LMO Detection Workshop

5-8 November 2019

Chris Viljoen

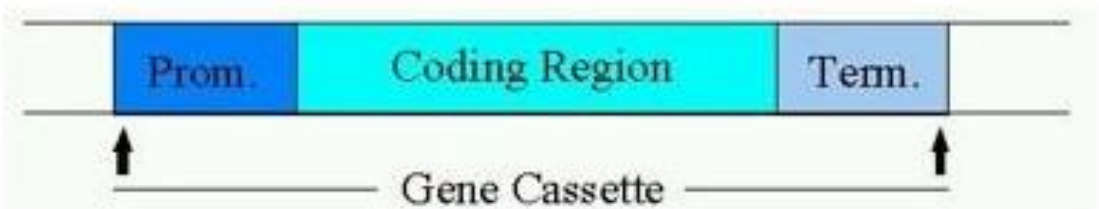
SANGL

SOUTHERN AFRICAN NETWORK FOR GM DETECTION LABORATORIES

Contents

Introduction to LMOs and LMO detection

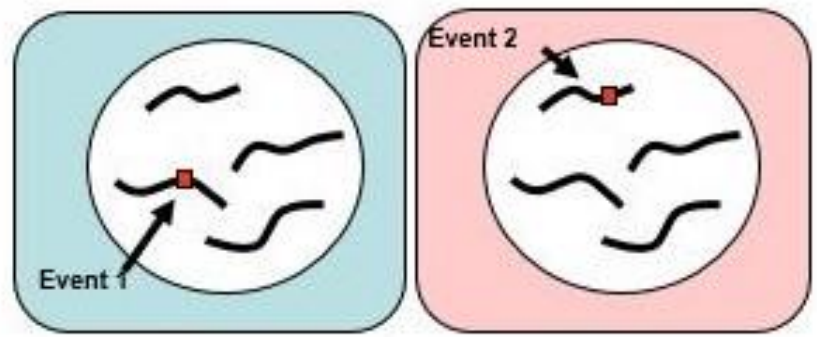
The transgene construct



Promoter	IR Genes	HT Genes	Terminator
35S *FMV*	*Cry1AB* *Cry1F* *Cry3Aa2* *Cry3Bb1* *Cry34Ab1* *Cry35Ab1* *Cry9c*	*EPSPS* *Pat* *Bar*	*NOS*

GM events

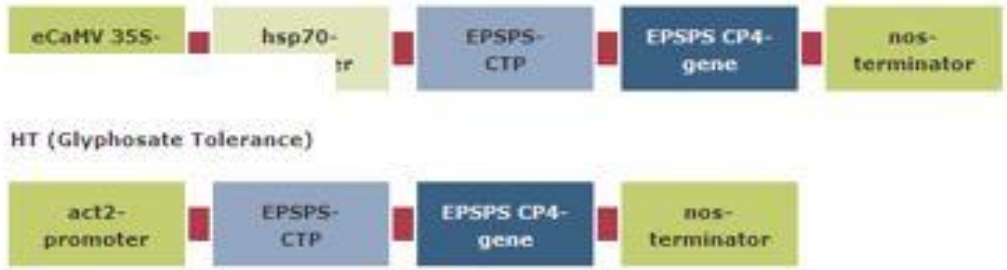
- Gene transformation inserts genes into the genome randomly
- Each transformation is unique and creates a different event
- Different insertion “events” of the same gene



Maize: MON810

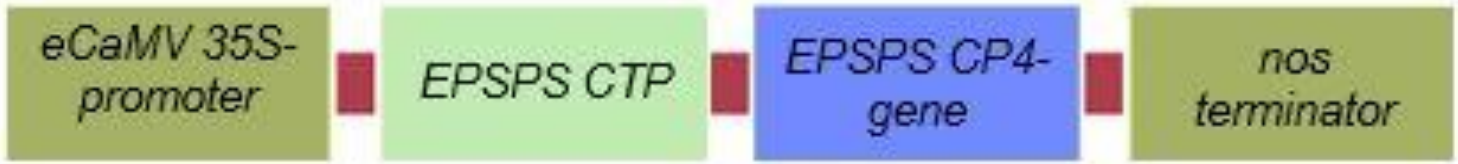
Code	Name	Type	Promoter, other	Terminator	Copies	Form
cry1Ab	*Cry1Ab delta-endotoxin (Btk HD-1) (Bacillus thuringiensis subsp. kurstaki (Btk))*	*IR*	*enhanced CaMV 35S, maize HSP70 intron*	*None. Lost through 3' truncation during integration*	*1*	*Truncated*

Maize: NK603



Code	Name	Type	Promoter, other	Terminator	Copies	Form
CP4 epsps	*5-enolpyruvyl shikimate-3-phosphate synthase (Agrobacterium tumefaciens CP4)*	*HT*	*P-ract1/ract1 intron containing rice actin 1 promoter, transcription start site chloroplast transit peptide from A. thaliana EPSPS gene (CTP2)*	*A. tumefaciens nopaline synthase (nos) 3'-untranslated region*	*1*	*CP4 EPSPS gene modified for plant-preferred codons*
CP4 epsps	*5-enolpyruvyl shikimate-3-phosphate synthase (Agrobacterium tumefaciens CP4)*	*HT*	*enhanced CaMV 35S, maize HSP70 intron chloroplast transit peptide from A. thaliana EPSPS gene (CTP2)*	*A. tumefaciens nopaline synthase (nos) 3'-untranslated region*	*1*	*CP4 EPSPS gene modified for plant-preferred codons*

Soybean: GTS40-3-2



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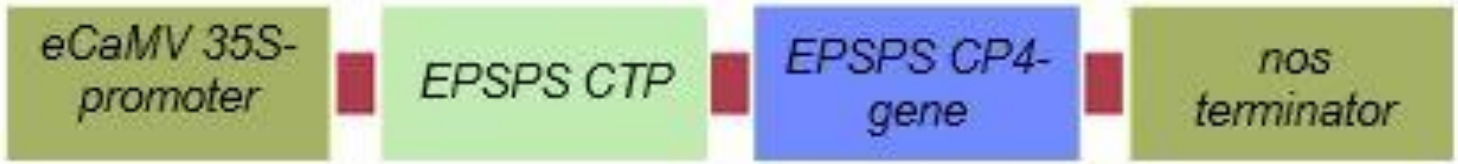
CP4 epsps	*5-enolpyruvyl shikimate-3-phosphate synthase*	*HT*	*enhanced CaMV 35S chloroplast transit peptide from Petunia hybrida*	*A. tumefaciens nopaline synthase (nos) 3'-untranslated region*	*1*	*Native; Also 2 partial gene sequences (250 bp; 72 bp)*

Soybean: GTS40-3-2



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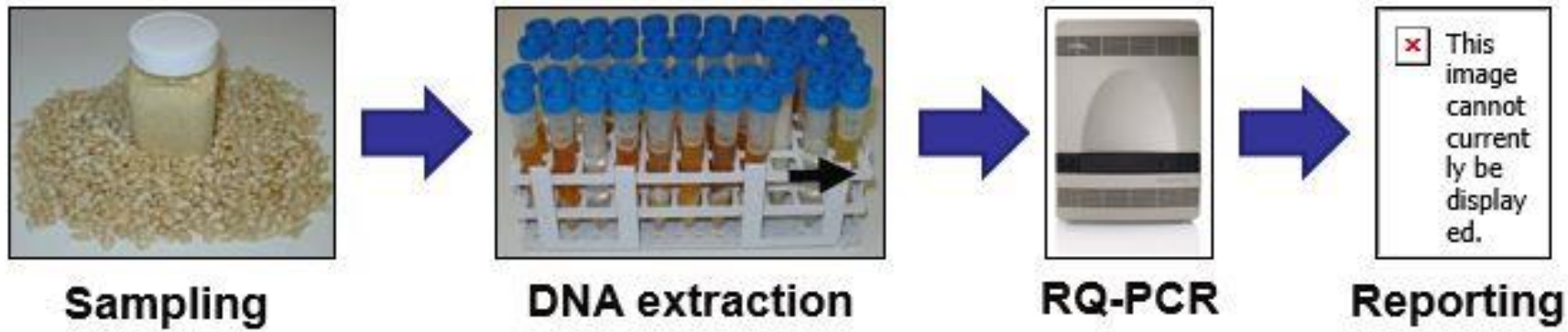
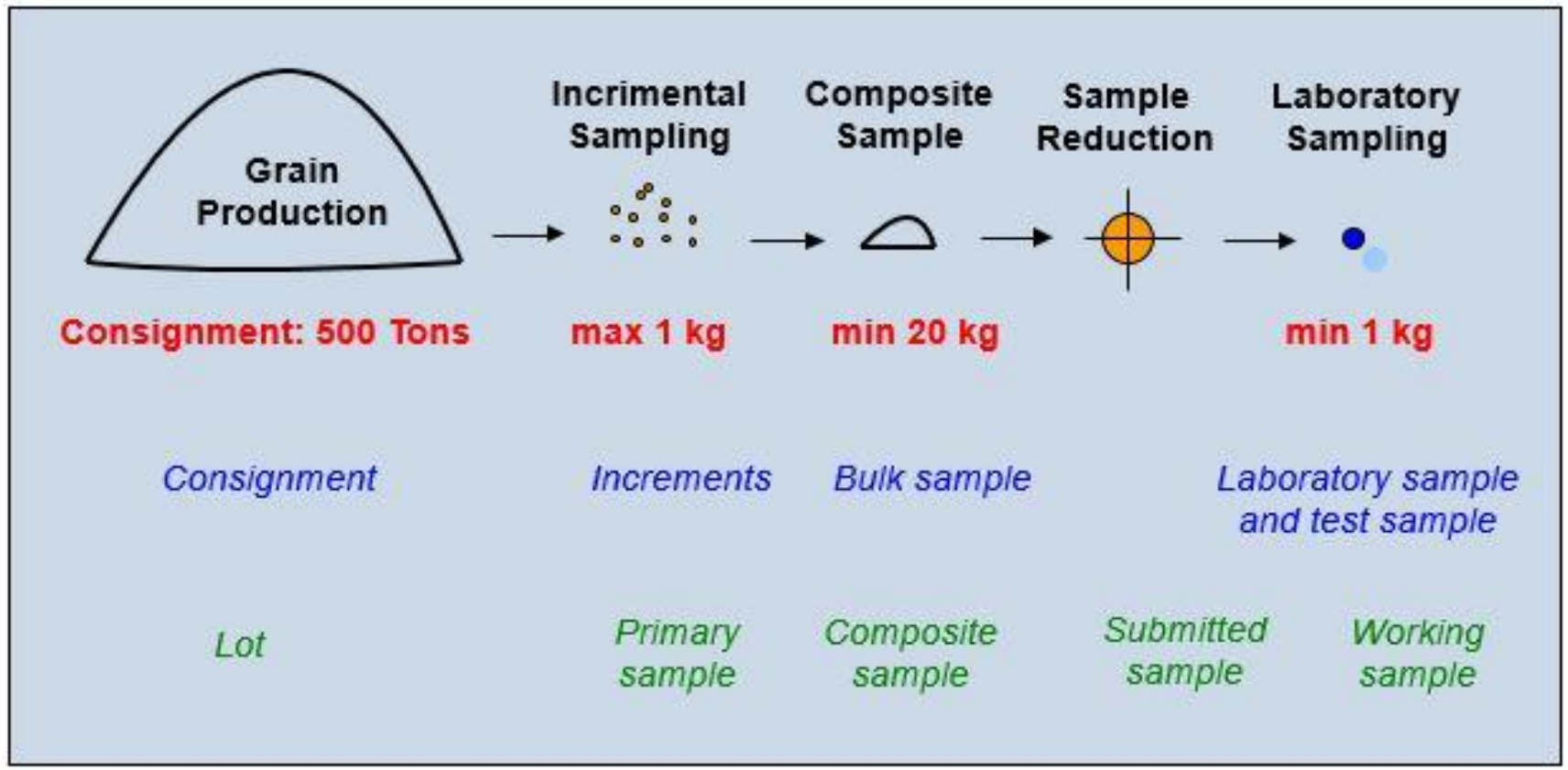
LMO detection process



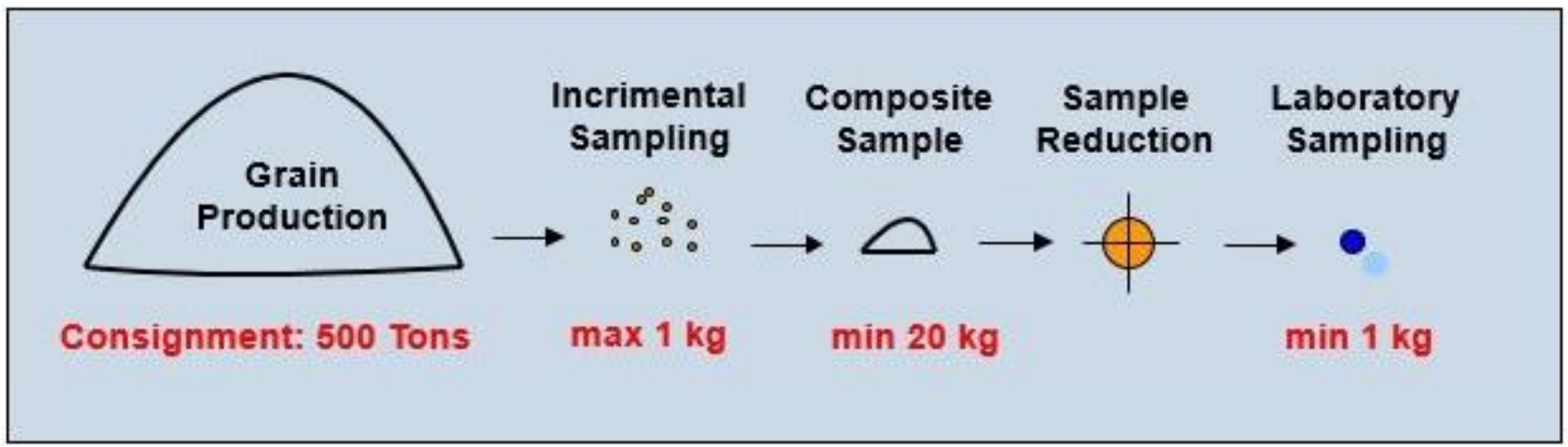
Sampling and sample preparation

LMO sampling



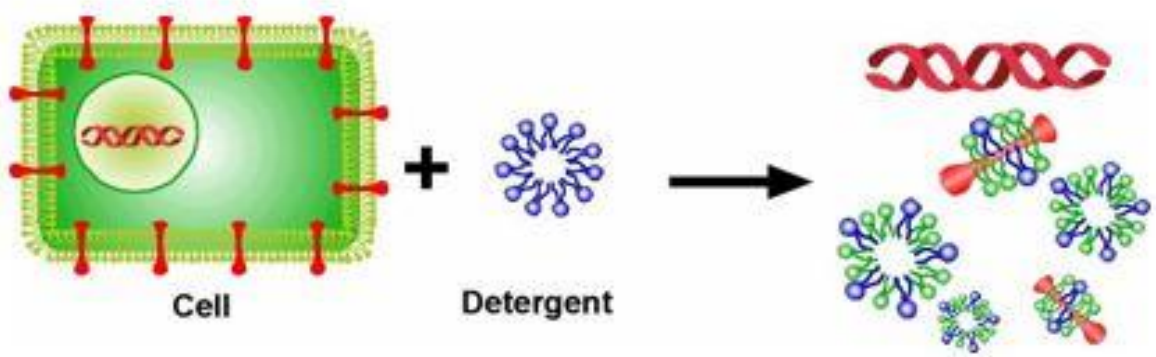
Sample reduction

1. Combine sufficient numbers of increments forming the bulk sample
2. Reduction of the bulk sample to the laboratory sample
3. Homogenisation and reduction of the particle size by appropriate means to form the test sample



DNA extraction

- **CTAB Method (cetyltrimethylammonium bromide)**



Introduction to DNA extraction

Basic steps in DNA extraction

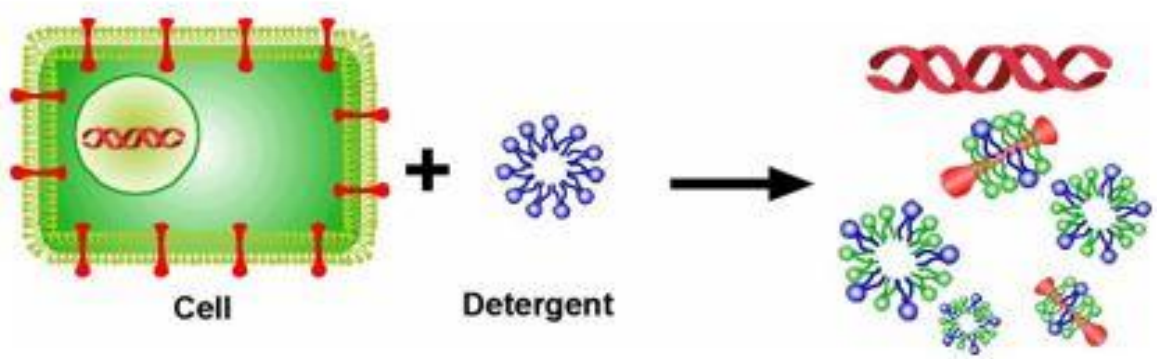
- Cell Lysis
- DNA Extraction
- DNA Purification

Cell lysis

- Mechanical disruption (e.g. grinding)
- Chemical treatment (e.g. detergent lysis, chaotropic agents)
- Enzymatic digestion (e.g. proteinase K)

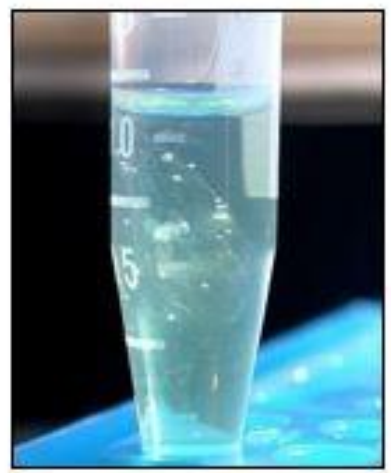
DNA extraction

- **CTAB Method (cetyltrimethylammonium bromide)**



DNA purification

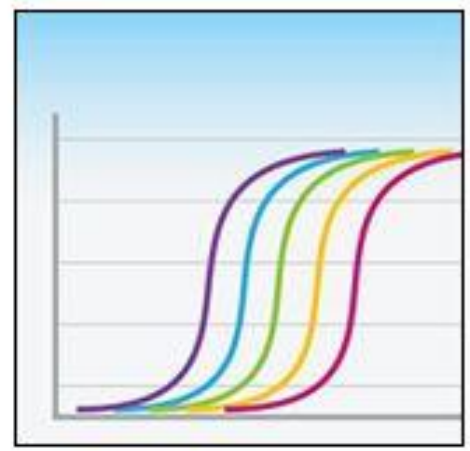
- DNA precipitation
- Column purification



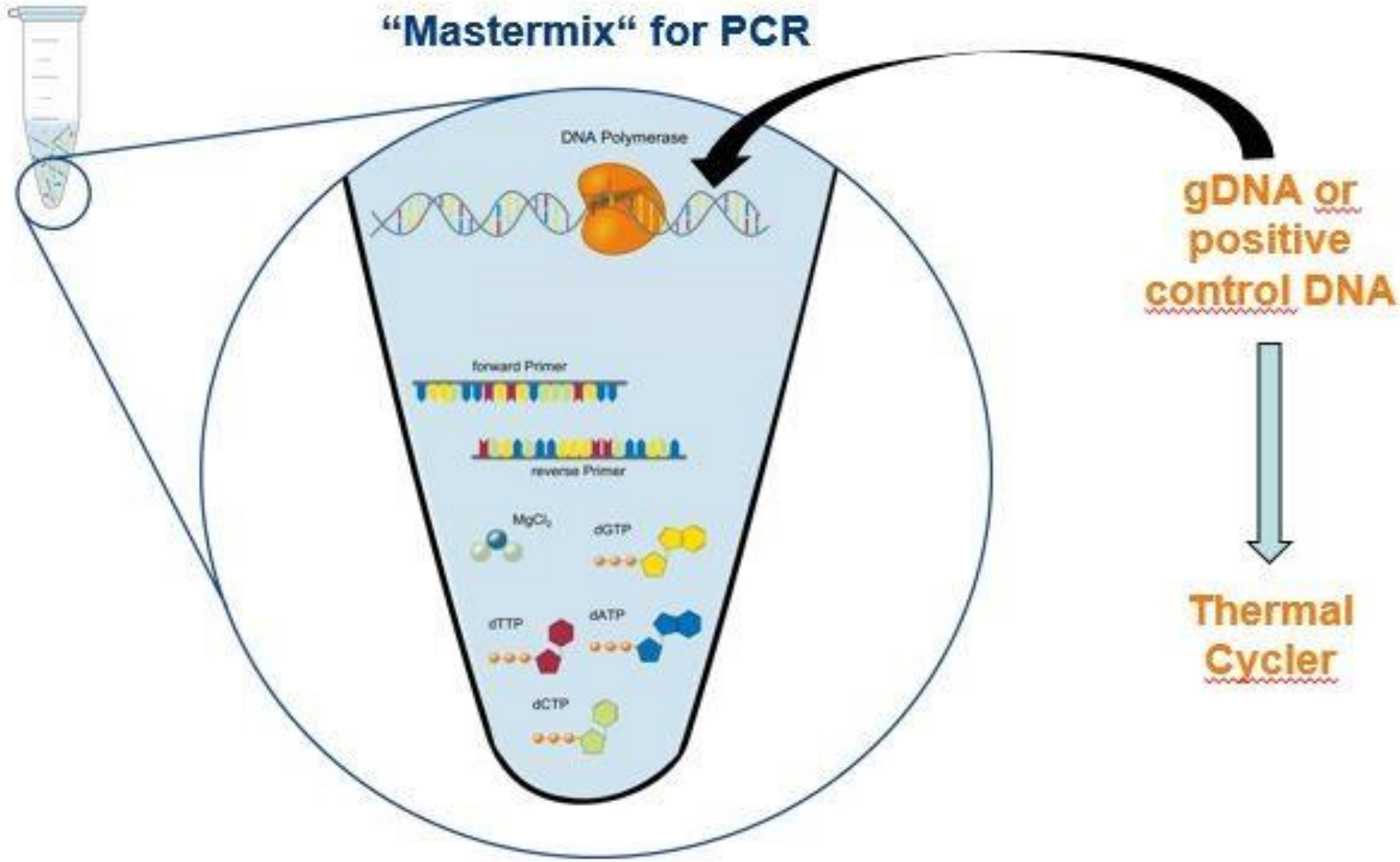
Considerations

- Combination of mechanical disruption, chemical treatment and enzymatic digestion
- Quantity and quality of extracted DNA is important

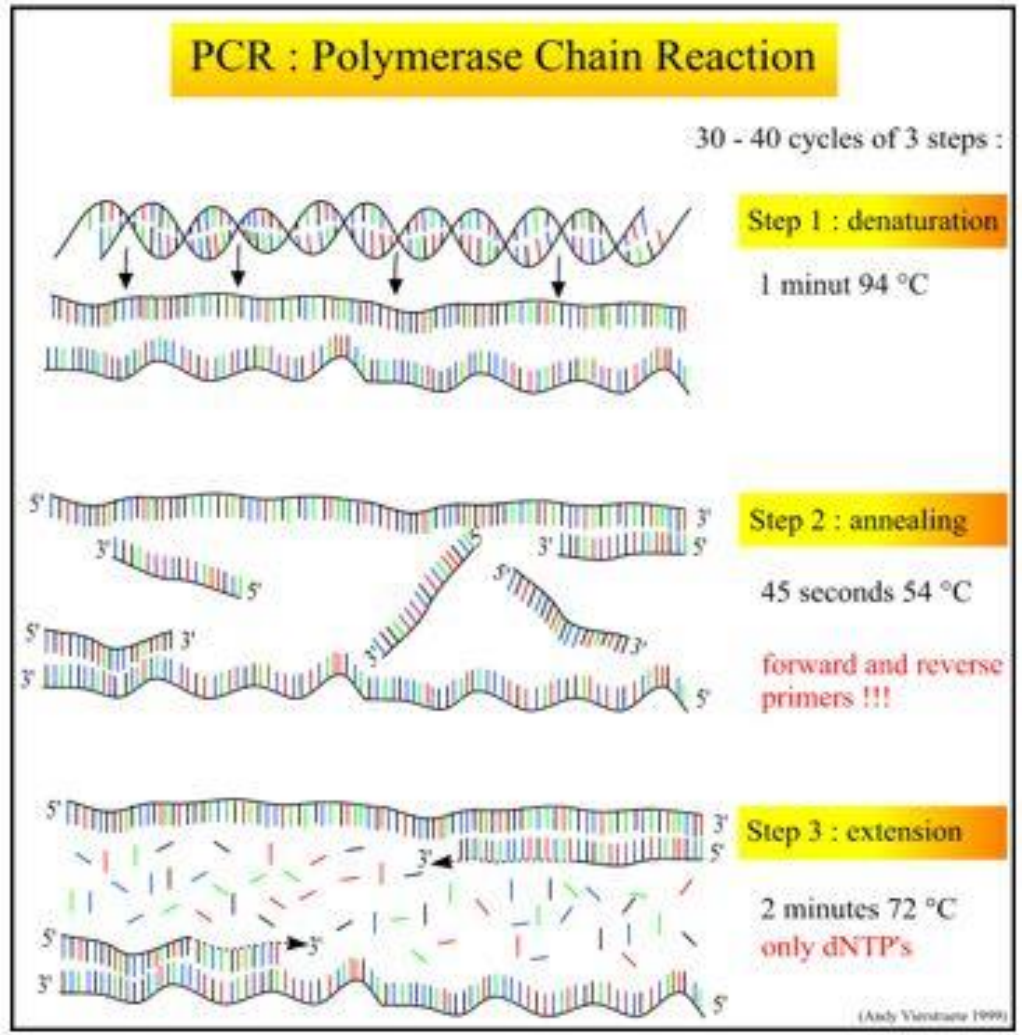
Introduction to PCR and preventing contamination



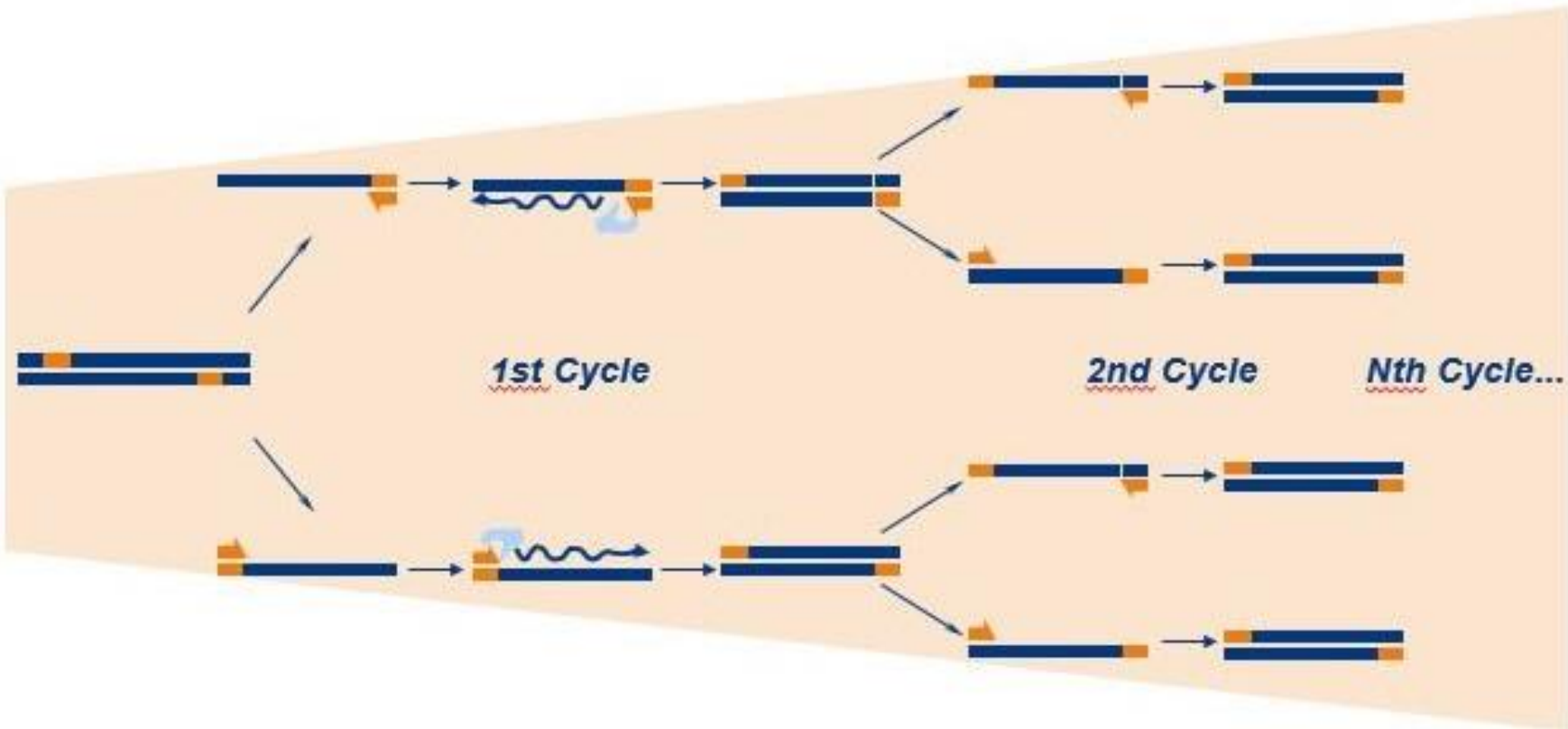
Reagents for PCR



PCR Cycling


PCR : Polymerase Chain Reaction
30 - 40 cycles of 3 steps :
Step 1 : denaturation
1 minut 94 °C
Step 2 : annealing
45 seconds 54 °C
forward and reverse primers !!!
Step 3 : extension
2 minutes 72 °C
only dNTP's

Polymerase Chain Reaction



Sources of contamination during PCR

- Previous DNA extractions on laboratory benches and equipment
- Cross-contamination between samples
- Previous PCR amplifications

Methods to prevent contamination

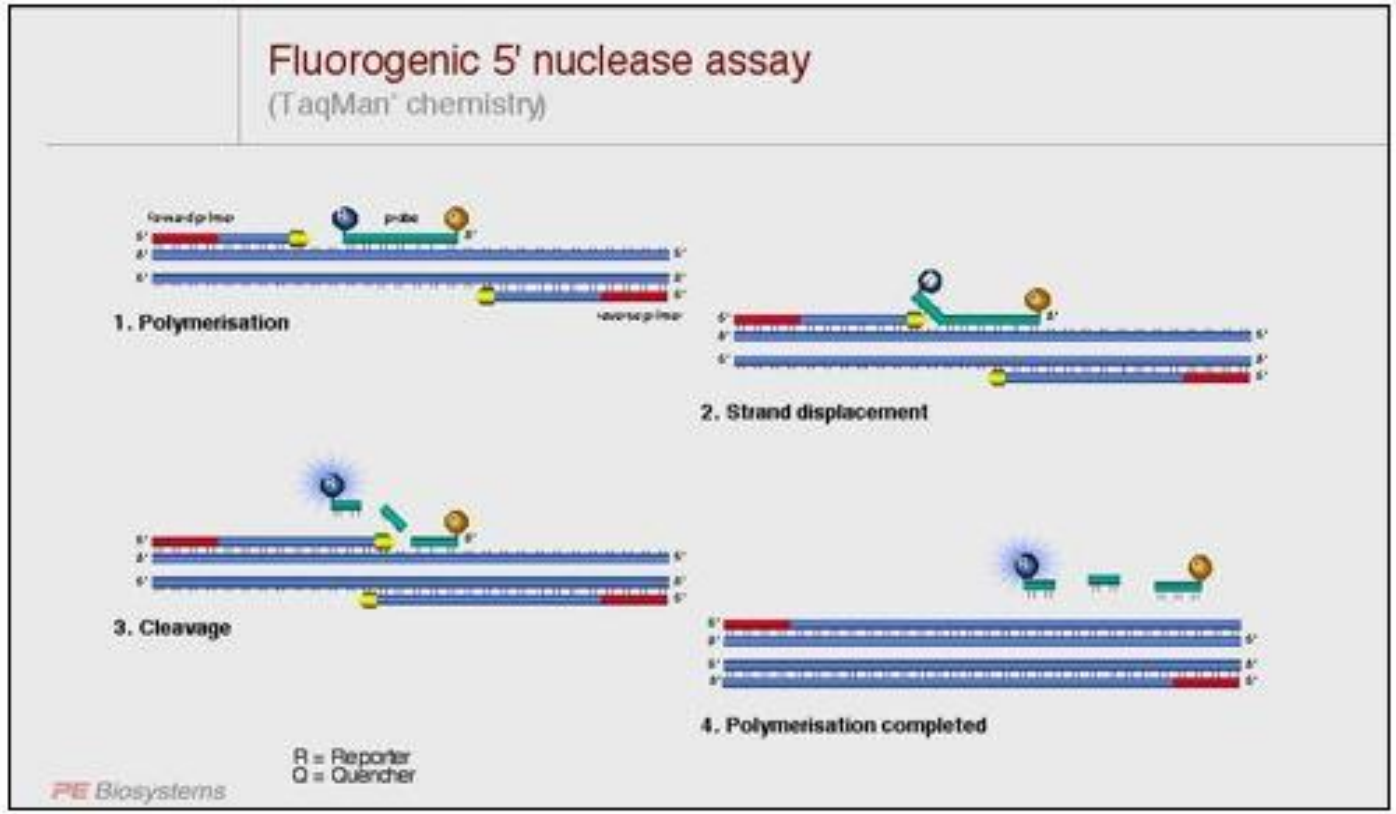
- Separate activities into different physical areas
- Use dedicated equipment in different areas
- Use a one way flow system
- Wash benches with 10% bleach and 70% ethanol
- Use sterile plasticware and filter tips
- Aliquot reagents
- Check contamination using controls

Introduction to Real-time PCR

Real-time PCR

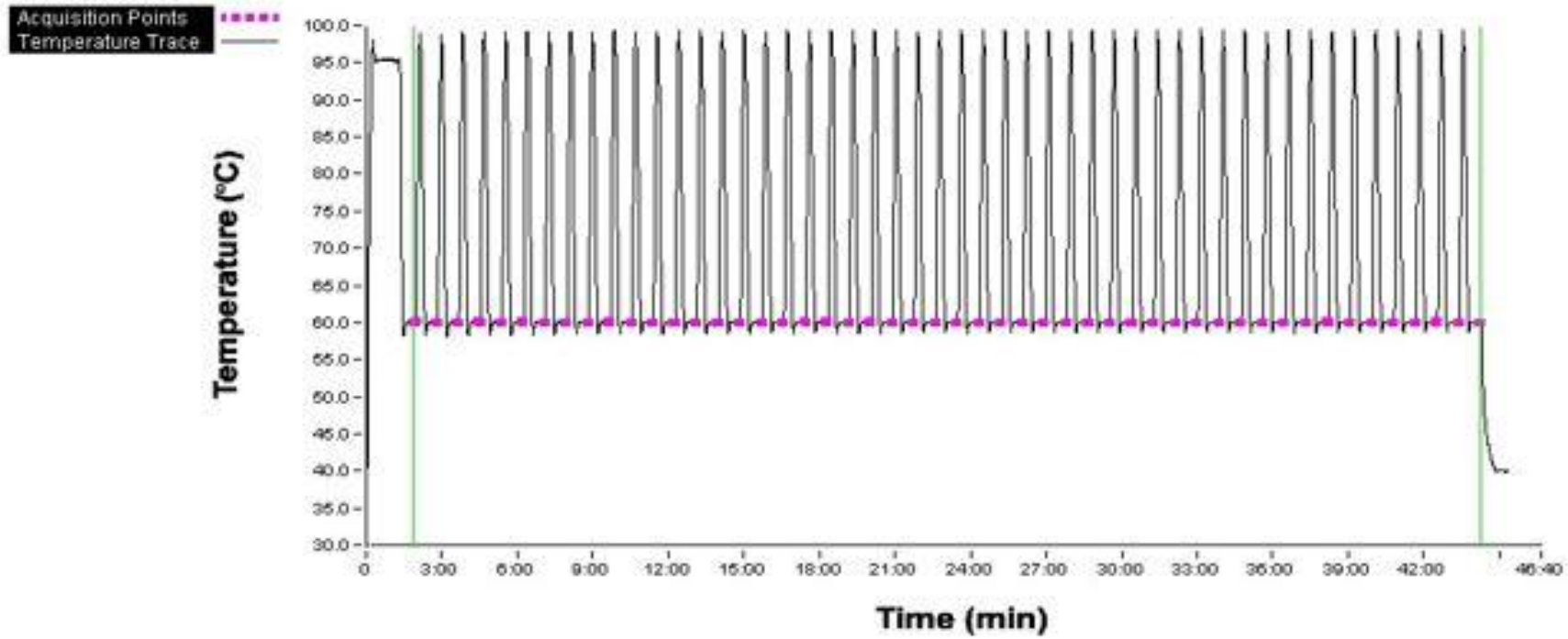
- PCR amplification and detection in one reaction
- Detection of amplicon using fluorescence

Real-time PCR probe detection



Principle of FRET

PCR thermal cycling



RT-PCR terminology

Baseline

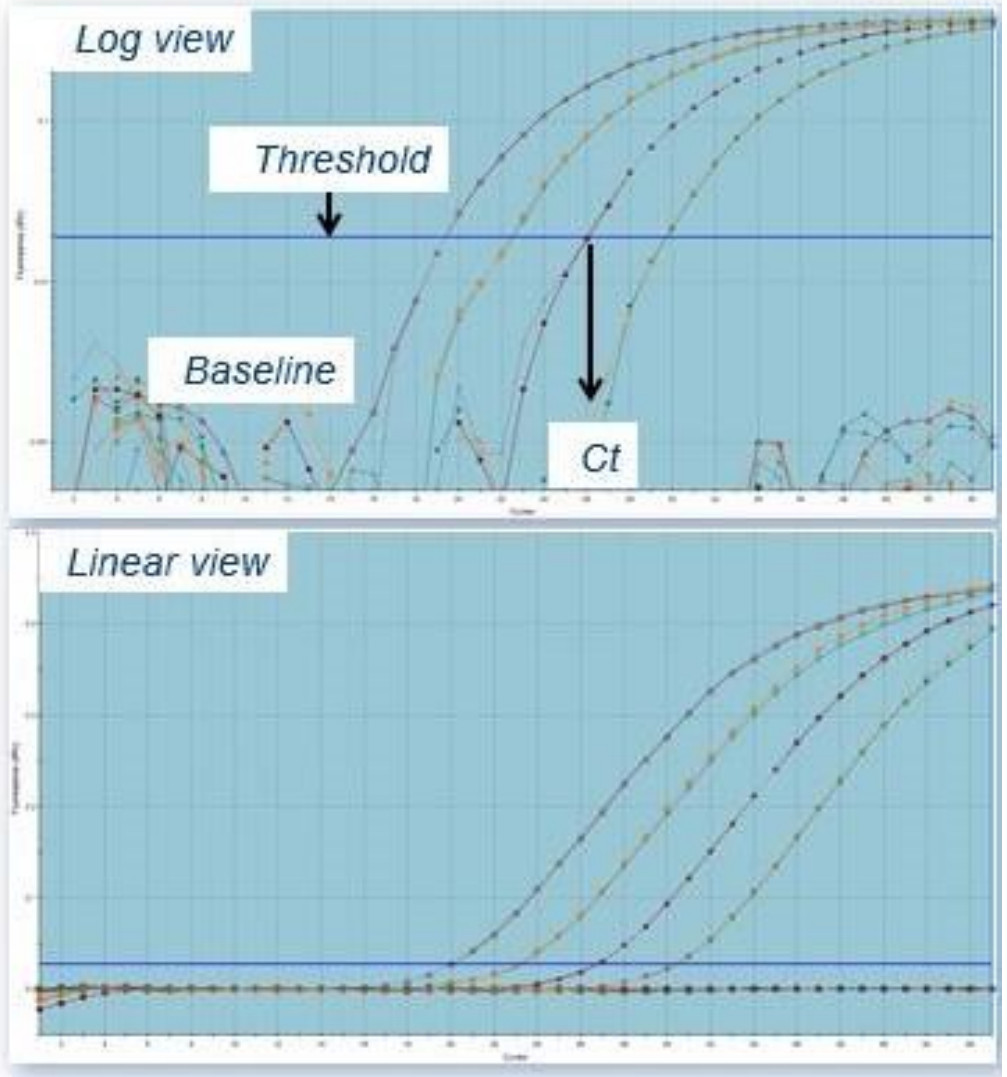
- *Eliminate background*
- *Cycles with amplification*

Threshold

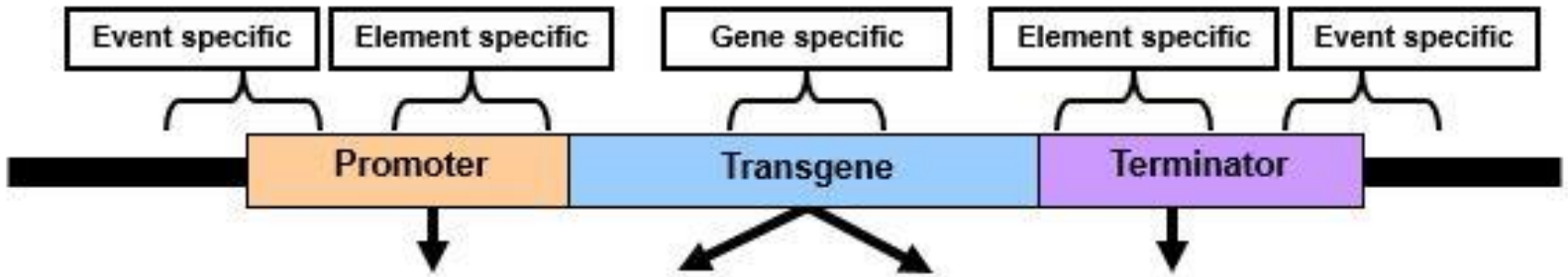
- *Point above baseline*
- *Set in the exponential phase of PCR*

C_t

- *Threshold cycle number at which the fluorescence signal crosses the threshold*
- *Inversely related to starting amount of target DNA*



Transgene construct



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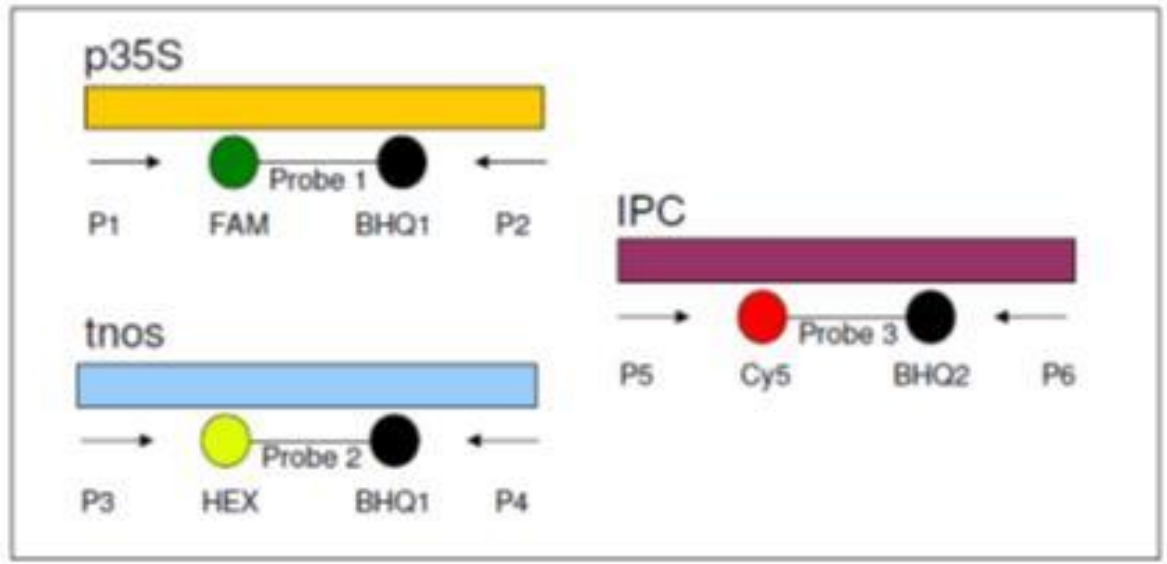
35S/NOS/IPC RT-PCR Screening



Considerations

- Real-time PCR when using detection probes combines PCR and verification in one reaction
- Real-time PCR can be used for qualitative LMO detection
- Qualitative methods do not give any indication of LMO content

LMO detection process