A novel NGS target enrichment technology: Improved speed, selectivity, and uniformity

Joel Pel[1], Wendy Choi[1], Milenko Despotovic[1], Laura Gelinas[1], Amy Leung[1], Lloyd Ung[1] and Andre Marziali[1,2]

[1] Boreal Genomics Inc, Vancouver, BC Canada, [2] University of British Columbia, Vancouver, BC Canada

I. Linked Target Capture (LTC)

LTC offers a novel, rapid, high specificity target capture method with broad NGS applications

- **High on-target fraction & uniformity**: reduces required sequencing depth & cost
- **Simple, single day workflow**: by combining PCR & capture workflow steps, reduces library prep time to < 1 day compared to multi-day workflows for high coverage commercial panels
- **Highly scalable**: from 100bp - Mb+ sized panels
- **Compatible with molecular barcodes**: UMIs and independent capture of both senses of the starting template enables duplex sequencing

II. Workflow

Ligation: 2.5 hours

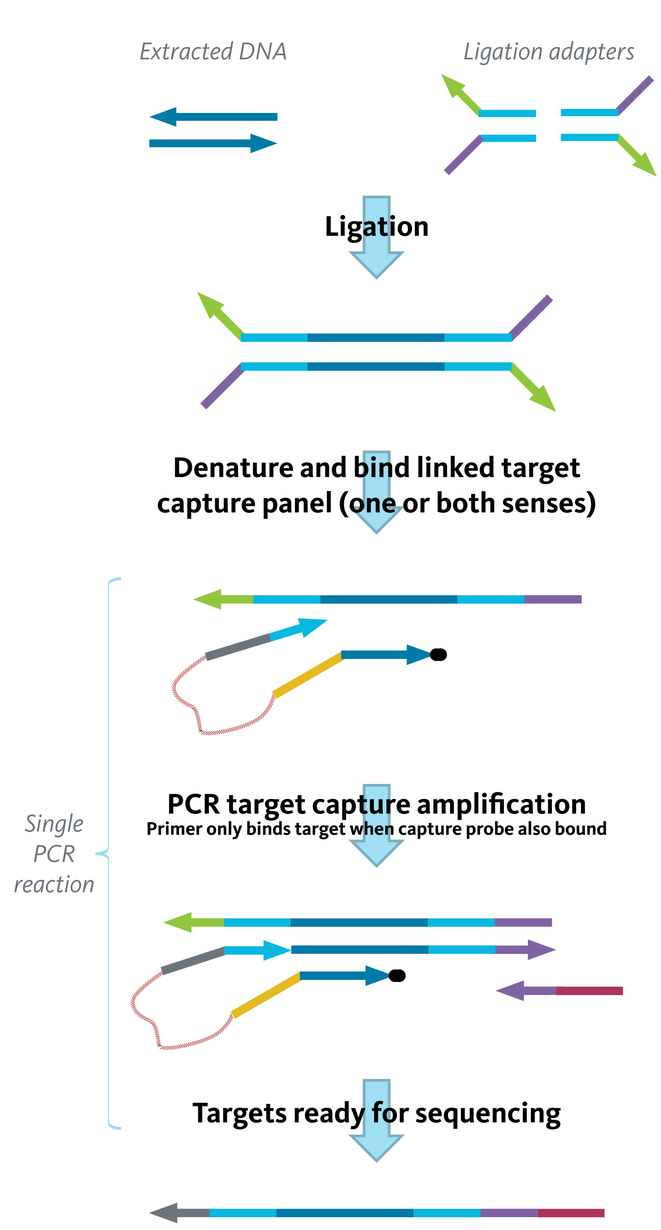
- Standard ligation with custom adapters
- Compatible with UMIs and sample barcodes

Target Capture Amplification: 4-6 hours *(depending on # of PCR cycles)*

- Enrichment achieved through proprietary Probe-Dependent Primer (PDP) PCR *in one or both directions*
- Universal (light blue) portion of PDP only binds and extents if probe is bound to template, decoupling thermodynamics of sequence recognition and extension
- Number of PCR cycles defines specificity, equivalent to multiple captures, but without loss
- *Long capture, complicated pull downs and pre/post PCR all eliminated*

Library Clean-up: ~1 hour

- Library is cleaned up prior to quantification and then is ready for sequencing



Linked Target Capture workflow

III. Panel Design

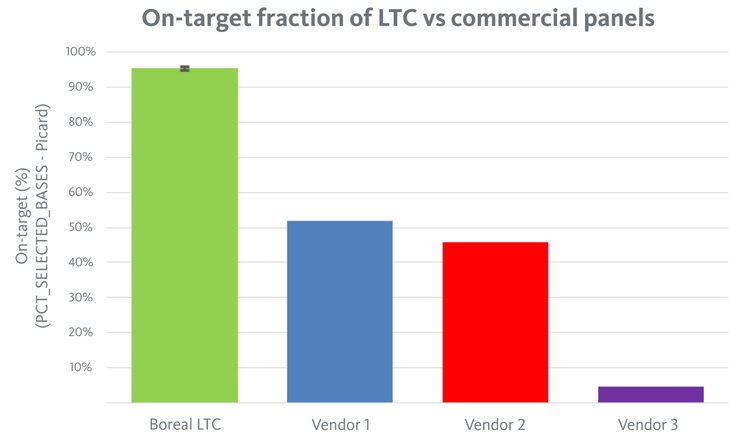
LTC panels are highly scalable and simple to design. The following 31-gene panel was used for comparison with commercial target capture products of similar coverage.

Targeted coverage of 31 genes										
AKT3	ALK	APC	AR	ATM	BRAF	CDH1	CDK4	DDR2	EGFR	EGFR
ERBB4	ESR1	FBXW7	FGFR2	IDH1	JAK1	JAK2	KDR	KIT	KRAS	MAP2K1
MAPK1	MET	MLH1	NRAS	PDGFRA	PIK3CA	PIK3R1	PTEN	TP53		

IV. LTC Performance

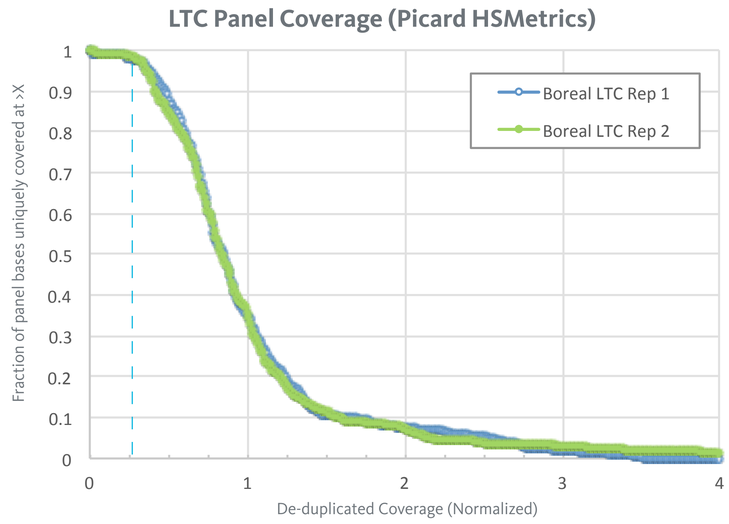
On-target Fraction

- Boreal LTC demonstrates very high on-target fraction compared to other methods, especially for small panels (<50kb)
- On-target % calculated without inflation from flanking target regions



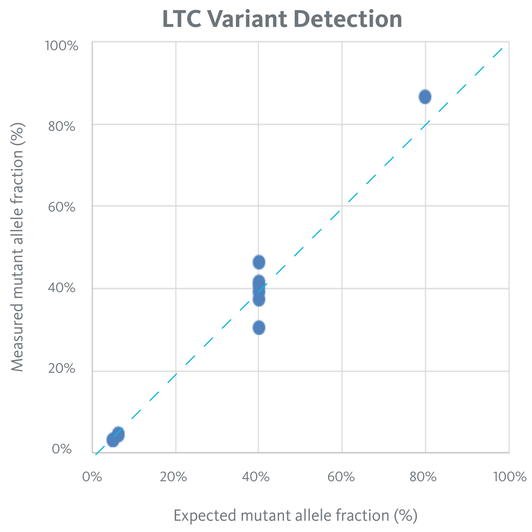
Panel Uniformity

- >98% of panel bases covered >0.25x of mean coverage (Picard HSMetrics)
- High uniformity translates to more coverage with fewer reads
- Probe balancing is expected to increase uniformity further



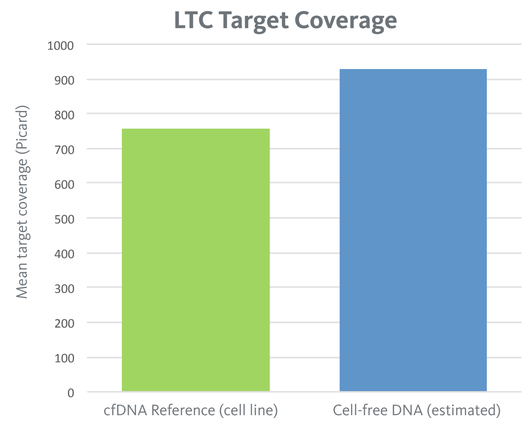
Variant Detection

- Correlation of measured and expected mutant allele fraction from various commercial cell lines
- Mutant allele abundances were determined from characterized cell lines, or as a mixture of cell line dilutions
- Integration of UMIs lowers detection threshold beyond data shown



Depth of Coverage

- Mean target coverage with 30ng input into library construction
- Cell-line and cell-free DNA results are comparable to date (testing ongoing)



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