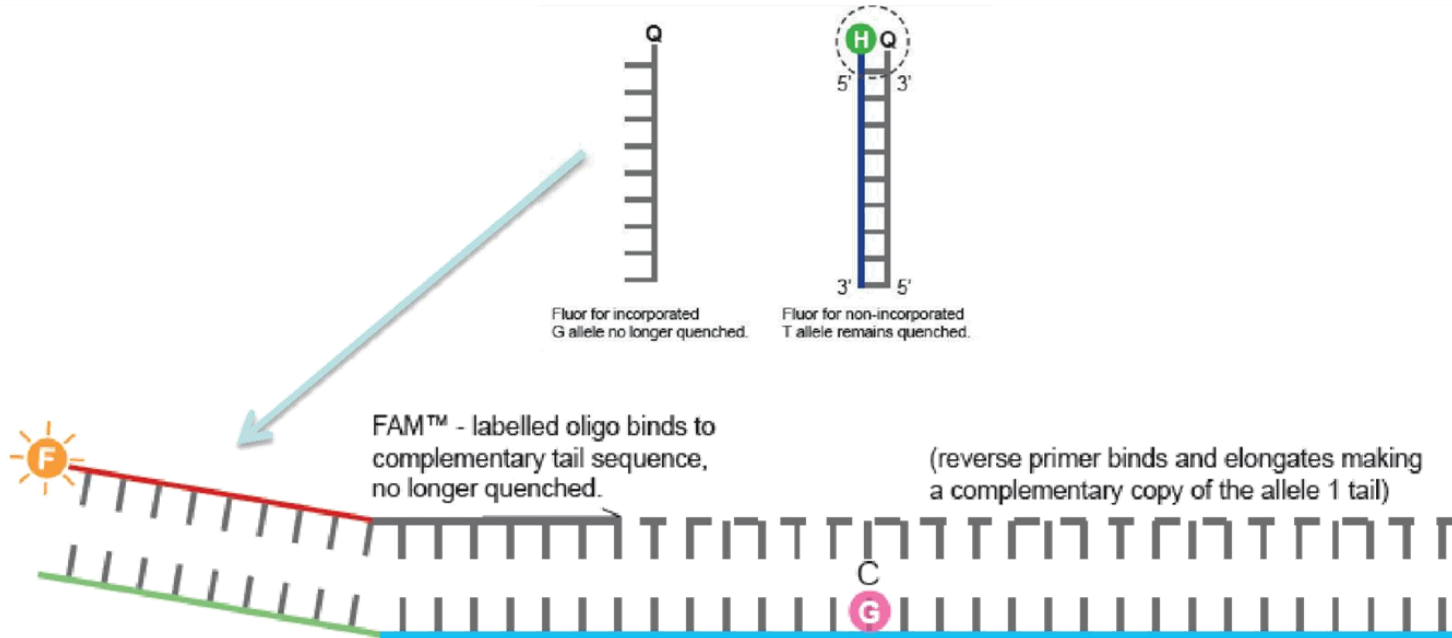
# Denatured template and annealing components – PCR Round 1



## Denatured template and annealing components – PCR Round 2

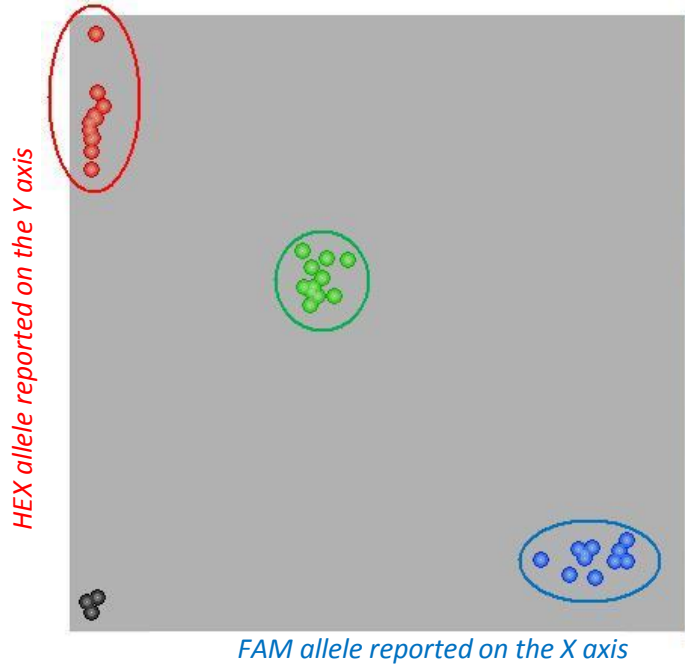


# Signal Generation – PCR round 3





# KASP Results



**RED** Sample is homozygous for the HEX allele

**GREEN** Sample is heterozygote: One FAM allele and one HEX allele

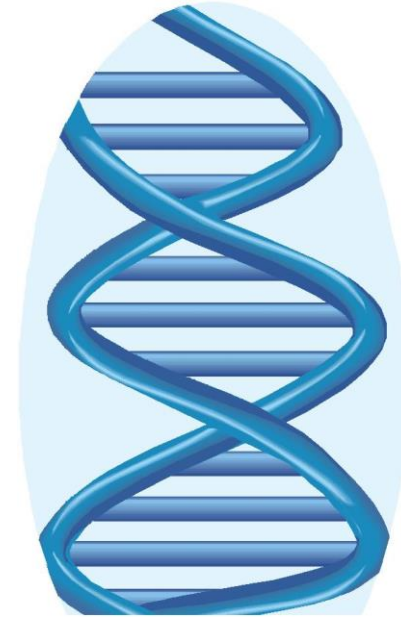
**BLUE** Sample is homozygous for the FAM allele

- Signal values generated at the end of the KASP thermal cycle are plotted as a graph.
- Each data point represents an individual DNA sample
- Samples of the same genotype cluster together

## DNA requirements for KASP

- Most KASP assays will function with 2.5 – 50 ng DNA per reaction
- For human DNA (genome = 3000 Mb), we would recommend using 5 ng /  $\mu$ L concentration (wet DNA) as a starting point
- The purity of DNA is important but no more so than for standard PCR
- Crude extractions – inhibitors can remain
- May require optimisation using a dilution series

2.5 – 50 ng /  $\mu$ L



# Polymorphism types

–the types of polymorphisms that can be detected using KASP



- SNP differentiation — **A** — or — **G** — ?
- Single base insertion — **A** — or — . — ?  
or deletion
- Small insertions — **AA** — or — **AGCTGGTCA** — ?  
or deletions
- Large insertions — **AA** — to — **A** — ..... — **A** — ?  
n = 26 – 200,000 bases  
or deletions

# BHQ probe portfolio

An array of engineered probes tailored to your application

## BHQ<sup>®</sup>

Fluorophore 3' BHQ



## BHQplus<sup>®</sup>

Fluorophore 3' BHQ



## BHQnova<sup>™</sup>

Fluorophore 3' BHQ



### Advantages

Simplicity of design for broad application and great value with powerful multiplexing

Engineered to provide the highest fidelity for superior mismatch discrimination

Optimal quenching efficiency, low background, enhanced signal detection

### Common Applications

- Multiplexing
- Robust
- All purpose

- CNV
- SNP Genotyping
- Multiplexing
- AT-rich

- AT or GC-rich
- Long templates
- Multiplexing

### Structure

- Dual-labeled linear probe
- Align up to 25 bases

- Compact dual-labeled linear probe
- Align up to 25 bases

- Double-quenched dual-labeled probe
- Align with sequences over 25 bases

# qPCR design collaborations

REALTIMEDESIGN™

Main Menu ▶

Sequence Entry

## Help

Enter your sequence(s) or accession Number(s) into the sequence entry field. ▶ [More...](#)

## Sequence Entry

Enter nucleotide sequence(s) or NCBI accession number(s). ▶ [More...](#)

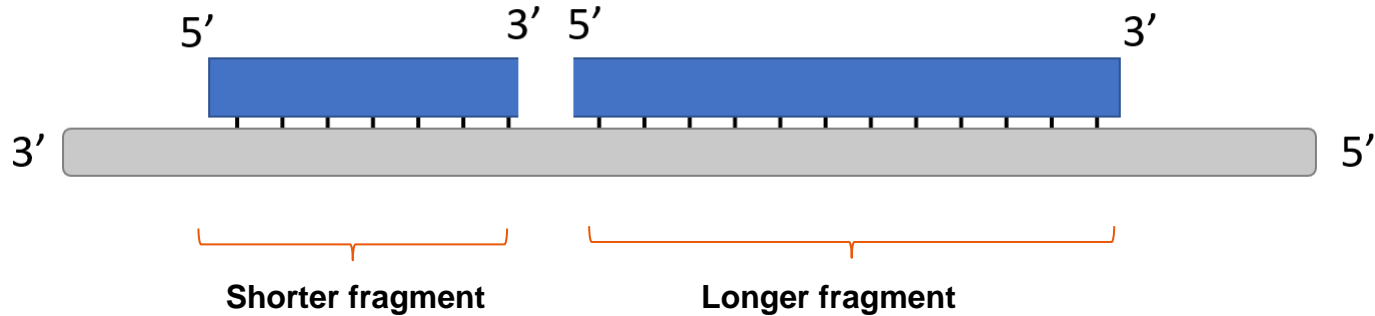
Upload a File

Express: qPCR - BHQ Probe

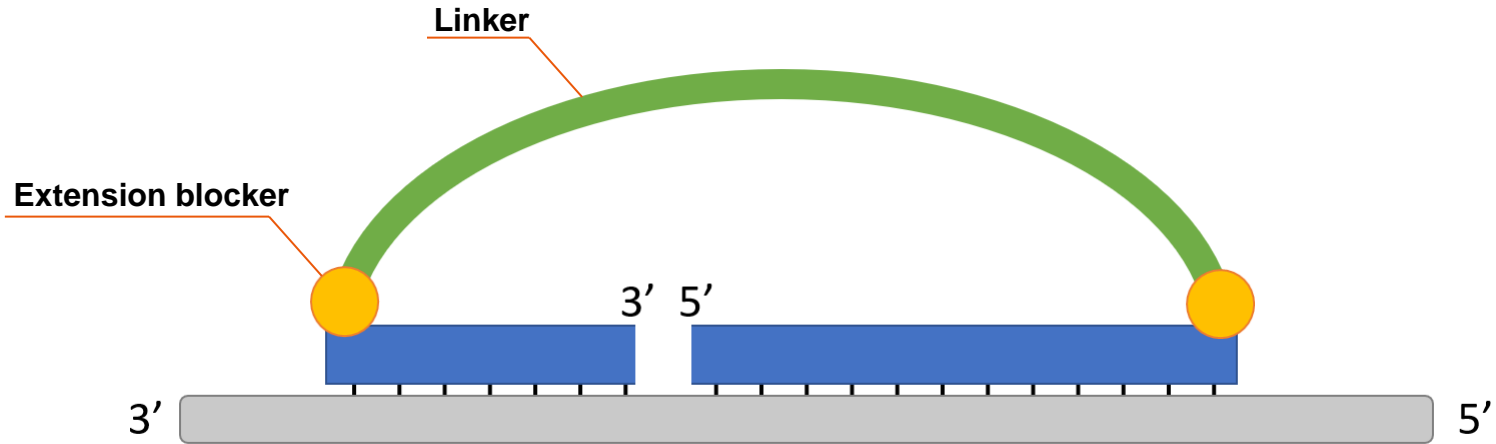
NCBI Database Search Options - Human (Homo sapiens)

Next >>

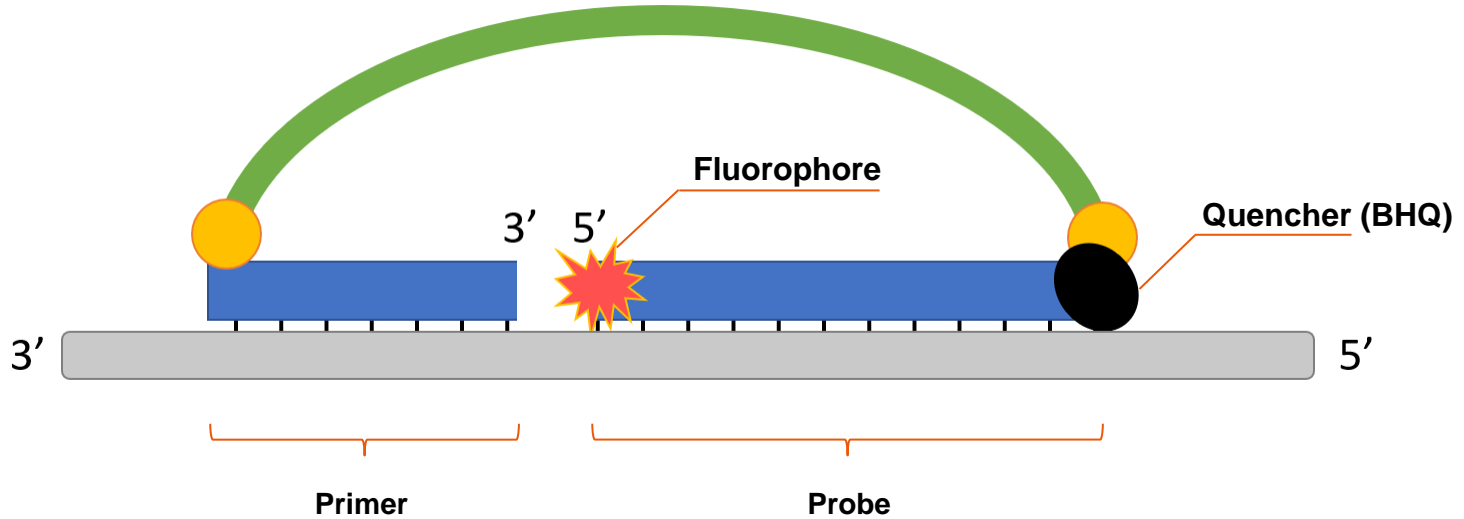
# What are Cooperative Primers (CoPrimers) ?



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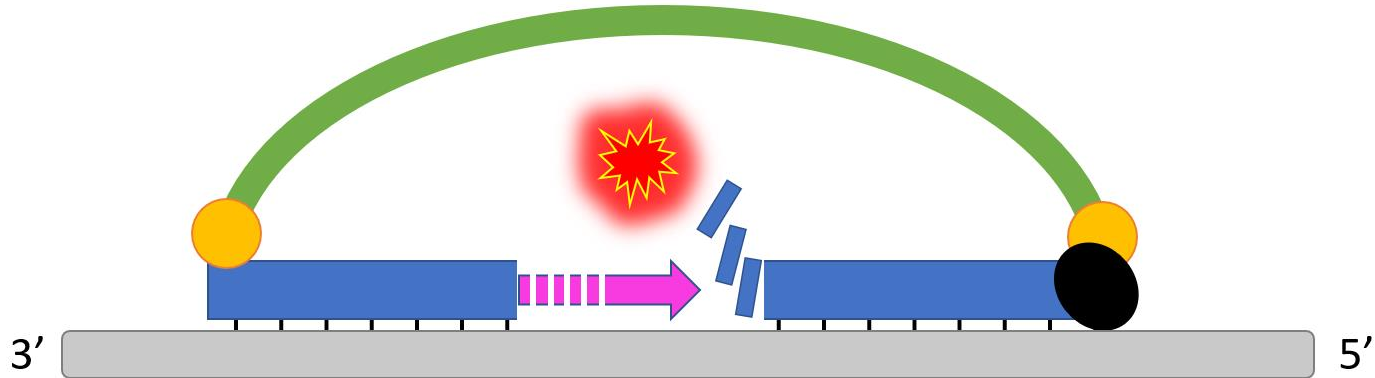


# What are Cooperative Primers (CoPrimers) ?





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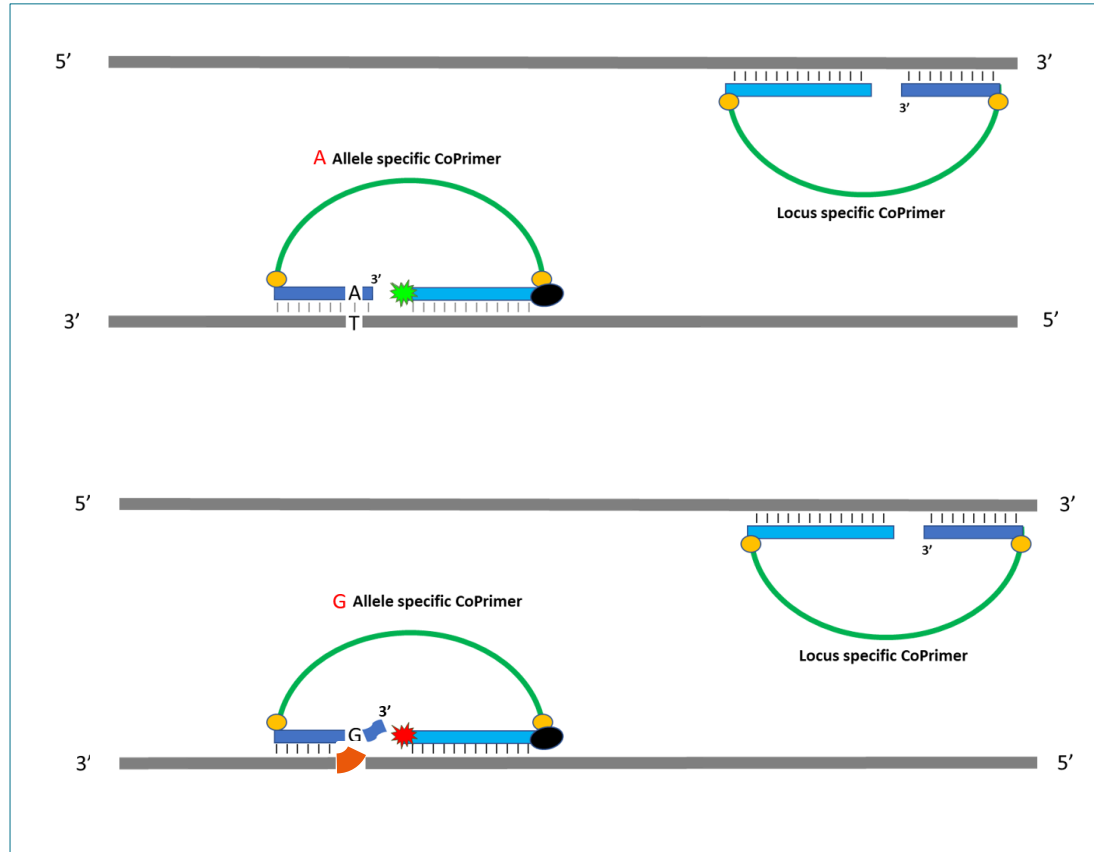
**Each extension results in fluorophore cleavage**

# Allele-specific CoPrimer design

**A>G**

**LEGEND:**

- Allele-specific Priming segments (forward primer)
- Locus specific Priming segment (reverse primer)
- Capture segments (forward and reverse primers)
- Flexible linker
- Fluorophores
- Quencher
- Primer extension

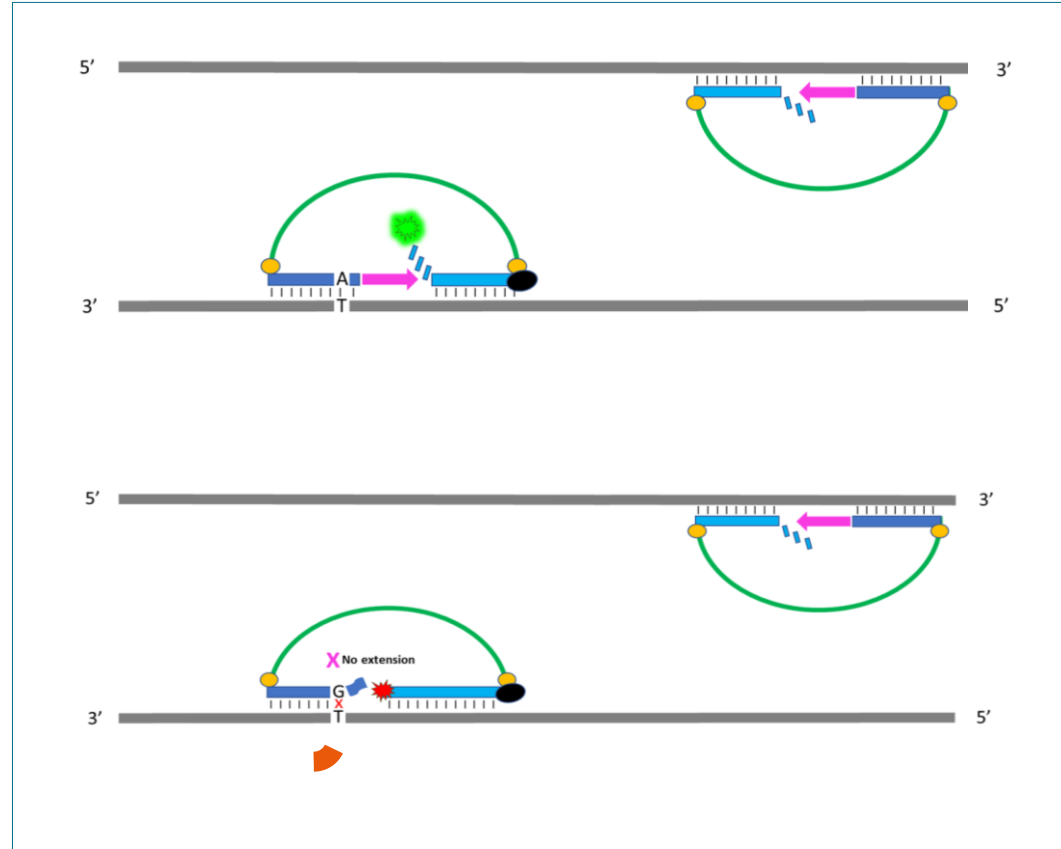


# Allele-specific CoPrimer design

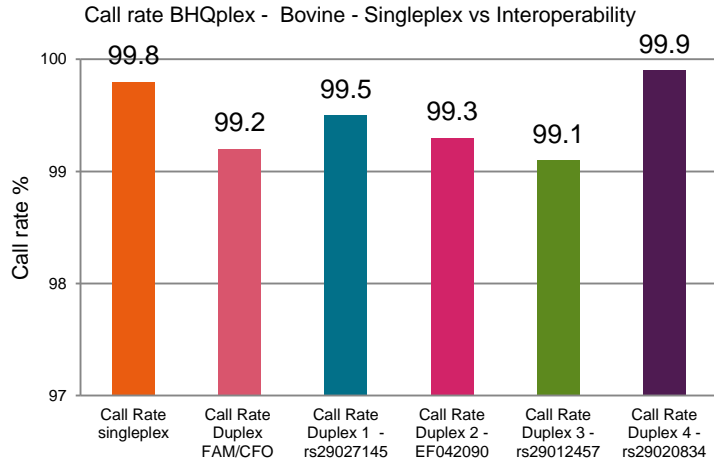
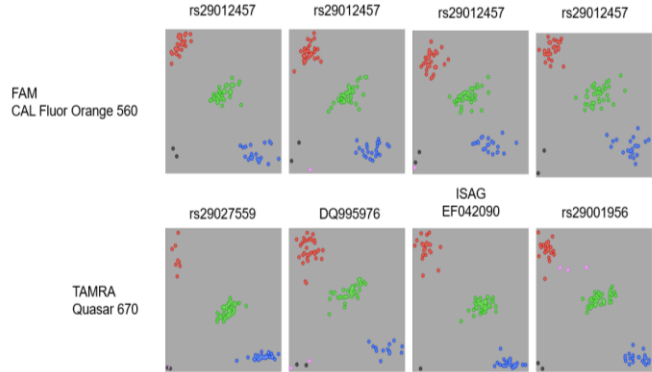
**A>G**

**LEGEND:**

- Allele-specific Priming segments (forward primer)
- Locus specific Priming segment (reverse primer)
- Capture segments (forward and reverse primers)
- Flexible linker
- Fluorophores
- Quencher
- Primer extension



# Assay Interchangeability



- **Methods:**

- > 7 Bovine SNPs designed and tested in both singleplex and duplex
- > Run in 4x4 interchangeability matrix (hold 1 SNP assay constant and switch the 2<sup>nd</sup> SNP)
- > Run on SNPLine in 384 plates with BHQMM, analyzed in Kraken

- **Results:**

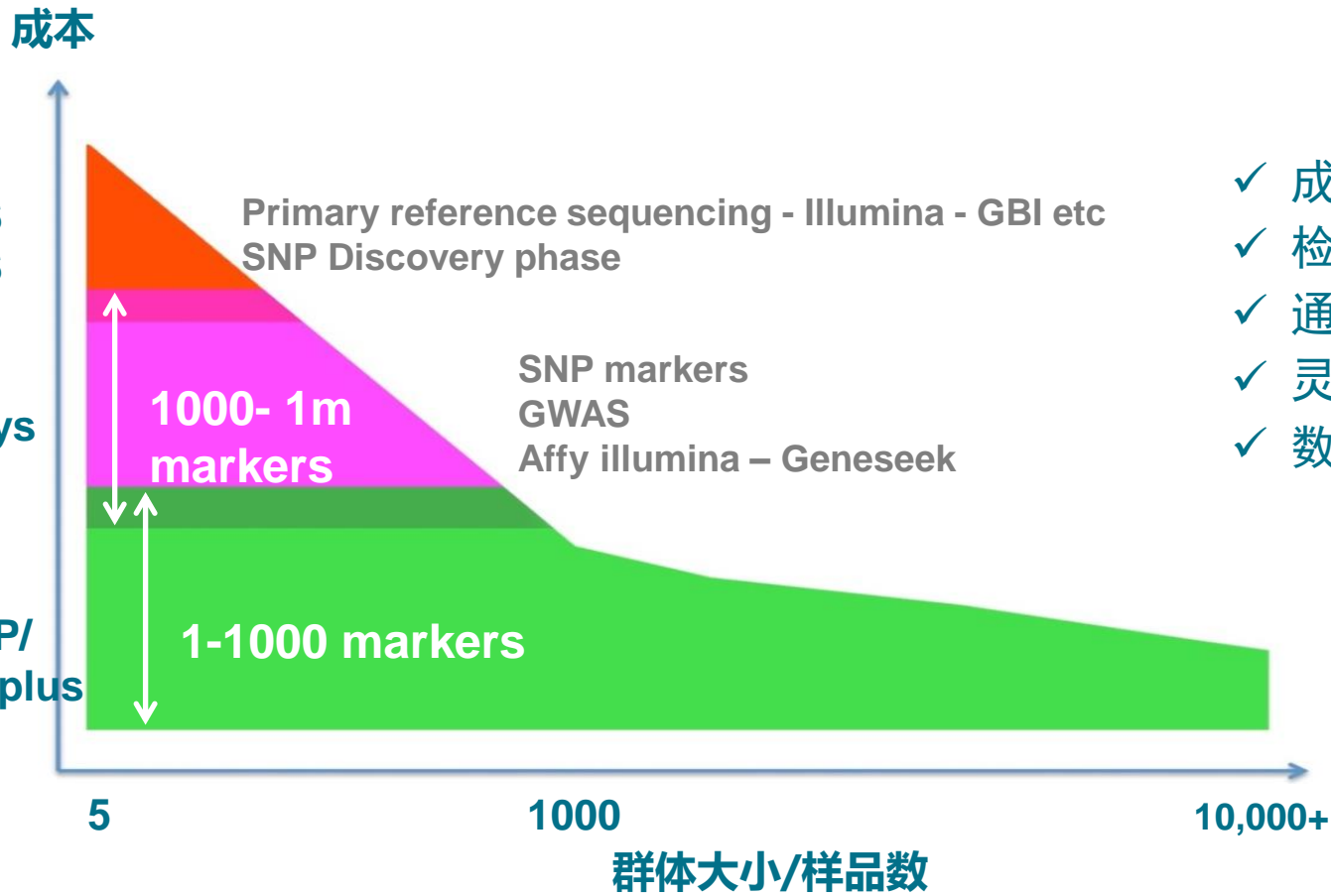
- > >99% call rate and 100% concordance with KASP
- > NO optimization done between pairs

- **Conclusions:**

- > Technology is robust and enables assay interchangeability with **minimal to no optimization** of assay pairs

# Value Proposition

# 单位点基因分型技术优势



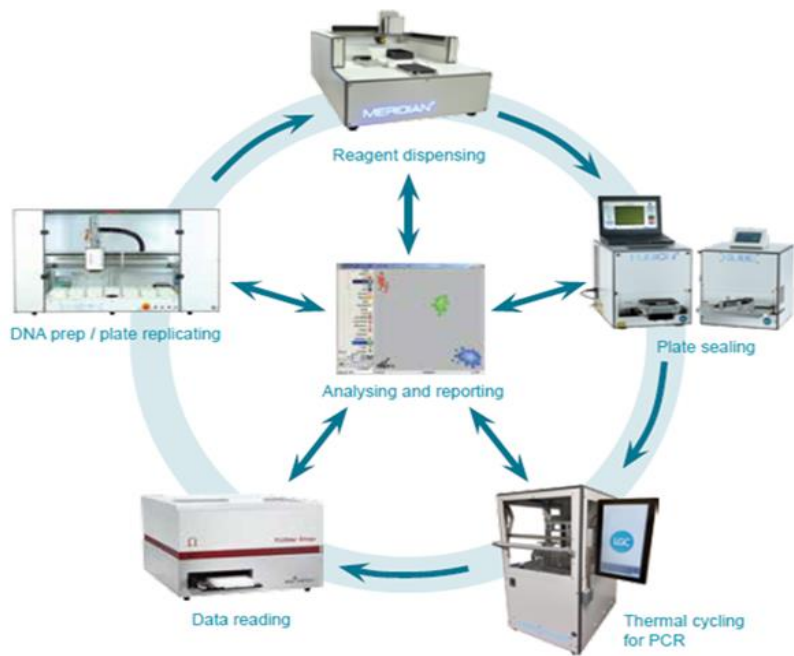
- ✓ 成本低
- ✓ 检测周期短
- ✓ 通量高
- ✓ 灵活性强
- ✓ 数据分析处理简单

# KASP vs 探针法 成本

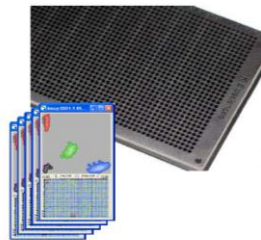
	探针法 LGC	探针法多重/ LGC	KASP
试剂成本/test (1ul)	0.085	0.0425	0.14
新位点合成探针起始成本	2000	4000	200
<b>1000样品均成本</b>	2.085	4.0425	<b>0.34</b>
<b>5000样品均成本</b>	0.485	0.8425	<b>0.18</b>
<b>5万样品均成本</b>	<b>0.125</b>	<b>0.1225</b>	0.144
<b>500万样品均成本</b>	0.105	<b>0.0825</b>	0.14

# 使用自动化仪器平台的优势

- ✓ PCR体系构建精准高效
- ✓ 避免人为操作误差
- ✓ 高通量
- ✓ 反应体系低至1uL



1536 plates



DNA 提取

反应体系  
配置

PCR

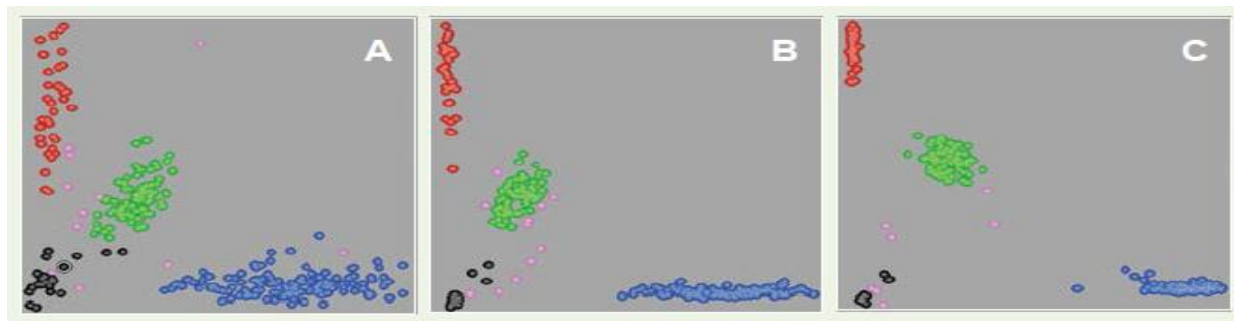
数据读取  
及分析



# Troubleshooting

# 加循环（每次3个循环）

Protocol Stage	Temperature	Duration	Number of cycles for each stage
Stage 1 Amplification	94°C	20 seconds	x 3 cycles
	57°C	60 seconds	
Optional Stage 2 (read stage for qPCR instruments only)	30°C (any temperature below 40°C is suitable for the read stage)	60 seconds	x 1 cycle



- 加1次（3个）循环-图B; 加2次（6个）循环-图C
- **LGC** 建议最多加4次循环

# 分型分散

- DNA样品污染

- 检查DNA提取
- 检查DNA储存

- DNA样品板之间质量及浓度差异过大

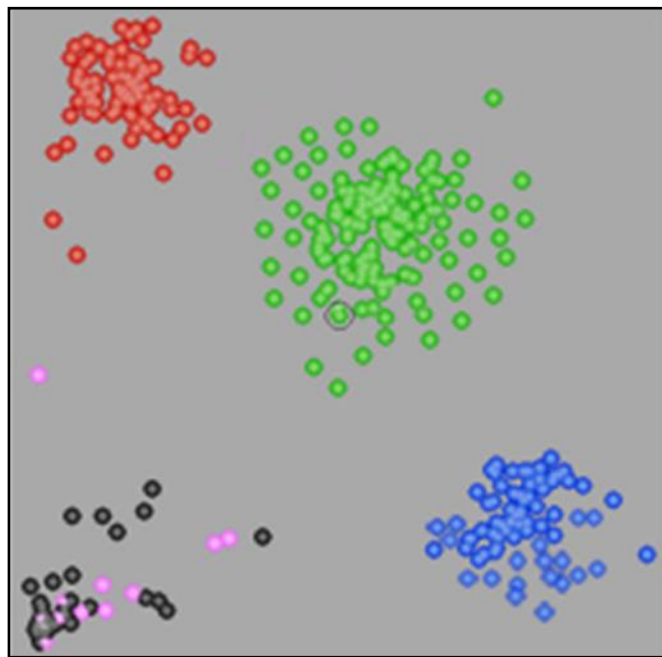
- 基因分型前进行均一化
- 样品进行浓度梯度稀释

- 镁离子浓度过高或过低

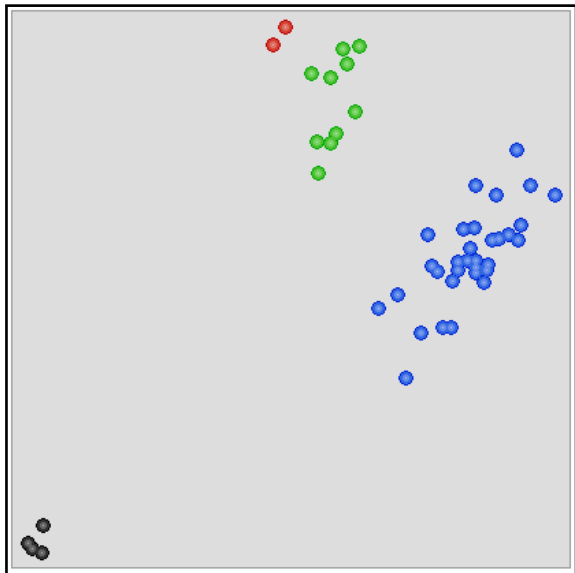
- 降低或升高镁离子浓度

- 非最优的循环条件

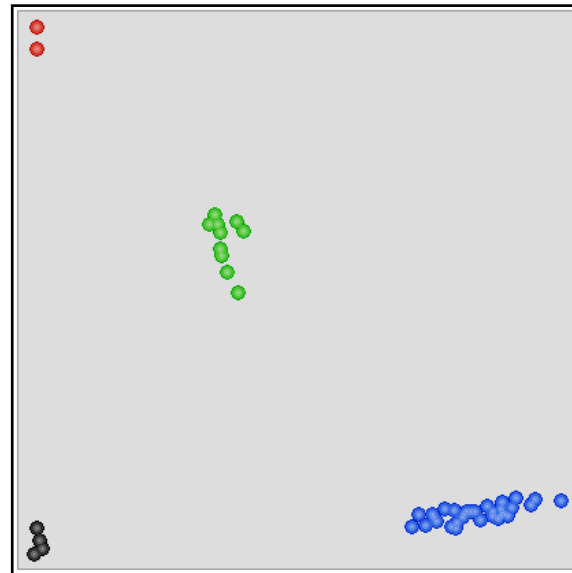
- 使用其他循环条件(参考GC%含量指导手册)



# 优化案例 – 68-62°C touchdown 循环程序



61-55°C cycling conditions



68-62°C cycling conditions

# 复杂序列的引物设计

烟草，同源序列干扰

Species/Abbrv	***** * ***** * ***** * ***** * ***** *
1. isoT-WT	TCGACACAAAAG--GGAGAGGATTTTTTAGGCAAAAATCAATGGCCACTGAAACACCGATAGAGGCGACGGAGGTTCTGCCGGCGC-----CGGATACGGTGGAGAAGCAGCCGC
2. isoT-Del_A	TCGACACAAAAG--GGAGAGGATTTTTTAGGCAAAAATCAATGGCCACTG--AGCACCGATAGAGGCGACGGAGGTTCTGCCGGCGC-----CGGATACGGTGGAGAAGCAGCCGC
3. isoT-Del_GA	TCGACACAAAAG--GGAGAGGATTTTTTAGGCAAAAATCAATGGCCACT--AGCACCGATAGAGGCGACGGAGGTTCTGCCGGCGC-----CGGATACGGTGGAGAAGCAGCCGC
4. isoS-WT	TCGACACAAAAGGGAGGAGAGGATTTTTTAGGCAAAAATCAATGGCCACTGAAACACCGATAGAGGCGACGGAGGTTCCGCCGGCGTCAGCGACGGAGACGGTGGCGAAGCAGCCAC
5. isoS-Del_A	TCGACACAAAAGGGAGGAGAGGATTTTTTAGGCAAAAATCAATGGCCACTG--AGCACCGATAGAGGCGACGGAGGTTCCGCCGGCGTCAGCGACGGAGACGGTGGCGAAGCAGCCAC

iosT型下游公共引物区

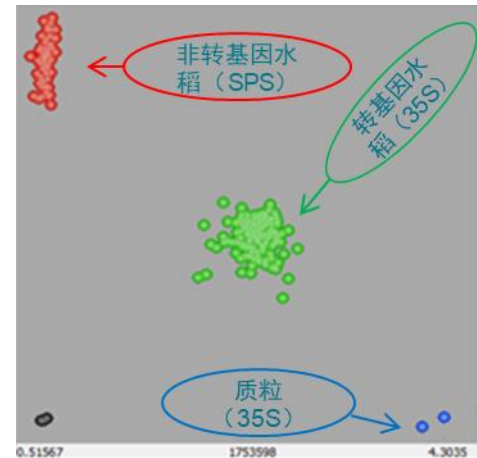
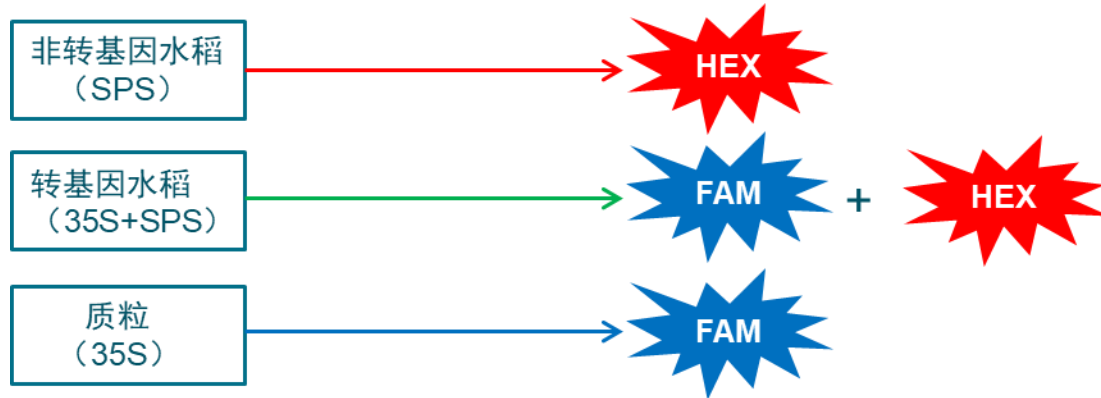
野生型上游引物区

突变位点，  
突变型上游引物识别区

iosS型下游公共引物区

# 复杂序列的引物设计

水稻，转基因检测



# 应用案例

# 植物





# SNP标记用于分子标记辅助选择

**MSV: Maize Streak Virus/玉米条锈病**  
**PVA: Provitamin A/维生素A**

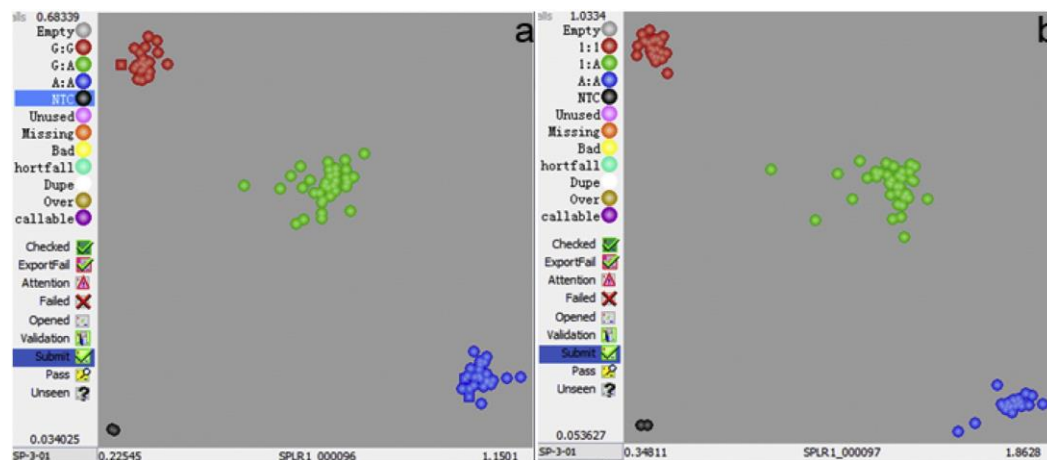


MSV_SNP	Resistance	Susceptible	Line1	Line2	Line3	Line4
PZE0186365075	C:C	A:A	C:C	A:A	A:A	C:C
PZE-101093951	A:A	G:G	A:A	G:G	G:G	A:A
PZE0186065237	C:C	T:T	C:C	T:T	T:T	C:C
MSV_Selection			Select	Reject	Reject	Select

PVA_SNP	Favorable	Unfavorable	Line1	Line2	Line3	Line4
S10_134583972	G:G	C:C	G:G	G:G	C:C	C:C
S10_134655704	C:C	T:T	C:C	C:C	T:T	T:T
SYN11355	A:A	G:G	A:A	A:A	G:G	G:G
PZE-110083653	G:G	A:A	G:G	G:G	A:A	A:A
S10_136072513	G:G	T:T	T:T	T:T	G:G	G:G
S10_136840485	C:C	T:T	C:C	C:C	T:T	T:T
S10_137904716	C:C	T:T	C:C	C:C	T:T	T:T
PVA_Selection			Select	Select	Reject	Reject

# SNP标记用于高油酸花生检测

山东省农业科学院生物技术研究中心王兴军教授带领其团队开发了KASP标记，用于检测高油酸含量花生。



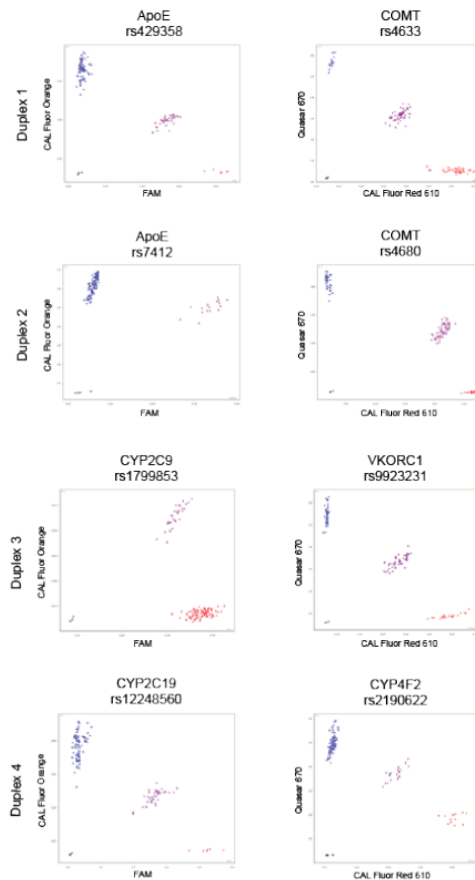
# 人类

**BIOSEARCH™**  
**TECHNOLOGIES**  
GENOMIC ANALYSIS BY LGC



# BHQplus 探针在药物基因组学及疾病易感性检测方面的应用

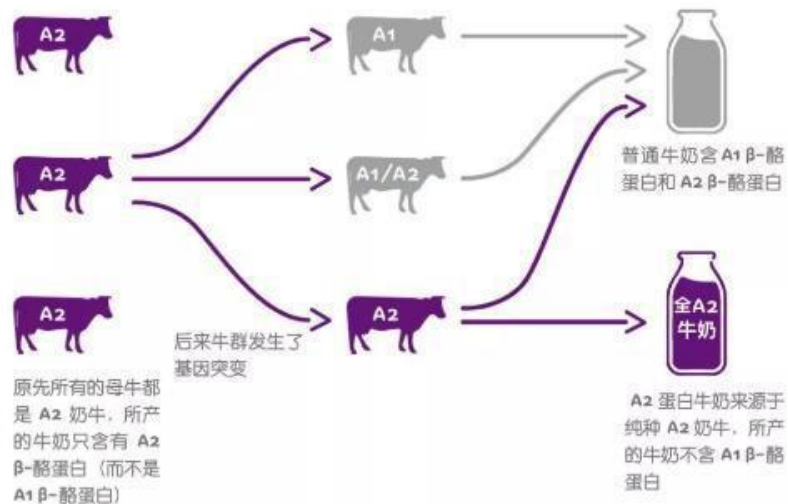
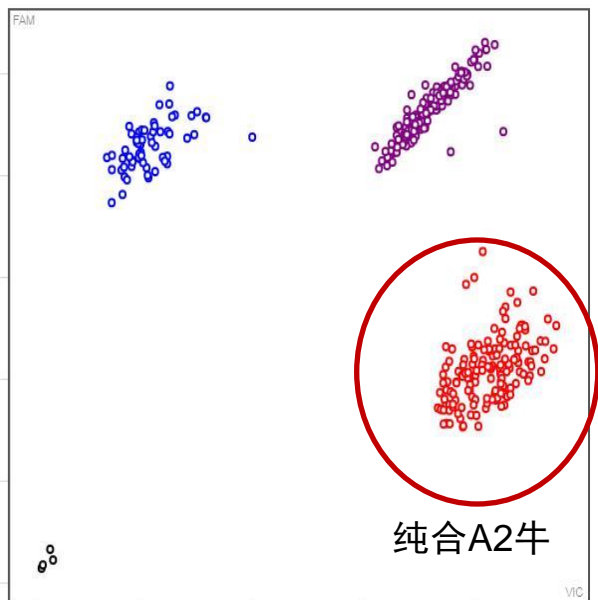
SNP rs #	Gene	等位基因意义
rs1799853	CYP2C9	与华法林代谢不良相关
rs12248560	CYP2C19	超快代谢表型，药物代谢
rs2108622	CYP4F2	与华法林代谢不良相关
rs9923231	VKORC1	与华法林敏感性相关
rs429358	ApoE	影响阿尔茨海默病的风险
rs7412	ApoE	影响老年痴呆症的风险
rs1801131	MTHFR	与多种类型脑癌的风险增加相关
rs1801133	MTHFR	与多种类型脑癌的风险增加相关
rs4633	COMT	精神分裂症易感性，疼痛反应/耐受性
rs4680	COMT	精神分裂症易感性，疼痛反应/耐受性



# 动物



# A2牛筛选



挪威和澳洲的牧场以及中国的部分第三方检测公司都在利用单位点基因分型技术检测来进行A2牛分子标记辅助选择

# Thank you

胡鹏果 Pengguo.hu@lgcgroup.com  
周斌 Albert.zhou@lgcgroup.com