



# Variety Tracer

# 品种追踪

*Application of DNA technology in the enforcement of  
Plant Breeders' Rights*

*DNA技术在育种者权利执法领域的应用*

Hedwich Teunissen, Naktuinbouw R&D, IP-Key CHINA, December 2, 2020  
Hedwich Teunissen, 荷兰新品办研发处, IP Key 中国, 2020年12月2日



molecular biologists  
分子生物学家

# The Team 团队



botanists – crop specialists  
植物学家 — 作物专家



instructing party,  
clients & legal  
experts  
指导方、客户及  
法律专家



Bioinformatic experts  
生物信息学专家





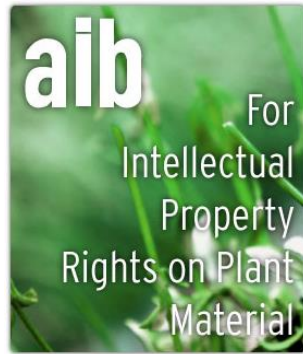
# Applications 应用

- Swaps/Mix-ups 互换/混合
- Identification issues 识别组织
  - True-to-type QC in propagation process
  - 繁殖过程中进行纯种质量控制
- Research in case of suspicion of fraud
- 怀疑存在欺诈时，进行研究
  - Suspected material is identical to protected material
  - 可疑材料与受保护材料完全一致
  - Unauthorized use of protected parental line
  - 未经授权使用受保护的亲本品系
  - Counterfeit seeds
  - 假冒种子
  - EDV
  - 实质性衍生品种





# Track down fraud 追踪欺诈



Police Officers in the world of Plants  
植物保护界的警察



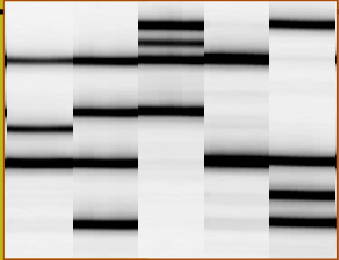
**Crime Plant Investigation**  
**植物犯罪调查**



# Variety Tracer 品种追踪

The 'Sherlock Holmes' concept  
in infringement matters

侵权追查领域的“福尔摩斯”理念



# How does Variety Tracer work...

## 品种追踪的工作方式...

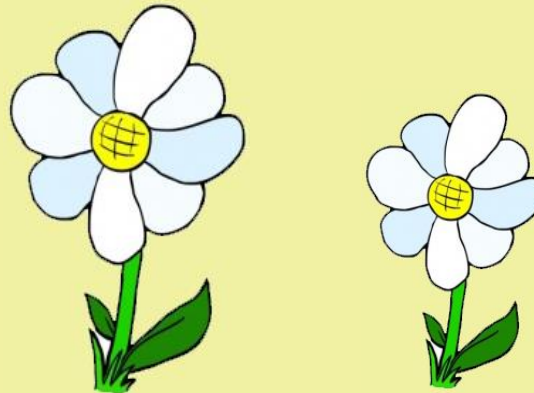
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品种A = 品种B

Var A  $\neq$  Var B

品种A  $\neq$  品种B

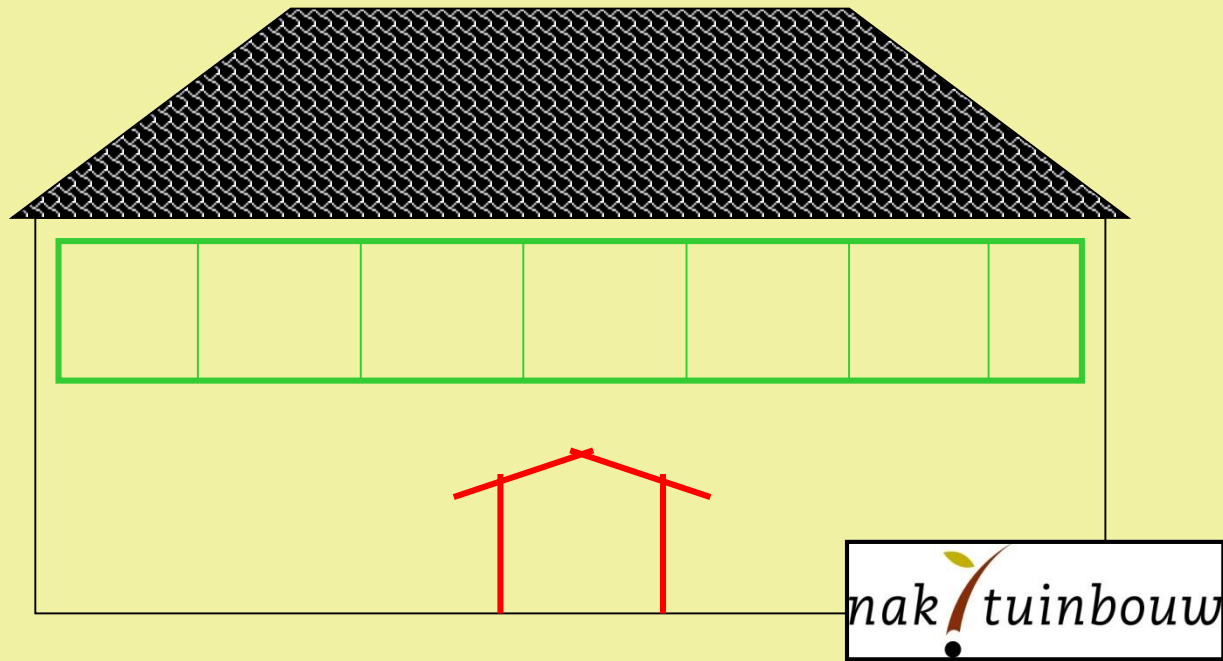
Var B  
品种B



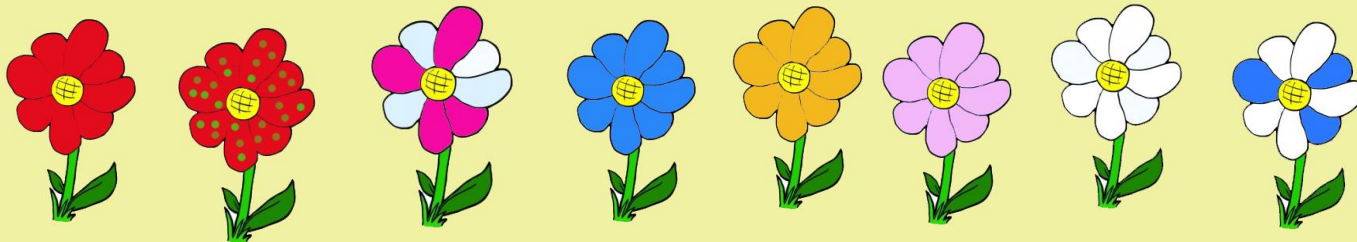
Var A  
品种A

# Naktuinbouw Variety Tracer

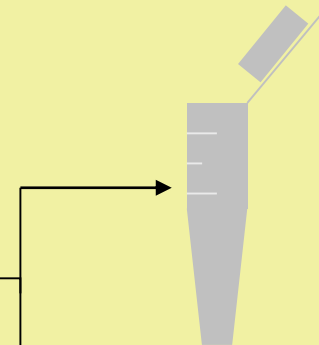
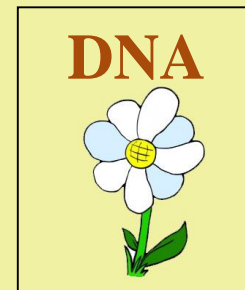
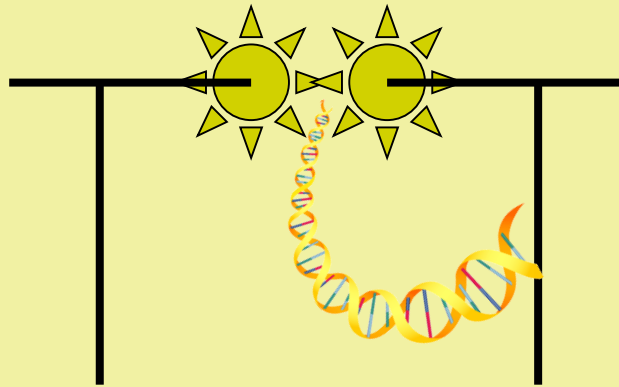
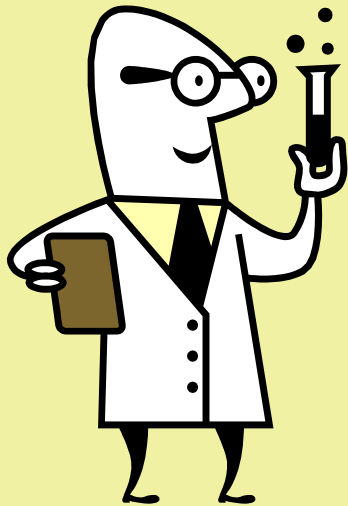
## Naktuinbouw 品种追踪



Collect reference varieties... 收集参考品种...

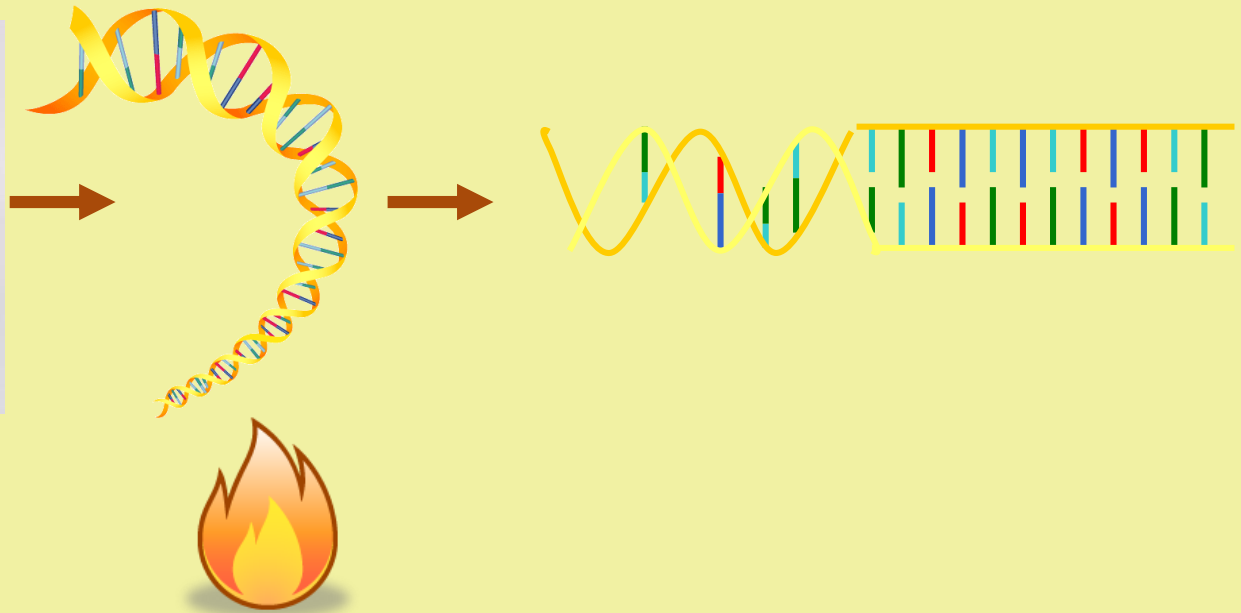


# In the lab... 实验室...



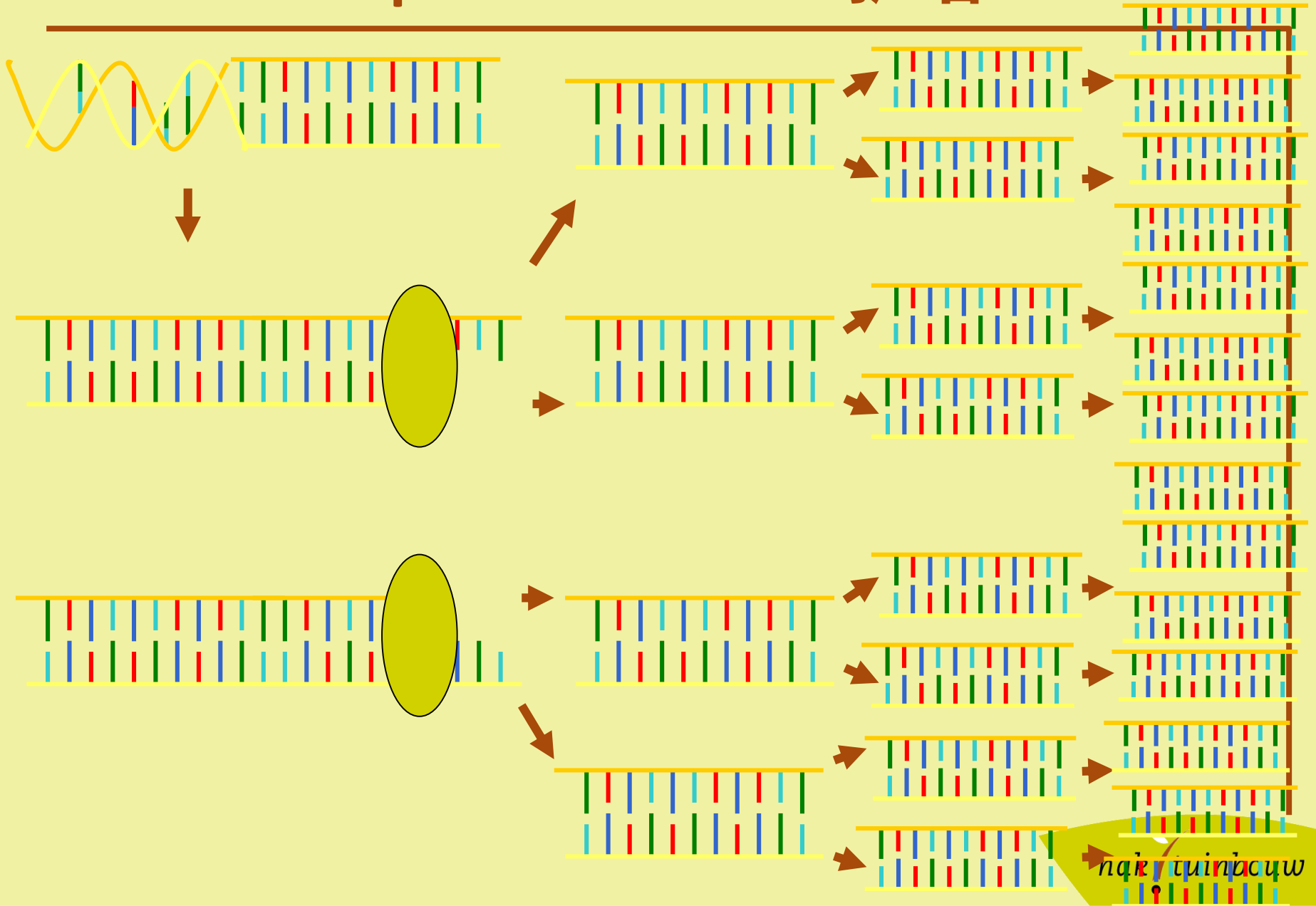


# DNA profiling... DNA鉴定...



Based on PCR technology  
根据聚合酶链式反应技术

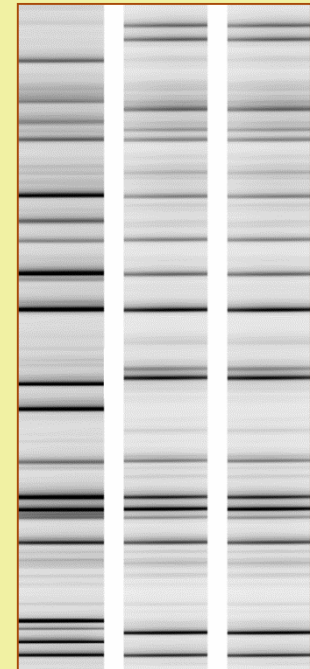
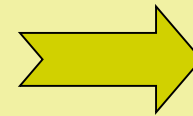
# DNA multiplication...DNA扩增...



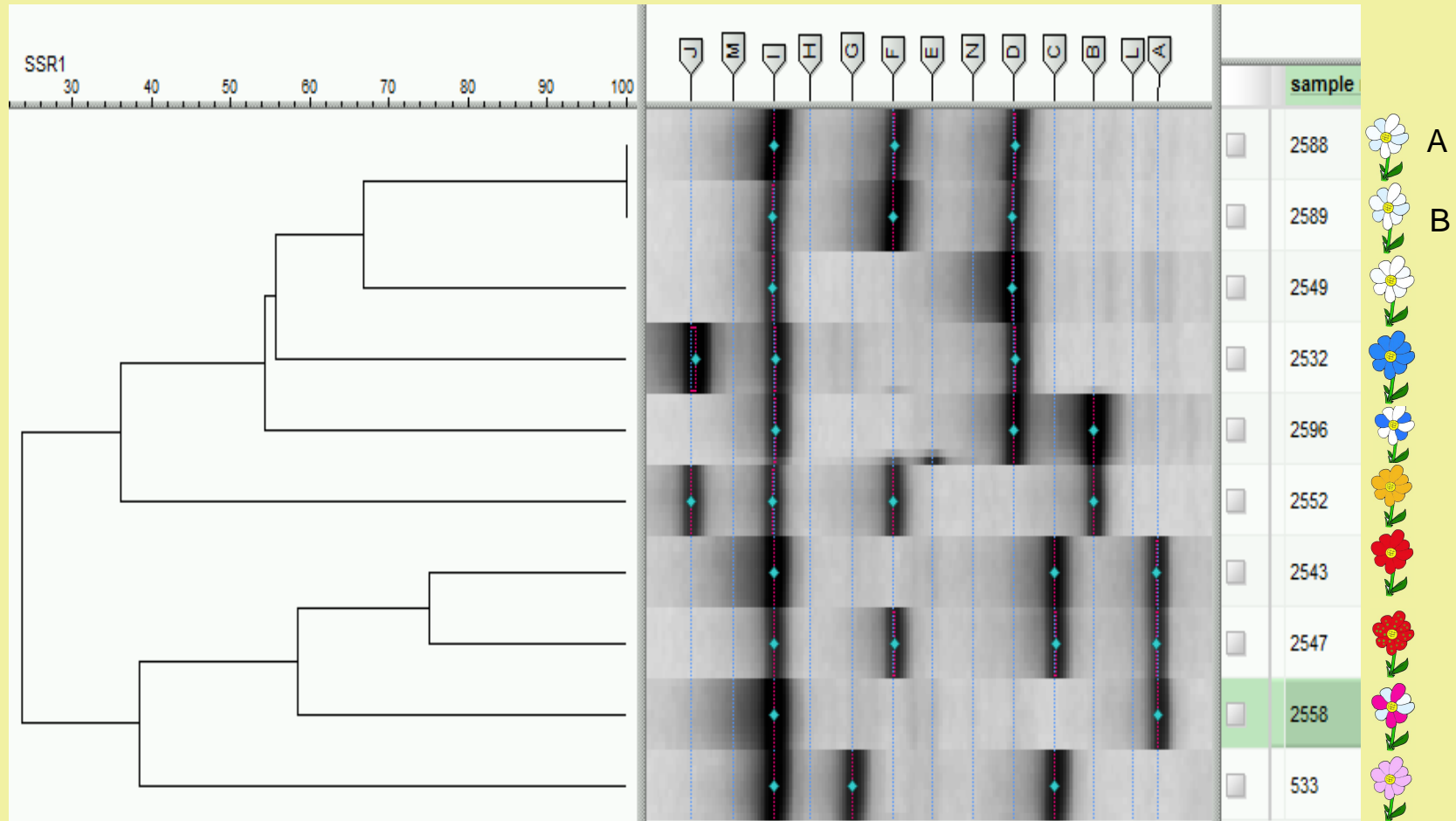
# DNA visualization... DNA可视化

After selective multiplication of DNA, visualization on gel.  
DNA fragments can be separated based on size. This results  
in the bar-code DNA profile

对DNA进行选择性扩增后，在凝胶上进行可视化处理。  
可根据大小分离DNA碎片，从而产生条码型DNA图谱。

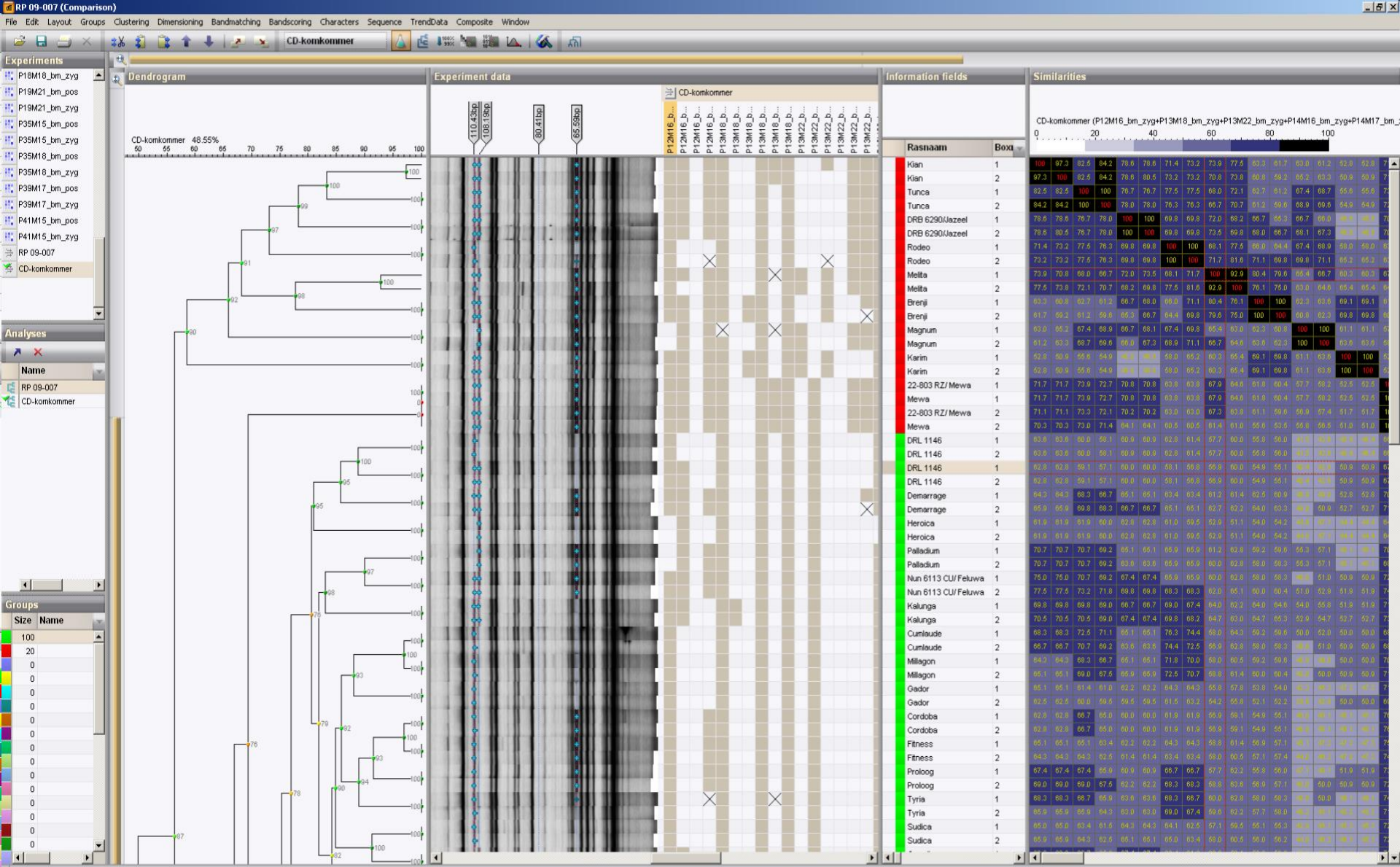


# Genetic relationships... 遗传关系...



# Bionumerics – Applied Maths

# Bionumerics – 应用数学





# Every VT project is unique 每个品种追踪项目都是独一无二的

- Background information 背景信息
- Independent sampling 独立取样
- Morphologic comparative research 形态对比研究
- Genetic Research 遗传研究
- Report 报告





# Comparing is relative 对比是相对的



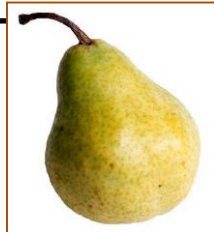
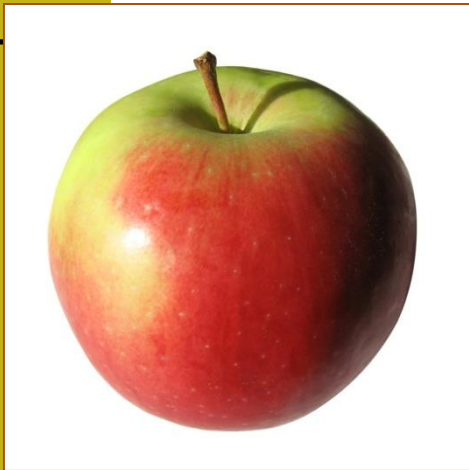
Choice of Marker Technology  
How deep?  
(number of markers?)  
标记物选择深度?  
(标记物数量?)



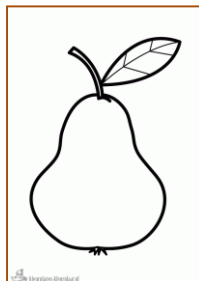
Choice of reference framework  
How wide?  
(which references are informative?)  
参考范围选择宽带?  
(哪些参考物是信息性的?)

# Choice of reference framework

## 参考范围选择

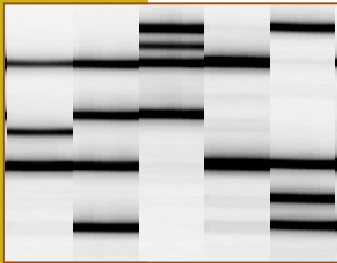


Genetic relationship?? 遗传关系?



Reference framework not relevant  
参考范围不相关

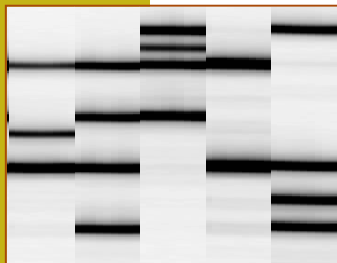
# Selection of samples 样本选择



- Genetic width 基因宽度：
  - Reference collection representative for the morphological diversity within the genus/species.
  - 收集可代表植物属/种内形态多样性的参考样本
- Fine tune genetic similarity of known related varieties:  
微调已知相关物品的遗传相似度：
  - Varieties that are related but not essentially derived e.g. seedlings, (half) sibs and parental lines
  - 相关但并非实质性衍生的品种，例如，秧苗、（半）姊妹品系和亲本品系
- Fine tune genetic similarity of known derived varieties:  
微调已知衍生品种的遗传相似度：
  - Accepted mutants/derivations + original variety
  - 经认可的变异品种/衍生品中+原始品种



# Selection of samples 样本选择



- Samples from the varieties in question from different origin
- 对应品种来自不同产地的样本
  - Selection and drift
  - 选择和漂移
- Original identity sample (DUS application)
- 原始身份样本（特异性、一致性和稳定性测试）
- All samples *in duplo* as technical control
- 所有样本都采用双倍数，进行技术对照



# Comparing is relative 对比是相对的



Choice of Marker Technology

How deep?

(number of markers?)

标记物选择深度?

(标记物数量?)



Choice of reference framework

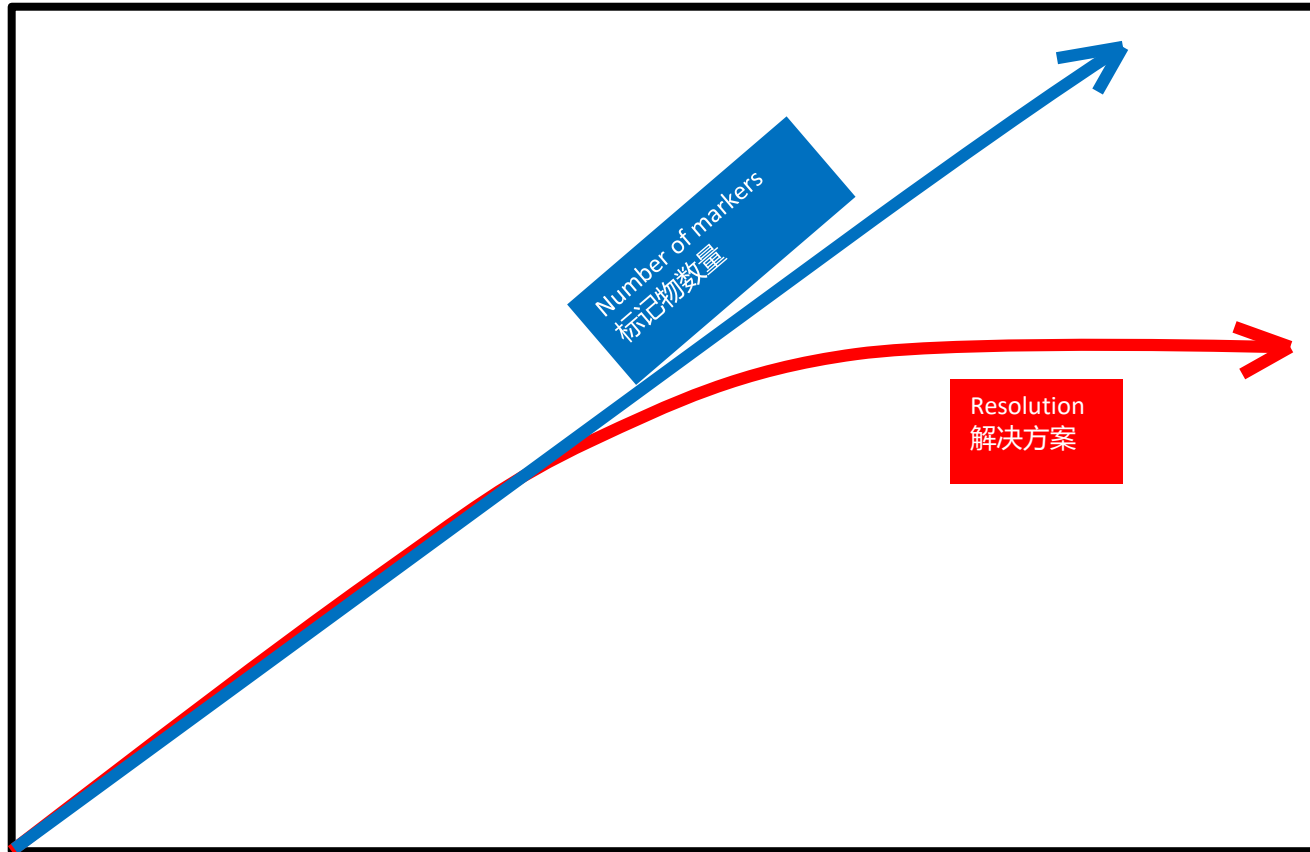
How wide?

(which references are informative?)

参考范围选择宽带?

(哪些参考物是信息性的?)

# What resolution do we need 我们需要的解决方案





# Genetic resolution - choice of technology

## 遗传学解决方案 — 技术选择



### AFLP

扩增片段长度  
多态性

Random 随机

Generally applicable  
普遍适用

Bi-allelic 双等位基因

Dominant 单显性

### SNPs

单核苷酸多态性

Targeted 定向

Crop specific 作物特异性

Bi-allelic 多等位基因

Co-dominant 等显性

SSR 简单序列重  
复:

Targeted 定向

Crop specific 作物特异性

Multi-allelic 多等位基因

Co-dominant 等显性

NGS 新一代测序:

Random and Targeted 随  
机与定向

Generally applicable 普  
遍适用

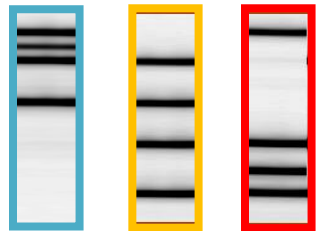
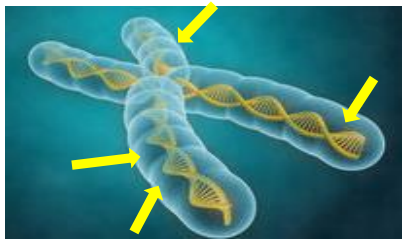
Multi-allelic 多等位基因

Co-dominant 等显性



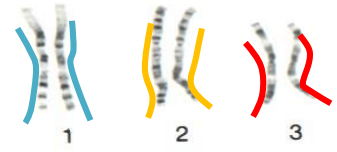
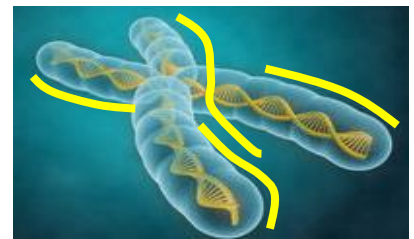
# Next Generation Sequencing 新一代测序

## Variety Tracer 1.0 品种追踪1.0版



## from Variety Tracer 1.0 to 2.0 从1.0版到2.0版

## Variety Tracer 2.0 品种追踪2.0版



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# Example from real life 实例



# Harrogate 英国哈罗盖特市

现在蔬菜展也要进行欺诈检测：展会中的番茄要接受DNA测试，以确保品种正确

## Now even veg shows are testing for cheats: Tomatoes in contest will have DNA examined to ensure they are correct variety

- Harrogate Autumn Flower Show is using DNA technology to ensure a £1,000 giant tomato prize is not won by a cheating grower
- The show is running a Gigantomo class, with a £1,000 top prize at stake
- Organiser will use Dutch specialists to DNA test plants to make sure the entries are what they claim to be
- Winner could scoop a further £5k if heaviest fruit sets a new world record

By DAILY MAIL REPORTER

PUBLISHED: 00:58 GMT, 8 September 2015 | UPDATED: 03:33 GMT, 8 September 2015



You would think a set of scales and an expert eye would be enough to judge something as genteel as a giant vegetable contest.

But the competition has grown so fierce in one picturesque town that organisers have introduced DNA testing to weed out any imposters.

Adjudicators at the Harrogate Autumn Flower Show will be relying on Dutch specialists to ensure that entries to their Gigantomo tomato class really are from that strain of the plant.

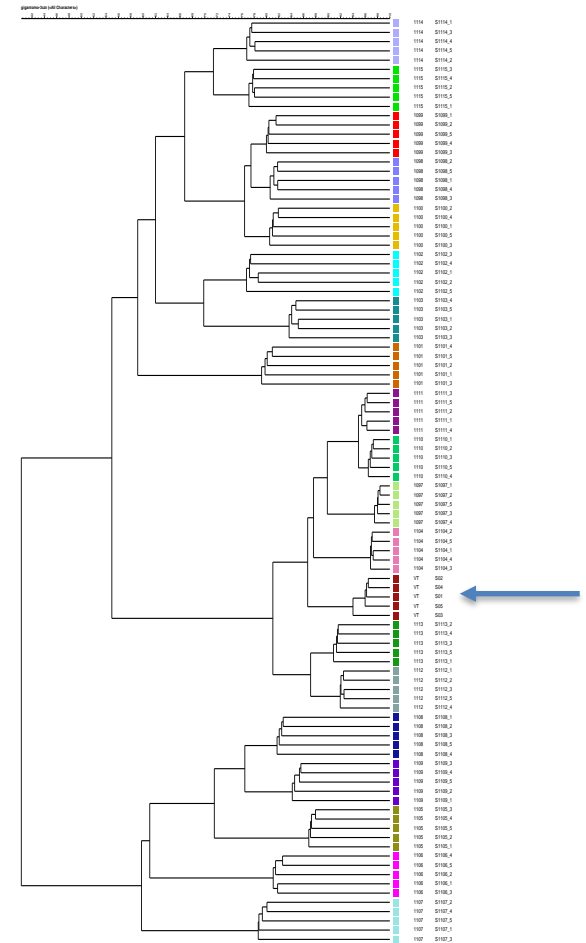
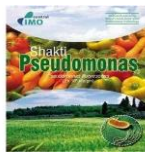
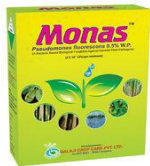






# Unexpected discovery 意外发现

Sample 样品	Nr reads 读长数	Nr reads mapped 映射读长数	% mapped 映射比例
S01	14,891,765	14,691,276	98.7
S02	19,300,543	19,026,261	98.6
S03	16,093,446	6,221,944	<b>38.7</b>
S04	18,251,261	18,075,659	99.0
S05	17,633,012	17,358,359	98.4







*Quality in Horticulture*  
*园艺质量*