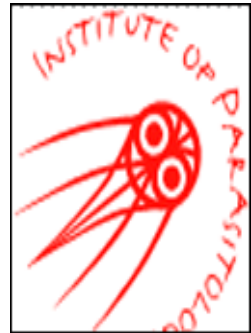


Molecular Diagnosis for Benzimidazole Resistance-Associated SNPs in Human Soil- Transmitted Helminths

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Institute of Parasitology

McGill University



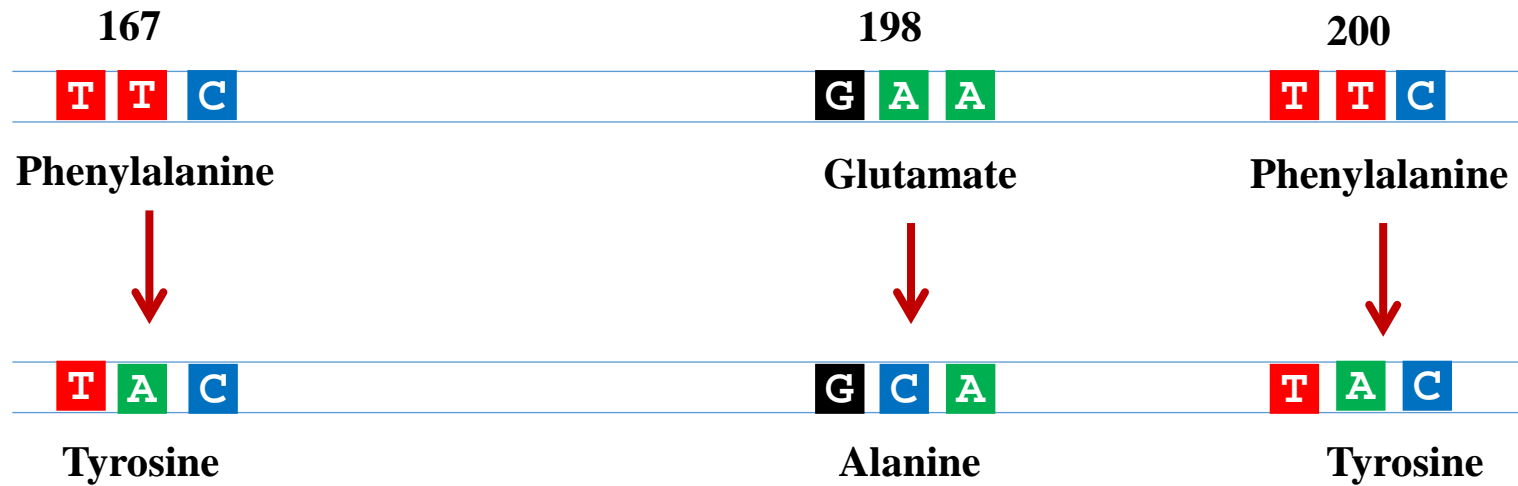
Risk of BZ Resistance Development in MDA Programs for STH

In human **hookworms**: reduced mebendazole efficacy, but genetic evidence lacking

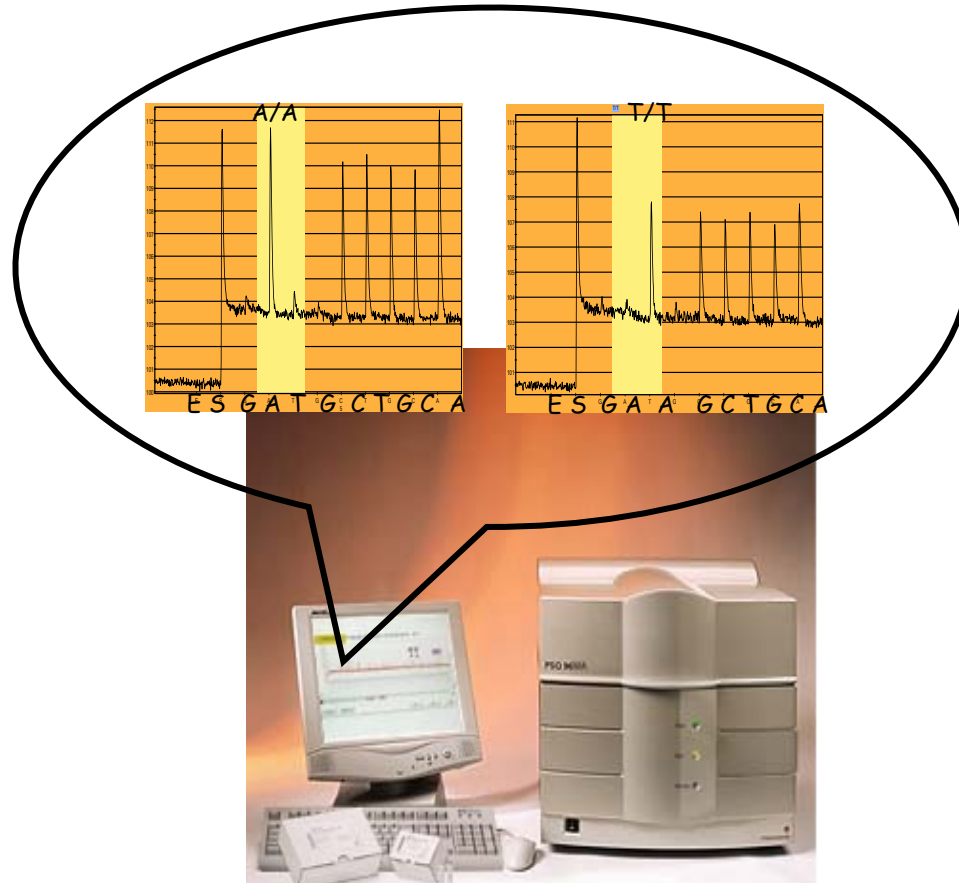
- Mali (De Clercq *et al.*, 1997)
- Zanzibar (Albonico *et al.*, 2003)
- Vietnam (Flohr *et al.*, 2007)
- *T. trichiura* 200SNP detected in stool samples from:
 - Kenya and Panama (Diawara *et al.*, 2009)
 - Haiti where 200SNP increased following treatment (Diawara *et al.*, 2013)
 - Moreover, 200SNP was very high in samples from Haitian subjects who responded poorly to ABZ, but was very low in subjects who responded very well to ABZ (Diawara *et al.*, 2013)
- *N. americanus*: 200SNP detected at low frequency in samples from Panama and Haiti (Diawara *et al.*, 2013)
- Huge MDA programs, with ABZ/MBZ around the world that treat repeatedly markedly increase the risk of BZ resistance being selected. This would jeopardize control efforts

Benzimidazole Resistance

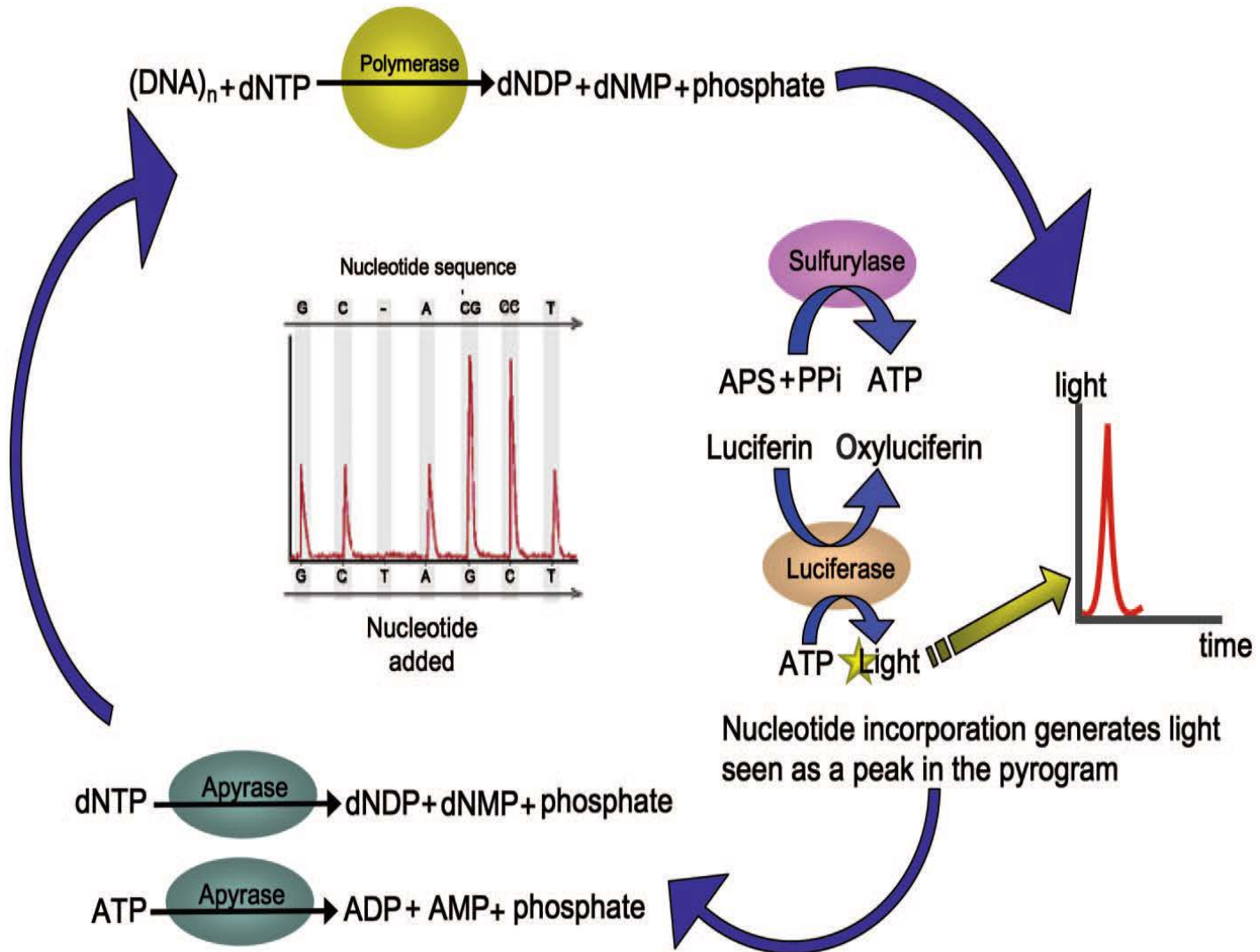
➤ β -tubulin isotype 1 gene



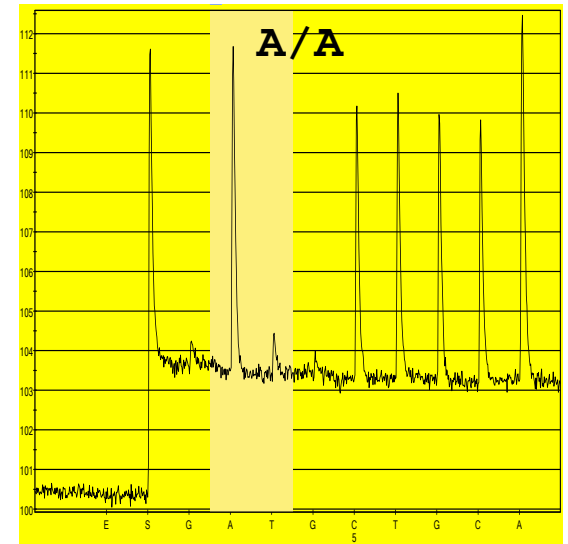
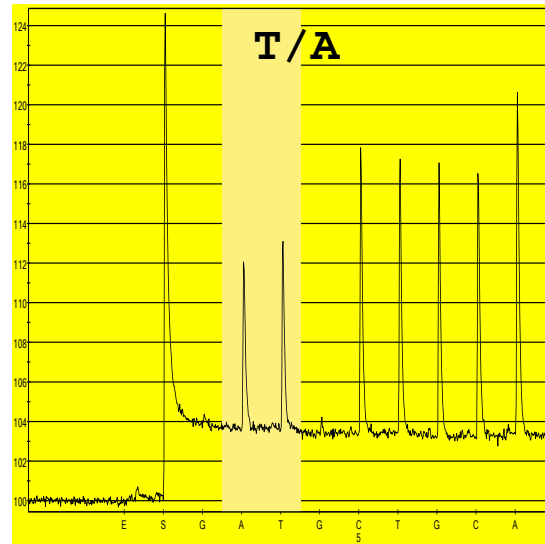
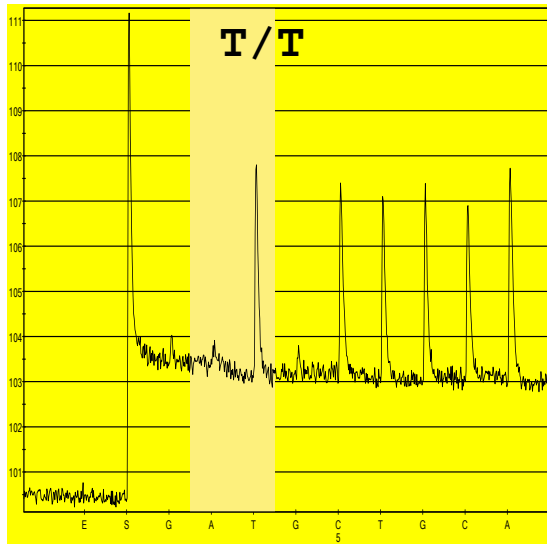
Pyrosequencing



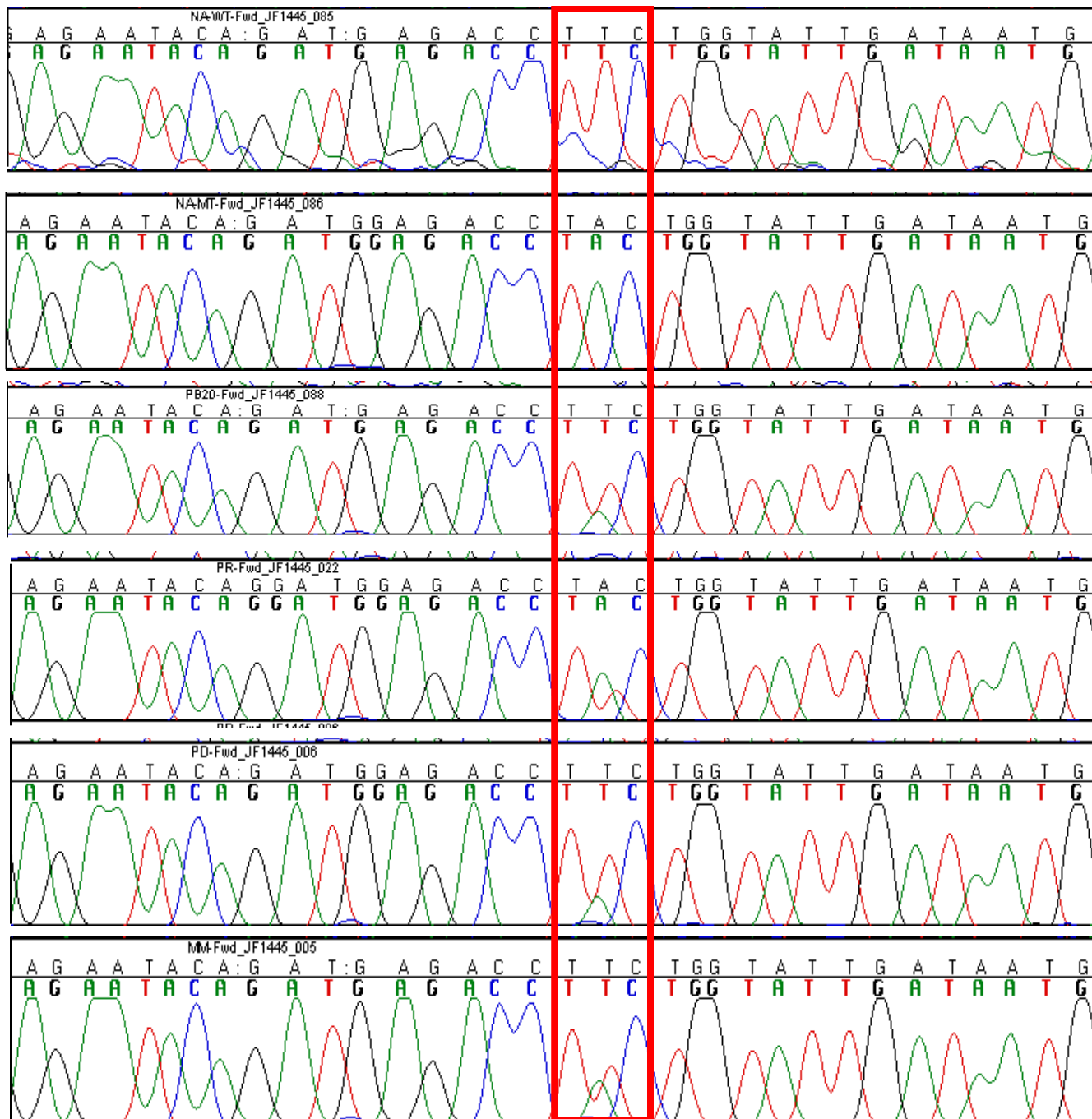
Pyrosequencing



Pyrosequencing Results



Pyrosequencing results
have been confirmed
by Sanger sequencing



Loop-mediated Isothermal Amplification (LAMP)

Loop-mediated Isothermal Amplification Method (LAMP)

A novel gene amplification method, uses a strand displacement DNA polymerase (*Bst* DNA polymerase) and a set of four specially designed primers

1. A **one-step** DNA amplification technology (amplification = detection)
2. **Rapid, sensitive** and **robust**: can amplify target DNA from partially processed samples)
3. **Highly specific** (four specific primers to amplify six DNA target regions)
4. Less expensive, less instrumentation and allows **visual detection** formats
5. **Isothermal** conditions (water bath or heating block is sufficient)

Smart Amplification Process (SmartAmp2)

Isothermal DNA amplification method for detection of SNPs or DNA target

1. **Asymmetrical primer design**

Turn-back and folding primers are used to minimize alternative mis-amplification pathways

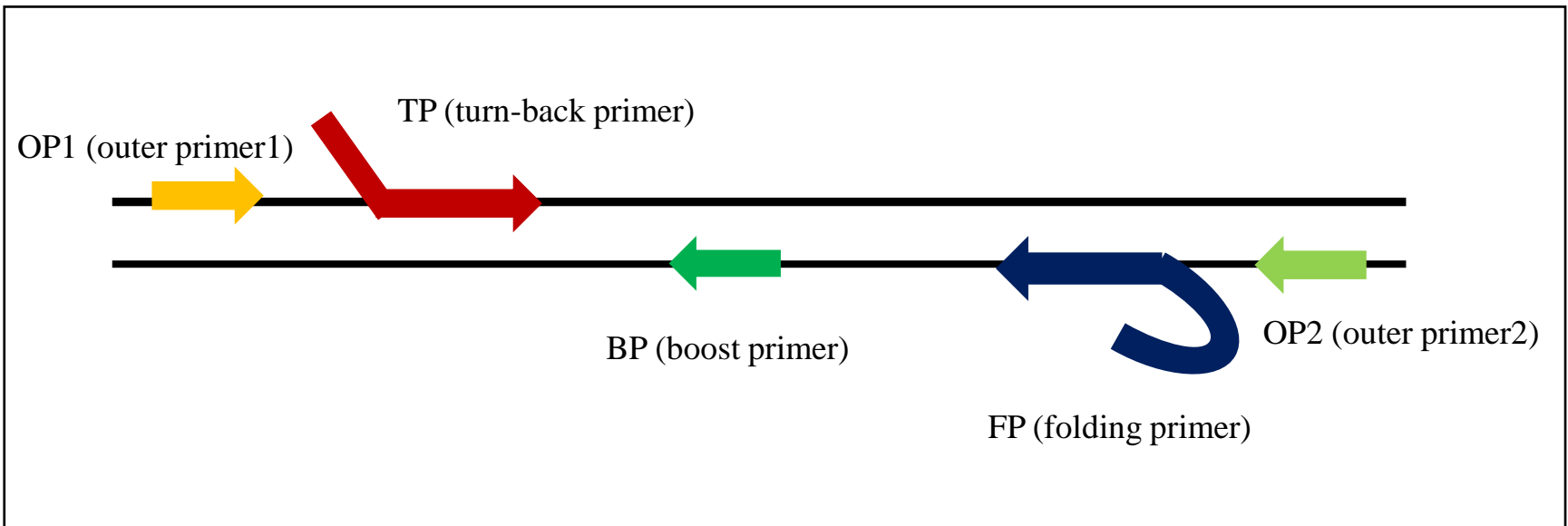
2. **DNA polymerase with strand-displacement activity**

3. **Mismatch binding protein**

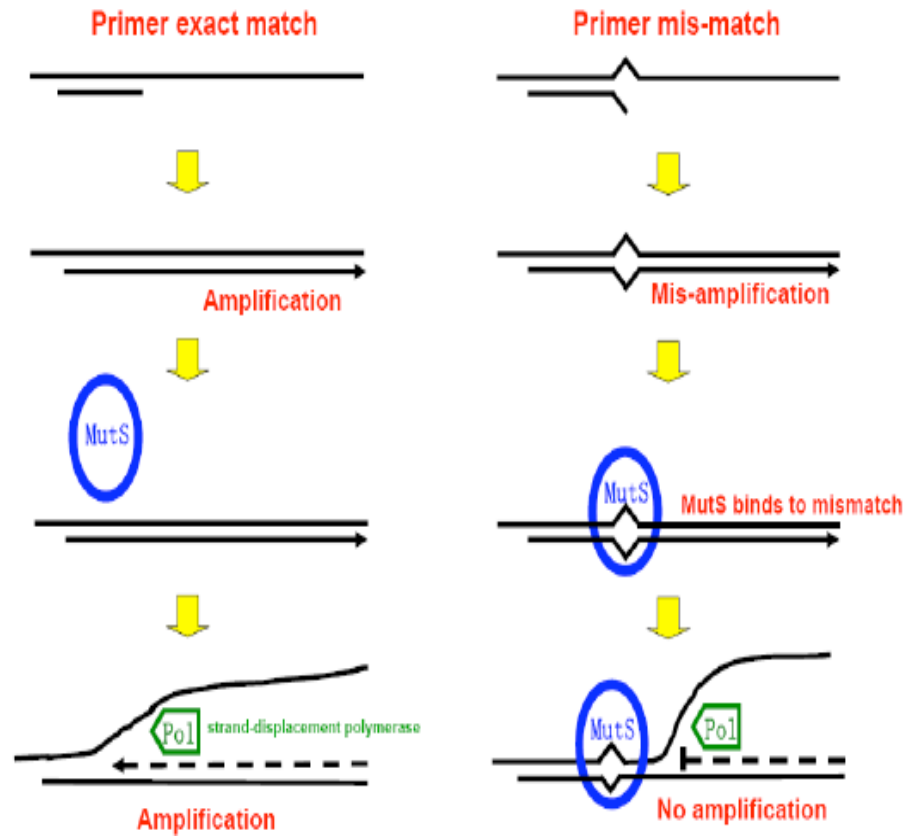
Inhibits background DNA from entering the amplification cycle, by specifically binding to mis-primed amplification products

Smart Amplification Process (SmartAmp2)

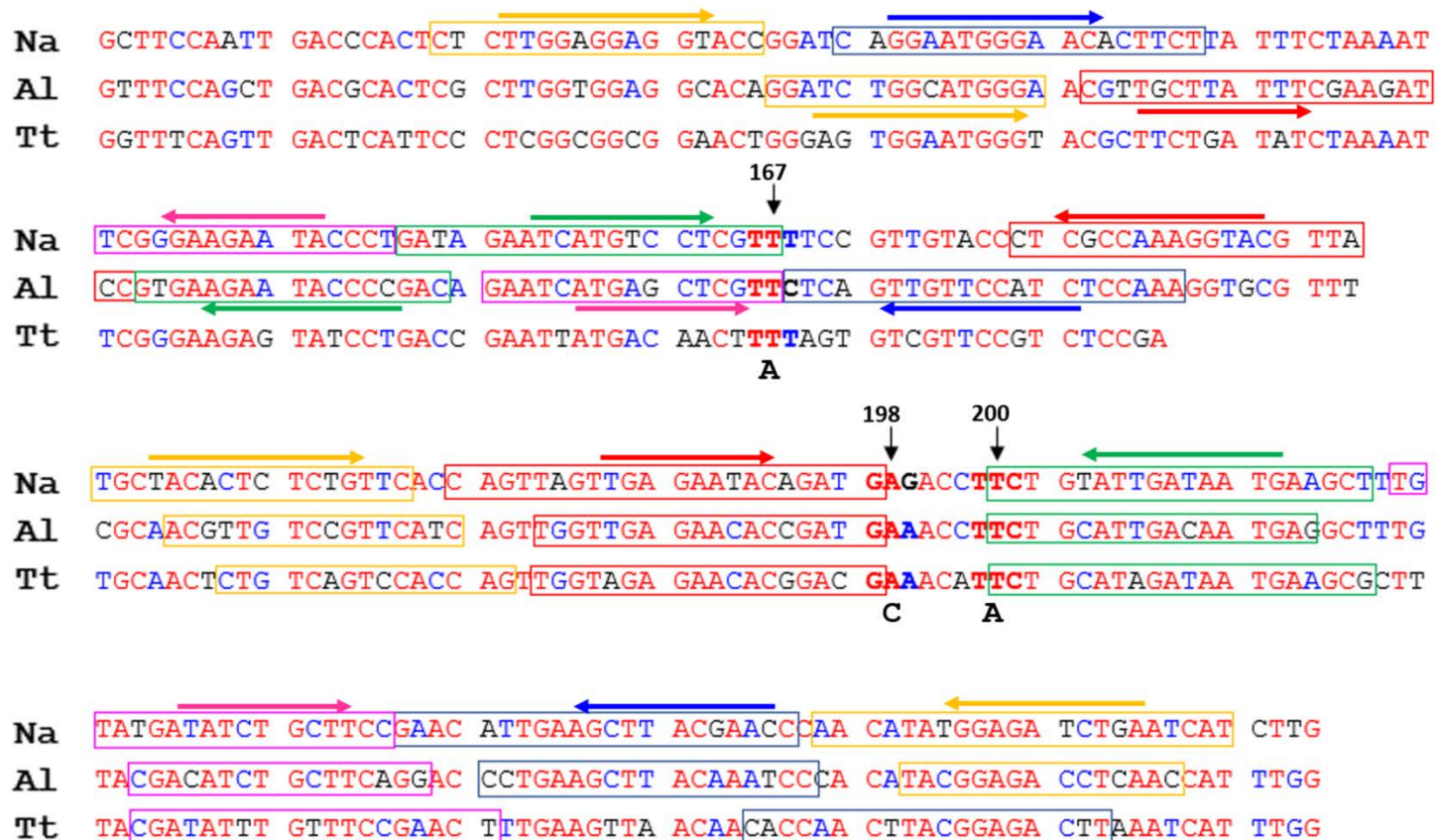
Asymmetrical primer design



Smart Amplification Process (SmartAmp2)

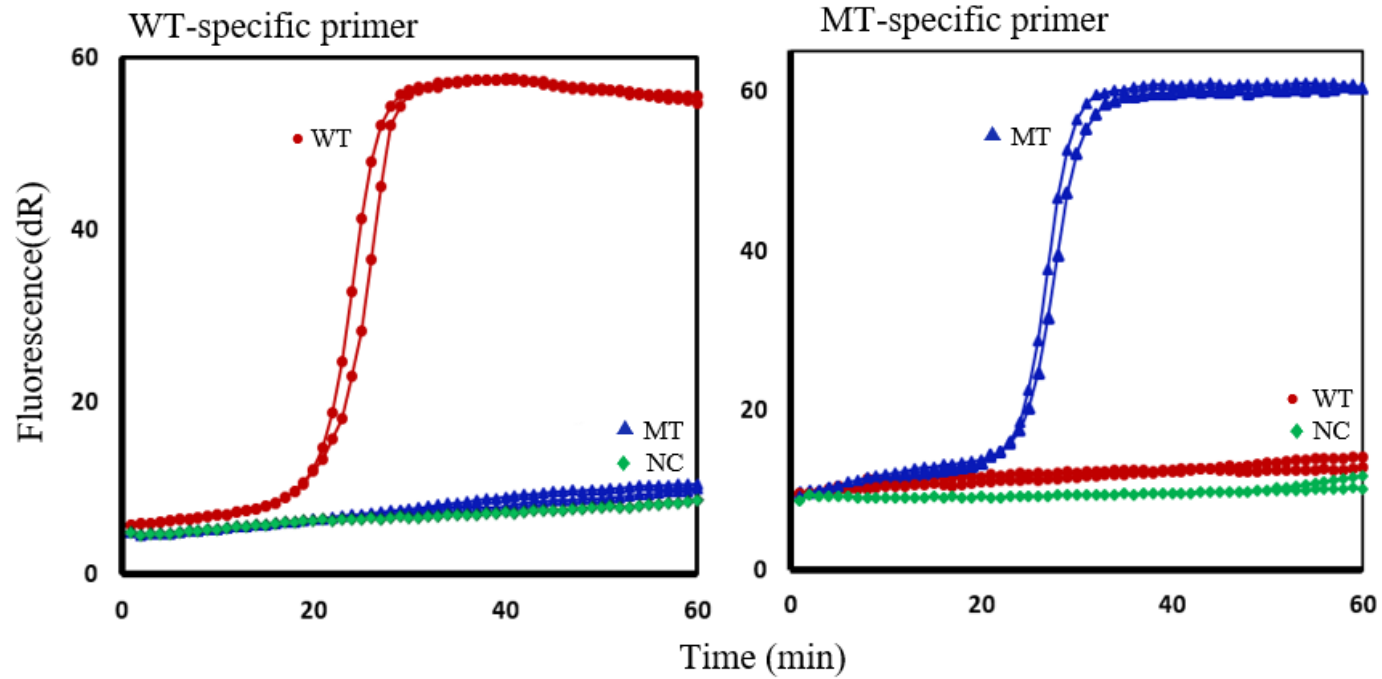


β -tubulin Isotype 1 Sequence Alignment SmartAmp2 Primer Design



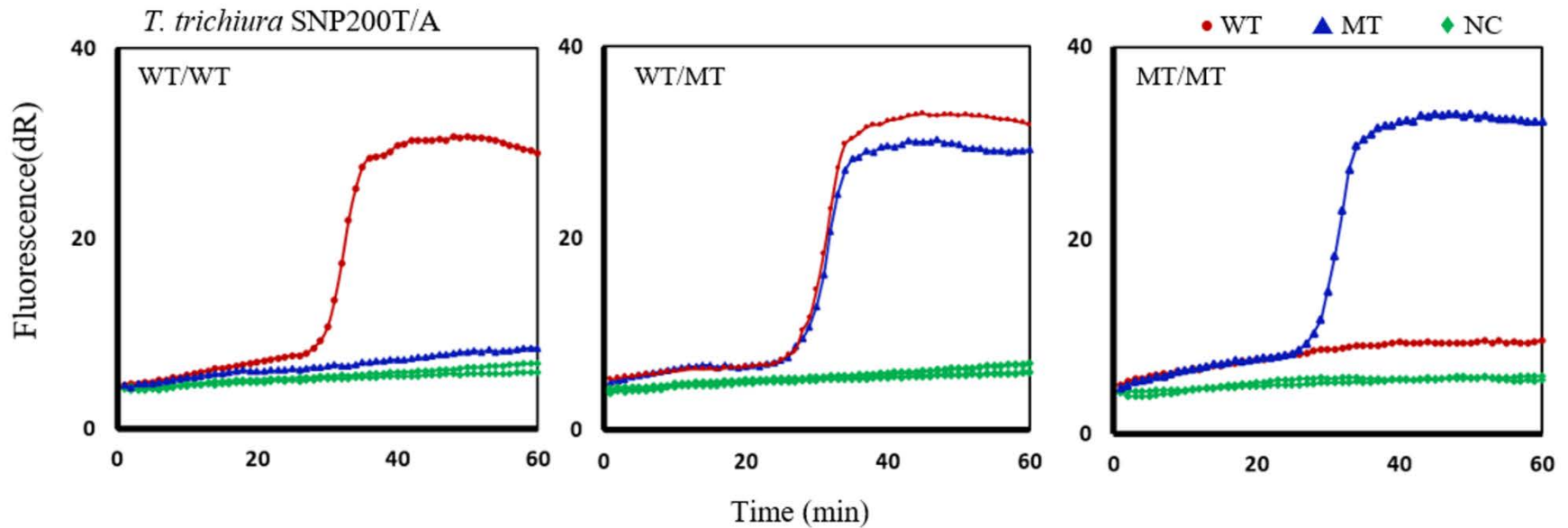
-  Outer primers (Op1, Op2)
-  Turn-back primer (TP-A)
-  Boost primer (BP)
-  Turn-back primer (TP-Bc)
-  Folding primer (FP)

I. Assay optimization



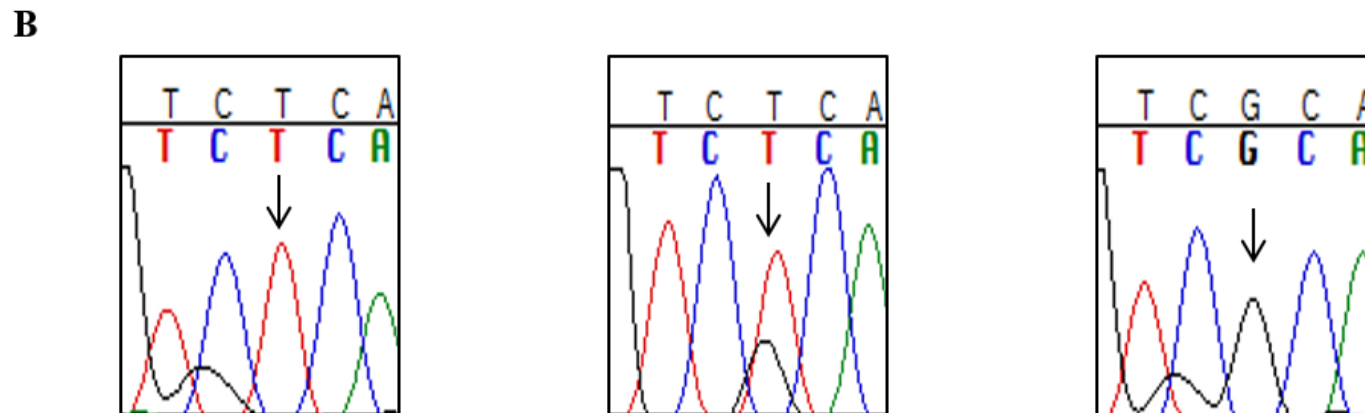
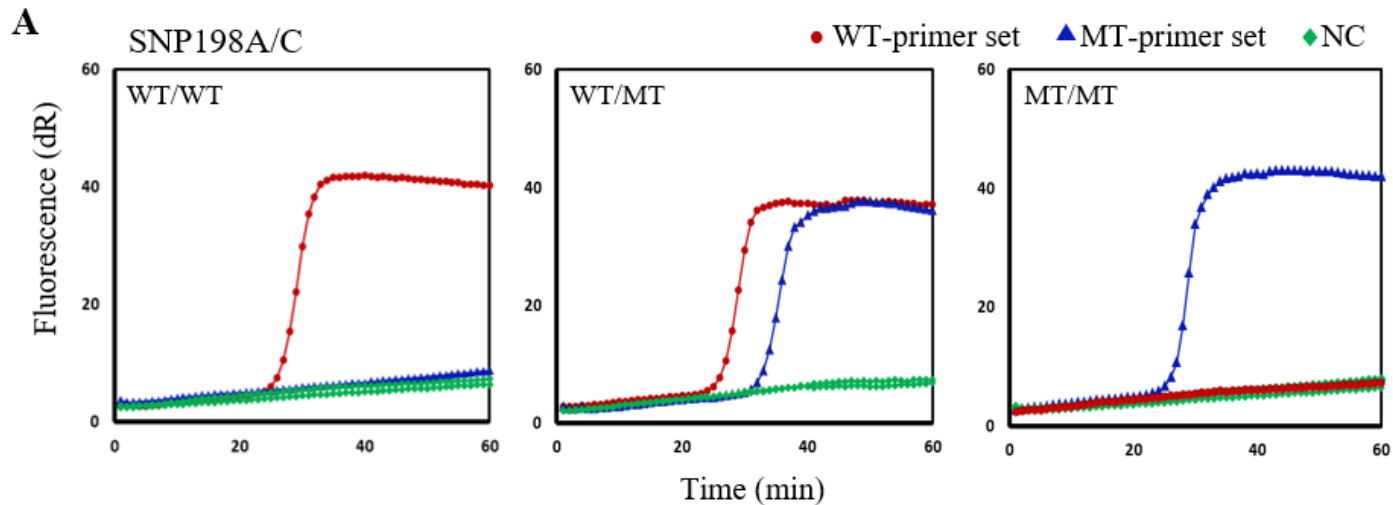
II. Assay validation

T. trichiura field samples (SNP200)



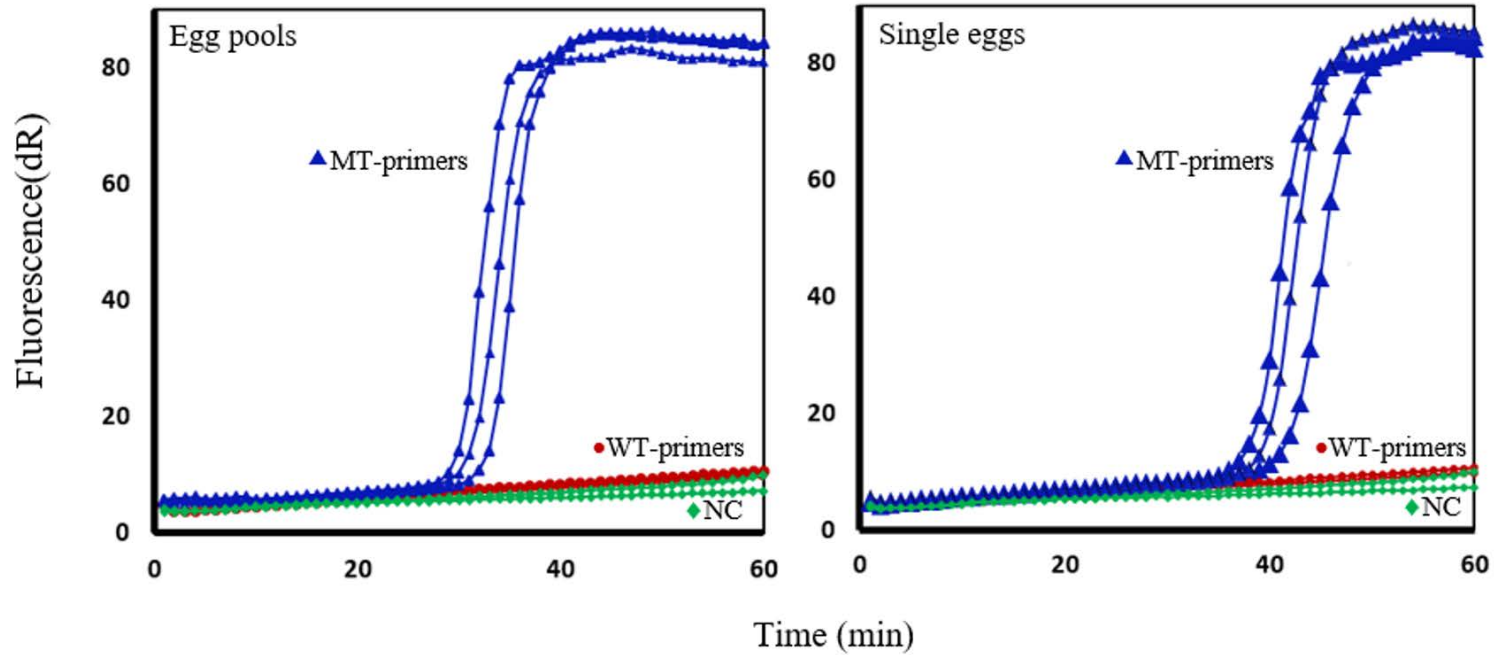
II. Assay validation

N. americanus field samples (SNP198)

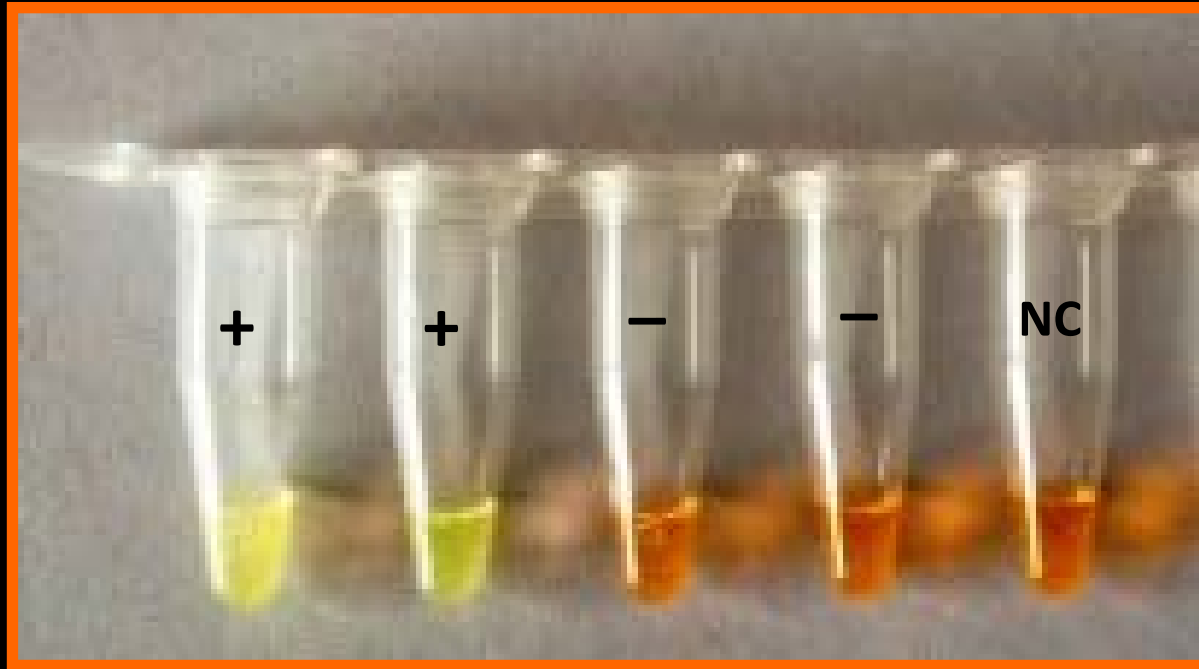


II. Assay validation

A. lumbricoides samples (SNP167)



Colorimetric assay SYBR Green



The color changes from orange (negative reaction) to yellow (positive reaction)

Summary

- Pyrosequencing assays established for SNPs associated with BZ resistance, for *A. lumbricoides*, *N. americanus* & *T. trichiura*, using egg pools
- Novel LAMP assays established for SNPs in the β -tubulin in *A. lumbricoides*, *N. americanus* & *T. trichiura*
 - These LAMP assays are highly specific and sensitive
 - Require minimum equipment – hot water
 - Readout can be by hand-held fluorimeter or optical readout
- In this project, the McGill team will focus on detection of BZ resistance associated SNPs and determine proportion of such SNPs in a sample
 - Molecular assessment of any resistance will be compared with FECRT evidence of resistance