Rapid Identification of Azole-Resistant *Candida tropicalis* (Clade 4)

1. Purpose & Scope

This protocol outlines the procedure for the rapid identification of the predominant azole-resistant genotype (Clade 4) in *Candida tropicalis*. This method utilizes TaqMan SNP Genotyping Assays to detect specific polymorphisms in the CTRG_05978 and SNQ2 genes, providing a faster alternative for detecting the clade 4 genotype.

2. Materials & Equipment

Equipment

Real-Time PCR System: ABI QuantStudio™ 6 Flex System (Applied Biosystems) or equivalent.

Reagents

Master Mix: TaqMan® Genotyping Master Mix (Applied Biosystems).

Primers & Probes: Custom TaqMan assays designed for the following targets:

CTRG_05978 Type IV	Forward Primer	CGTCATATACTCGGAAGGACTTTCTCTA	-62 – -33
	Reverse Primer	CGATCTCTTTATAGTTGTTGGAGAAGTGA	+13 – +42
	Probe 1 (ATGA)	CCCCTTAAATAATGATTAAAT	-11 – +10
	Probe 2 (ATAA)	CCCCTTAAATAATAATTAAAT	-11 – +10
SNQ2 A2977G	Forward Primer	TGAAAAGATCATTGATGTTTTGGATATGAAAGG	+2931 – +2963
	Reverse Primer	CGTTGCTCGACATTTAAACCATTACC	+2992 – +3017
	Probe 1 (A)	CCGATGCCATTGTTG	+2969 – +2983
	Probe 2 (G)	CCGATGCCGTTGTTG	+2969 – +2983

CTRG_05978: Targeting the start codon region (specifically the first 4-mer).

SNQ2: Targeting the SNP at position 2977.

3. Target Markers & Clade 4 Definition

To positively identify a strain as Clade 4, the isolate must exhibit the following

combined genotype:

Target Gene	**Target Locus / Region**	**Clade 4 Genotype Marker**
CTRG_05978	Start codon region (first 4-mer)	**ATRA**
SNQ2	Position 2977	**Mutation A2977G** (Amino acid I993V)

4. Experimental Procedure

Thermal Cycling Conditions

CRITICAL: The cycling conditions differ between the two target genes. Please ensure the correct protocol is selected for the specific assay being run.

Protocol A: Detecting CTRG_05978

Note: This protocol includes a specific annealing step at 54°C.

Stage	**Temperature**	**Time**	**Cycles**
Pre-Read / Hold	95°C	10 min	1
PCR Cycling	95°C	15 sec	**40**
	54°C	**20 sec**	
	60°C	1 min	
Post-Read / Hold	60°C	1 min	1

Protocol B: Detecting *SNQ2*

Note: This protocol follows a standard 2-step cycling procedure.

Stage	Temperature	Time	Cycles
Pre-Read / Hold	95°C	10 min	1
PCR Cycling	95°C	15 sec	**40**
	60°C	1 min	
Post-Read / Hold	60°C	1 min	1

5. Data Analysis & Interpretation

Analyze the data using the Allelic Discrimination plot on the QuantStudio™ software.

CTRG_05978 Analysis:

Look for the ATRA signature.

SNQ2 Analysis:

Look for the A2977G mutation.

Identify samples carrying the G allele (or G/G homozygous / A/G heterozygous depending on probe design).

Final Conclusion:

Positive for Clade 4: If the sample is identified as “CTRG_05978 ATRA” AND “*SNQ2* A2977G”.

Negative for Clade 4: If the sample lacks either or both markers.

6. Reference

Based on the methodology described in:

Tseng, K-Y., et al. (2024). Rapid identification of the predominant azole-resistant genotype in Candida tropicalis. FEMS Yeast Research, 24, foae025.