AmplideX® PCR/CE *TOMM40* Kit (RUO)

2500-0665

For Research Use Only. Not for use in diagnostic procedures

Types of Dementia

DEMENTIA

An "umbrella" term used to describe a range of symptoms associated with cognitive impairment.

ALZHEIMER'S
50%–75%

VASCULAR
20%–30%

LEWY BODIES
10%–25%

FRONTOTEMPORAL
10%–15%

MIXED DEMENTIA = >1 NEUROPATHOLOGY - PREVALENCE UNKNOWN
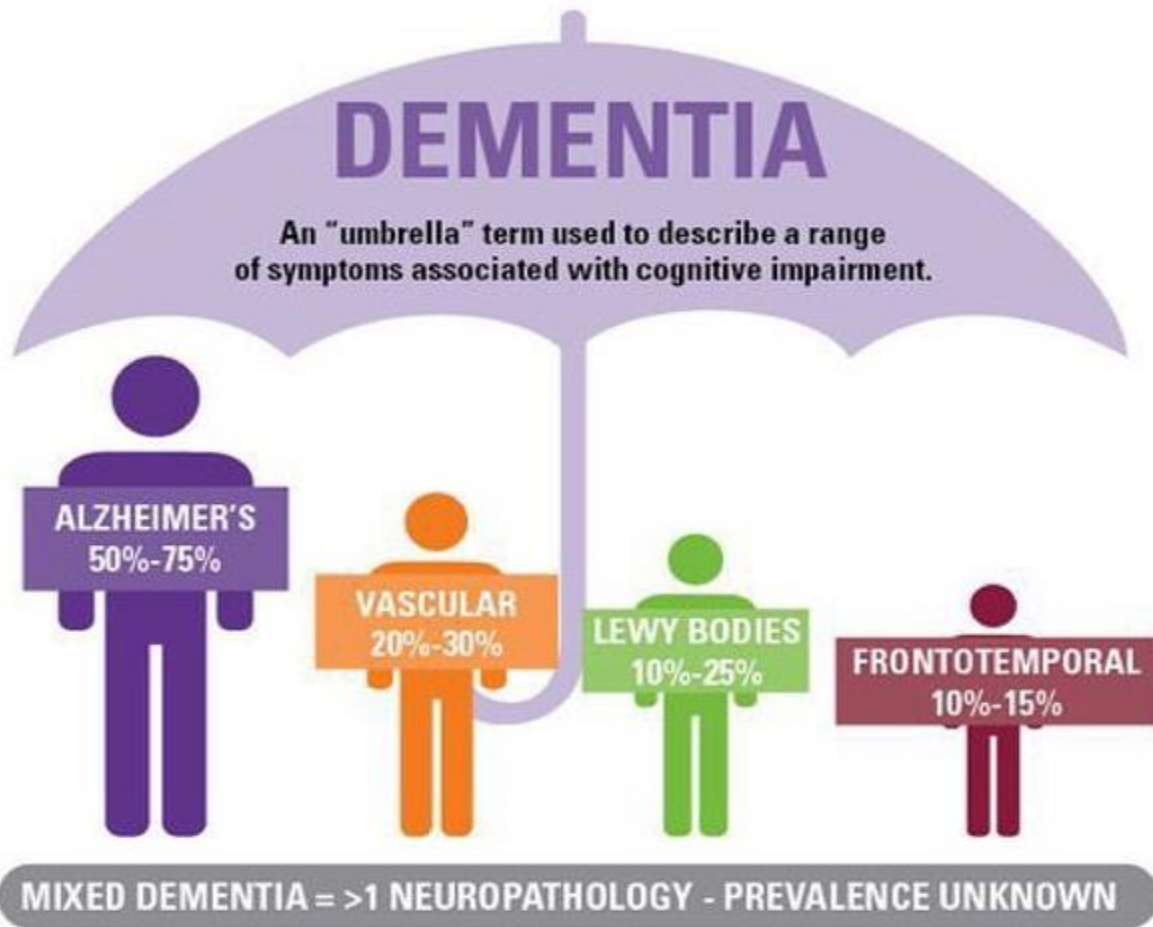
Alzheimer's
Deposits of the protein fragment beta-amyloid (plaques) and twisted strands of the protein tau (tangles)

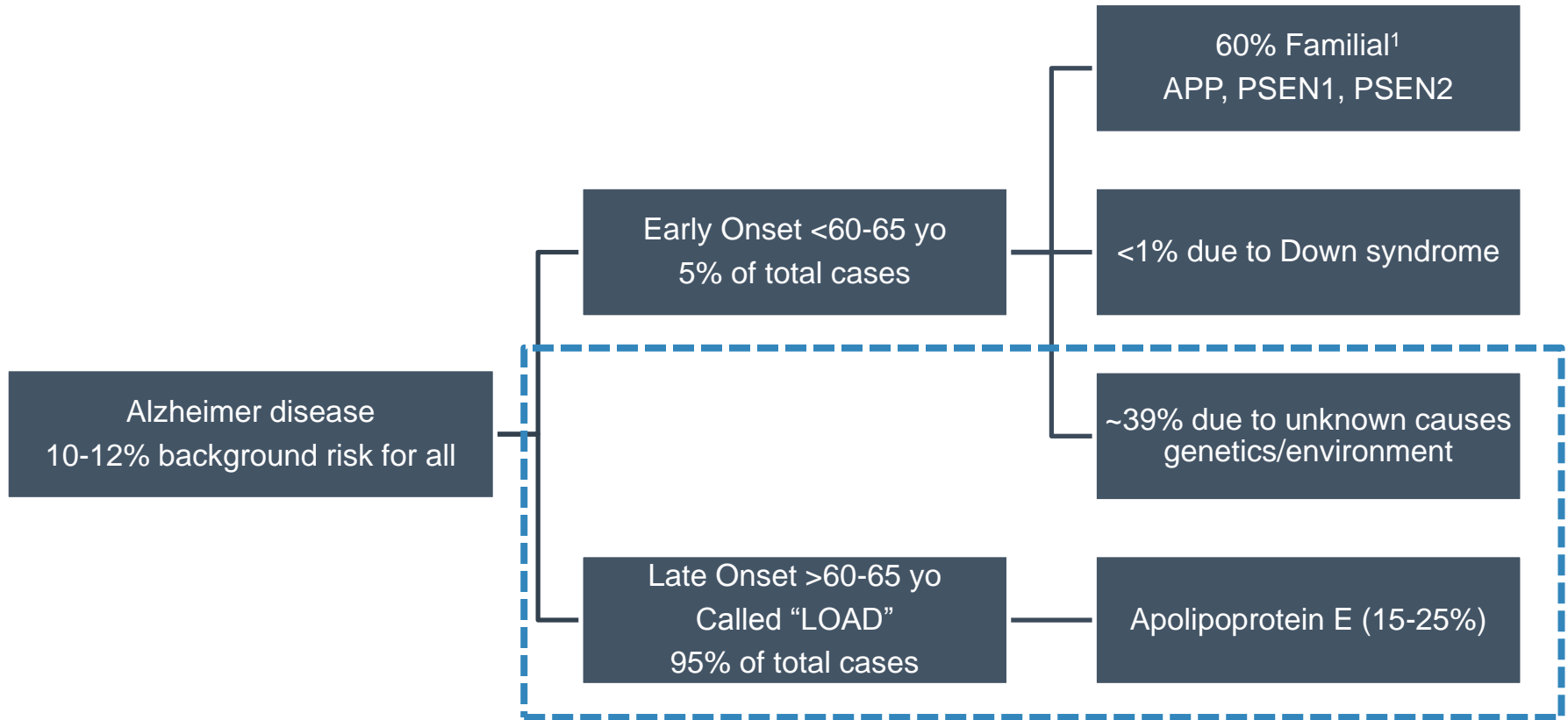**Vascular**
Occurs most commonly from blood vessel blockage

Lewy Bodies
Lewy bodies are abnormal aggregations (or clumps) of the protein alpha-synuclein

Frontotemporal
A mild to severe decrease in overall brain weight and atrophy of the frontal and temporal lobe

Asuragen®
Genetics

Alzheimer's Association. 2016 Alzheimer's disease facts and figures. Alzheimer's Dement. 2016 Apr;12(4):459-509.

Alzheimer's Disease Genetics

```
Alzheimer disease                    Early Onset <60-65 yo          60% Familial[1]
10-12% background risk for all       5% of total cases              APP, PSEN1, PSEN2

                                                                    <1% due to Down syndrome

                                                                    ~39% due to unknown causes
                                                                    genetics/environment

                                     Late Onset >60-65 yo
                                     Called "LOAD"
                                     95% of total cases             Apolipoprotein E (15-25%)
```

[1]Campion D. *et al*. Am J Hum Genet. 1999;65:664–70; Brickell KL, *et al*. Arch Neurol. 2006;63:1307–11.

Asuragen®
Genetics

3

Apolipoprotein E *(APOE)* Identified as AD Risk Factor

| Allele	Ɛ2	Ɛ3	Ɛ4
General Frequency	8.4%	77.9%	13.7%
AD Frequency	3.9%	59.4%	36.7%

- Principal cholesterol carrier in the brain
- Three alleles: Ɛ2, Ɛ3, and Ɛ4
  - Ɛ2 considered protective for AD
  - Ɛ4 associated with increased risk of AD

Farrer et al J*ama* 1997; 278 (16): 1349–56

APOE Alone Unable to Predict AD Risk

Overall Risk:

- Depends on combination of risk factors

- Presence of one or more Ɛ4 alleles does NOT mean AD

- Lifetime risk still associated with family history

  - Ɛ4 negative does not remove 2-3x increased risk

- Varies based on age, gender, and ethnicity

Asuragen®
Genetics

TOMM40: Missing Piece For AD Risk
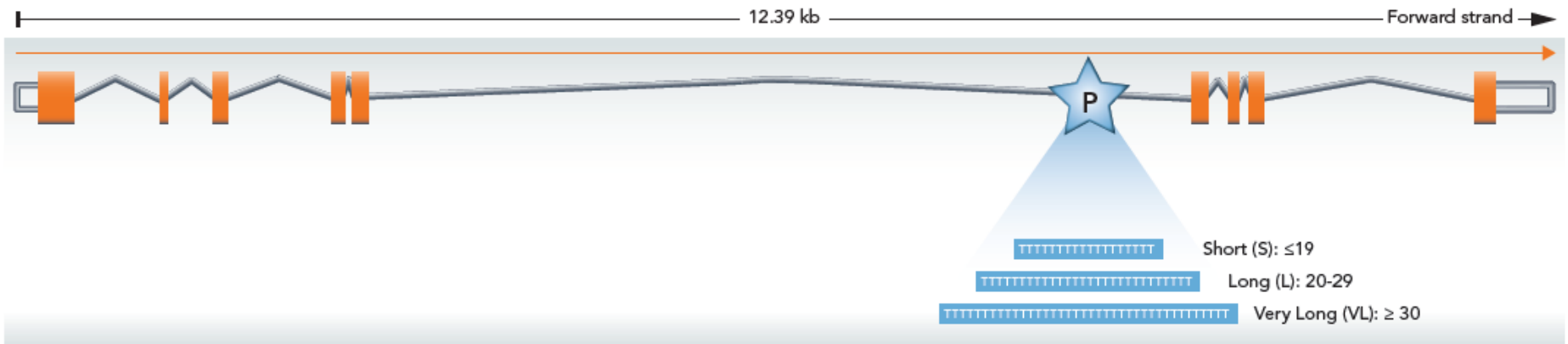
- Encodes Tom40, the translocase of the outer mitochondrial membrane pore subunit

- Mitochondrial Dysfunction: Early defect in LOAD pathogenesis and other age-related diseases

- Poly-T length repeats linked with LOAD

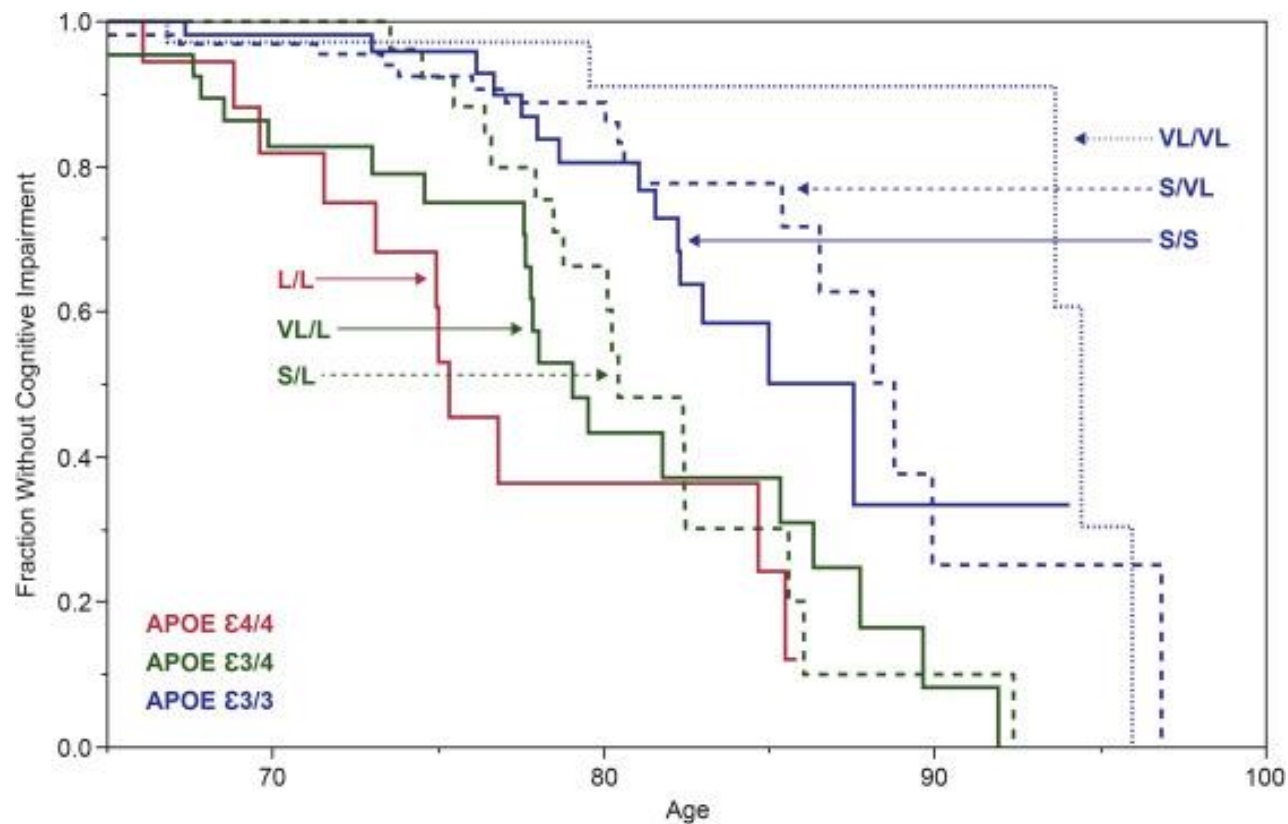# *APOE* + *TOMM40* Poly T Improves the PPV of AD Risk Prediction?
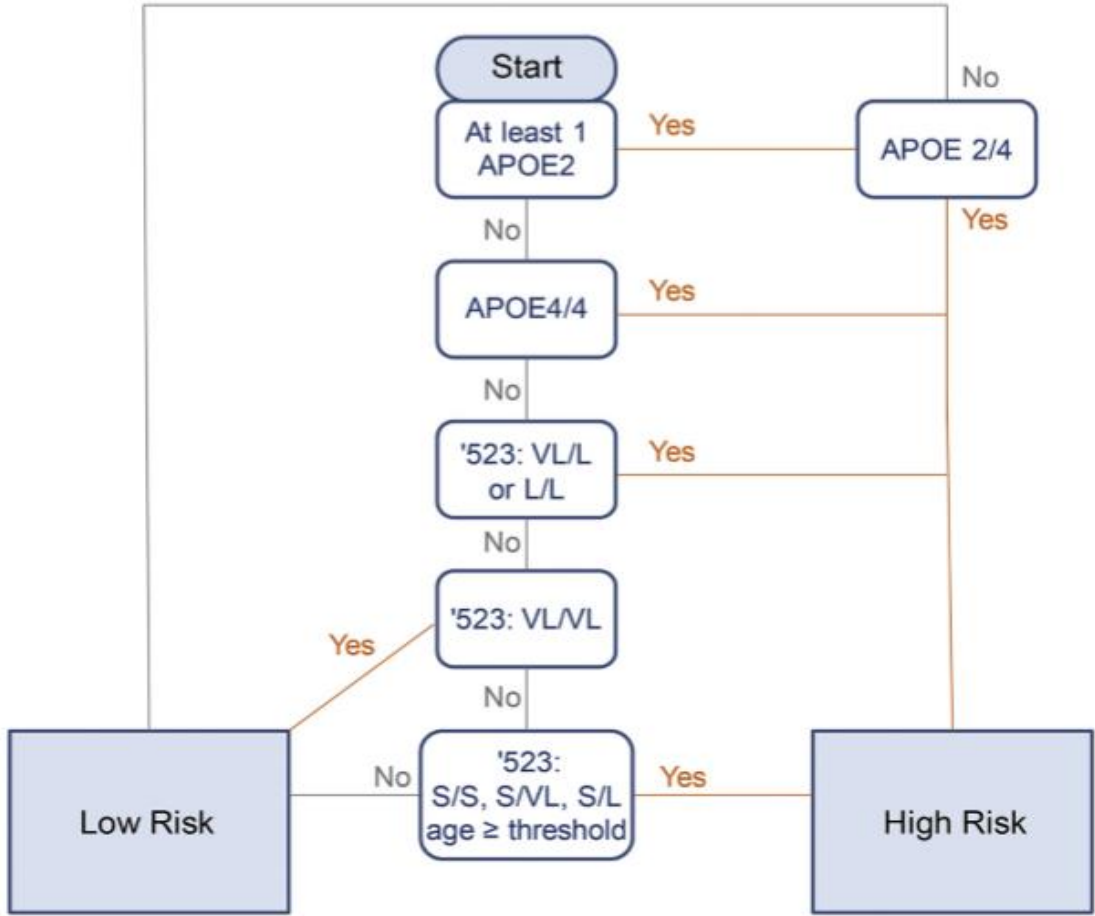
Short (S; ≤19Ts)
Long (L; 20–29 Ts)
Very Long (VL; ≥30Ts)

7

Association of *APOE/TOMM40* with AD Development
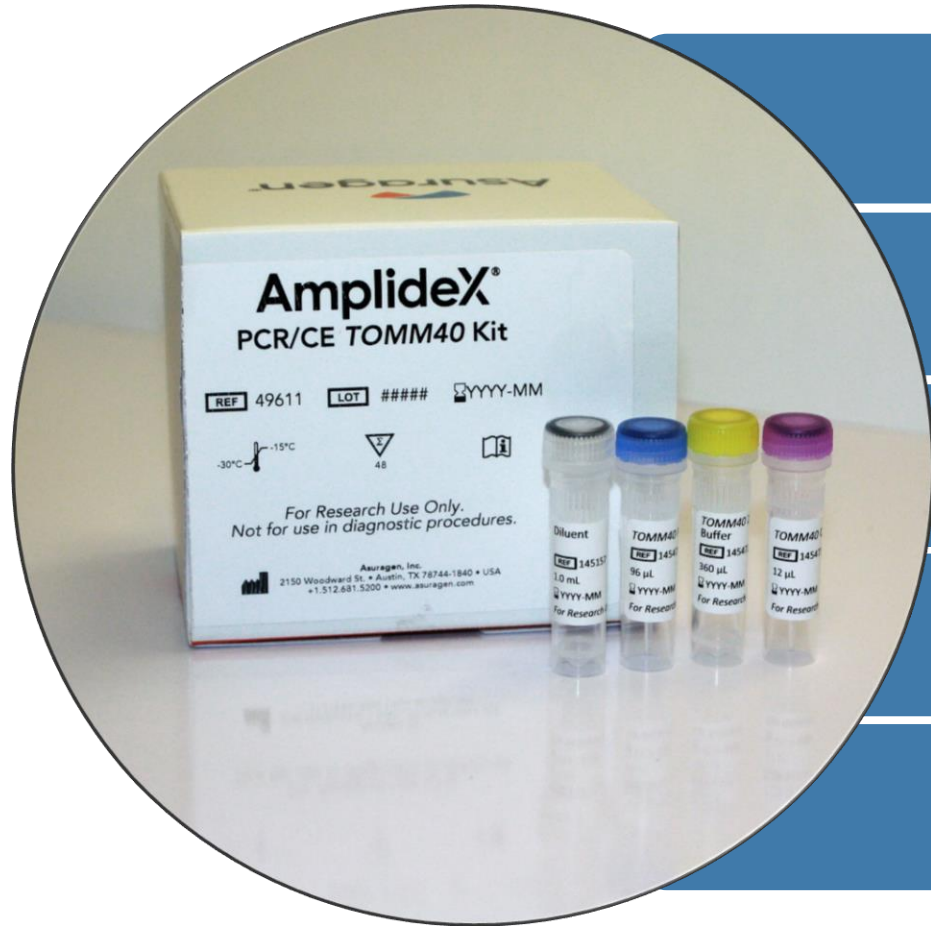
Challenges of *TOMM40* '523 Genotyping

AT Rich Region

- Challenging to amplify
- Hard to resolve (polymerase stutter and slippage)

Lack of Robust Standardized Assays

- Inaccurate results
- Inability to perform high throughput studies

Asuragen®
Genetics

Simple and Robust Test for Detection of *TOMM40* Poly T Repeats

Scalable run sizes

All-inclusive Reagents

Optimized TAT with Minimized HOT

PCR Calibrator for accurate sizing

Sizing accuracy down to 1 bp

Asuragen® Genetics

AmplideX® PCR/CE TOMM40 Kit
Enables Simple, Reliable TOMM40 Gene Research

Technology	Workflow	Performance

- Leverages proven AmplideX® chemistry for high homology sequences

- < 3 hr DNA to result
- 30 min Hands-on-Time
- Easy to interpret

- Repeatable
- Reproducible
- Accurate sizing w Single-Base Resolution

Asuragen®
Genetics

Simple workflow minimizes hands on time

Includes all PCR Reagents	**Detailed protocol guide**
• Calibrator for accurate sizing	• Master mix composition
• Primer mix	• PCR cycling conditions
• PCR enzyme and buffers	

Calibrator Enables Accurate Sizing

- Calculate mobility and correction factor (run or instrument specific)

- Use provided equation from protocol guide to calculate Poly T repeats
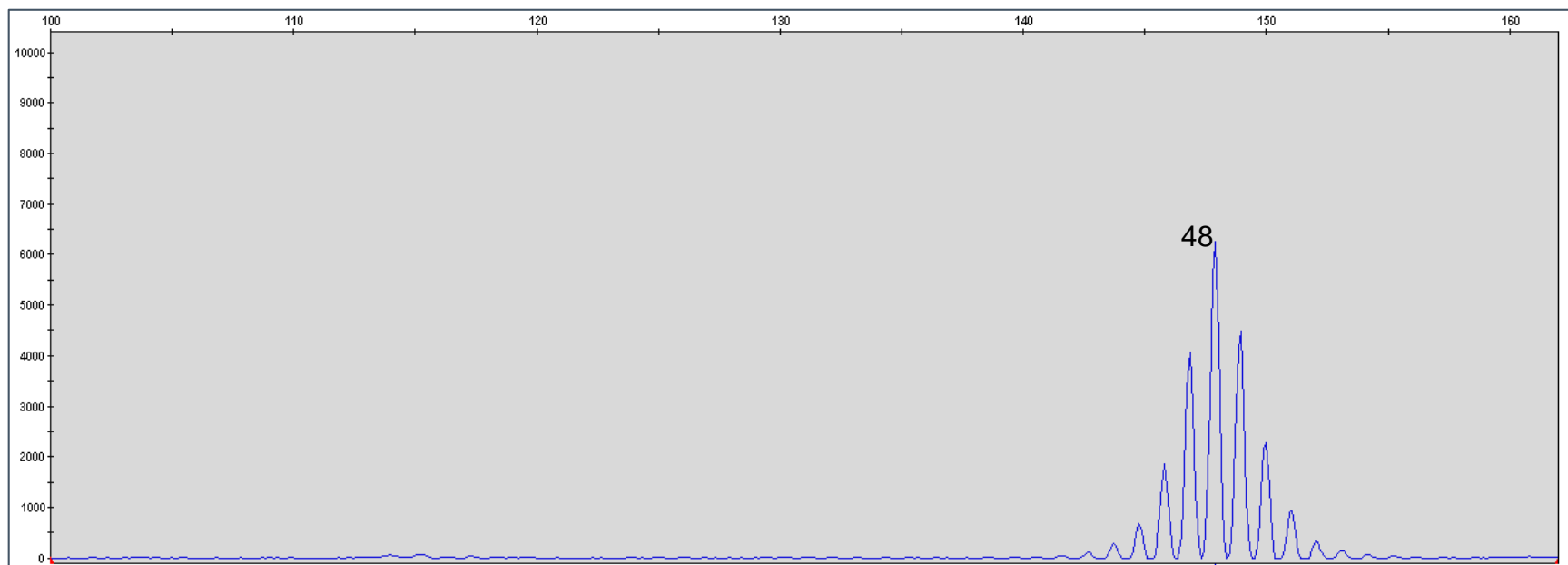

Asuragen *TOMM40* Calibrator
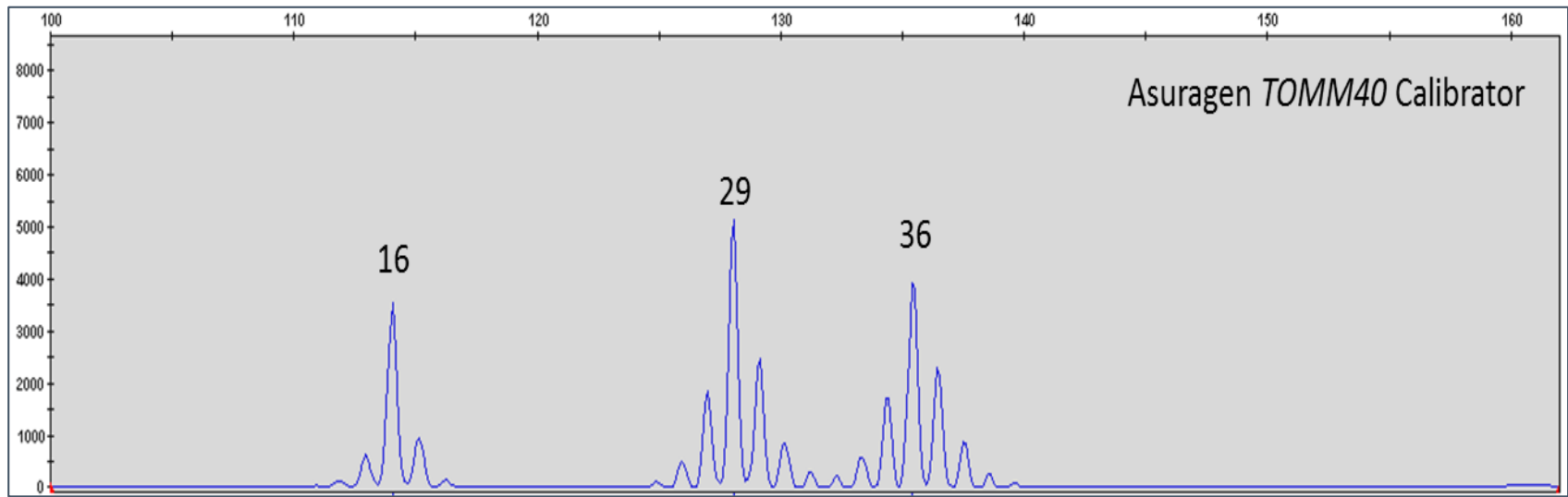
$$T_n = \frac{(CE\ Sizing - Intercept)}{Slope}$$
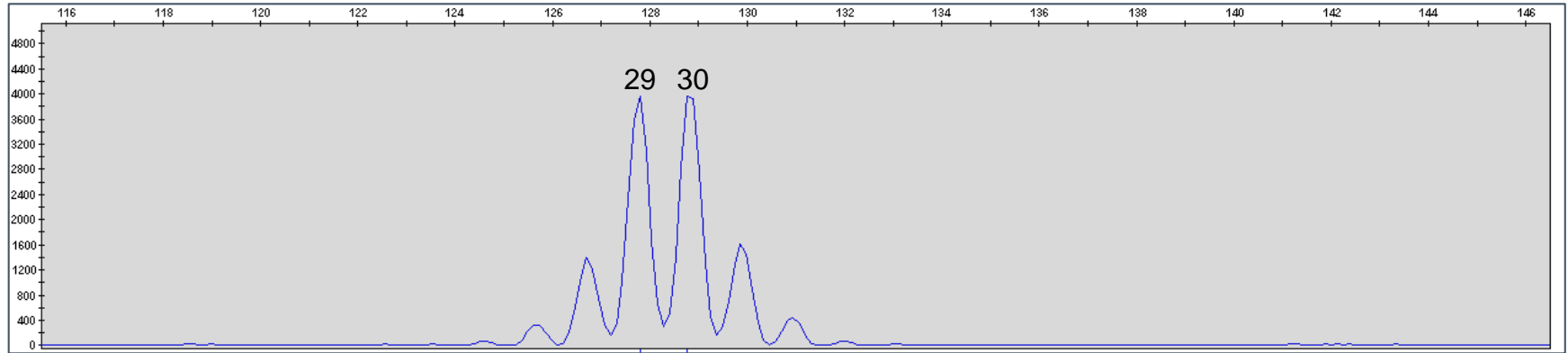
Configuration		C_0	m_0
3730, 3730xL	36 cm	96.92	1.07
3500, 3500xL	50 cm	96.96	1.07

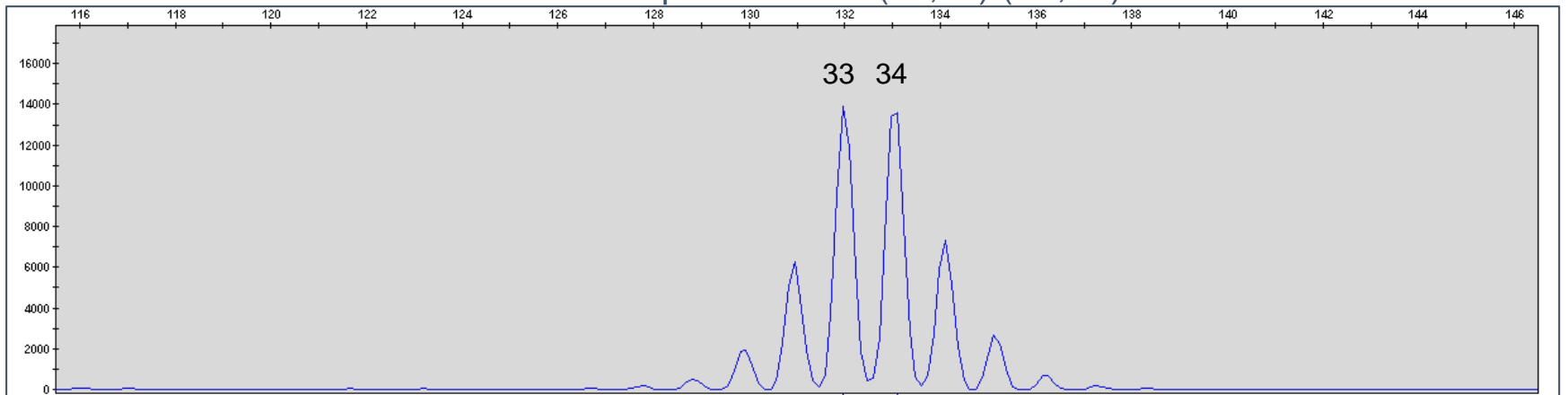# Accurate Sizing Enables Accurate Sample Categorization

A synthetic 48 mer was accurately sized

Single bp resolution allows ability to distinguish n,n+1 alleles

Coriell sample NA11325 (33,34) (VL,VL)

Compatibility with Different Sample Types

Sample Name	Blood	Buccal
F01	16/16	16/16
M02	16/34	16/34
M03	16/16	16/16
F04	16/22	16/22
F05	16/37	16/37
F06	16/29	16/29
M07	16/36	16/36
F08	16/16	16/16
M09	22/36	22/36
F10	16/38	16/38
M11	35/35	35/35
M14	16/36	16/36
M15	16/34	16/34
M18	16/34	16/34

*6 females and 8 males with matched blood and buccal samples

Asuragen®
Genetics

Inter-Operator and Multi-Day Runs Demonstrate Assay Reproducibility

Operator 1
Day 1

Operator 2
Day 2

Operator 3
Day 3
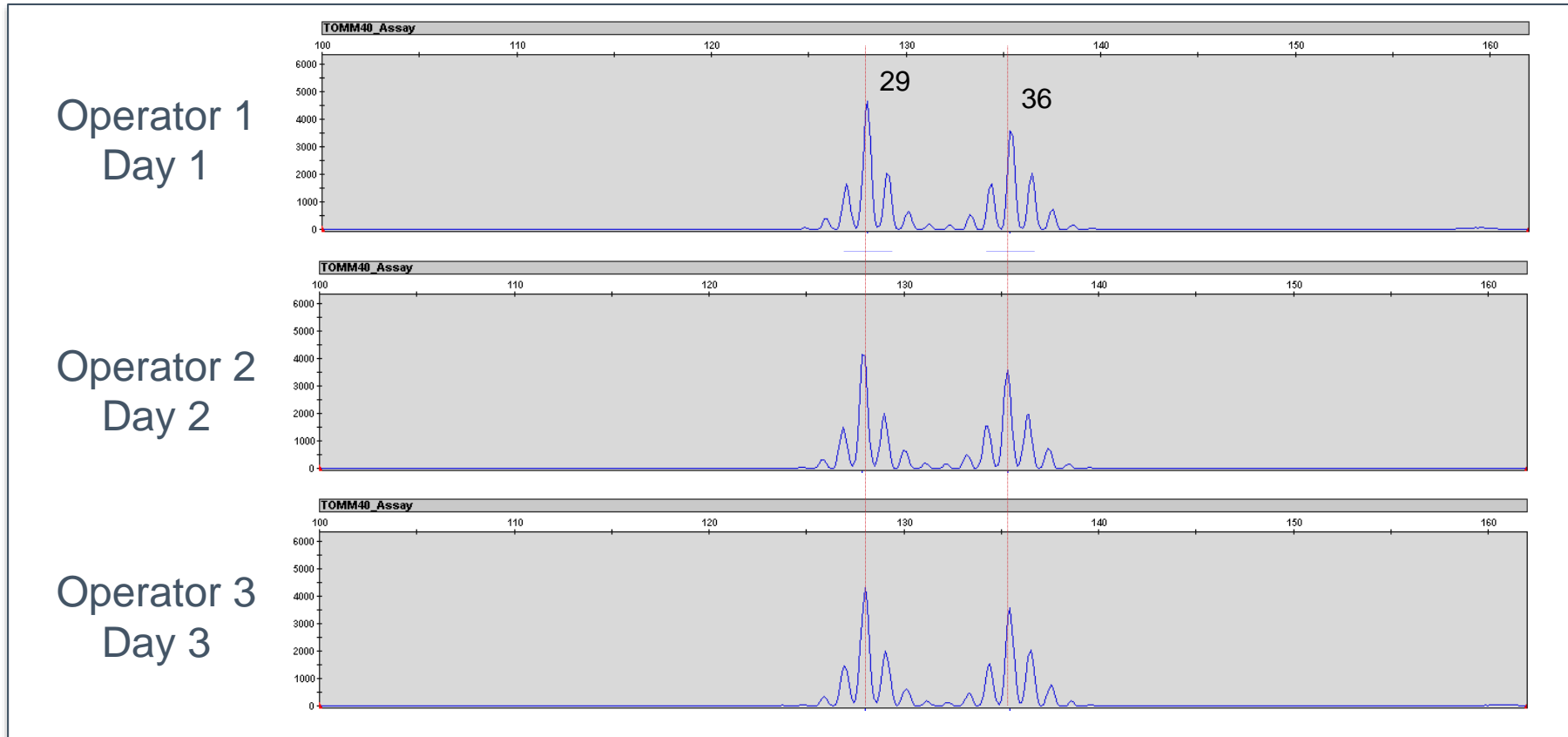
Sample: L/VL, 29/36 alleles

Assay Tolerates Wide Range of DNA Input

Recommended assay input range = 5-100 ng
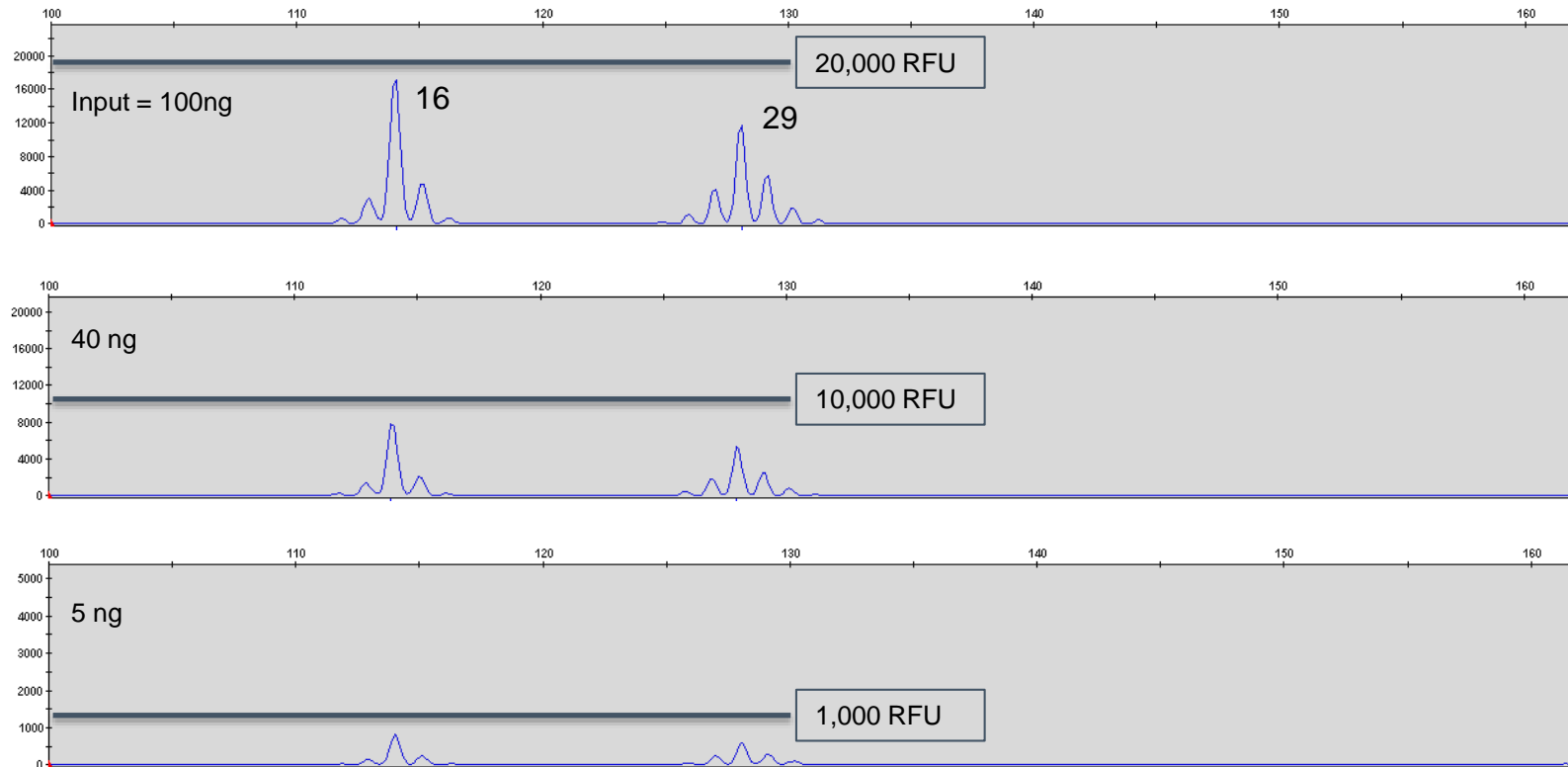
Simplified Workflow, Reliable Performance

Reducing Complexity	Optimizing Workflow	Quality Performance
Reagents and protocol enables easy assay set up	<30 mins of hands on time	1 bp Resolution
Clear data interpretation	<3 hrs of TAT	Reliable and accurate results

Asuragen®
Genetics