Linked Target Capture: Rapid and High Performance NGS Target Enrichment Ideal for Clinical and Custom Applications

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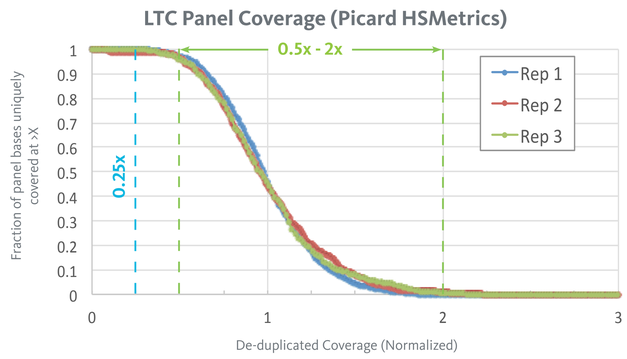
I. Linked Target Capture (LTC)

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 < 8 hours compared to multi-day workflows for commercial high coverage panels
- **Highly scalable & simple design**: from 100bp - Mb+ sized panels
- **Compatible with molecular barcodes**: UMIs and independent capture of both senses of the starting template enables duplex sequencing

Panel Uniformity

- >99% of panel bases covered with at least 25% of mean depth
- >95% of panel covered between 0.5x and 2x
- High uniformity translates into more coverage with fewer reads



II. Workflow

Ligation

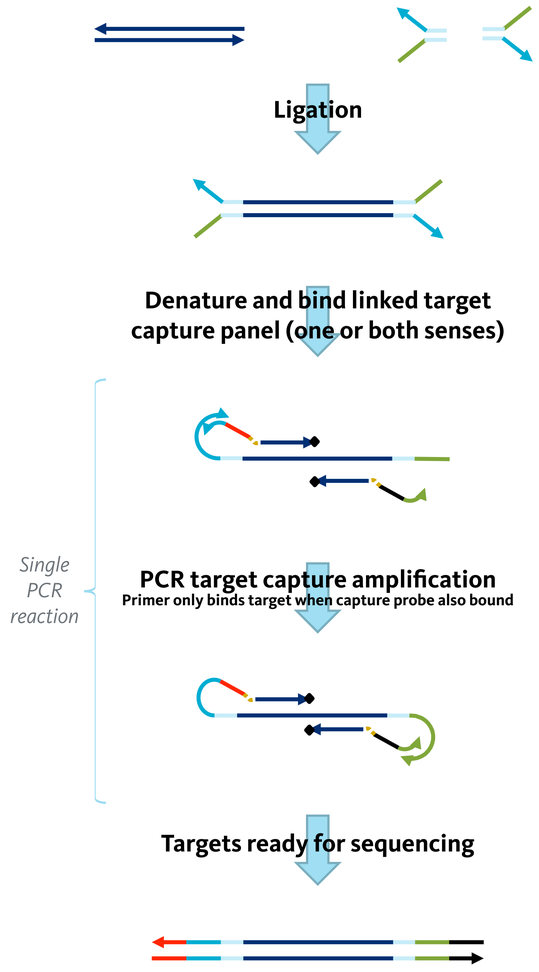
- Standard ligation with custom adapters
- Compatible with optional UMIs

Target Capture PCR

- Enrichment achieved through proprietary Probe-Dependent Primers (PDP) used in PCR
- Universal primer portion (blue/green) of PDP only binds and extends if probe (dark blue) is bound to template, decoupling thermodynamics of sequence recognition and extension
- *Long capture, complicated pull downs and pre/post PCR all eliminated*

Sequencing Clean-up & Quant

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



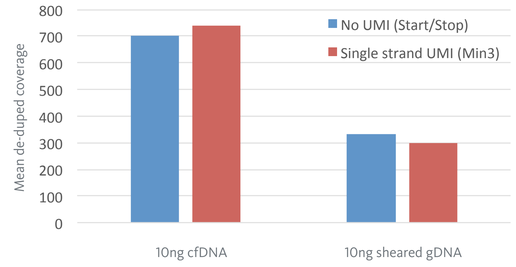
Purified DNA

- Library prep (ligation)
- Pre-amp (optional)
- Target Capture PCR
- Library quant (Qubit)
- Sequencer setup

Current workflow		*Optimized workflow*	
Total time	Hands-on	*Total time*	*Hands-on*
130 min	50 min	*105 min*	*50 min*
67 min	40 min	*(67 min)*	*(40 min)*
255 min	60 min	*120 min*	*30 min*
15 min	15 min	*15 min*	*15 min*
30 min	30 min	*30 min*	*30 min*
8 h 17 min	**3 h 15 min**	***4 h 30 min (5 h 37 min)***	***2 h 05 min (2 h 45 min)***

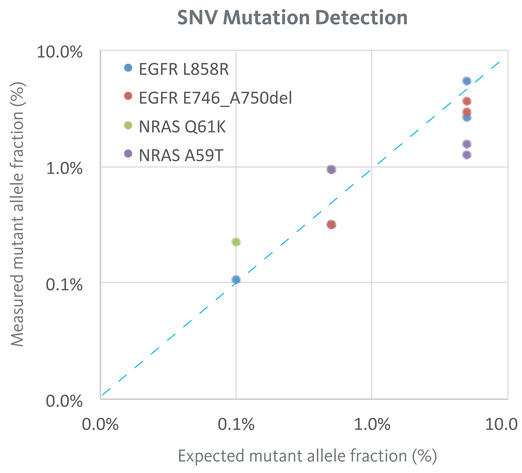
Depth of Coverage

- 10ng input into rapid library construction produces >700x de-duplicated coverage
- Coverage can be increased by extending ligation time beyond 15 min used in rapid workflow
- Lower coverage seen with sheared gDNA may be due to shearing-induced damage
- Coverage calculations made using Picard HsMetrics (start/stop) and single strand UMI[1]



Variant Detection

- All four classes of mutations detected: CNVs, fusions, indels, SNVs
- Using UMIs, SNV detection enabled down to 0.1% or below
- Error rate of ~1 x 10^{-6} with partial duplex UMIs
- Demonstrated detection of both MET amplification and a known CCDC6:RET gene fusion common in Lung Adenocarcinoma
- Gene fusions with unknown partners can be discovered with the linked ligation workflow described below



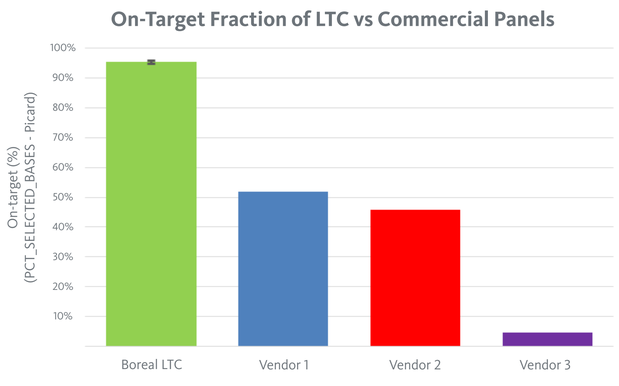
III. Panel Design & Performance

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		

On-Target Fraction

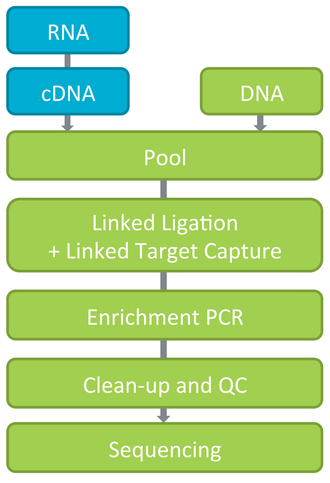
- Boreal LTC demonstrates very high on-target fraction compared to other methods, especially for small panels (<50kb)
- On-target % calculated without inflation from flanking target regions



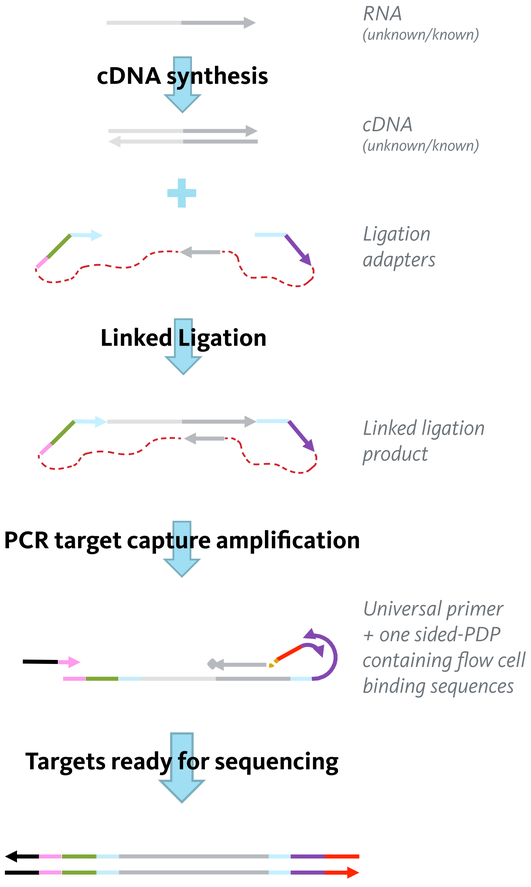
IV. Sequence-Targeted Ligation

Linked ligation combined with linked target capture enables a simple, combined gene fusion and target capture workflow that does not require prior knowledge of fusion partners

- Integrated UMIs & random start/stop sites improve sequencing accuracy and low frequency variant detection
- Demonstrated detection of CCDC6:RET fusion from cell line with the following linked ligation workflow



Combined gene fusion and target capture workflow



Linked ligation workflow for unknown gene fusion capture

With reagent support from Integrated DNA Technologies Inc. (IDT)

[1] *Fulcrum Genomics: https://github.com/fulcrumgenomics/fgbio*