ThermoFisher SCIENTIFIC

Ion AmpliSeq™ Technology

Dr. Franziska Freund
Sr. Sequencing Sales Specialist

26.06.2015

The world leader in serving science

Ion AmpliSeq™ Technology: As Simple As PCR

Your targets, your genome, your panel

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

Simple

- As little as10 ng of DNA per pool
- FFPE-compatible
- PCR-based target selection

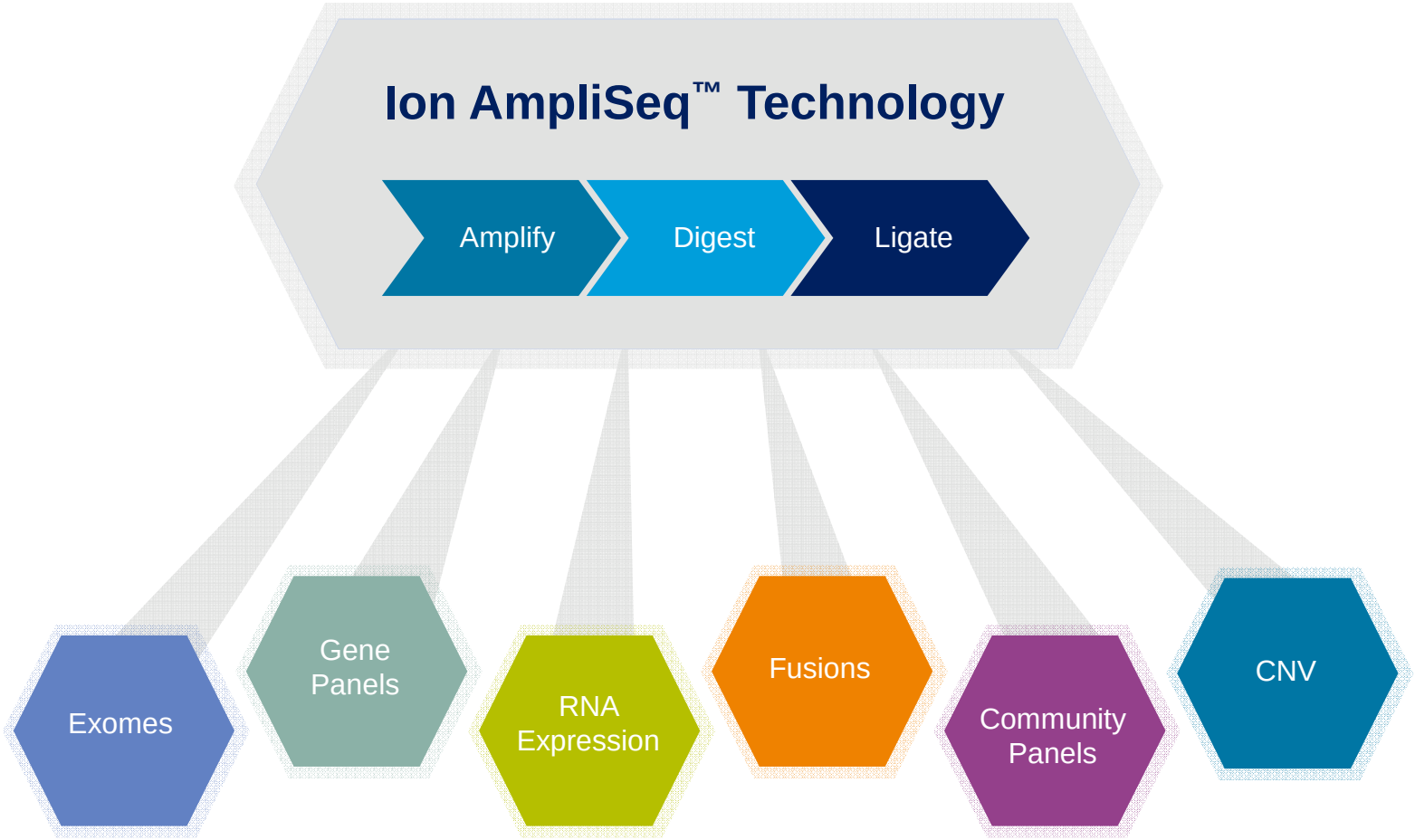
Scalable

- 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



ThermoFisher SCIENTIFIC

Ion AmpliSeq™ Target Selection Overview

(RNA only) Reverse transcribe the RNA

↓

Amplify targets

↓

Partially digest primer sequences

↓

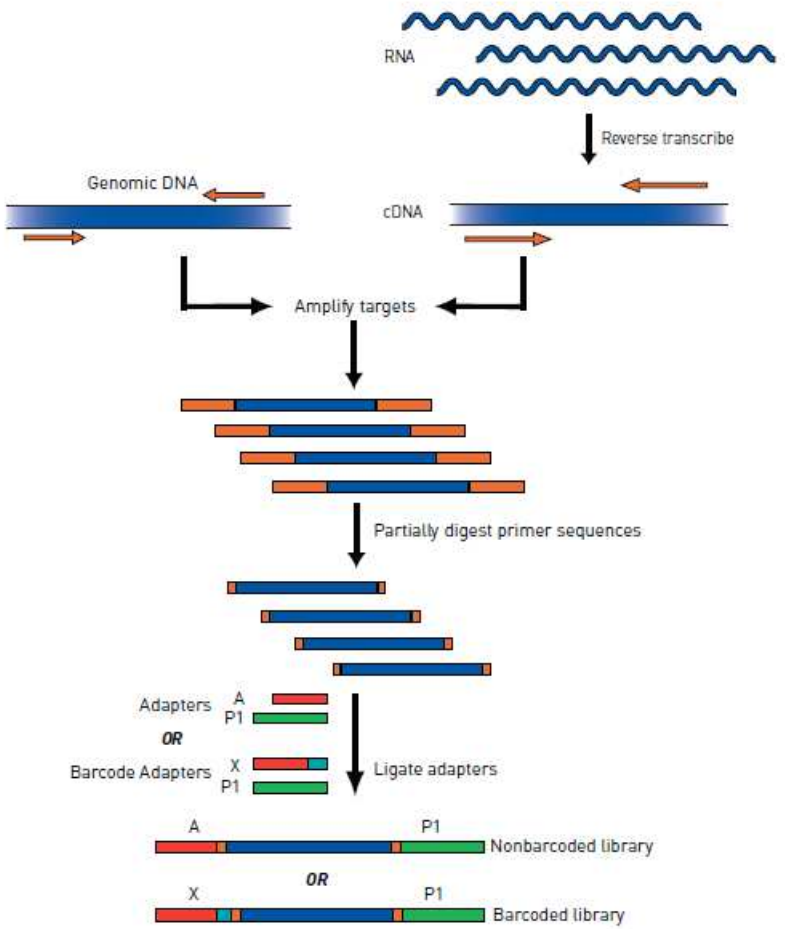
Ligate adapters to the amplicons and purify

↓

Option 1: Equalize the library (not recommended for libraries from RNA)	Option 2: Quantify the unamplified library by qPCR (recommended for libraries from RNA)	Option 3: Quantify the amplified library with the Qubit® 2.0 Fluorometer or Agilent® 2100 Bioanalyzer® instrument

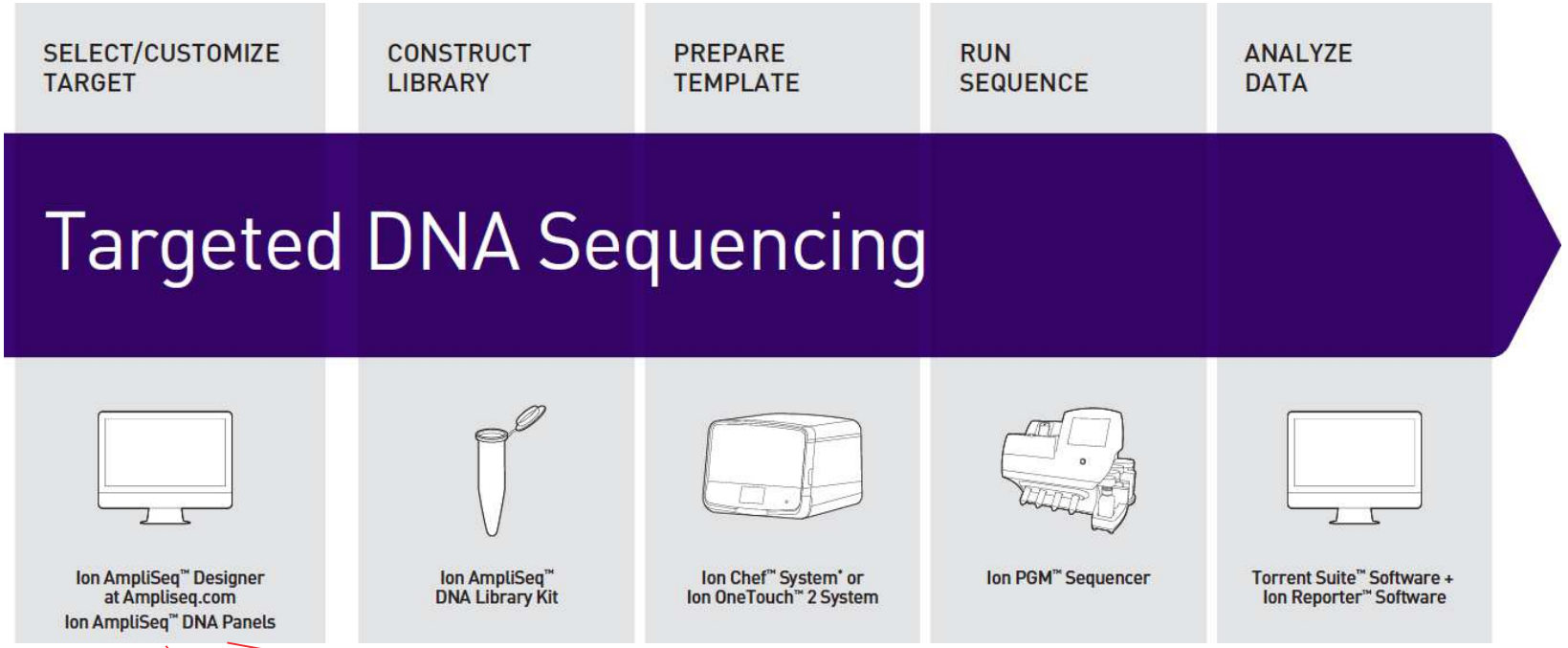
↓

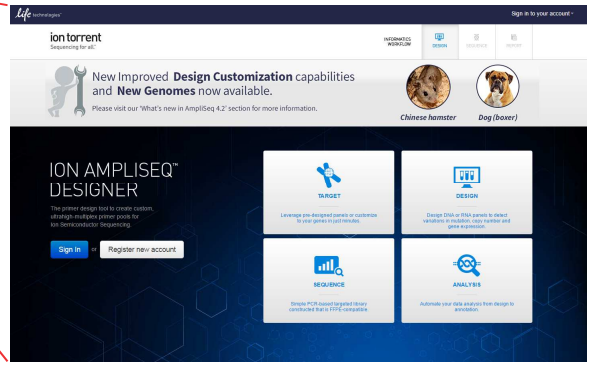
Combine libraries *(optional)* and proceed to template preparation.



ThermoFisher SCIENTIFIC

Technology Summary





http://www.ampliseq.com

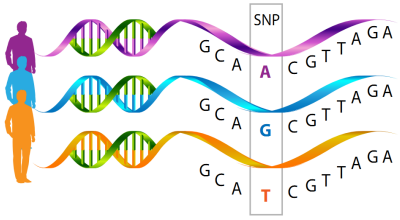
ThermoFisher SCIENTIFIC

AmpliSeq Designer Objectives

- 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:

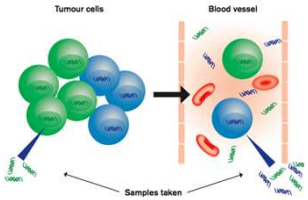
- RNA pre-calculated designs
- DNA gene designs
  - “Standard” AmpliSeq designs
- DNA hotspot designs
  - 1-pool designs for SNP’s



- 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



ThermoFisher SCIENTIFIC

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.

Ion AmpliSeq™ DNA panels

Ion AmpliSeq™ Cancer Hotspot Panel v2

The Ion AmpliSeq™ Cancer Panel targets 50 genes

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

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

As little as 10ng input
50 genes
207 amplicons

Gene list available www.lifetechnologies.com/ampliseqready

Ion AmpliSeq™ Comprehensive Cancer Panel (CCP)

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

Gene list available www.lifetechnologies.com/ampliseqready

ThermoFisher SCIENTIFIC

Ion AmpliSeq™ Exome

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

Prof. Orla Sheils
Trinity College Dublin,
Ireland

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

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

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

Oncomine™ Solid Tumour Fusion Transcript Kit

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

*according to PubMed

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

ThermoFisher SCIENTIFIC

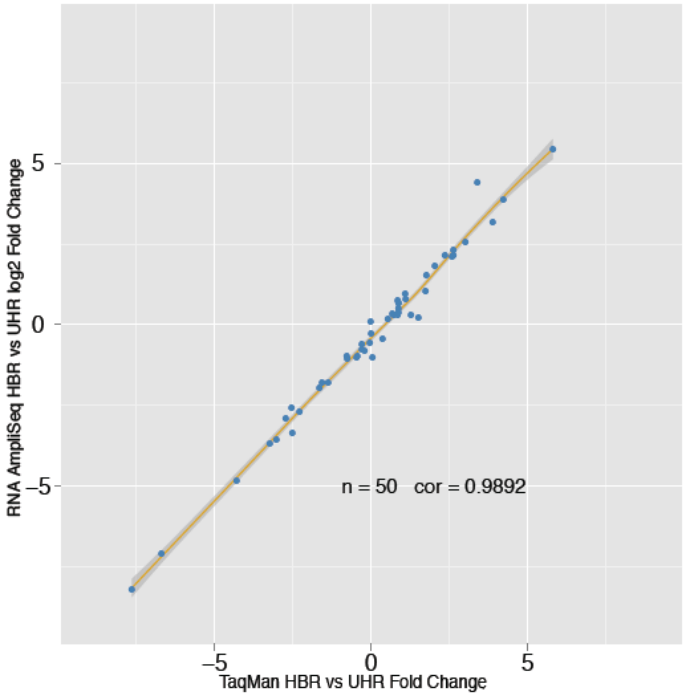
Ion AmpliSeq™ RNA Cancer Panel

50 genes, from 500 pg unfixed RNA, one tube

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

Targeted quantitative expression

Complement to Cancer Hotspot Panel

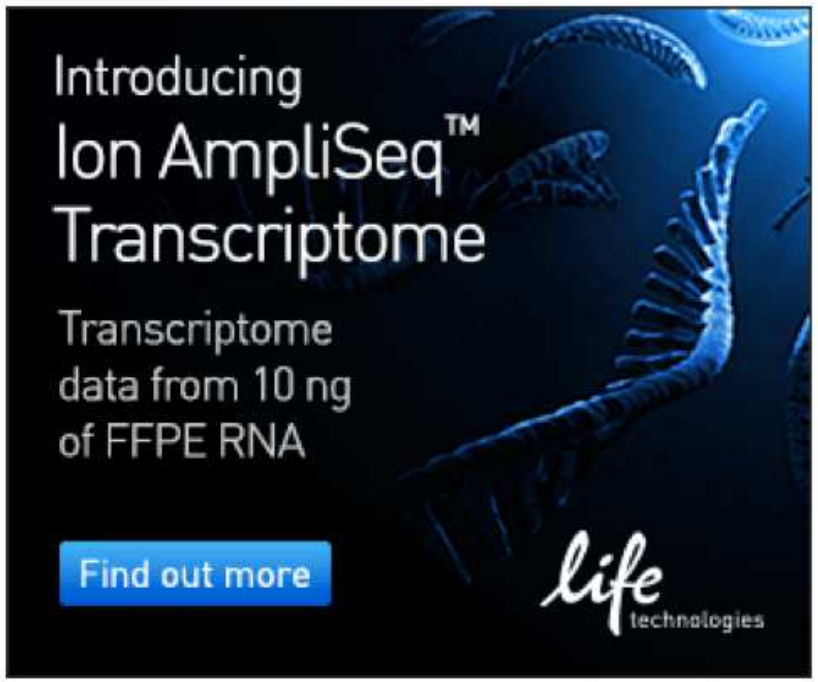


ThermoFisher SCIENTIFIC

Ion AmpliSeq™ Transcriptome

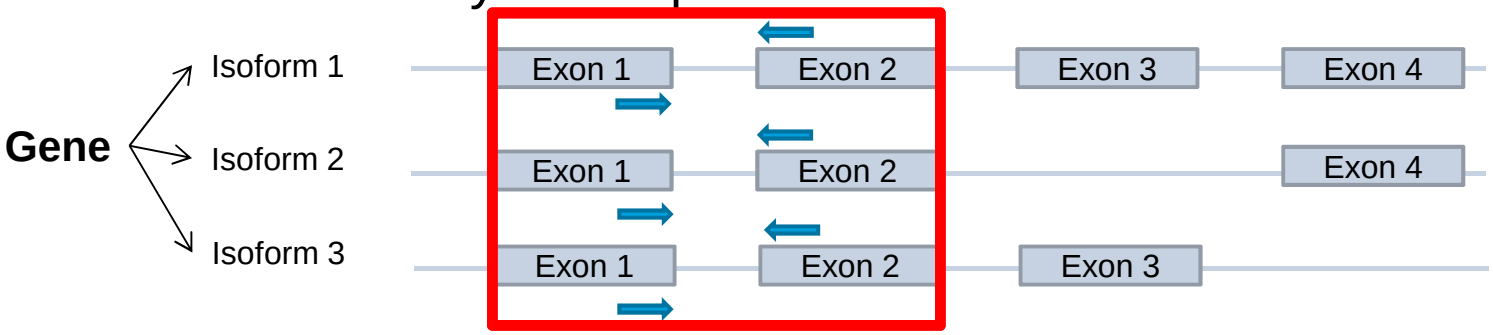
Rapid, cost-effective gene-level expression analysis with simple workflows compatible with FFPE samples

- **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



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

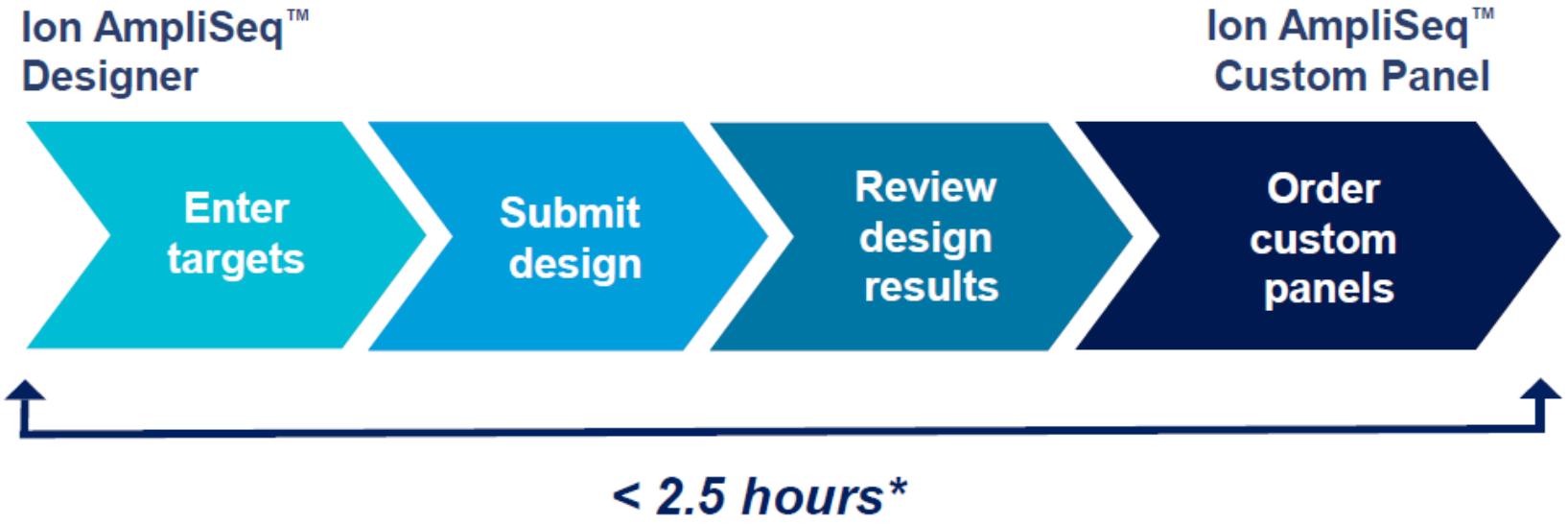


ThermoFisher SCIENTIFIC

Ion AmpliSeq™ Custom Design

Ion AmpliSeq™ Designer

Online panel design in four easy steps at ampliseq.com



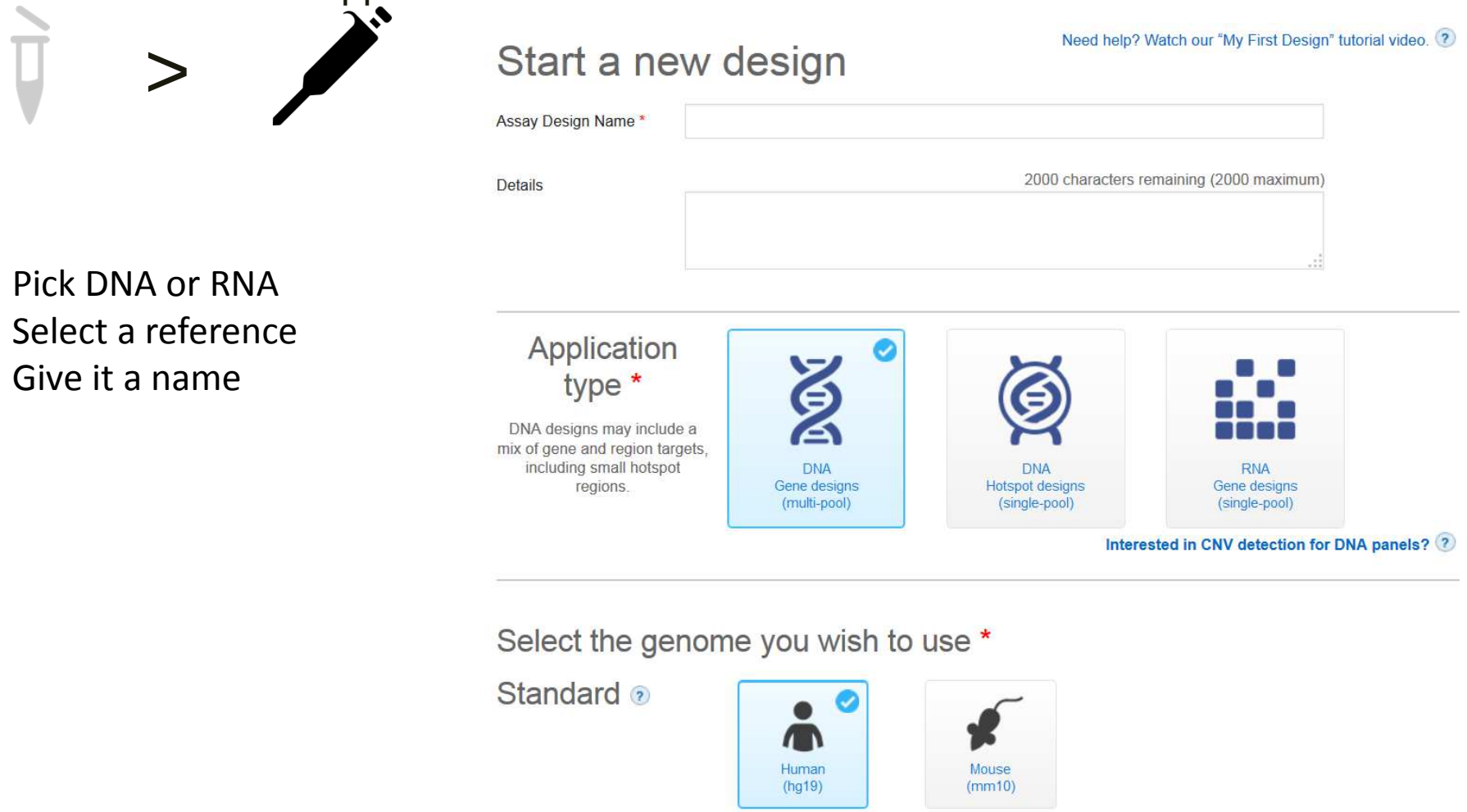
*2.5 hours based on 10 kb design. A 250 kb design can take up to 48 hours.

ThermoFisher SCIENTIFIC

Ion AmpliSeq™ Designer

Your Panel > Choose Application

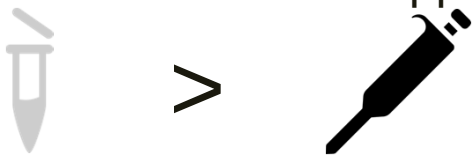
- Pick DNA or RNA
- Select a reference
- Give it a name



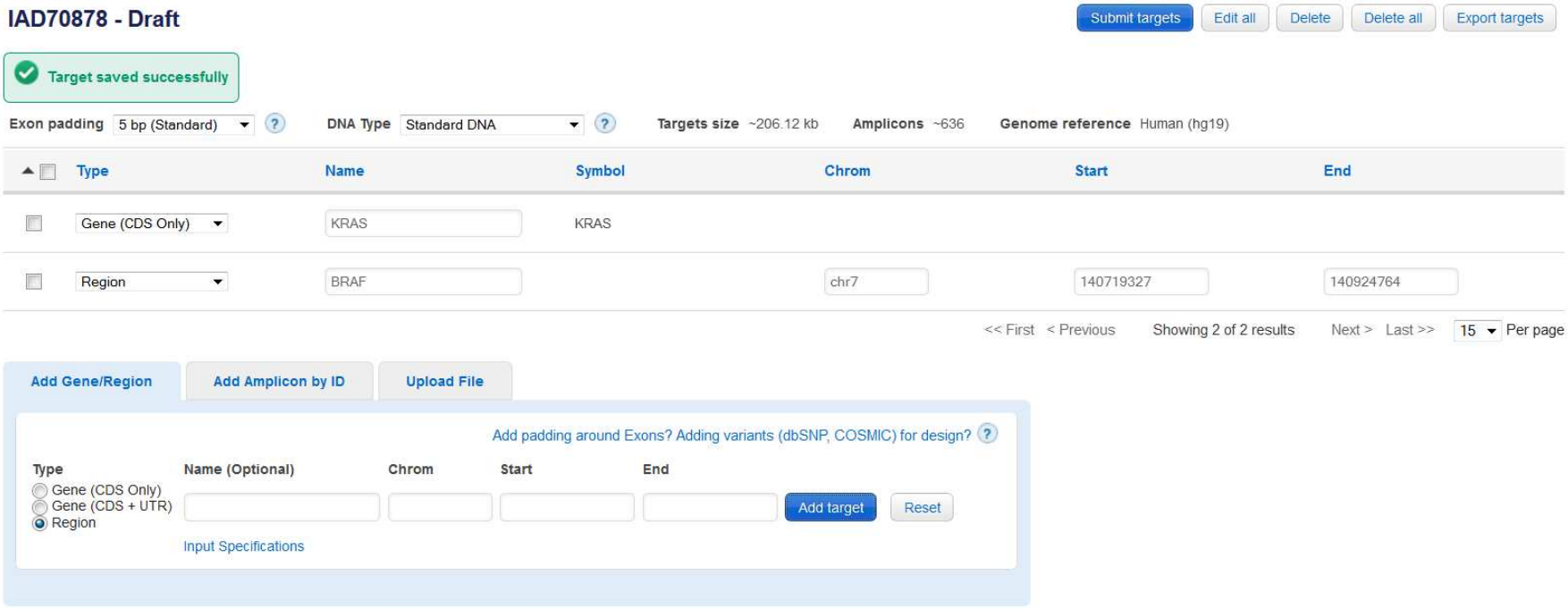
ThermoFisher SCIENTIFIC

Ion AmpliSeq™ Designer

Your Panel > Choose Application

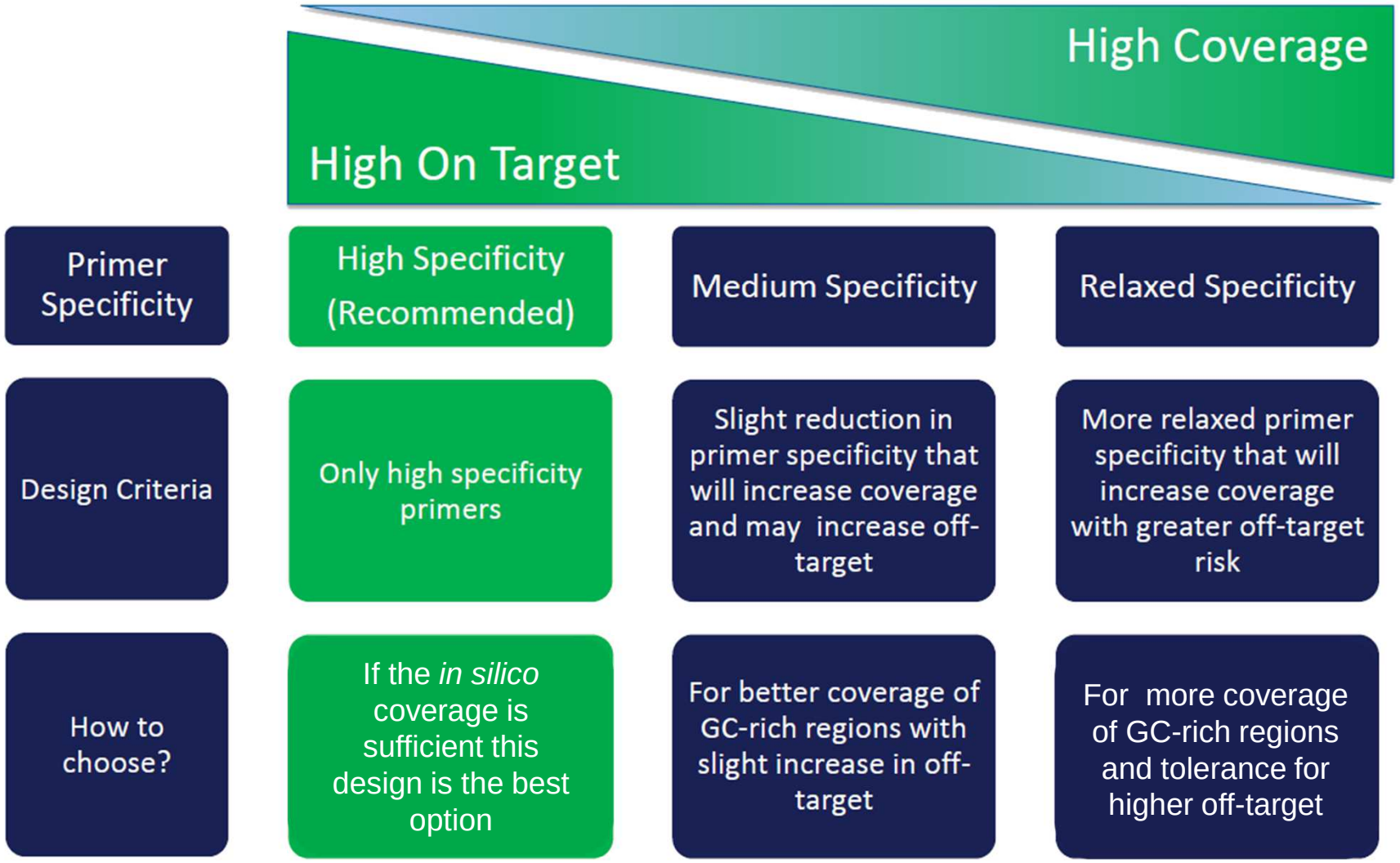


- Adding targets
  - Gene symbol
  - Genomic coordinates
  - dbSNP, COSMIC IDs
  - Upload BED/CSV file



ThermoFisher SCIENTIFIC

Choosing the Correct Design

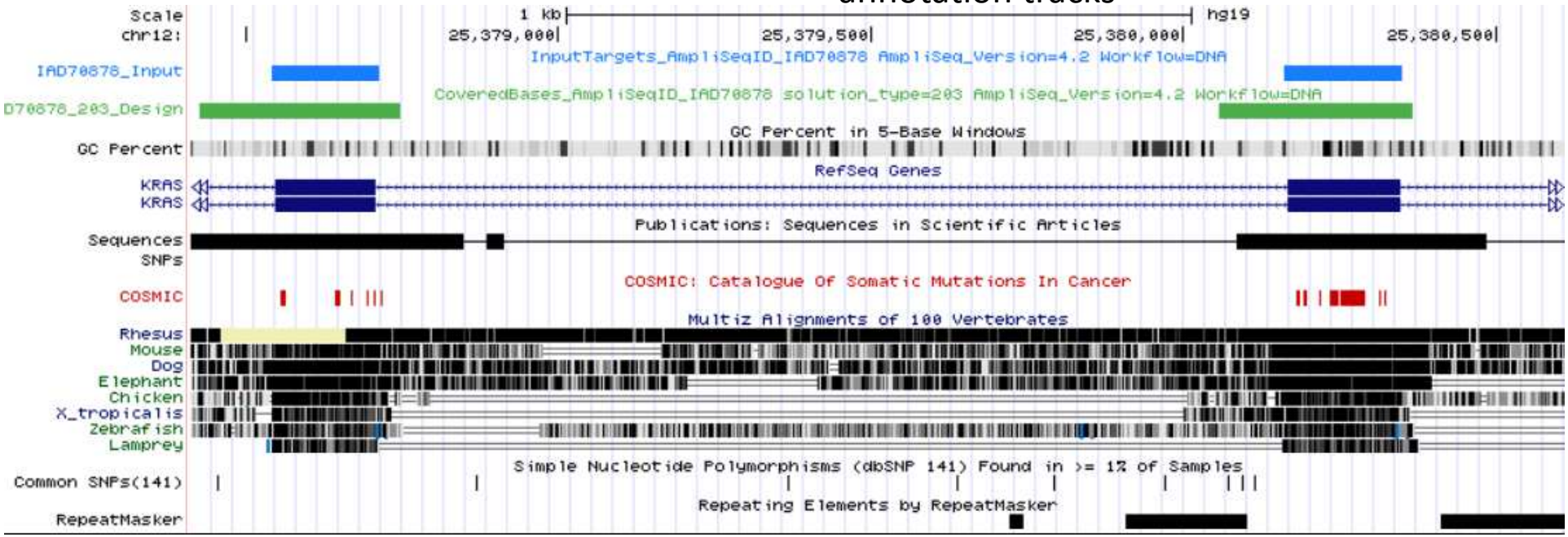


Ion AmpliSeq™ Designer

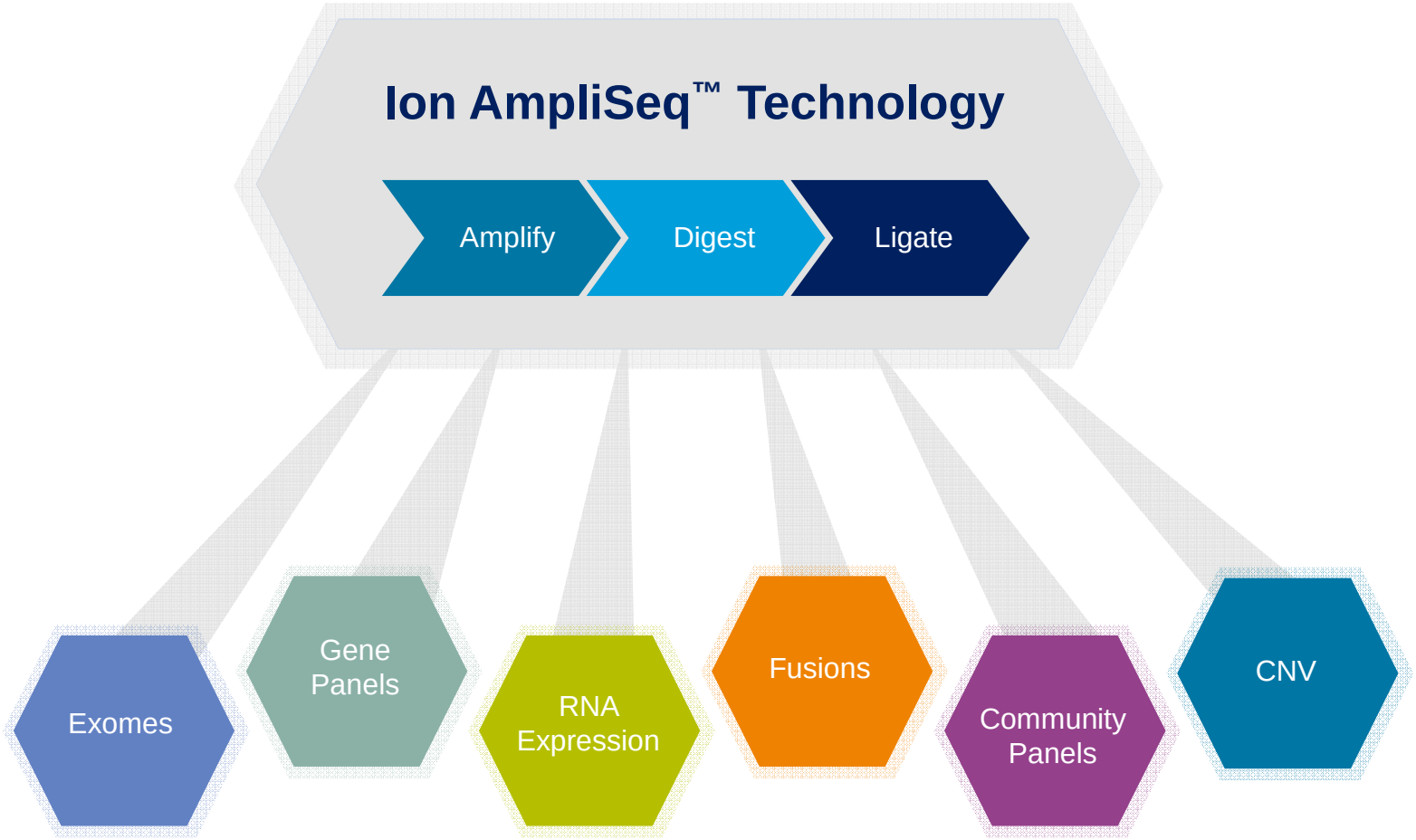
Review your Design Before Ordering

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



ThermoFisher SCIENTIFIC

Start sequencing now at lifetechnologies.com/ampliseq

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC