From Bench to Bedside: Translational Genomics

Rachid Karam, M.D. Ph.D.
Associate Director, Translational Genomics Lab

Disclosure: Rachid Karam is an employee of Ambry Genetics.

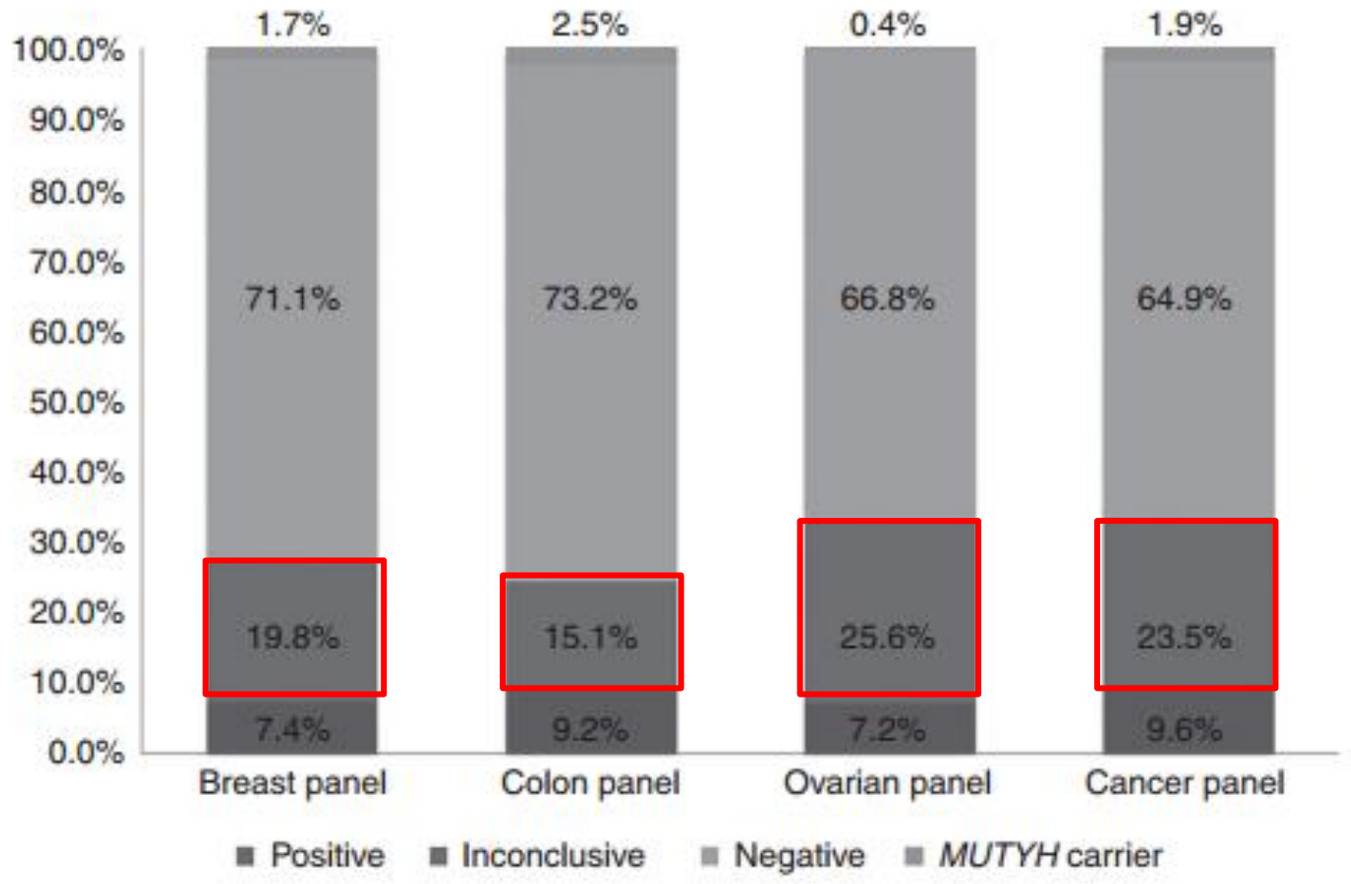
Ambry Genetics® | Overview

- The Problem: Variants of Unknown Significance
- RNA Studies
- Duplication Breakpoint Analysis

Translational Genomics is the field of genetics aiming at understanding the *clinical significance of genomic variance*.

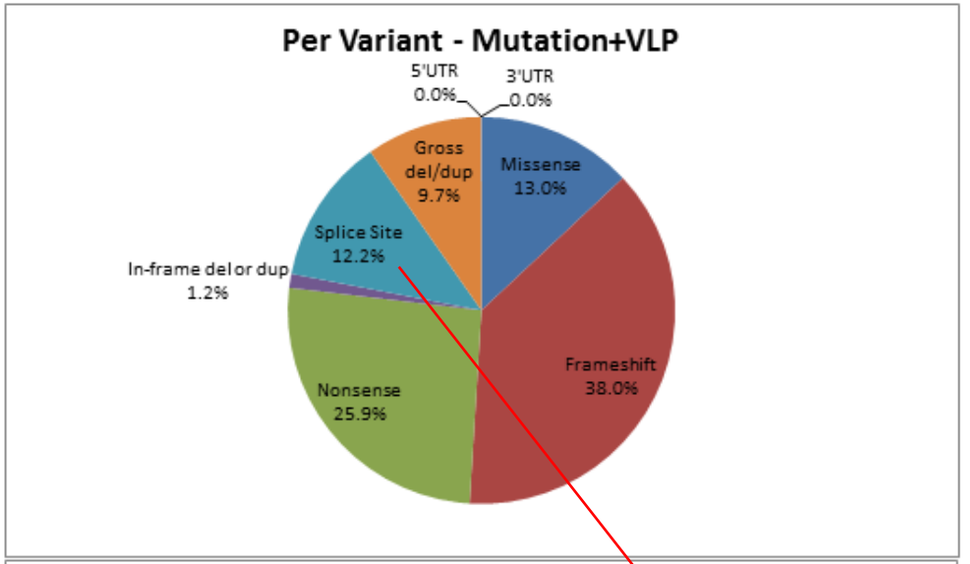
Variants of Unknown Significance - VUS

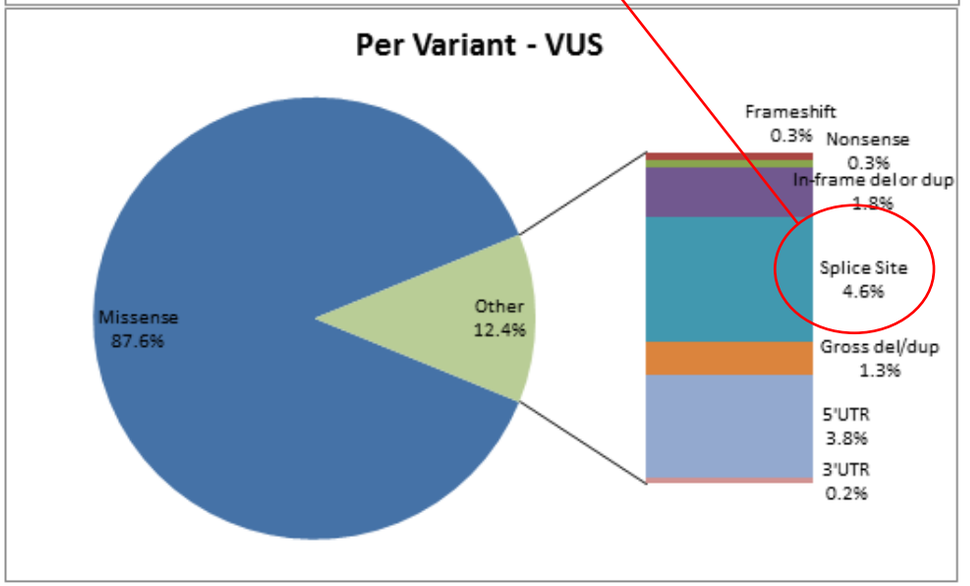
Percentage of positive, inconclusive, and negative results by panel



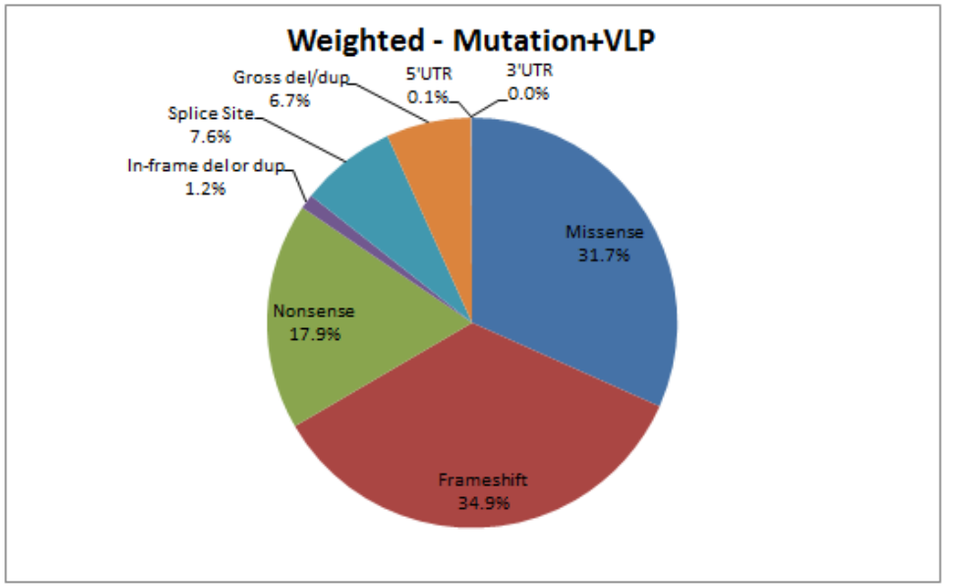
LaDuca *et al*. 2014

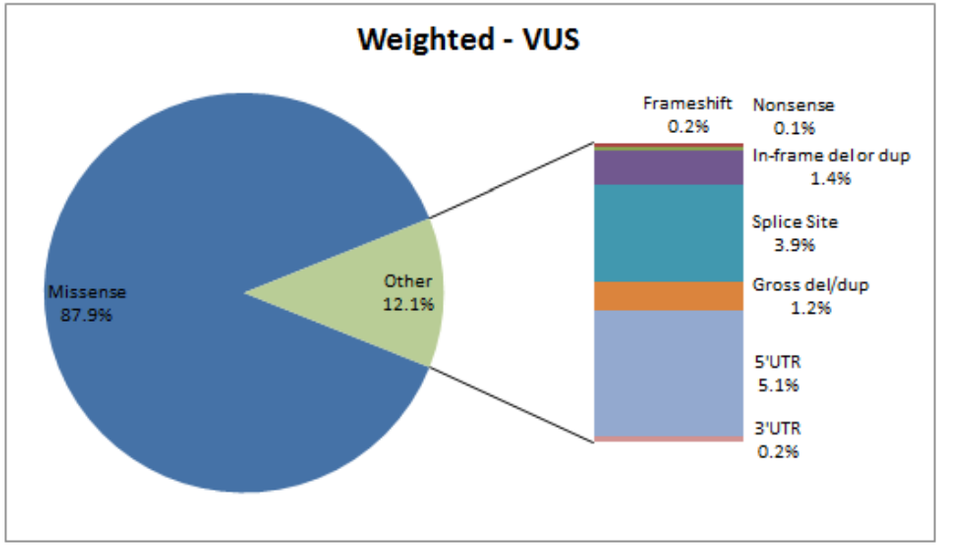
Ambry Genetics | Lessons from over 1,000,000 Tumor Suppressor Alleles sequenced

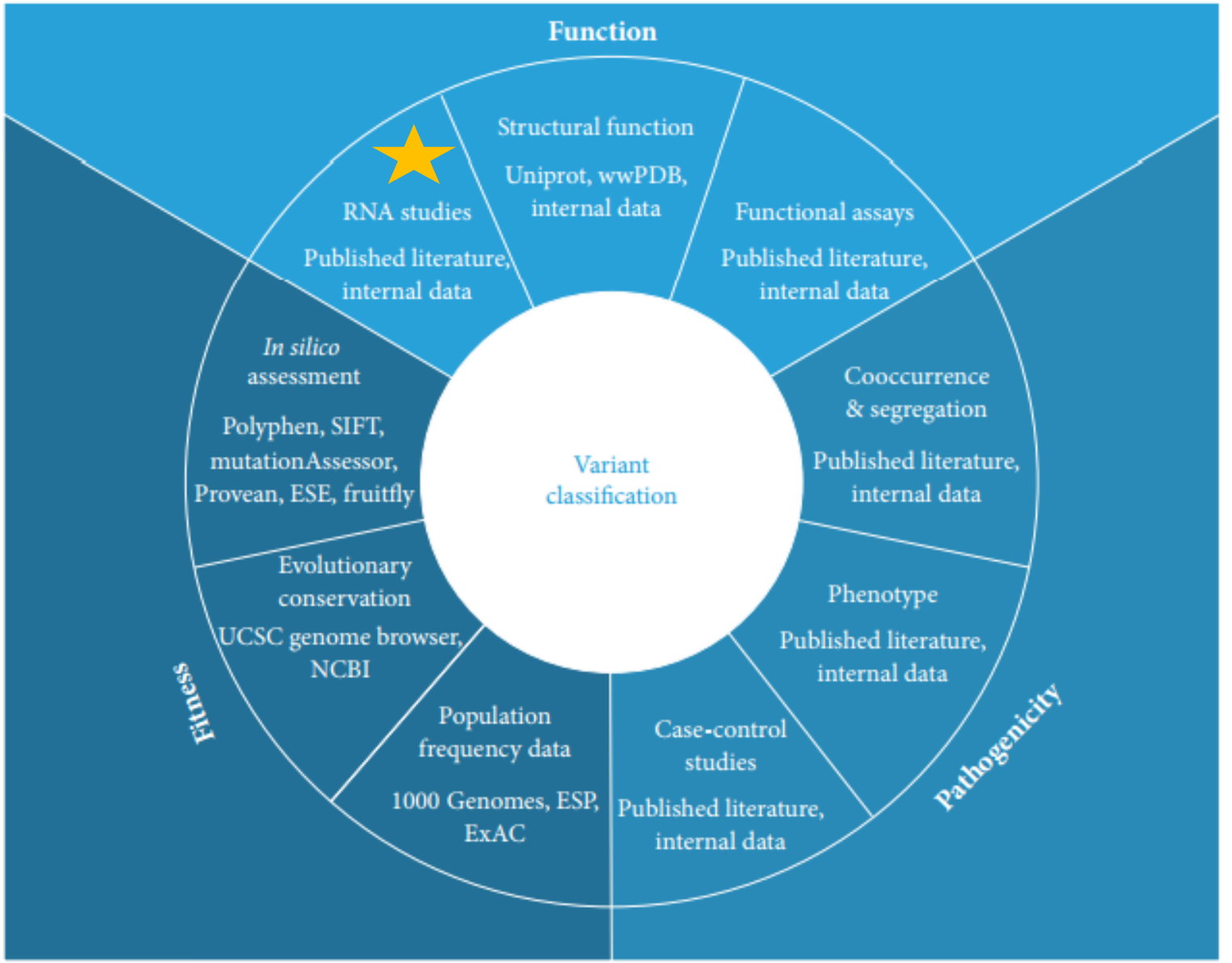




Here are the distributions of the type of mutations per variant, weighted to frequencies of the variants.

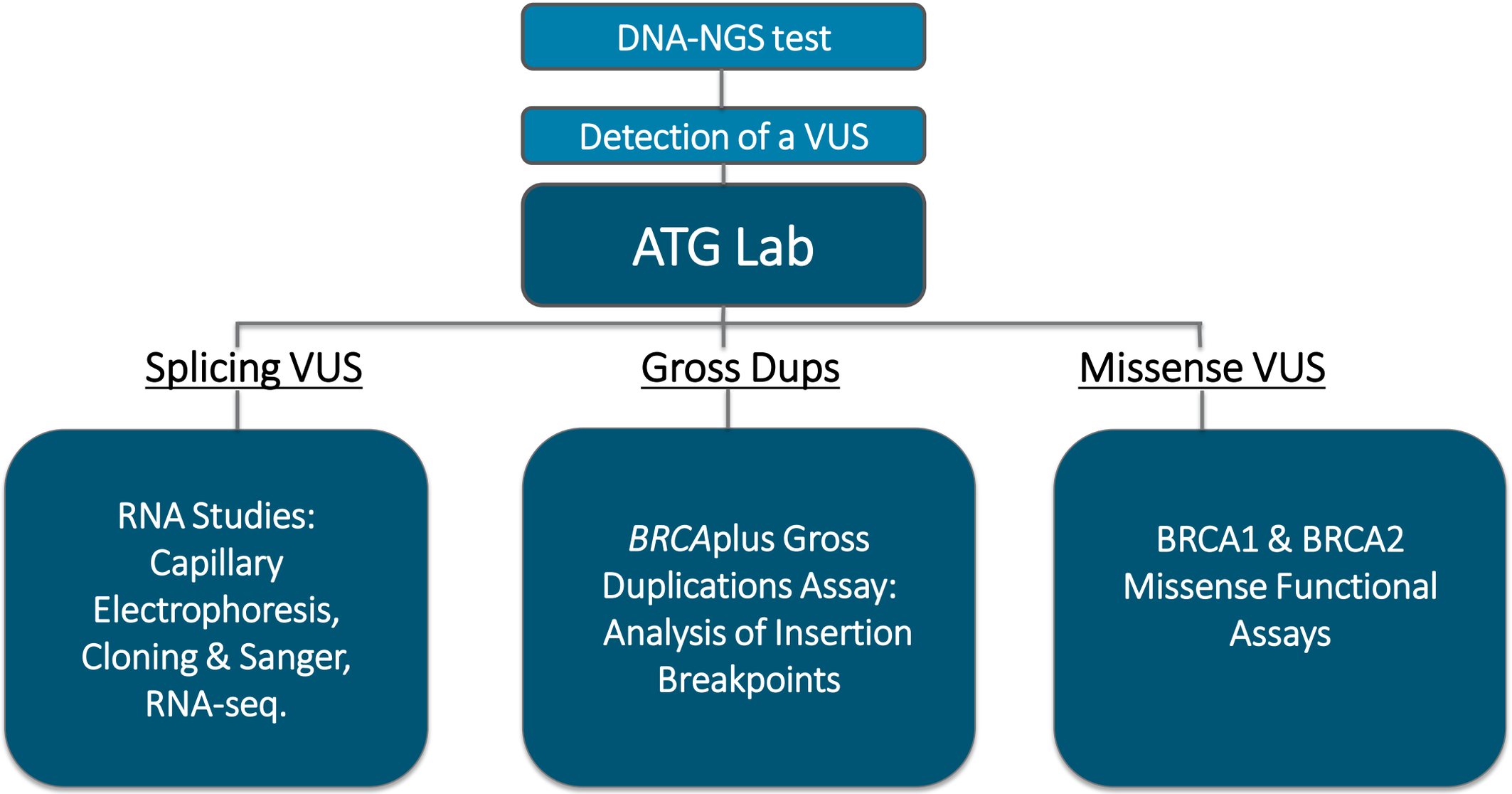




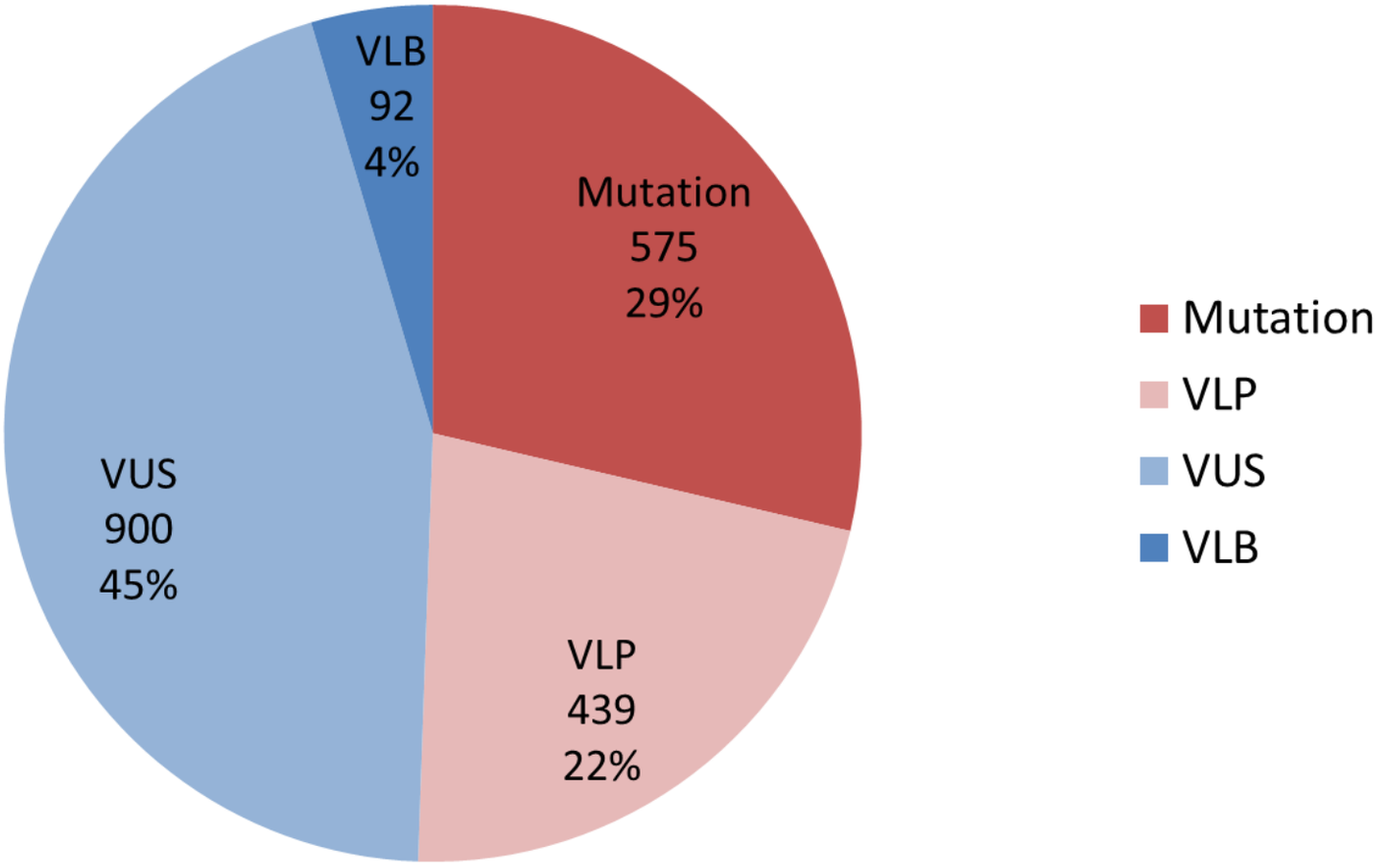


Pesaran *et al*. 2016

Ambry Translational Genomics Lab (*ATG Lab*)



Ambry Genetics® | Ambry Cohort - Classification of Splicing Variants (n=2,006)



Ambry Classification of Splicing Variants: Current Criteria

Pathogenic

1) Functionally-validated splicing mutation (demonstrating abnormal splicing leading to transcripts that (i) are out-of-frame and subject to nonsense mediated mRNA decay or (ii) coding an abnormal protein product affecting a functional domain).

2) Variants at IVS±1, IVS ±2, Exon last nucleotide **AND** more features suggestive of pathogenicity (i.e. RNA studies, clinical data)

Likely Pathogenic

3) Variants at IVS±1 or IVS±2, that are untested for splicing aberrations in vitro - without other features suggestive of pathogenicity

4) In-frame splicing not in a known protein functional domain

VUS

Others (First/Last nt, novel splice sites, synonymous with abnormal *in silico*, 5'SS+3,4,5 and 3'SS-3,4,5)

These will be covered in 1 above i.e. require additional evidence (RNA studies, clinical data, *in silico*)

RNA Studies Protocol & Validation

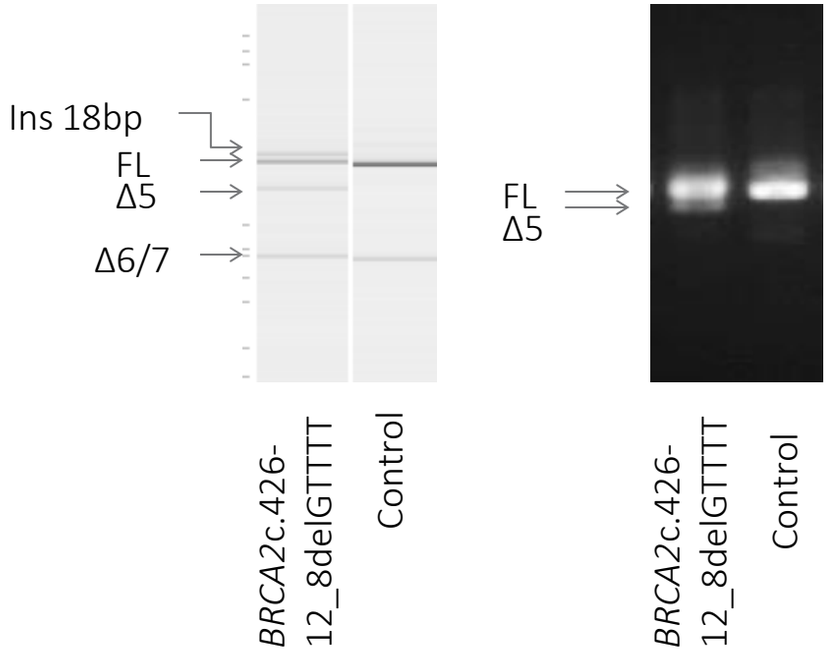
Clinical Chemistry 60:2
341–352 (2014)

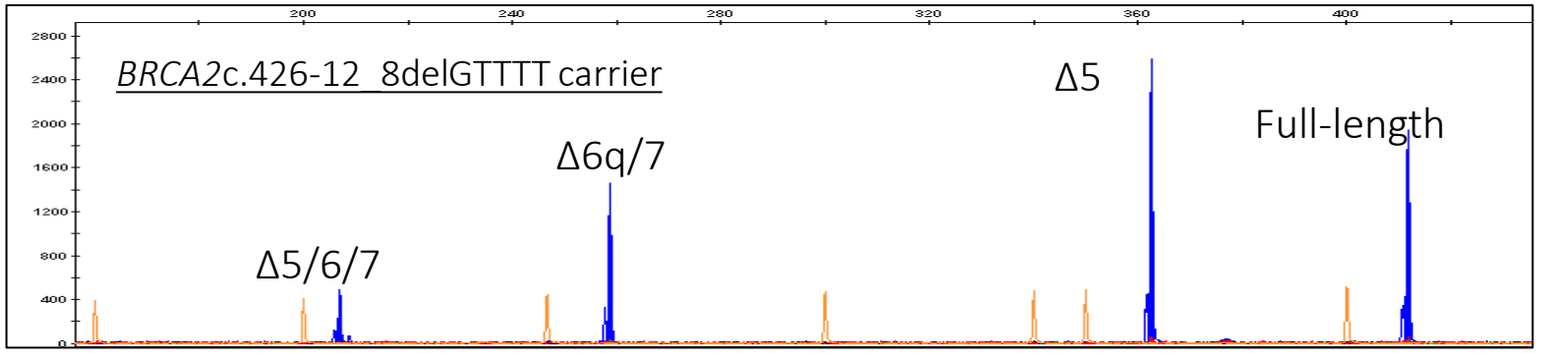
Molecular Diagnostics and Genetics

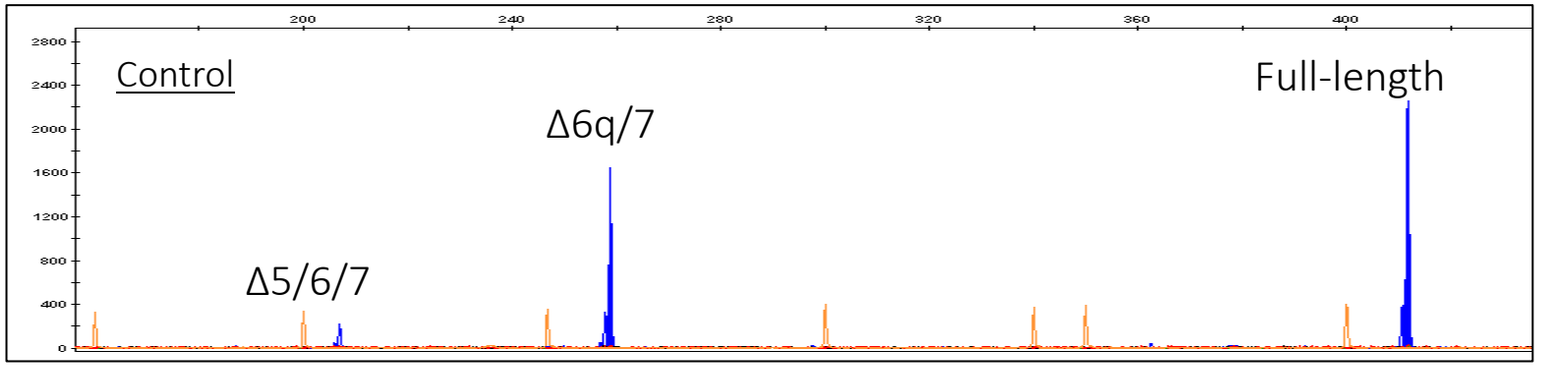
Comparison of mRNA Splicing Assay Protocols across Multiple Laboratories: Recommendations for Best Practice in Standardized Clinical Testing

Phillip J. Whiley,[1,2] Miguel de la Hoya,[3] Mads Thomassen,[4] Alexandra Becker,[5,6] Rita Brandão,[7] Inge Sokilde Pedersen,[8] Marco Montagna,[9] Mireia Menéndez,[10] Francisco Quiles,[10] Sara Gutiérrez-Enríquez,[11] Kim De Leeneer,[12] Anna Tenés,[11] Gemma Montalban,[11] Demis Tserpelis,[7] Toshio Yoshimatsu,[13] Carole Tirapo,[14] Michela Raponi,[15] Trinidad Caldes,[3] Ana Blanco,[16] Marta Santamariña,[17] Lucia Guidugli,[18] Gorka Ruiz de Garibay,[3] Ming Wong,[19] Mariella Tancredi,[20] Laura Fachal,[16] Yuan Chun Ding,[21] Torben Kruse,[4] Vanessa Lattimore,[22] Ava Kwong,[23] Tsun Leung Chan,[23] Mara Colombo,[24] Giovanni De Vecchi,[24] Maria Caligo,[19] Diana Baralle,[15] Conxi Lázaro,[10] Fergus Couch,[17] Paolo Radice,[24] Melissa C. Southey,[18] Susan Neuhausen,[21] Claude Houdayer,[14] Jim Fackenthal,[13] Thomas Van Overeem Hansen,[25] Ana Vega,[16] Orland Diez,[11] Rien Blok,[7] Kathleen Claes,[12] Barbara Wappenschmidt,[5,6] Logan Walker,[22] Amanda B. Spurdle,[1] and Melissa A. Brown[2]
on behalf of the ENIGMA consortium

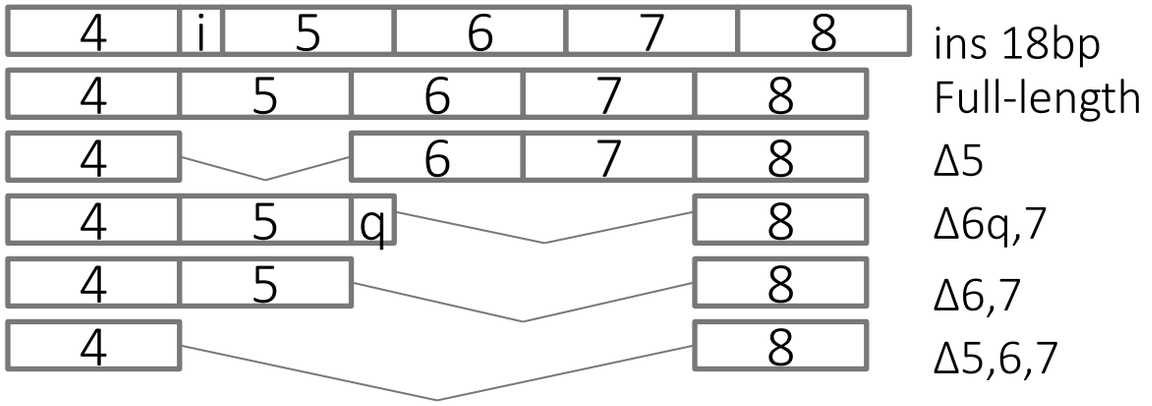
Digital gel visualisation (left), agarose gel electrophoresis (centre) and capillary EP (right) comparison for analysis of *BRCA2:* c.426-12_8delGTTTT. Capillary EP (CE) was the superior technique. Sequencing is necessary to characterize the transcripts (bottom).





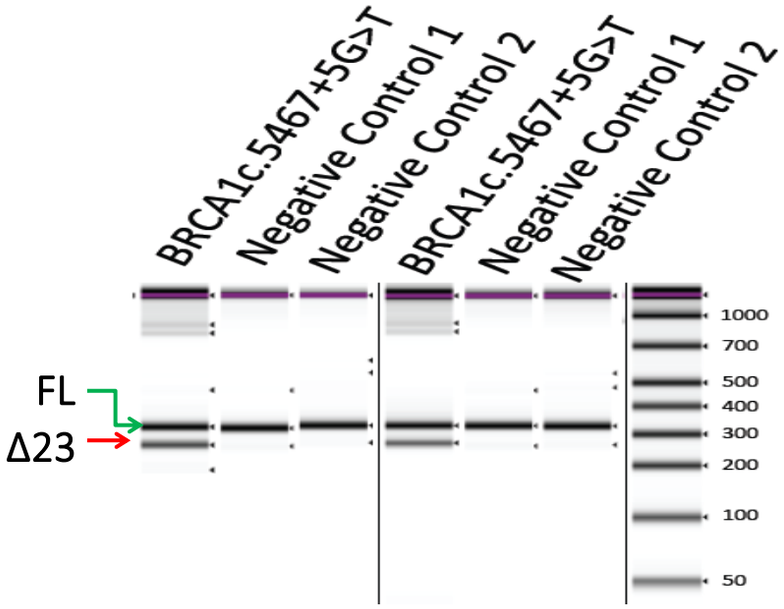


Schematic of Sanger characterized mRNAs:

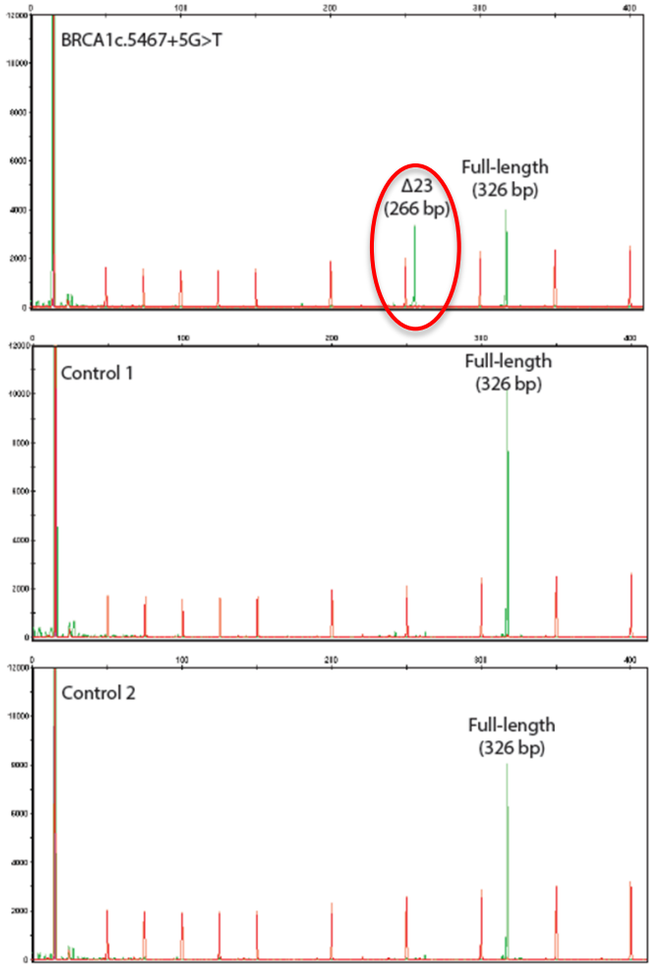


RNA Studies Validation:BRCA1c.5467+5G>T

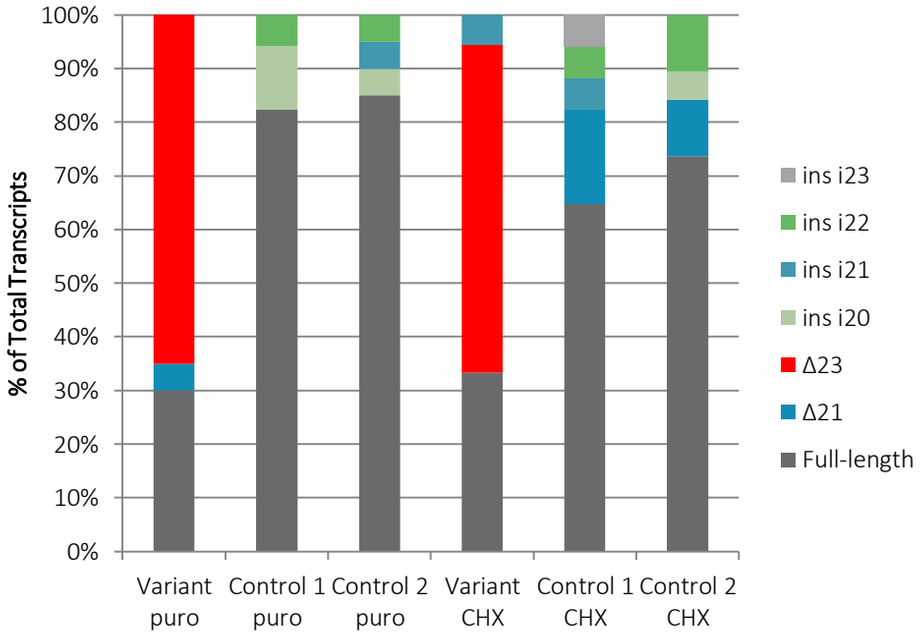
Gel Analysis



Capillary Electrophoresis



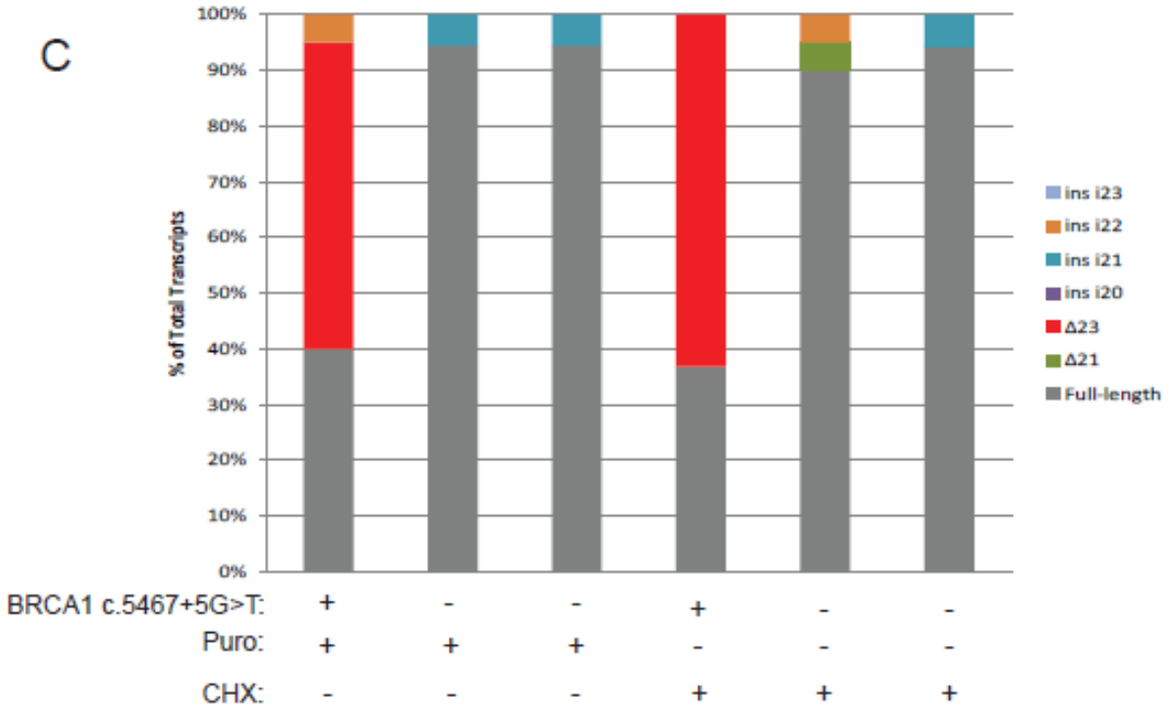
Cloning-Sanger



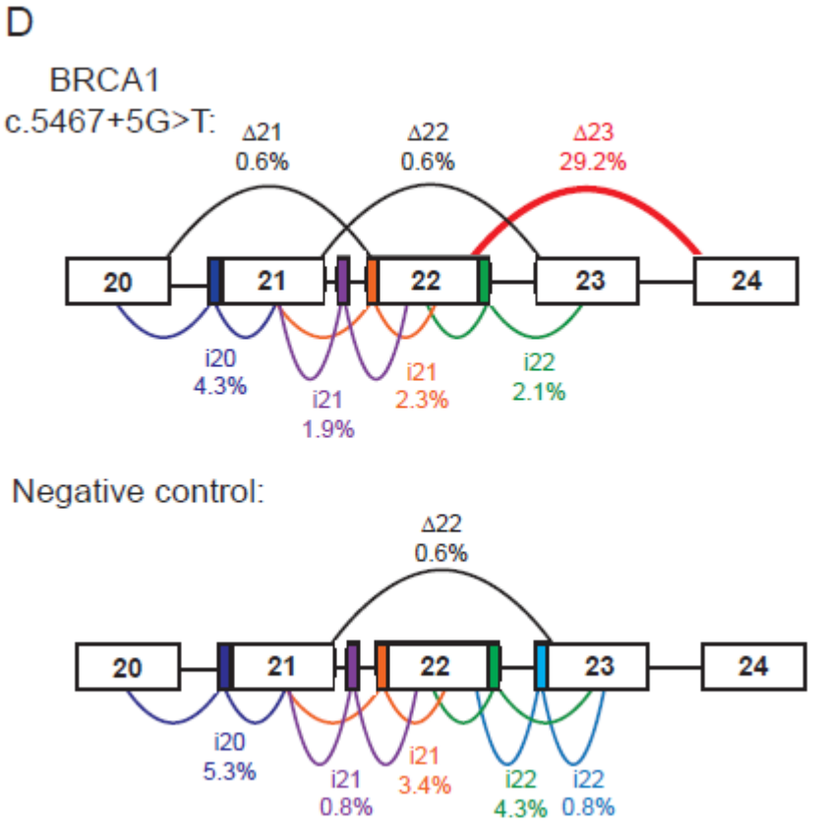
Ambry Genetics®

RNA Studies Validation:BRCA1c.5467+5G>T

Cloning-Sanger

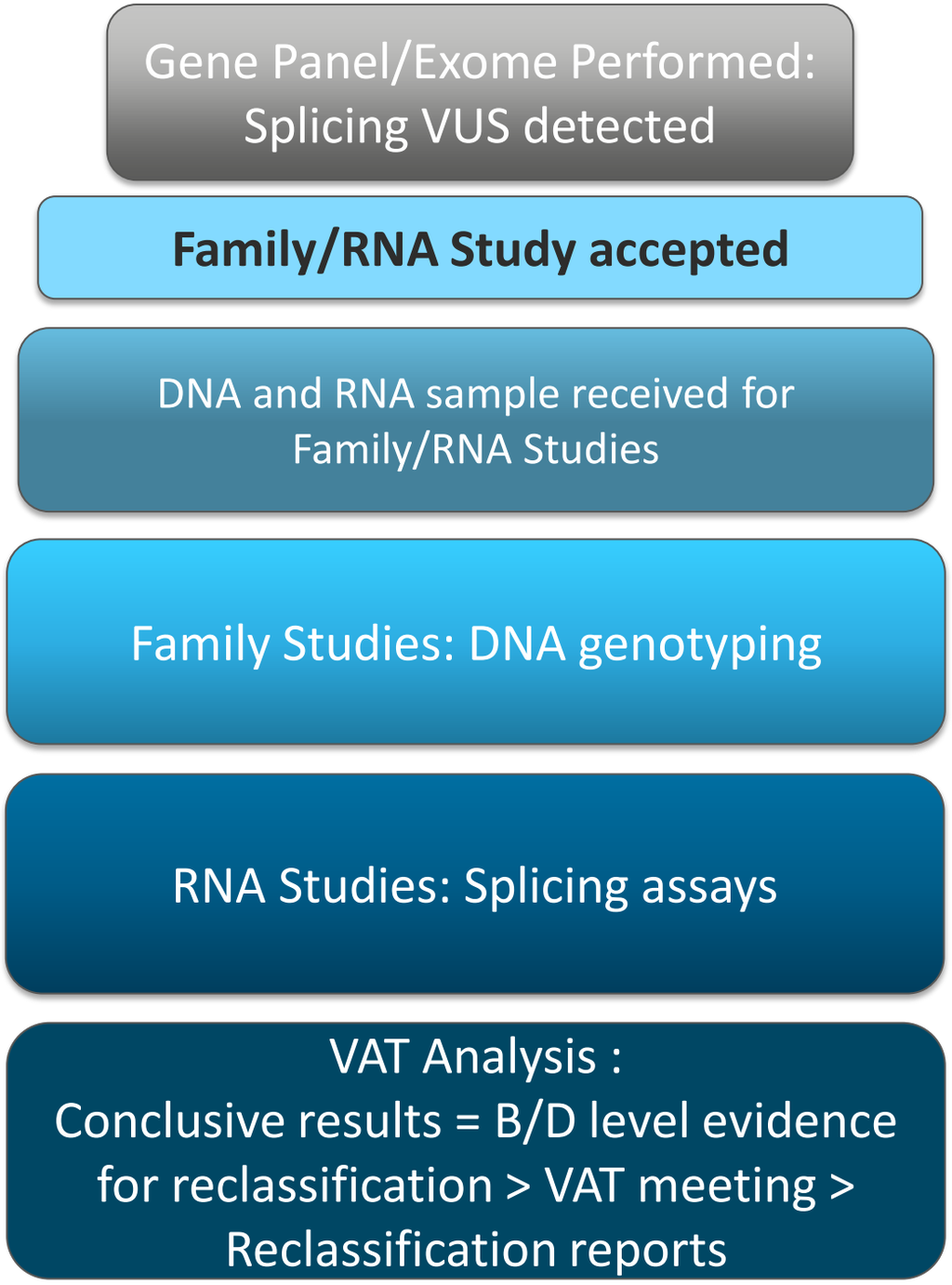


NGS



Ambry Genetics®

RNA Studies Workflow



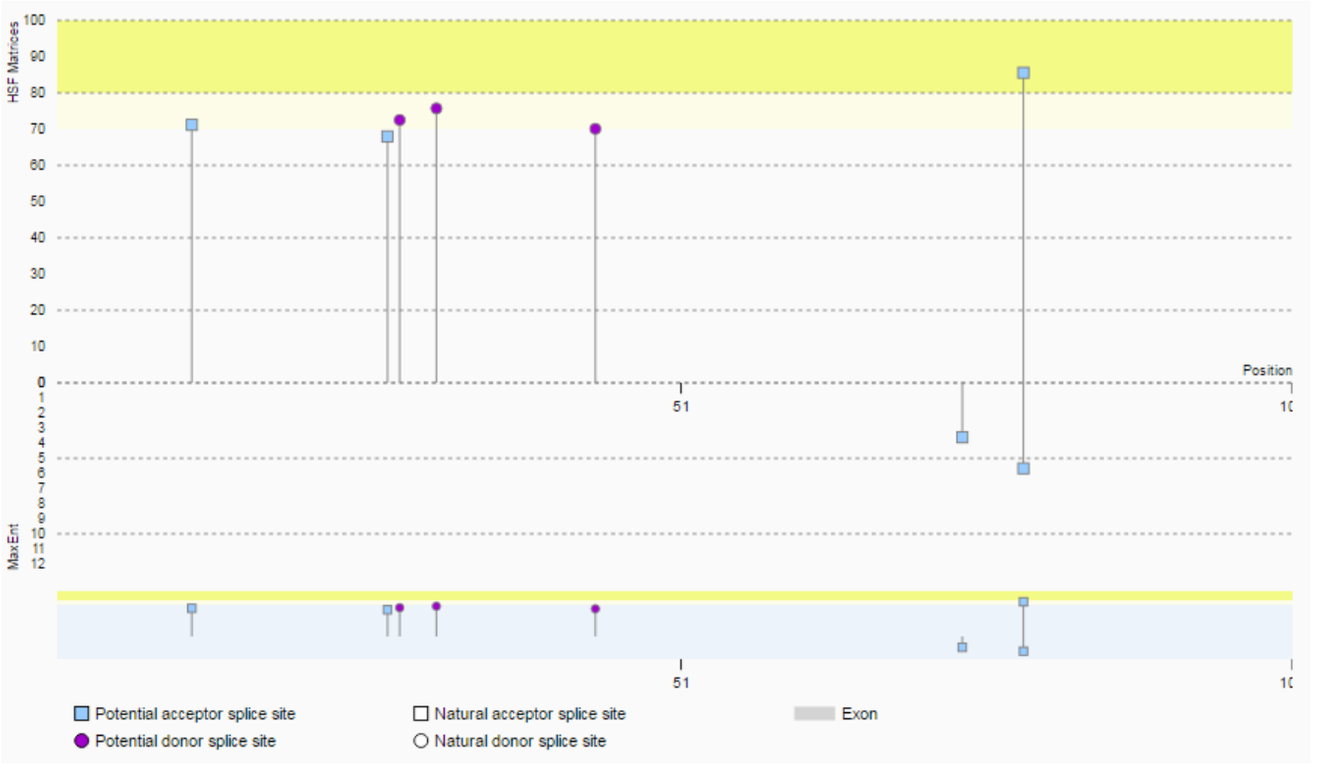
Ambry Genetics™

BRCA1 c.5152+5G>T

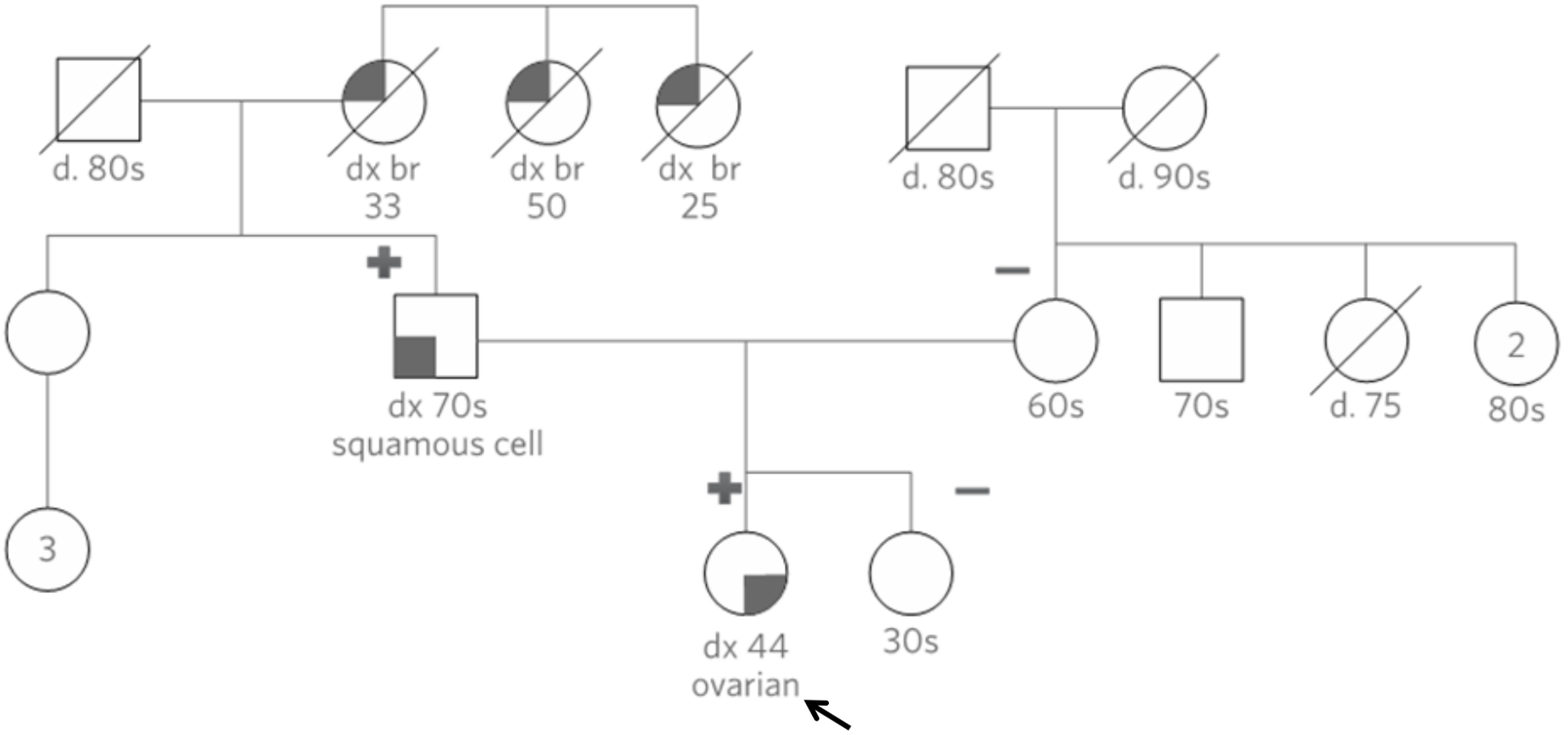
VUS to VLP

Ambry Genetics™

Ambry Genetics®

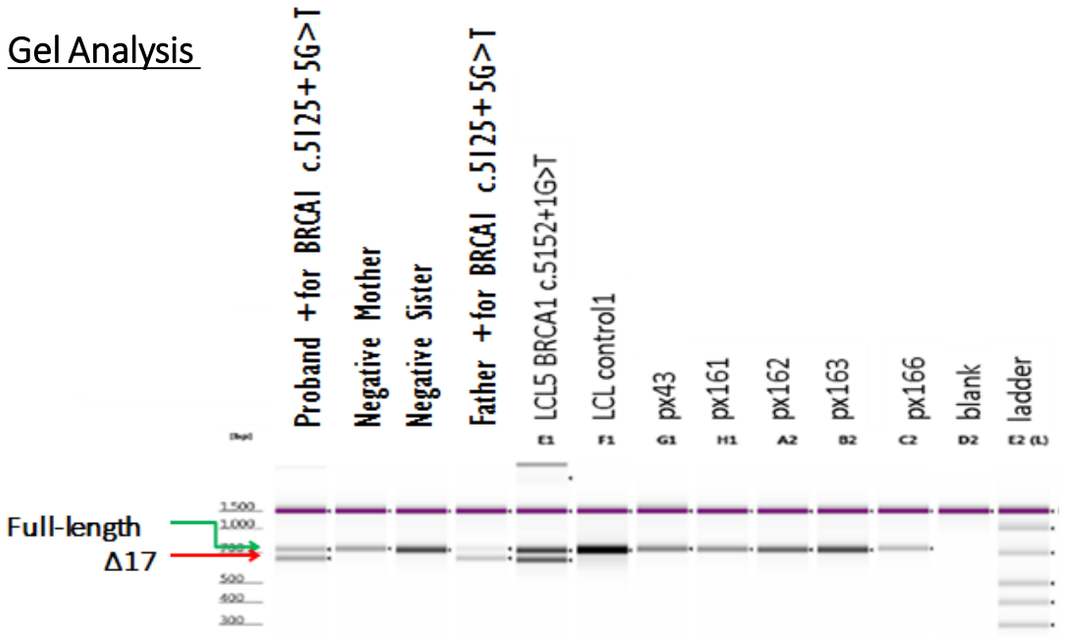


Ambry Genetics | Family Studies - BRCA1 c.5152+5G>T

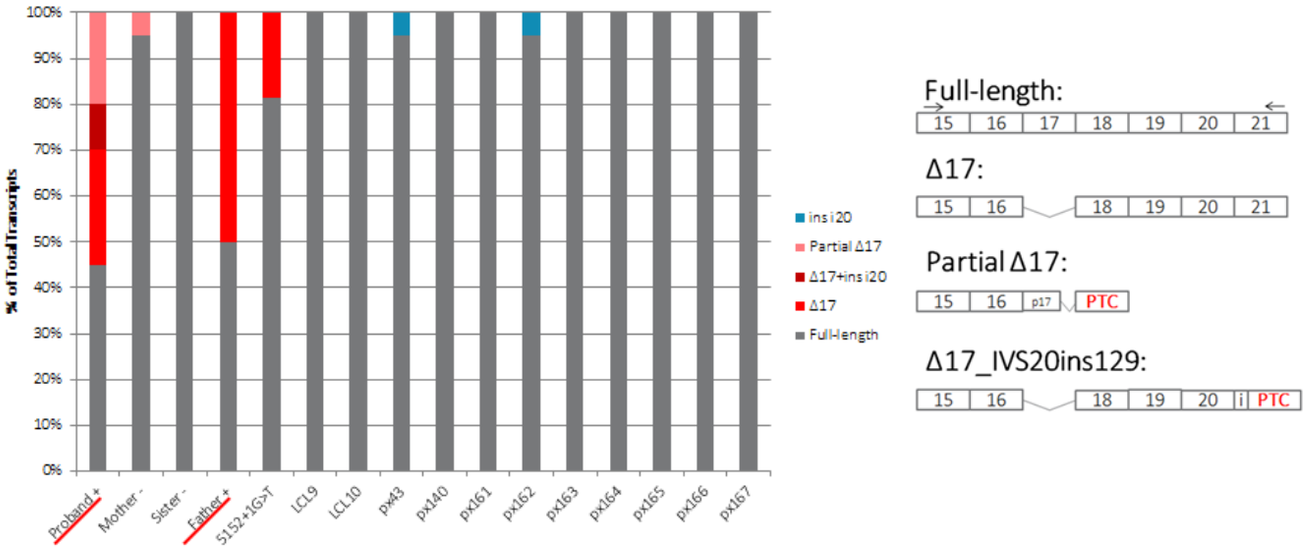


RNA Studies Reclassification VUS to VLP: BRCA1 c.5152+5G>T

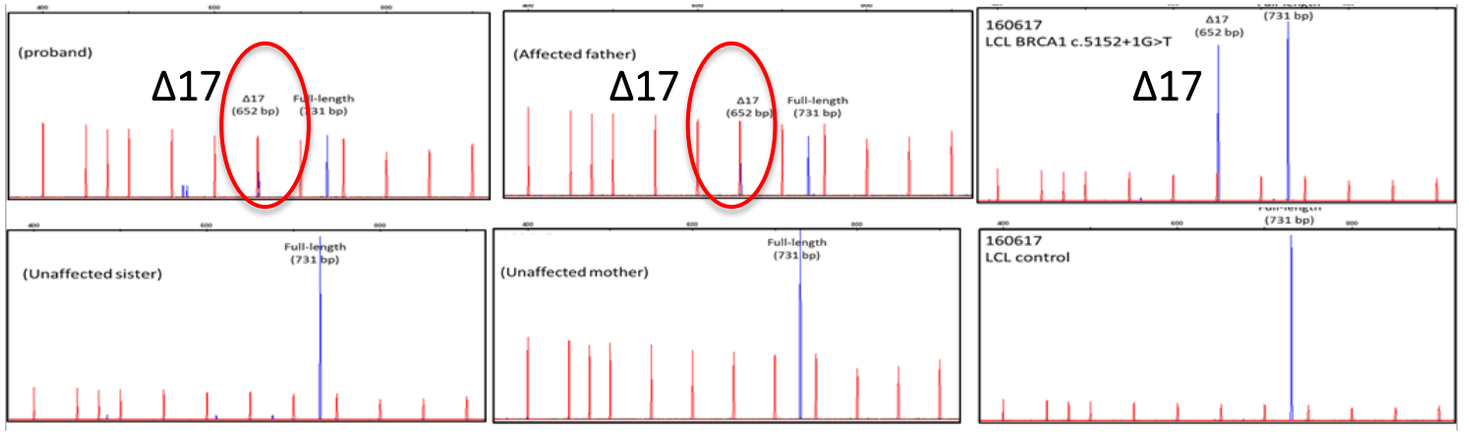
Gel Analysis



Cloning-Sanger



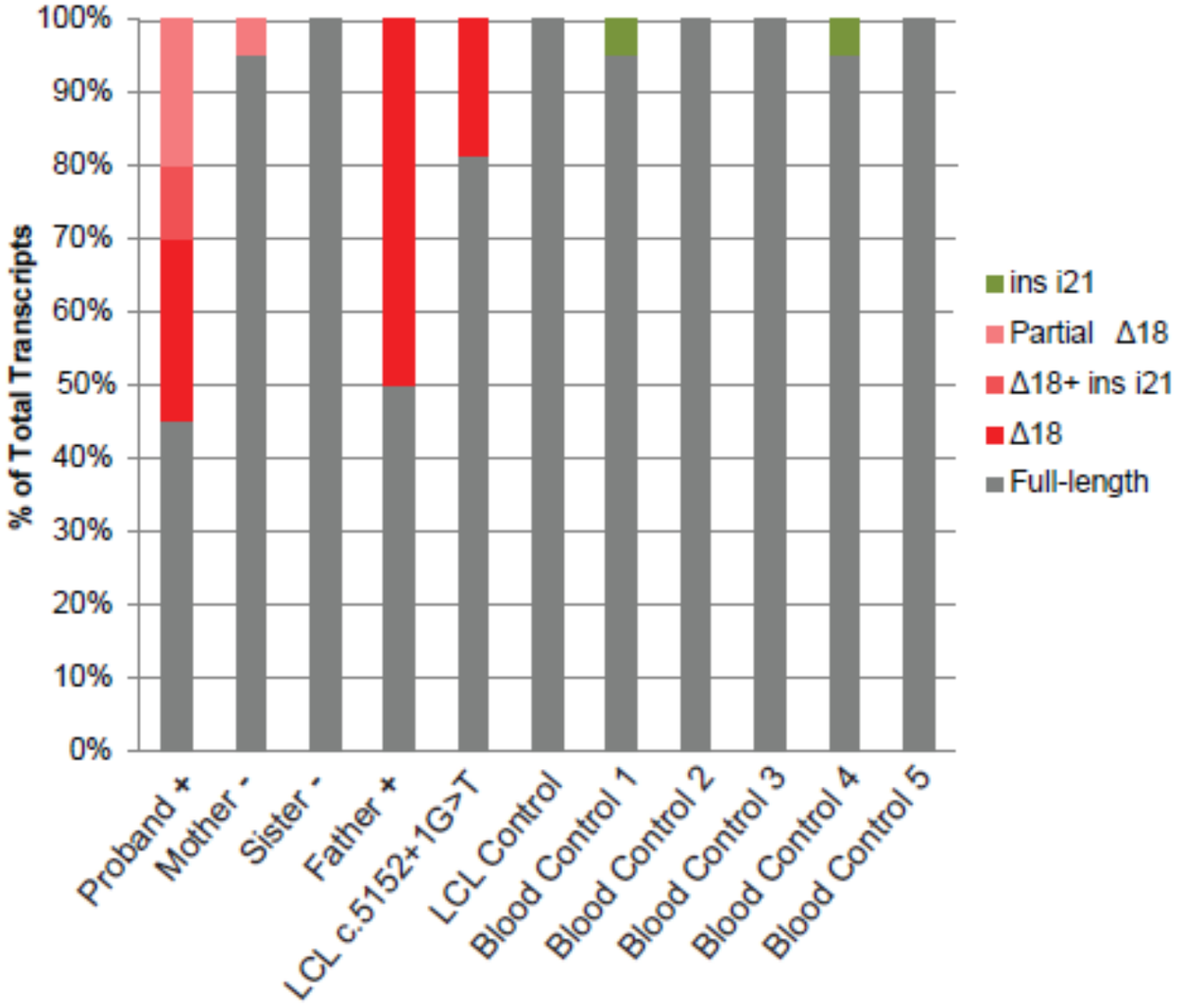
Capillary Electrophoresis



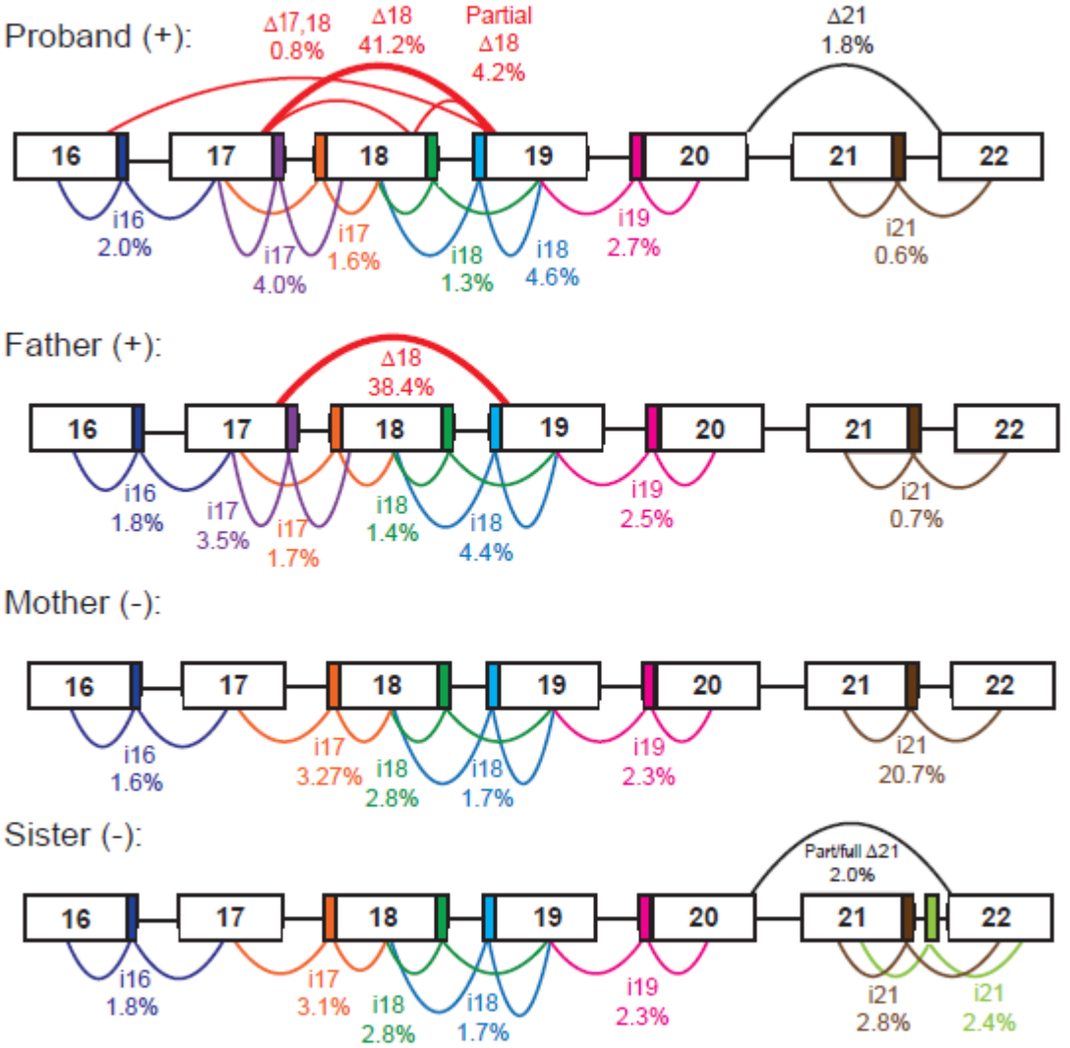
Ambry Genetics®

RNA Studies Reclassification VUS to VLP: BRCA1 c.5152+5G>T

Cloning-Sanger



NGS



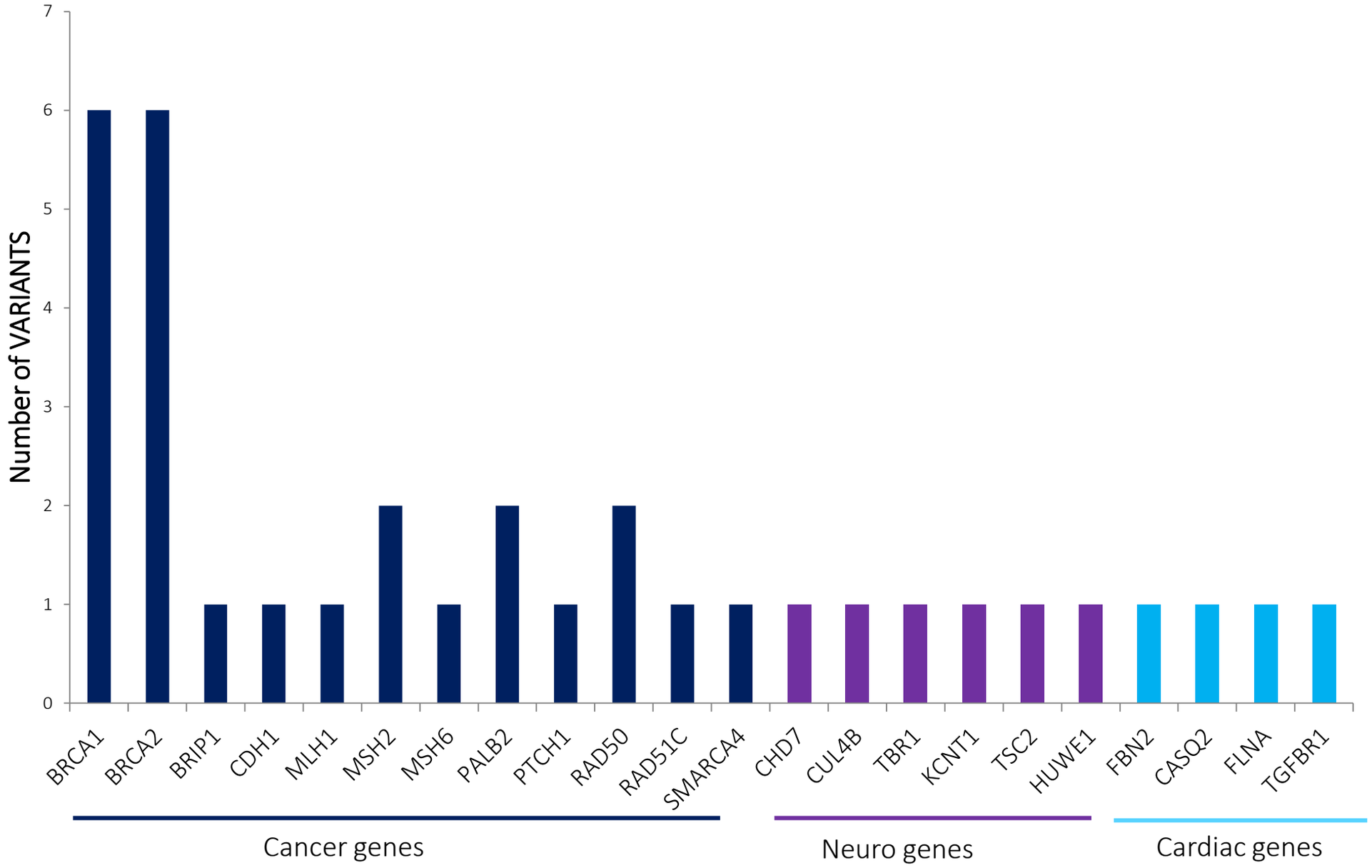
BRCA1 c.5152+5G>T: VUS to VLP

B – Validated RNA mutation

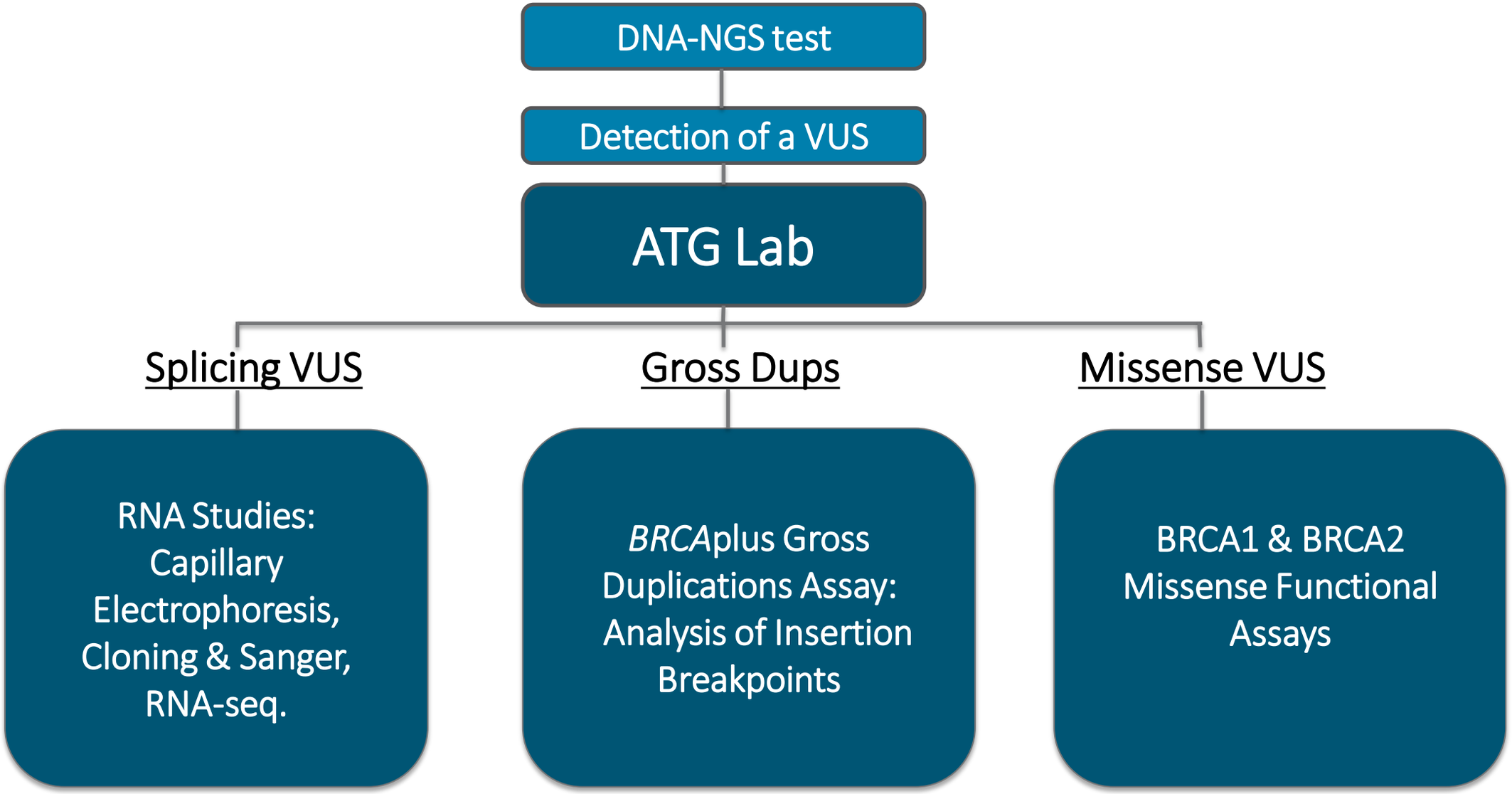
B – RNA Hotspot BRCA1 c.5152+1G>T (clinically validated mutation with *in silic*o predictions *AND* RNA data ≤ variant)

C – Splicing *in silico* in agreement (ESE+FF+HSF+MaxEnt)

Ambry Genetics® | RNA Studies



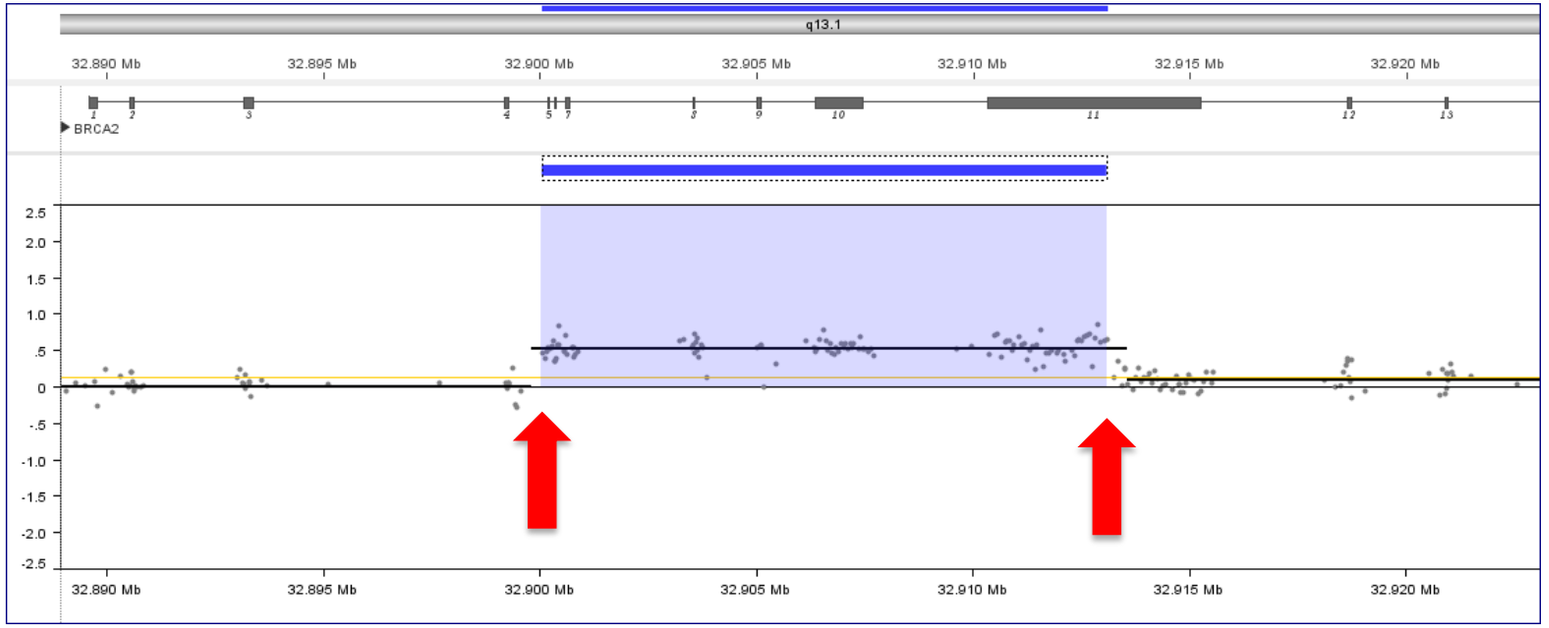
Ambry Translational Genomics Lab (*ATG Lab*)



Is the **duplication intragenic?**

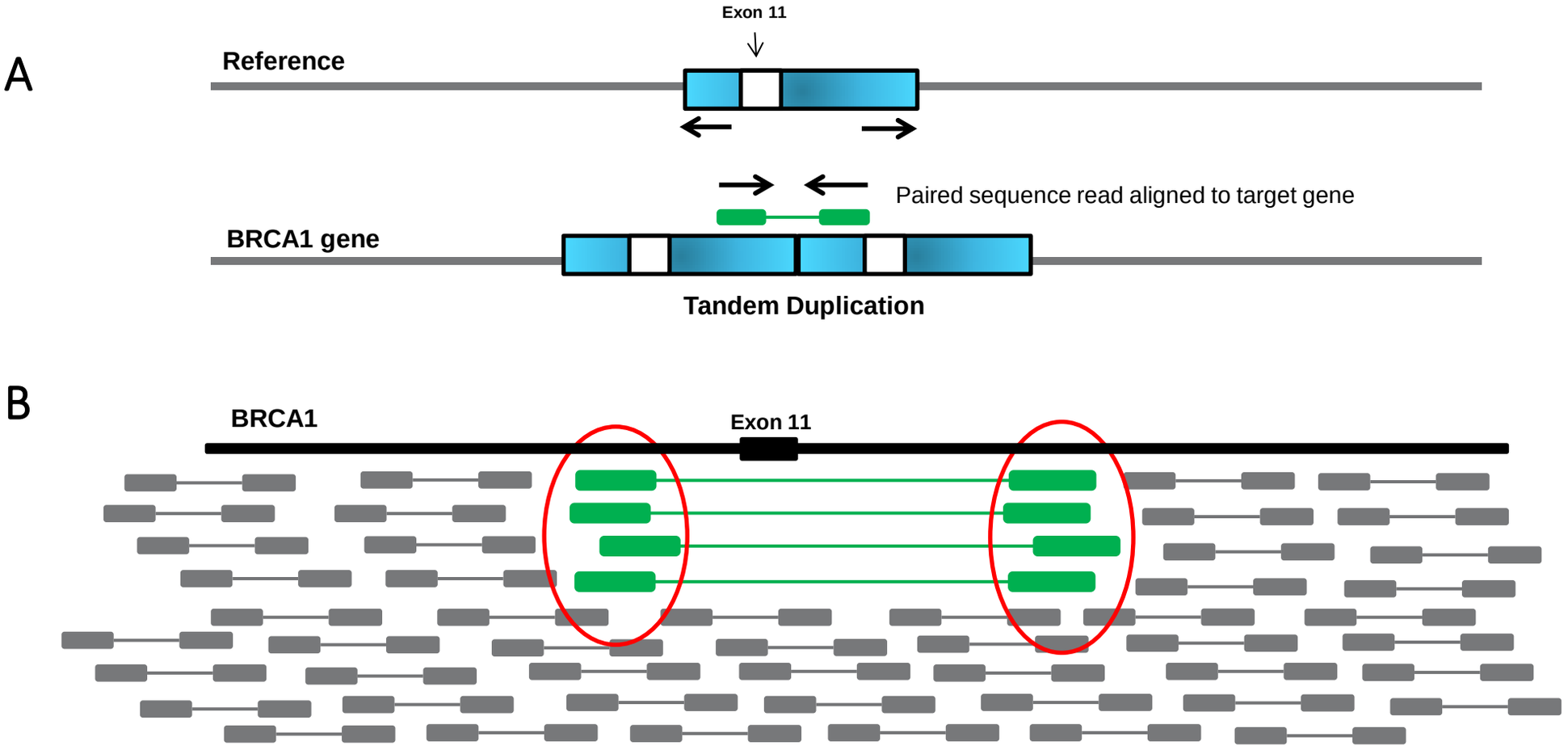
Is the **duplication in tandem?**

In-frame or **out-of-frame?**



Find the breakpoints of duplication!

Ambry Genetics

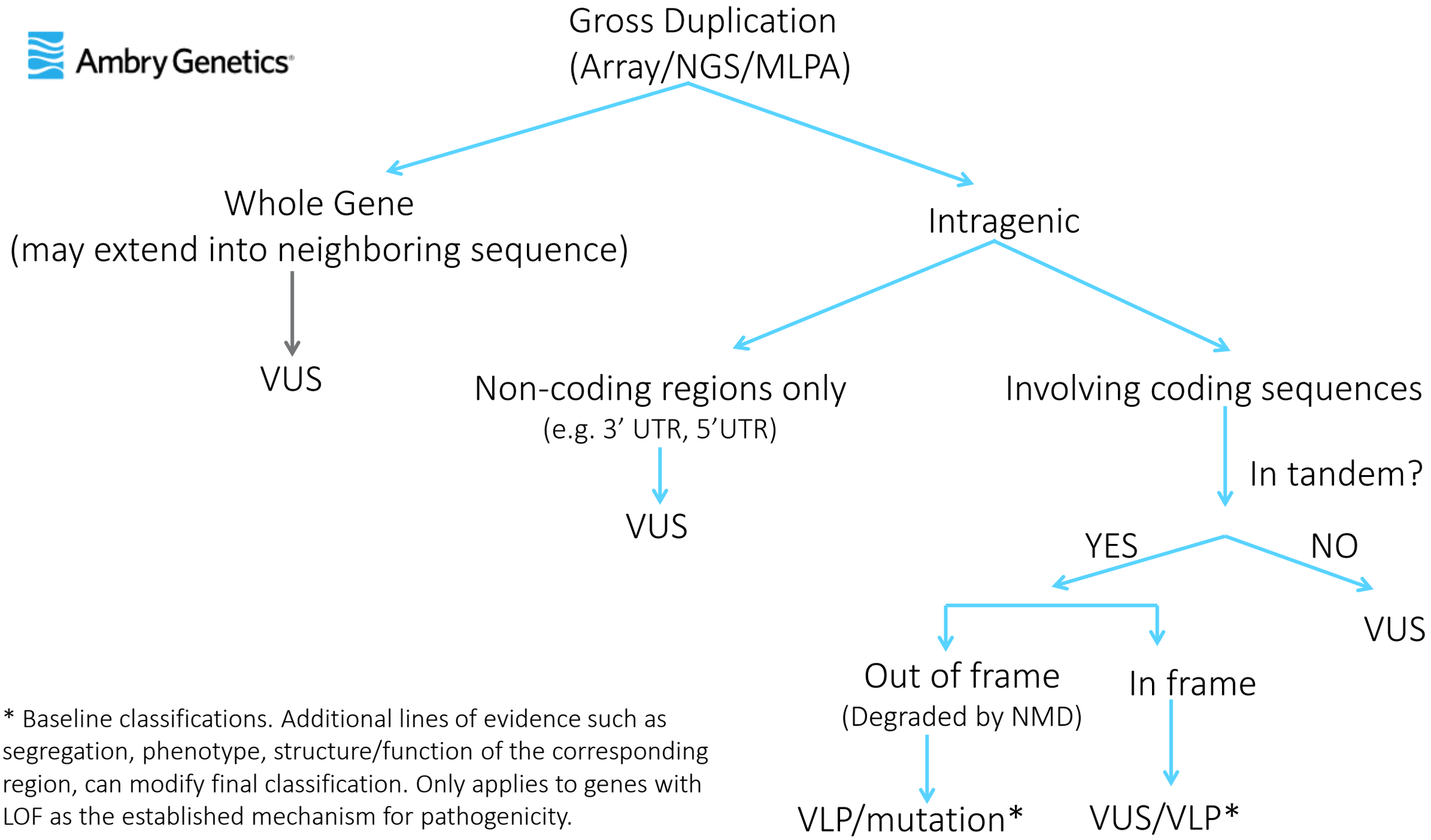


Ambry Genetics® | Gene Duplication Assay

Target Genes: BRCAplus Expanded Panel

BRCA1
BRCA2
CDH1
ATM
CHEK2
PALB2
PTEN
TP53

Zygosity	Classification	Gene	C_Variant
confirmed_HET	VUS	ATM	EX22_25DUP
confirmed_HET	VUS	ATM	EX6_61dup
confirmed_HET	VUS	ATM	EX61_3'UTRdup
confirmed_HET	VUS	ATM	EX61_62DUP
confirmed_HET	VUS	BRCA1	5'UTR_Ex19dup
confirmed_HET	VUS	BRCA1	5'UTR_EX1dup
confirmed_HET	VUS	BRCA1	5'UTR_EX20dup
confirmed_HET	VUS	BRCA1	5'UTR_EX6dup
confirmed_HET	VUS	BRCA1	5'UTR_EX9dup
confirmed_HET	VUS	BRCA1	5'UTRdup
confirmed_HET	VUS	BRCA1	EX11_12dup
confirmed_HET	VUS	BRCA1	EX12dup
confirmed_HET	VUS	BRCA1	EX16_18dup
confirmed_HET	VUS	BRCA1	EX21_3'UTRDUP
confirmed_HET	VUS	BRCA1	EX21dup
confirmed_HET	VUS	BRCA1	EX2dup
confirmed_HET	VUS	BRCA1	EX4_10dup
confirmed_HET	VUS	BRCA1	Ex6dup
confirmed_HET	VUS	BRCA1	IN14dup(partial)
confirmed_HET	VUS	BRCA2	5'UTR_3'UTRdup
confirmed_HET	VUS	BRCA2	5'UTR_EX2dup
confirmed_HET	VUS	BRCA2	EX11_12dup
confirmed_HET	VUS	BRCA2	EX11_17dup
confirmed_HET	VUS	BRCA2	EX12dup
confirmed_HET	VUS	BRCA2	EX14_17dup
confirmed_HET	VUS	BRCA2	ex19dup
confirmed_HET	VUS	BRCA2	EX4_10dup(partial)
confirmed_HET	VUS	CDH1	5'UTR_3'UTRdup
confirmed_HET	VUS	CDH1	**IN2_3dup**
confirmed_HET	VUS	CDH1	**In2_EX16dup**
confirmed_HET	VUS	CDH1	**in2dup**
confirmed_HET	VUS	CHEK2	**5'UTR_3'UTRdup**
confirmed_HET	VUS	CHEK2	**5'UTR_EX14dup**
confirmed_HET	VUS	CHEK2	**5'UTR_EX1dup**
confirmed_HET	VUS	CHEK2	**EX2_13dup**
confirmed_HET	VUS	CHEK2	**EX2_14dup**
confirmed_HET	VUS	CHEK2	**Ex2_3dup**
confirmed_HET	VUS	CHEK2	EX4_14dup
confirmed_HET	VUS	CHEK2	in1_ex13dup
confirmed_HET	VUS	CHEK2	IN2_IN13dup
confirmed_MOSAIC	VUS	PALB2	EX11dup
confirmed_HET	VUS	PALB2	EX13_3'UTRdup

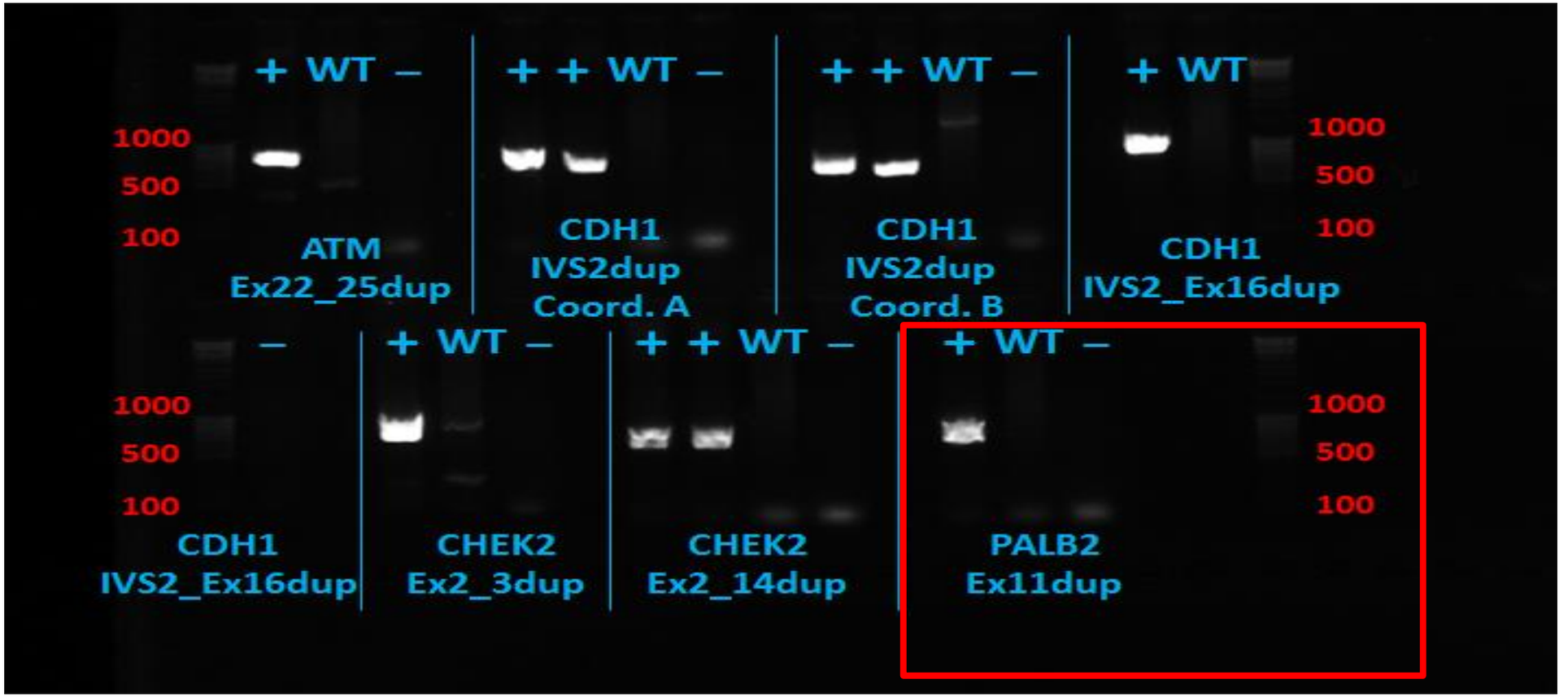


* Baseline classifications. Additional lines of evidence such as segregation, phenotype, structure/function of the corresponding region, can modify final classification. Only applies to genes with LOF as the established mechanism for pathogenicity.

Ambry Genetics

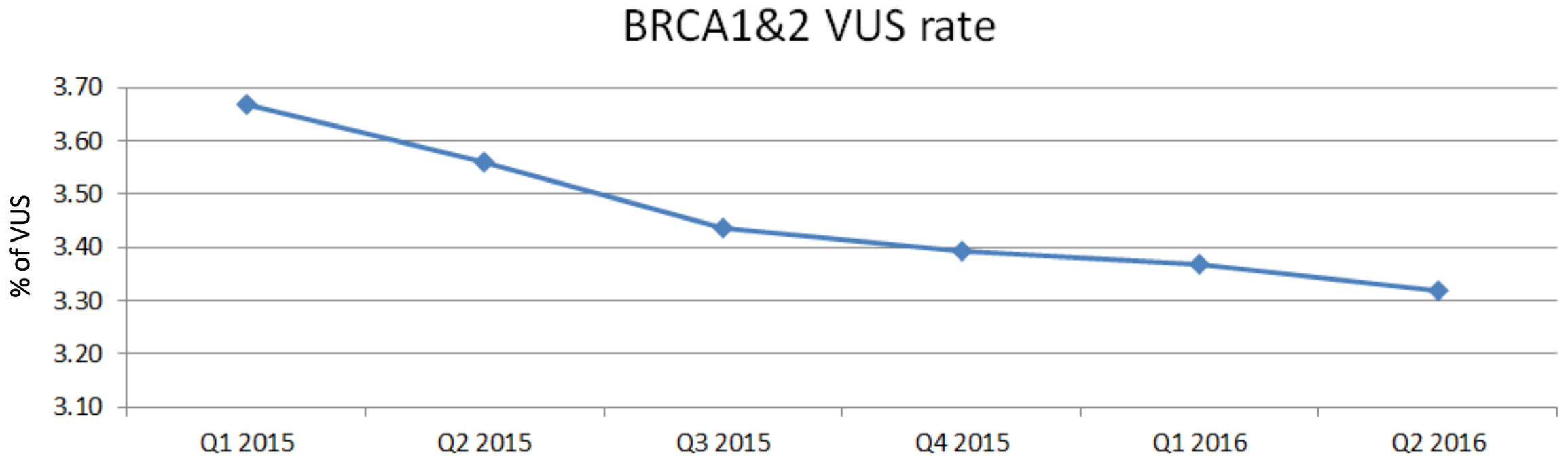
PALB2 EX11DUP: VUS to Pathogenic

PALB2 NM_024675 c.3113+1434_3201+1211dup	**PALB2:EX11Dup**	**Tandem Dup**	**p.G1068Efs*14**	**Frameshift**	**PCR+**



Ambry Genetics®

Reducing VUS rates



Ambry Genetics®

Summary

- Our RNA Studies protocol was validated using ENIGMA's gold-standard protocol.
- Duplication breakpoint analysis by NGS is a specific method to identify tandem duplications.
- RNA Studies and Dup analysis allow accurate classification of genomic variants, reducing VUS classifications.

Thank You

"So, umm, do we know where the genes are yet?"

Thank You