



## **Ion AmpliSeq™ Technology**

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Sr. Sequencing Sales Specialist

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# Ion AmpliSeq™ Technology: As Simple As PCR

*Your targets, your genome, your panel*

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Comprehensive gene coverage  
with the lowest amount of DNA or RNA Input

## Simple

- As little as 10 ng of DNA per pool
- FFPE-compatible
- PCR-based target selection



## Scalable

- Up to 24,000 primers per pool
- 1-1000s of genes
- 96 barcodes for multiplexing



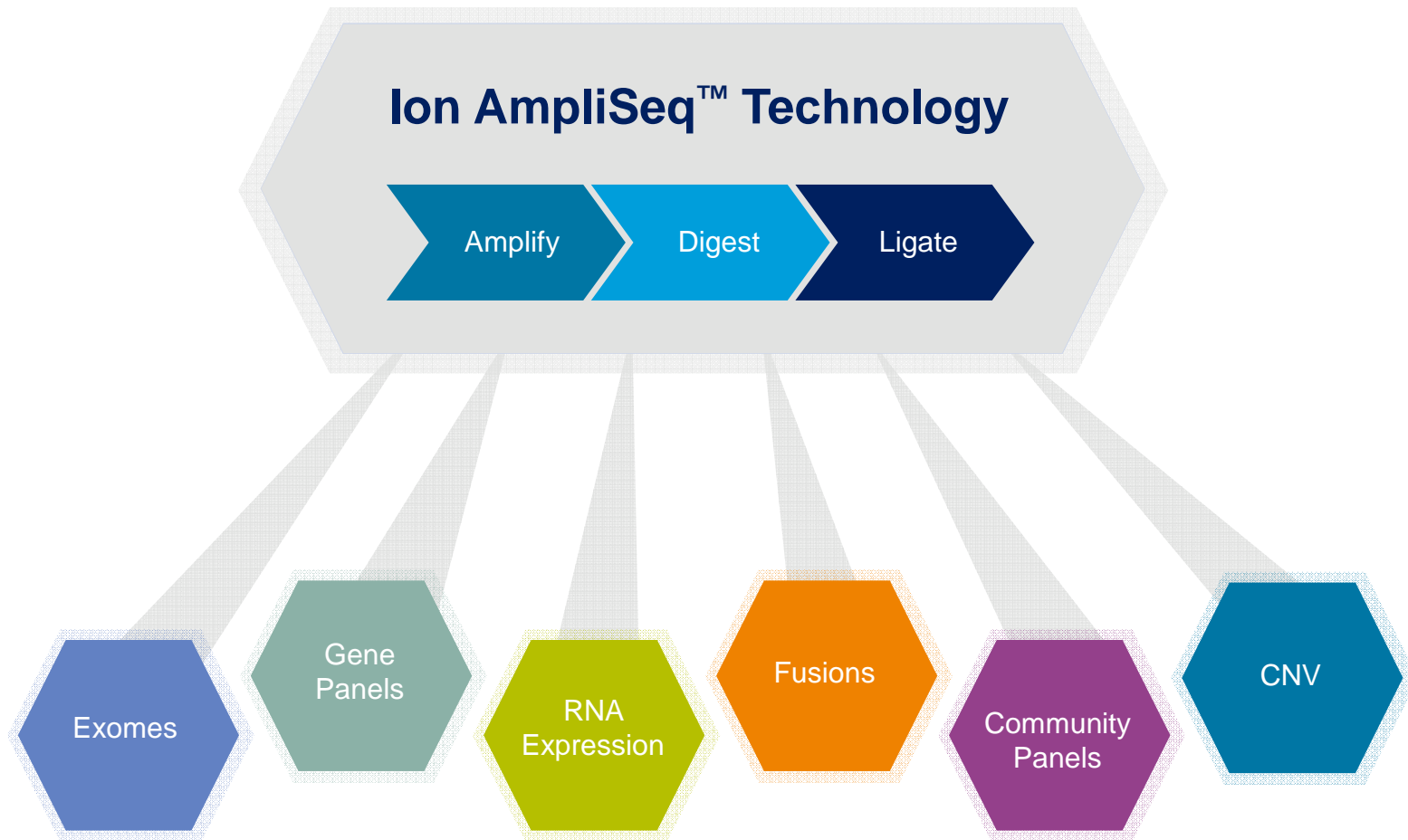
## Fast

- 1 day from DNA to results
- 2 hours to design custom panels
- 3.5 hours for target selection and library preparation

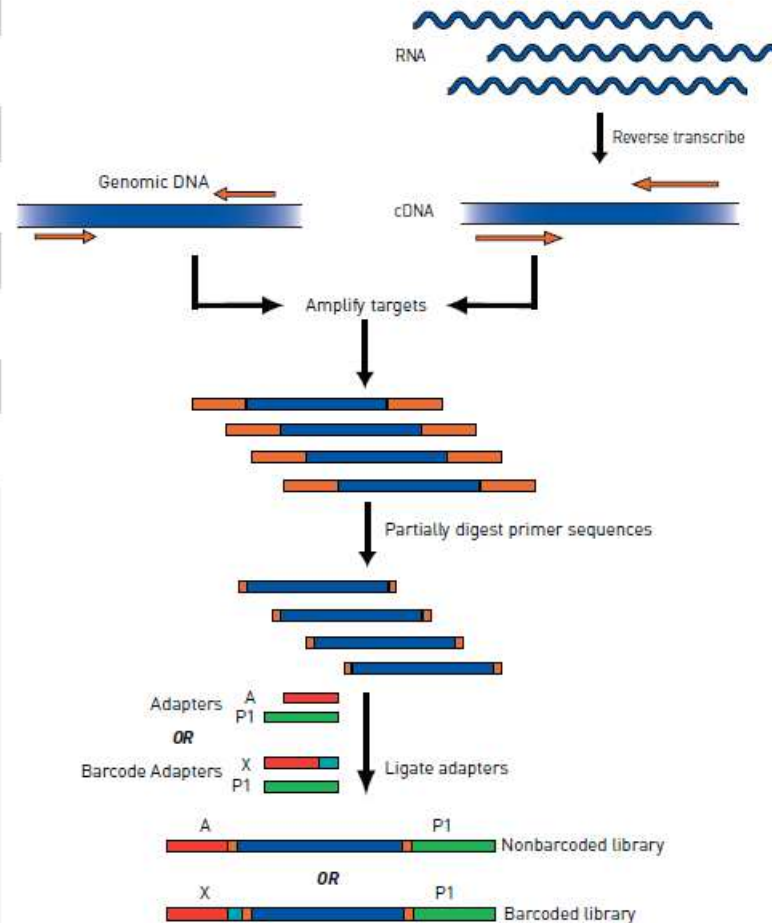
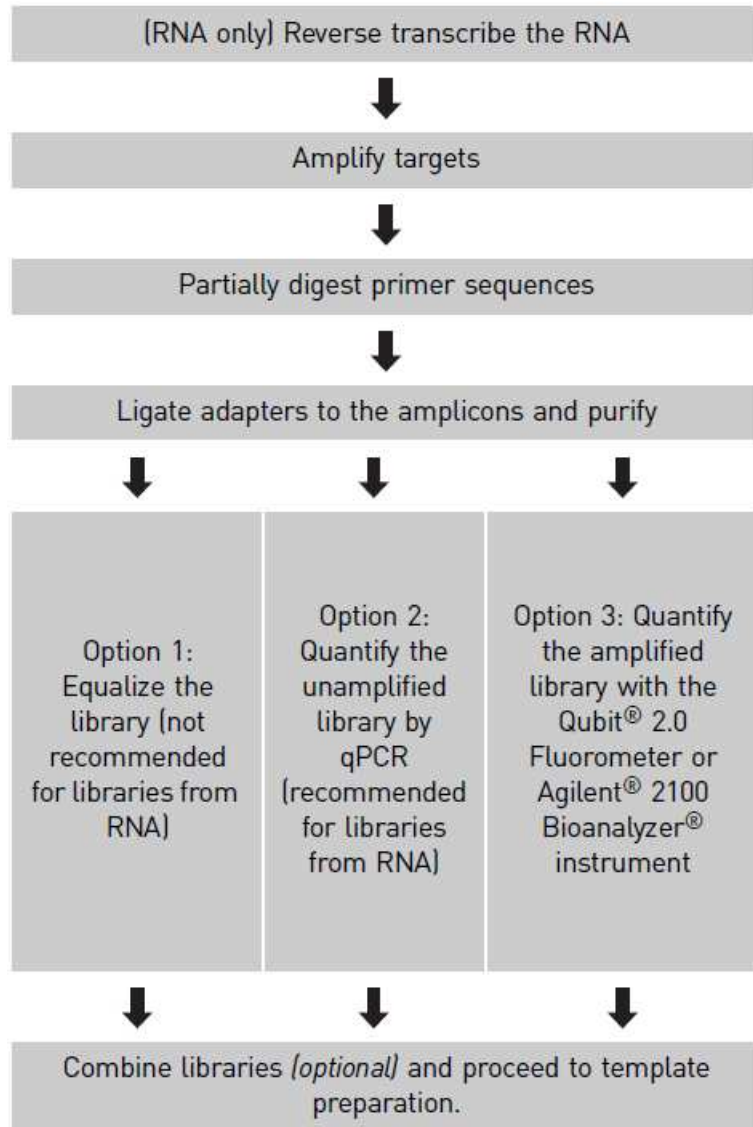


# Broad Applications with Ion AmpliSeq™ Technology

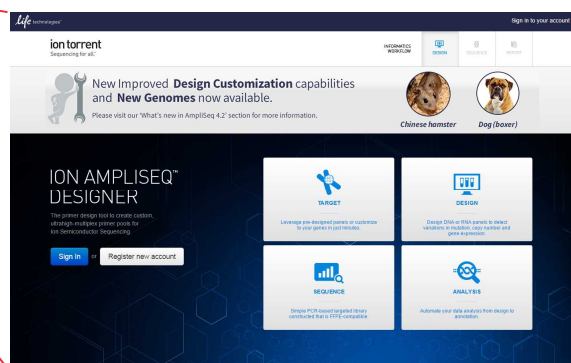
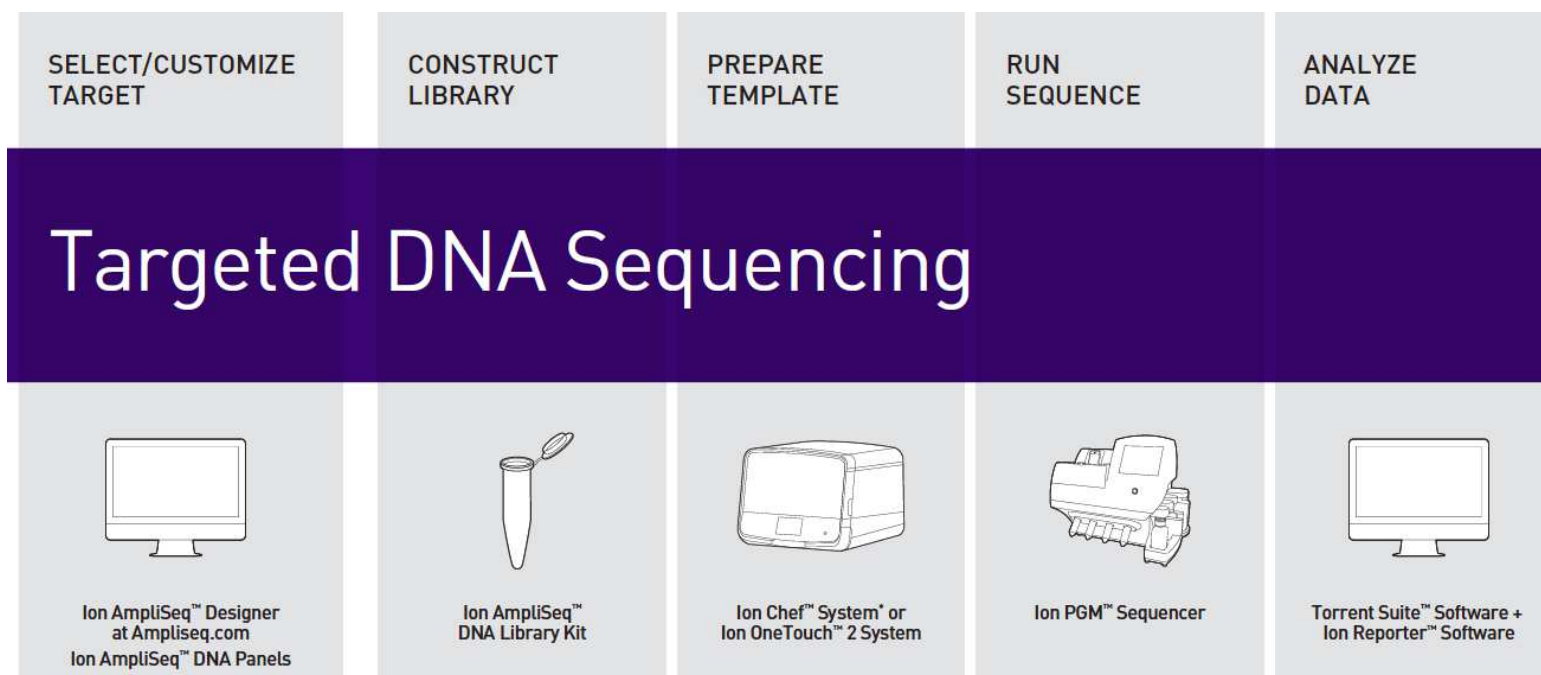
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# Ion AmpliSeq™ Target Selection Overview



# Technology Summary



<http://www.ampliseq.com>

# AmpliSeq Designer Objectives

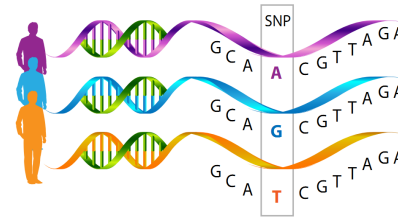
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- Initial step of AmpliSeq workflow
- Repository for Panels and Designs
- Design creation for human and non-human organisms
  - Development of highly specific primers
- Designs target genomic gene regions or SNP's
- Pre-design panels are:
  - Ready-to-Use
  - Community
- Easy to set up new designs
- Easy to share with collaborators



# Key technology features:

- RNA pre-calculated designs
- DNA gene designs
  - “Standard” AmpliSeq designs
- DNA hotspot designs
  - 1-pool designs for SNP’s



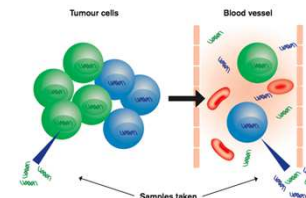
- Designs for “Any Genome”
  - AgBio applications and more



- Design customization
  - Sub-setting
  - Mix and match
  - Whitelist



- Support for Cell Free DNA (cfDNA) designs
  - 140bp amplicon sizes now available



# Ion AmpliSeq™ Panels



For DNA analysis and RNA expression measurement

Ready-to-use  
**Human Identity Panel**

Ready-to-use  
**Human Ancestry Panel**

Ready-to-use  
**RNA Apoptosis Panel**

Ready-to-use  
**RNA Cancer Panel**

Ready-to-use  
**Cancer Hotspot Panel**

Ready-to-use  
**Comprehensive Cancer Panel**

Ready-to-use  
**Inherited Disease Panel**

Community  
**RNA Fusion Lung Cancer Research Panel**

Community  
**BRCA 1 & 2 Research Panel**

Community  
**Hearing Loss Research Panel**

Community  
**CFTR Research Panel**

Community  
**TP53 Research Panel**

Community  
**AML Research Panel**

Community  
**Cardio Research Panel\***

Community  
**Dementia Research Panel**

Community  
**Colon & Lung Cancer Research Panel**

\*The content provided herein may relate to products that have not been officially released and is subject to change without notice.



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# Ion AmpliSeq™ DNA panels



# Ion AmpliSeq™ Cancer Hotspot Panel v2



As little as 10ng input  
50 genes  
207 amplicons

The Ion AmpliSeq™ Cancer Panel targets 50 genes

<i>ABL1</i>	<i>EZH2</i>	<i>JAK3</i>	<i>PTEN</i>
<i>AKT1</i>	<i>FBXW7</i>	<i>IDH2</i>	<i>PTPN11</i>
<i>ALK</i>	<i>FGFR1</i>	<i>KDR</i>	<i>RB1</i>
<i>APC</i>	<i>FGFR2</i>	<i>KIT</i>	<i>RET</i>
<i>ATM</i>	<i>FGFR3</i>	<i>KRAS</i>	<i>SMAD4</i>
<i>BRAF</i>	<i>FLT3</i>	<i>MET</i>	<i>SMARCB1</i>
<i>CDH1</i>	<i>GNA11</i>	<i>MLH1</i>	<i>SMO</i>
<i>CDKN2A</i>	<i>GNAS</i>	<i>MPL</i>	<i>SRC</i>
<i>CSF1R</i>	<i>GNAQ</i>	<i>NOTCH1</i>	<i>STK11</i>
<i>CTNNB1</i>	<i>HNF1A</i>	<i>NPM1</i>	<i>TP53</i>
<i>EGFR</i>	<i>HRAS</i>	<i>NRAS</i>	<i>VHL</i>
<i>ERBB2</i>	<i>IDH1</i>	<i>PDGFRA</i>	
<i>ERBB4</i>	<i>JAK2</i>	<i>PIK3CA</i>	

TaqMan® Mutation Detection Assays are available for the genes listed above.

Gene list available [www.lifetechnologies.com/ampliseqready](http://www.lifetechnologies.com/ampliseqready)

# Ion AmpliSeq™ Comprehensive Cancer Panel (CCP)

ion torrent  
by life technologies™

**Ion AmpliSeq™**  
**Comprehensive Cancer Panel**

Extensive survey of over 400 genes with only 40 ng of DNA

- Targets coding exons in 409 human oncogenes and tumor suppressor genes
- ~16,000 amplicons
- Detection of known COSMIC somatic mutations

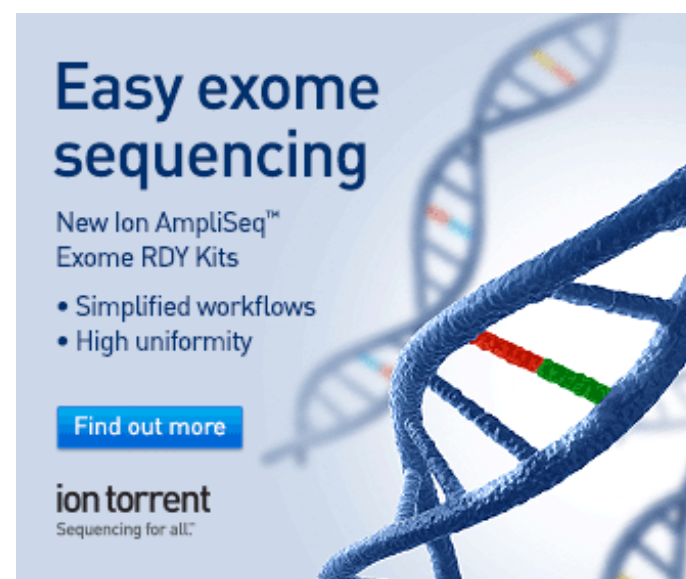
IDH1	KRAS	MLH1	NFKB2	PIK3CB	RARA	SOC51	TOP1
IDH2	LAMP1	MLL	NIN	PIK3CA	RB1	SOX11	TP53
IDF1R	LCK	MLL2	NKX2-1	PIK3CB	RECOL4	SOX2	TPR
IDF2	LIFR	MLL3	NLRP1	PIK3CD	REL	SRC	TRIM24
IDF2R	LPHN3	MLL10	NOTCH1	PIK3CG	RET	SSK1	TRIM3
IKBKB	POT1	MMP2	NOTCH2	PIK3R1	RHOH	STK11	TRIP11
IKBKE	LFP	MN1	NOTCH4	PIK3R2	RNASEL	STK36	TRRAP
IKZF1							
IL2							
IL21R							
IL4ST							
IL7R							
INO4							
IRF4							
IRS2							
ITGA10							
ITGA9							
ITGB2							
ITGB3							
JAK1							
JAK2							
JAK3							
JUN							
KAT5A							
KAT4B							
KDM5C							
KDM6A							
KDR							
KEAP1							
KIT							
KLF6							
ABL1	AURKA	BMFR1A	CDK4	CTNNB1	EPHB4	FANCD2	FZR1
ABL2	AURKB	BRAF	CDK6	CYLD	EPHB6	FANCF	GAPD
ACVR2A	AURKC	BRD3	CDK8	CYP2C19	ERBB2	FANGC	GATA1
ADAMTS20	AXL	BRIP1	CDKN2A	CYP2D6	ERBB3	FAS	GATA2
AFF1	BAI3	BTX	CDKN2B	DAXX	ERBB4	FBXW7	GATA3
AFK3	BAP1	BUB1B	CDKN2C	DCC	ERCC1	FGF1R	GDNF
AKAP9	BCL10	CARD11	CEBPA	DDB2	ERCC2	FGF2R2	GNA11
AKT1	BCL11A	CASC5	CHEK1	DDIT3	ERCC3	FGF3R	GNAQ
AKT2	BCL11B	CBL	CHEK2	DDR2	ERCC4	FGF4	GNAS
AKT3	BCL2	CCND1	CIC	DEK	ERCC5	FH	GPR124
ALK	BCL2L1	CCND2	CKS1B	DICER1	ERG	FLCN	GRM8
AFC	BCL2L2	CCNE1	CMPK1	DNMT3A	ESR1	FLJ1	GUCY1A2
AR	BCL3	CD79A	COL1A1	DPYD	ETS1	FLT1	HCA1
ARID1A	BCL6	CD79B	CRBN	DST	ETV1	FLT3	HIF1A
ARID2	BCL9	CDC73	CREB1	EGFR	ETV4	FLT4	HLF
ARNT	BCR	CDH1	CREBBP	EML4	EXT1	FN1	HNF1A
ASXL1	BIRC2	CDH11	CRKL	EP300	EXT2	FOXO2	HOOK3
ATF1	BIRC3	CDH2	CRTC1	EP400	EZH2	FOXO1	HRAS
ATM	BIRC5	CDH20	CSP1R	EPHA3	FAM123B	FOXO3	HSP90AA1
ATR	BLM	CDH5	CSMD3	EPHA7	FANCA	FOXP1	HSP90AB1
ATRX	BLNK	CDK12	CTNNA1	EPHB1	FANCC	FOXP4	ICK

Gene list available [www.lifetechnologies.com/ampliseqready](http://www.lifetechnologies.com/ampliseqready)

# Ion AmpliSeq™ Exome

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- 293,903 primer pairs across 12 primer pools
  - >24,500-plex PCR!
  - ~2.4 M PCRs per plate of 8 exomes!!
- Total DNA input as low as 50ng
- Covers >97% of CCDS (Release 12)
  - >19,000 coding genes >198,000 coding exons (no UTRs, miRNAs, or ncRNAs)
  - ~85% of human disease-causing variants found in coding regions or splice junctions



- Amplicon size range 225-275 bp
  - Average insert size is ~202 bp

# OncoNetwork Global Consortia

**Prof. Harriet Feilotter**  
Department of Pathology at  
Queen's University, Ontario Canada

**Prof. Ian Cree**  
Warwick Medical School  
United Kingdom

**Marjolijn J.L. Ligtenberg,  
Arjen R. Mensenkamp**  
Radboud University Nijmegen  
Medical Centre, The Netherlands

**Cecily P. Vaughn**  
ARUP Institute for Clinical and  
Experimental Pathology

**Dr. Cristoph Noppen &  
Dr. Henriette Kurth**  
VIOLLIER AG Basle,  
Switzerland

**Prof. Orla Sheils**  
Trinity College Dublin,  
Ireland

**Prof. Prof. Kazuto Nishio**  
Faculty of Medicine, Kinki  
University Osaka, Japan

**Prof. Pierre Laurent Puig**  
Université Paris Descartes,  
France

**Prof. Aldo Scarpa**  
ARC-NET University of  
Verona Italy

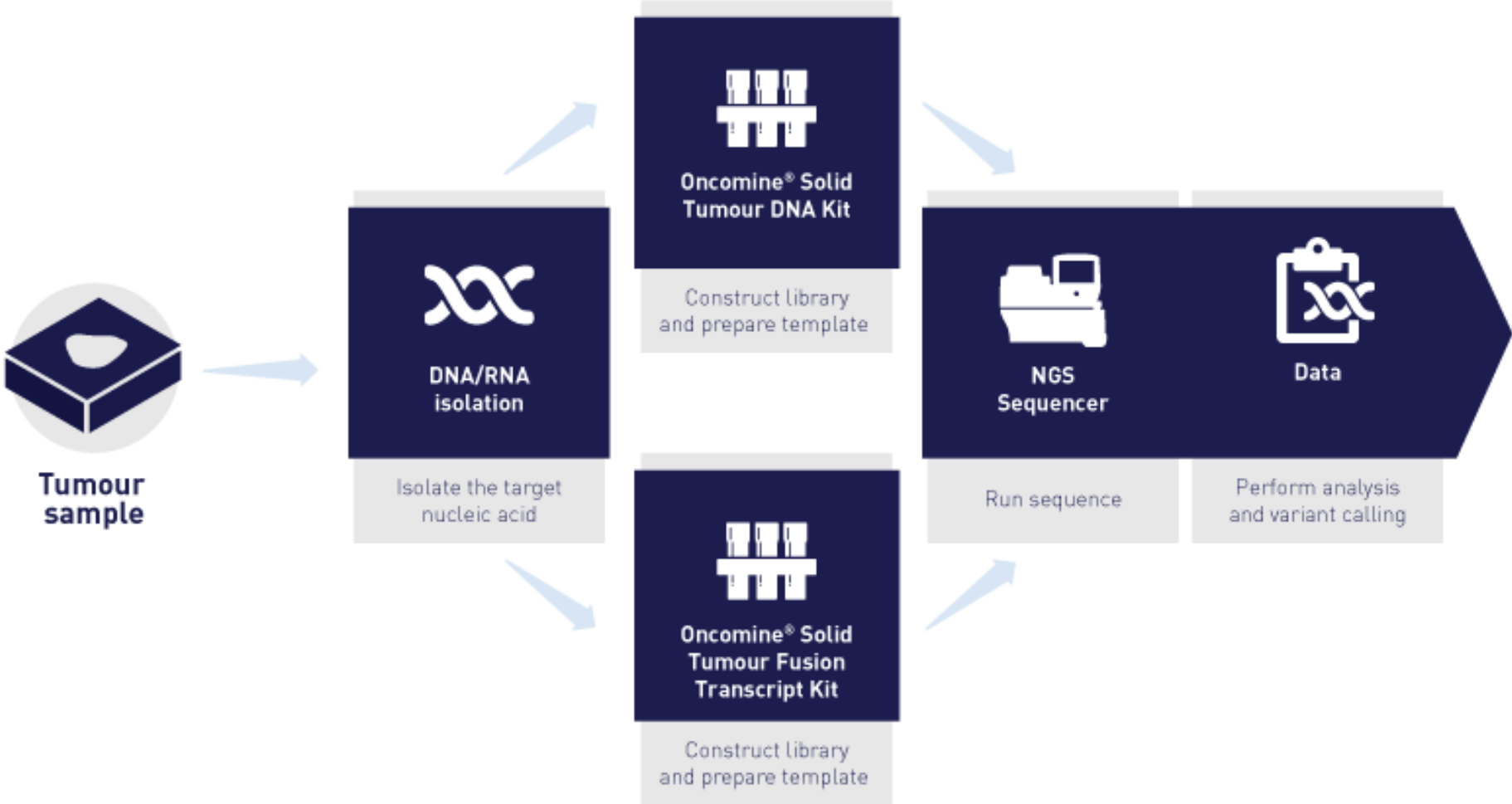
**Dr. Ludovic Lacroix**  
Institut Gustave Roussy  
Paris, France

**Dr. Jose Costa**  
IPATIMUP Medical Faculty of Porto.  
Portugal

**Dr. Nicola Normanno**  
Centro Ricerche  
Oncologiche  
Mercogliano, Italy



# Parallel DNA & RNA Analysis from single specimen



# Oncomine™ Solid Tumour DNA Kit

<b>Application</b>	DNA somatic mutation detection (substitutions, insertions, deletions and inversions)
<b>Sample type</b>	extracted human DNA samples (including those from FFPE tissue)
<b>Input DNA required</b>	10 ng or more.
<b>Genes</b>	EGFR, ALK, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7, TP53
<b>Mutations</b>	>1,800 cancer related mutations as supported by COSMIC database*
<b>Kit content</b>	Amplification oligonucleotides, library preparation reagents, x16 barcodes, DNA Equalizer
<b>Kit size</b>	96 tests (split into 6 packs of 16 tests per pack)
<b>Multiplexing</b>	Possible to run up to 16 samples in a single run, as supported by sample "bar coding"
<b>SKU</b>	A26761

\*<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>

# Oncomine™ Solid Tumour Fusion Transcript Kit

<b>Application</b>	RNA fusion transcript detection
<b>Sample type</b>	extracted human RNA samples (including those from FFPE tissue)
<b>Input RNA required</b>	10 ng or more of total RNA
<b>Genes</b>	ALK, RET, ROS1, NTRK1
<b>Fusion Transcripts</b>	>75 targets of specific designs for cancer relevant fusions* + <b>“imbalance” assay for non-targeted ALK fusions</b>
<b>In-run +’ve control genes</b>	Amplification of housekeeping genes to show successful reaction and avoid false –’ves (MYC, ITGB7, LMNA, HMBS, TBP)
<b>Kit content</b>	Amplification oligonucleotides, library preparation reagents, x16 barcodes
<b>Kit size</b>	96 tests (6 packs of 16 tests per pack)
<b>Multiplexing</b>	Possible to run up to 16 samples in a single run, as supported by sample “bar coding”
<b>SKU</b>	A26762

\*according to PubMed



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# Ion AmpliSeq™ RNA panels



# From Single Gene Quantification to Discovery



## qPCR/ Digital PCR

- Expression levels for single gene targets



## Targeted Ion AmpliSeq™ Panels

- Panels for gene-level expression and fusion detection



## Ion AmpliSeq™ Transcriptome

- Differential gene expression/ profiling + sensitivity for low-abundance RefSeq genes

Number and breadth of expression targets surveyed

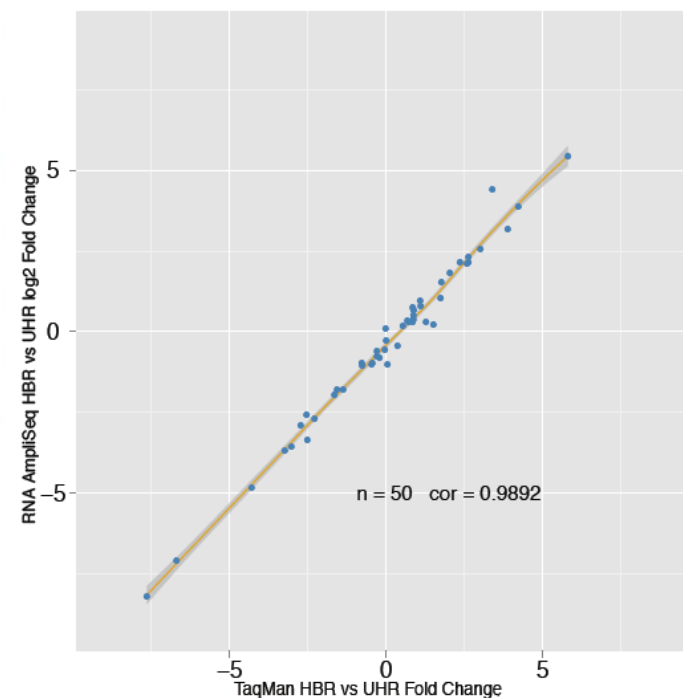
# Ion AmpliSeq™ RNA Cancer Panel

*50 genes, from 500 pg unfixed RNA, one tube*

<i>ABL1</i>	<i>EZH2</i>	<i>JAK3</i>	<i>PTEN</i>	<i>AKT1</i>
<i>FBXW7</i>	<i>IDH2</i>	<i>PTPN11</i>	<i>ALK</i>	<i>FGFR1</i>
<i>KDR</i>	<i>RB1</i>	<i>APC</i>	<i>FGFR2</i>	<i>KIT</i>
<i>RET</i>	<i>ATM</i>	<i>FGFR3</i>	<i>KRAS</i>	<i>SMAD4</i>
<i>BRAF</i>	<i>FLT3</i>	<i>MET</i>	<i>SMARCB1</i>	<i>CDH1</i>
<i>GNA11</i>	<i>MLH1</i>	<i>SMO</i>	<i>CDKN2A</i>	<i>GNAS</i>
<i>MPL</i>	<i>SRC</i>	<i>CSF1R</i>	<i>GNAQ</i>	<i>NOTCH1</i>
<i>STK11</i>	<i>CTNNB1</i>	<i>HNF1A</i>	<i>NPM1</i>	<i>TP53</i>
<i>EGFR</i>	<i>HRAS</i>	<i>NRAS</i>	<i>VHL</i>	<i>ERBB2</i>
<i>IDH1</i>	<i>PDGFRA</i>	<i>ERBB4</i>	<i>JAK2</i>	<i>PIK3CA</i>

Targeted  
quantitative  
expression

Complement to  
Cancer Hotspot Panel



# Ion AmpliSeq™ Transcriptome

*Rapid, cost-effective gene-level expression analysis with simple workflows compatible with FFPE samples*

- **Survey precious or degraded samples** with as little as 10 ng input RNA
- Perform **gene-level expression** surveys in **less than 1 hour hands-on time** (library prep), enabling RNA to gene expression levels in as little as 2 days
- **Achieve better performance than arrays** with wider dynamic range and detection of more genes at lower expression levels
- Obtain gene-level differential expression analysis with **simple automated workflows** that leverage existing microarray or NGS analysis pipelines



Introducing  
Ion AmpliSeq™  
Transcriptome

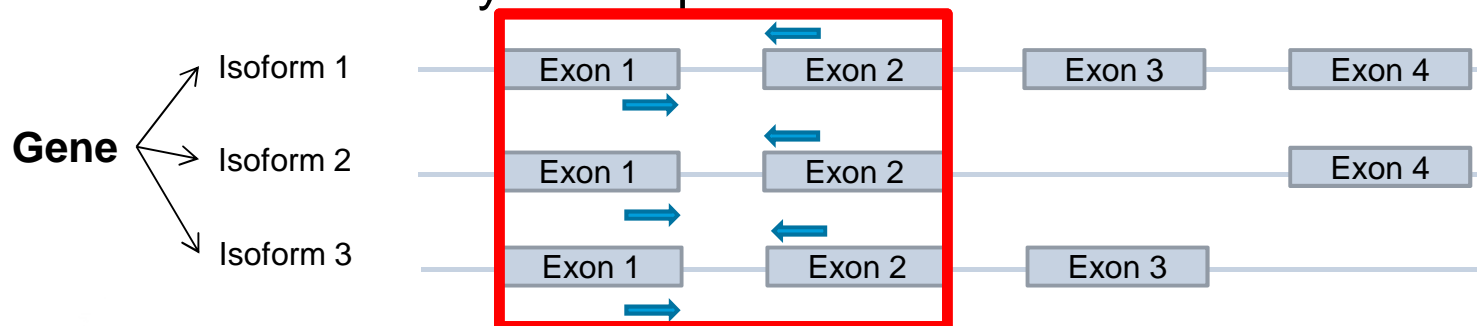
Transcriptome  
data from 10 ng  
of FFPE RNA

[Find out more](#)

life  
technologies

# Ion AmpliSeq™ Transcriptome – Overview

- **Comprehensive** coverage of RefSeq - 20,802 genes targeted
  - Single primer pool – simple workflow
  - Reports at **gene-level**
- **FFPE-compatible** as little as 10 ng RNA
  - Total RNA input from FFPE and other sources – **no selection or enrichment**
- Assay design
  - One amplicon per gene
    - ~150 bp amplicon size with ~110 bp insert size
  - Crosses exon boundary where possible

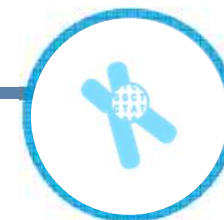


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# Ion AmpliSeq™ Custom Design



# Ion AmpliSeq™ Designer



Online panel design in four easy steps at [ampliseq.com](http://ampliseq.com)

Ion AmpliSeq™  
Designer

Ion AmpliSeq™  
Custom Panel



# Ion AmpliSeq™ Designer



Your Panel



Choose Application



## Start a new design

Need help? Watch our "My First Design" tutorial video. [?](#)

Assay Design Name \*

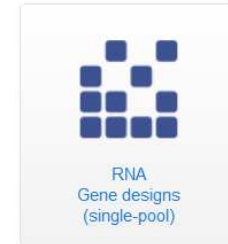
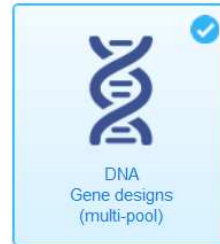
Details

2000 characters remaining (2000 maximum)

- Pick DNA or RNA
- Select a reference
- Give it a name

### Application type \*

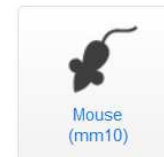
DNA designs may include a mix of gene and region targets, including small hotspot regions.



Interested in CNV detection for DNA panels? [?](#)

### Select the genome you wish to use \*

Standard [?](#)





# Ion AmpliSeq™ Designer



Your Panel



Choose Application



- Adding targets
  - Gene symbol
  - Genomic coordinates
  - dbSNP, COSMIC IDs
  - Upload BED/CSV file

IAD70878 - Draft

[Submit targets](#) [Edit all](#) [Delete](#) [Delete all](#) [Export targets](#)

✓ Target saved successfully

Exon padding 5 bp (Standard) ? DNA Type Standard DNA ? Targets size ~206.12 kb Amplicons ~636 Genome reference Human (hg19)

Type	Name	Symbol	Chrom	Start	End
<input type="checkbox"/> Gene (CDS Only)	KRAS	KRAS			
<input type="checkbox"/> Region	BRAF		chr7	140719327	140924764

<< First < Previous Showing 2 of 2 results Next > Last >> 15 Per page

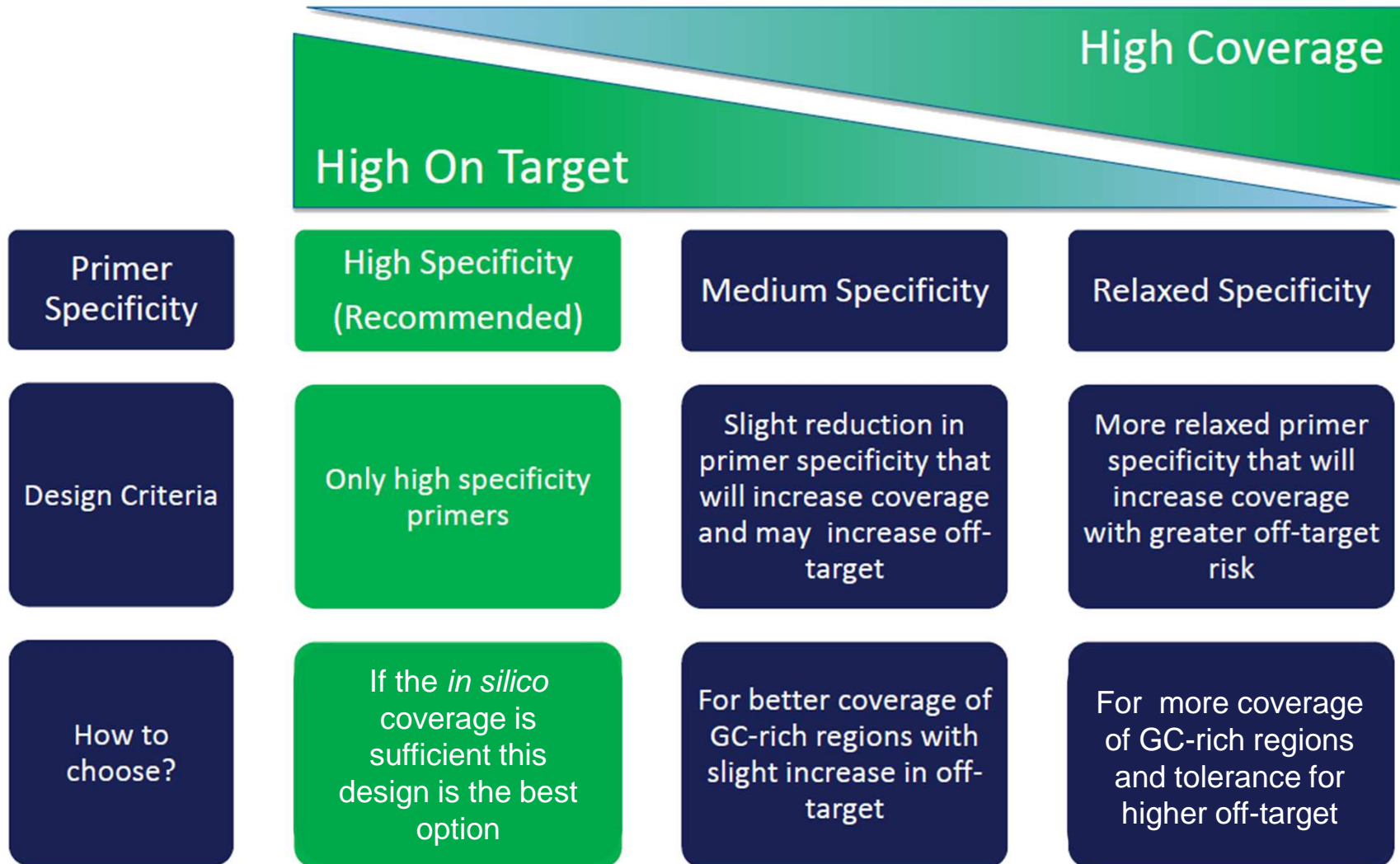
[Add Gene/Region](#) [Add Amplicon by ID](#) [Upload File](#)

Add padding around Exons? Adding variants (dbSNP, COSMIC) for design? ?

Type	Name (Optional)	Chrom	Start	End	
<input type="radio"/> Gene (CDS Only)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="button" value="Add target"/>
<input type="radio"/> Gene (CDS + UTR)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="button" value="Reset"/>
<input checked="" type="radio"/> Region	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	

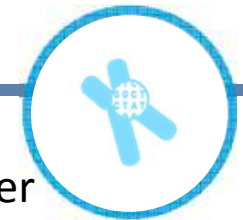
[Input Specifications](#)

# Choosing the Correct Design



# Ion AmpliSeq™ Designer

*Review your Design Before Ordering*



Your Panel



Choose Application

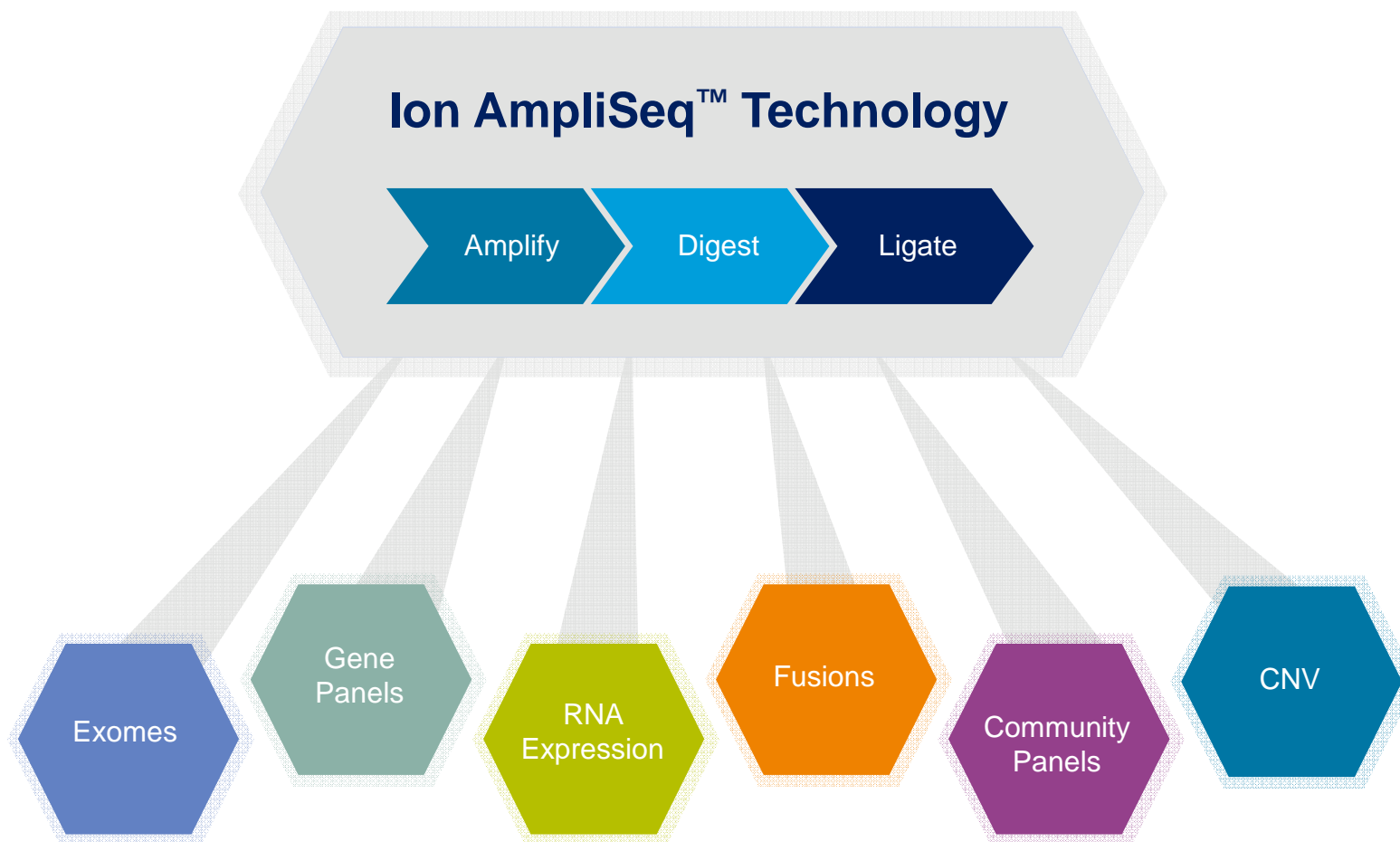


- Import it into a genome browser
- Coverage Summary
- Primer Details
- Amplicon ID, Fwd & Rev primer sequences
- Visualise *in silico* design in context with annotation tracks



# Broad Applications with Ion AmpliSeq™ Technology

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Start sequencing now at [lifetechnologies.com/ampliseq](http://lifetechnologies.com/ampliseq)

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