# NGS Facilitates Identification of Retrotransposon Insertional Mutations in Hereditary Cancer Genes

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Retroelements (REs)

- A subclass of transposons
- Only non-LTR retrotransposons are active
  - SINEs, Alu ~11%, ~300 bp
  - LINEs, L1 ~17%, ~6 kb or truncated

- Pathogenesis of REs
  - Mediating deletion/ duplication by NAHR
  - Transposition into critical gene regions

Adapted from Gregory TR. *Nat Rev Genet.* 2005; 6:699
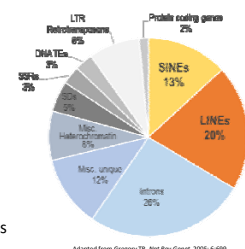
Pathogenic RE insertions

- ~ 100 RE insertion mutations have been reported to be associated with human disorders, including cancer.

- Due to challenges in detecting RE insertions, pathogenic RE insertions are likely underestimated.

- Here we show the utility of Next Generation Sequencing (NGS) in the identification of RE insertions in cancer predisposition genes.


Methods: NGS Panel Testing

- 25-gene hereditary cancer panel using PCR-based NGS.
  - *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53*

- NGS dosage analysis (NGS LR) to identify large rearrangement mutations.

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- NGS dosage analysis (NGS LR) to identify large rearrangement mutations.
- Apparent deletions undergo further investigation
  - Targeted PCR and sequencing analyses
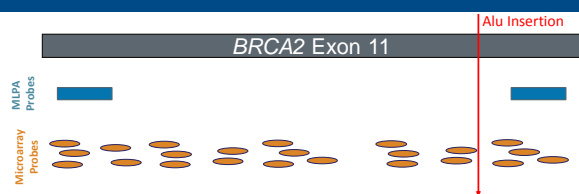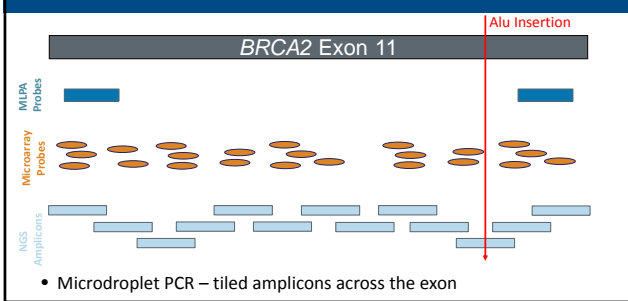
Limitations of MLPA and Microarray

Alu Insertion

BRCA2 Exon 11

Limitations of MLPA and Microarray

Alu Insertion

BRCA2 Exon 11
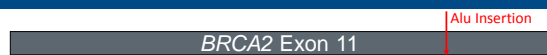
MLPA Probes

- MLPA probes provided limited coverage, especially in large exons.

Limitations of MLPA and Microarray

Alu Insertion
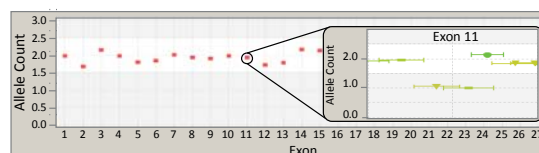
BRCA2 Exon 11

MLPA Probes

Microarray Probes

- Microarray probes have better coverage, but are not designed to bind to foreign sequence.
  - Good for duplications and deletions; Not good for RE insertions

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Limitations of MLPA and Microarray

Alu Insertion

BRCA2 Exon 11

MLPA Probes

Microarray Probes

NGS Amplicons
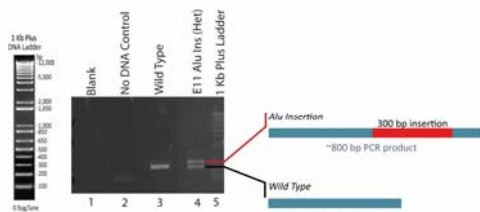
- Microdroplet PCR – tiled amplicons across the exon

An example: *BRCA2* c.5007_5008insAlu

- RE insertion causes appearance of decreased dosage in exon 11 due to preferential amplification of shorter wild type allele (reduced ability to produce the larger mutant product).
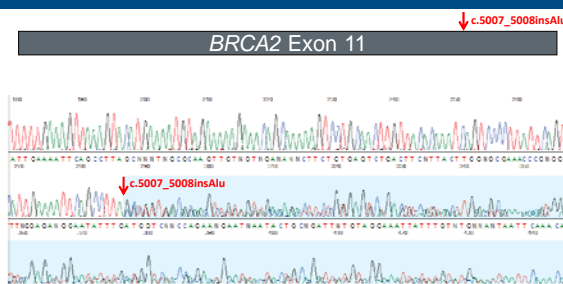
Allele Count

Exon 11

Allele Count

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27

Exon

An example: *BRCA2* c.5007_5008insAlu

- Long range PCR analysis shows a larger fragment in exon 11 (Lane 4)

1 Kb Plus DNA Ladder

Blank

No DNA Control

Wild Type

E11 Alu Ins (Het)

1 Kb Plus Ladder

Alu Insertion

300 bp insertion

~800 bp PCR product

Wild Type

~500 bp PCR product

1 2 3 4 5

An example: *BRCA2* c.5007_5008insAlu

c.5007_5008insAlu

BRCA2 Exon 11

c.5007_5008insAlu

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Identification of RE Insertions

Gene	RE	Unique N	Total N (%)
APC	Alu	1	1 (0.6%)
ATM	Alu	5	26 (14%)
BARD1	Alu	1	1 (0.6%)
BRCA1	Alu	2	3 (1.7%)
BRCA2	Alu	14	139 (77%)
MLH1	L1	2	5 (2.8%)
MSH2	Alu	2	5 (2.8%)
PALB2	Alu	1	1 (0.6%)
Total		28	181 (100%)
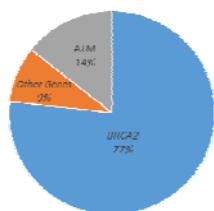
- 28 unique pathogenic RE insertions have been identified by our laboratory

NGS Identification of RE Insertions

- 10 novel RE insertions have been identified since the introduction of NGS, which accounts for 36% of all unique RE insertions identified to date.

- The increase in the number of RE insertions identified since the launch of our NGS gene panel is related to:
  - Increased coverage across exons
  - Testing more genes

Prevalence of RE Insertions by Gene
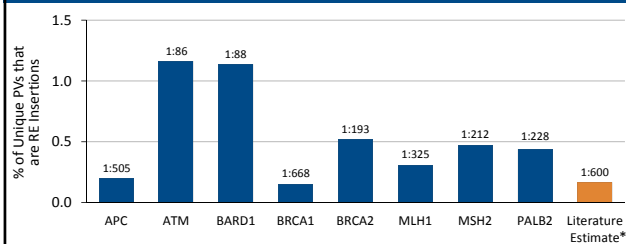
*Kazazian et al. Nature Genetics. 1999;22:130.

Possible Founder Mutations

Mutation	Total cases	Distinct Haplotypes (Lineages)	Dominant Ancestry*
BRCA2 c.156_157insAlu	33	1 (16)	Latin American (Portuguese Founder Mutation**)
BRCA2 c.3407_3408insAlu	46	2 (32)	African
BRCA2 c.5007_5008insAlu	5	2 (4)	Latin American
BRCA2 c.9327_9328insAlu	9	2 (2)	Central/ Eastern European
BRCA2 c.9451_9452insAlu	22	3 (8)	Western/ Northern European
ATM c.7374_7375insAlu	19	n/a	Western/ Northern European[†]

*Based on self-identified ancestry from the test request form   [†]Possible French-Canadian founder mutation
**Teugels Et al Human Mutation. 2005;26(3):284

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Conclusions

- PCR-based NGS, in conjunction with confirmatory assays, facilitates the identification of pathogenic RE insertions.

- This analysis provides evidence that the incidence of RE insertional mutations in human cancers may be higher than previously known.

- This added knowledge is of great importance for early diagnosis and preventive management for high risk patients and their families, particularly for patients whose mutations may have been missed using traditional technologies.

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