NGS e piattaforme integrate nella diagnostica oncologica

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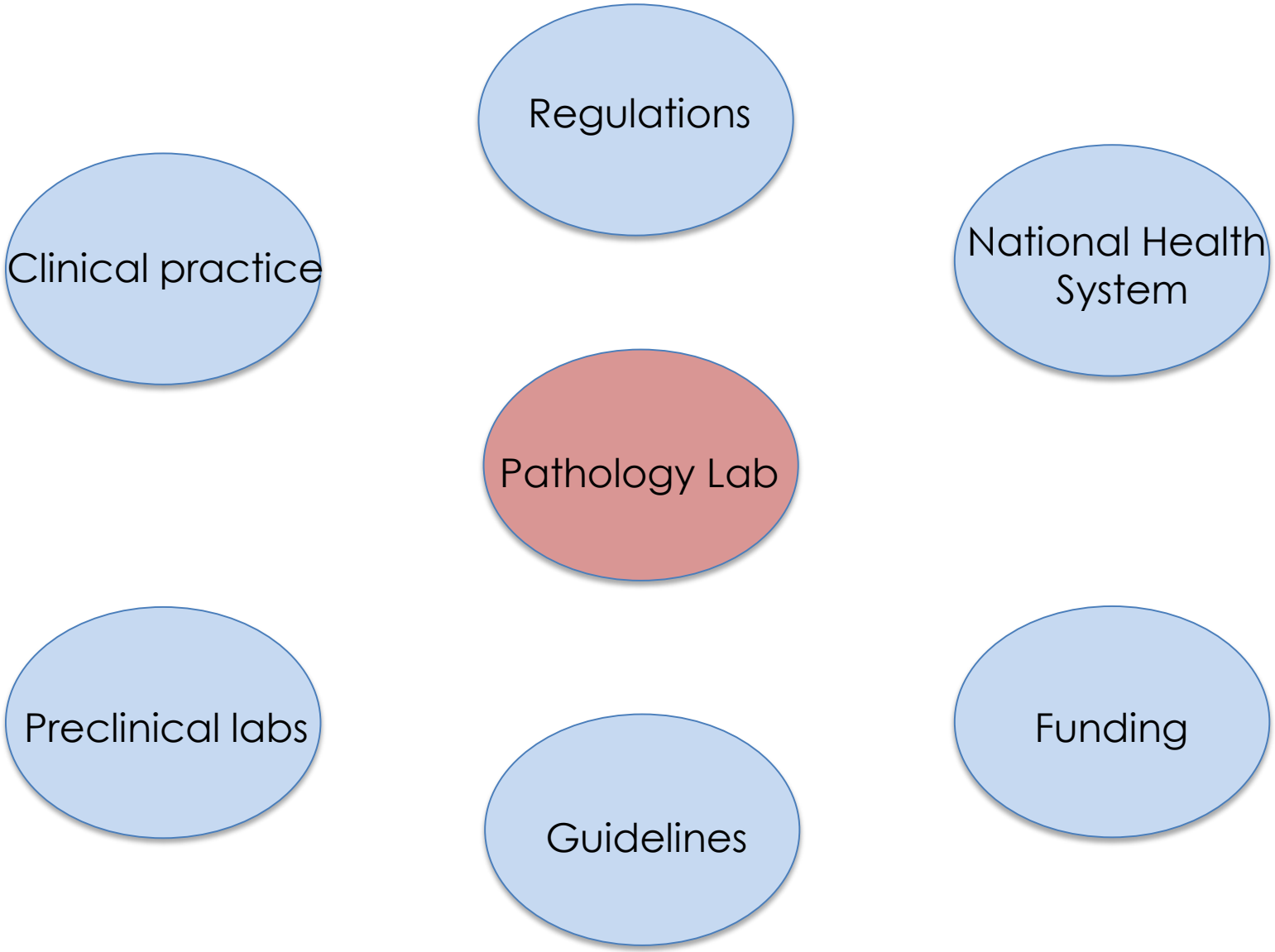
Pathology is a high tech hub for translational medicine

Setting the scene



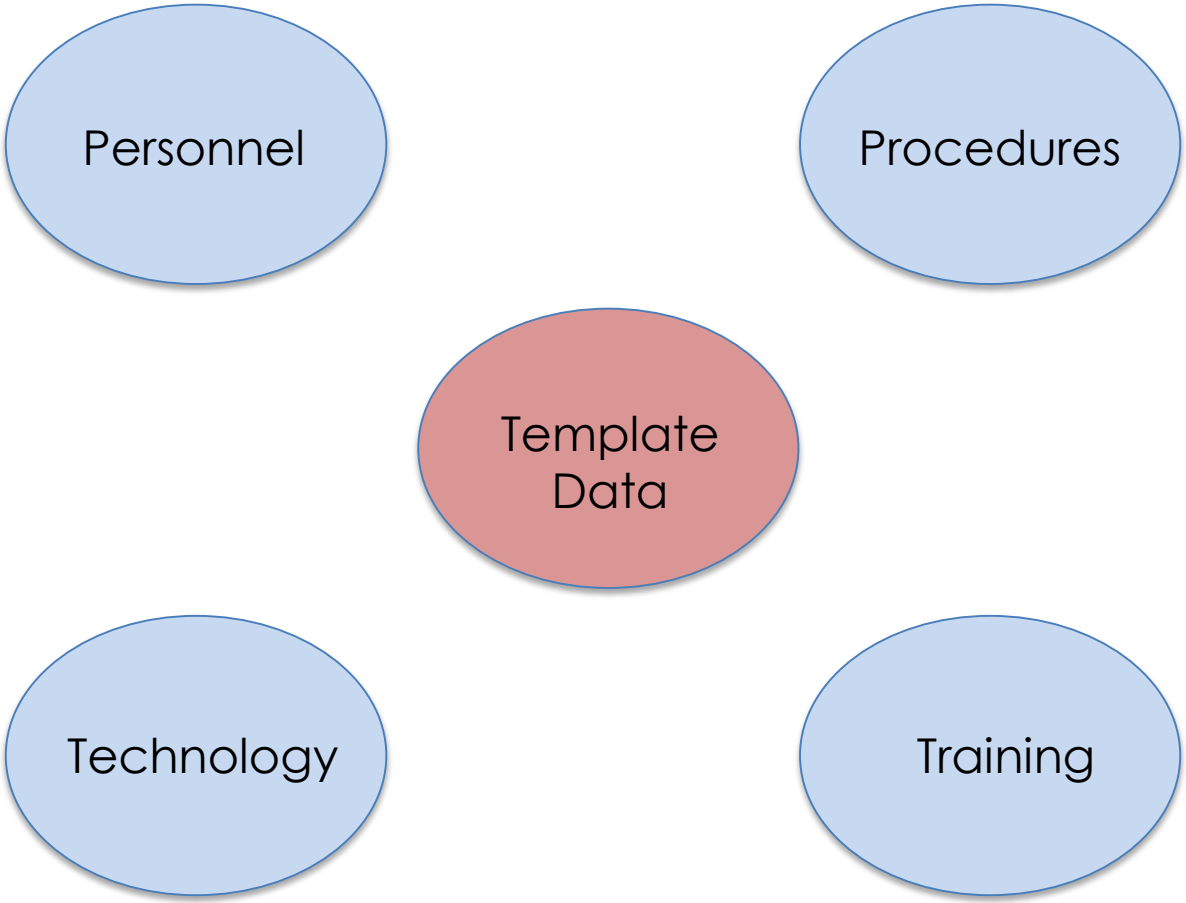
Biobanking

- Logistic
- FFPE
- Digital Pathology
- Fresh frozen
- Fresh
- Ethics

Data landscape –> Clinic/Research

- Tumor classification
- Data collection
- Laser capture microdissection
- Tissue microrray
- Digital Pathology
- IHC
- ISH, FISH
- Western blot
- RT-PCR
- Gene expression
- Gene sequencing
- NGS
- Cytogenetics
- Bioinformatic

Pathology for translational research



The times they are a changing’

Surgery -> Diagnosis -> Treatment

A paradigm shift

Surgery/Biopsy/Plasma/Saliva/Urine

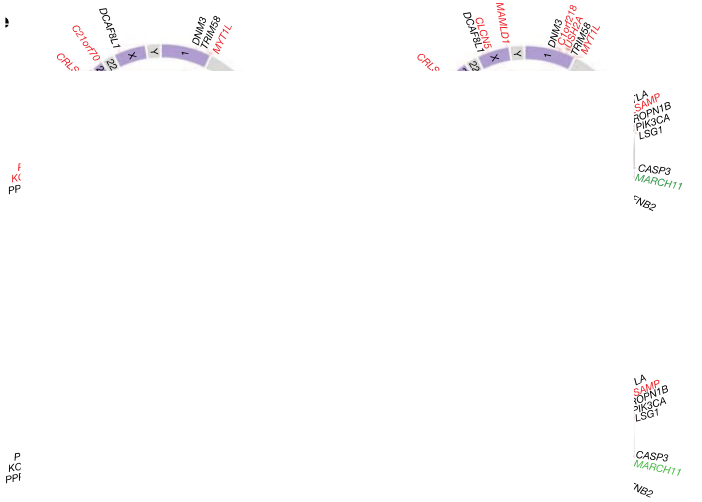
Diagnosis

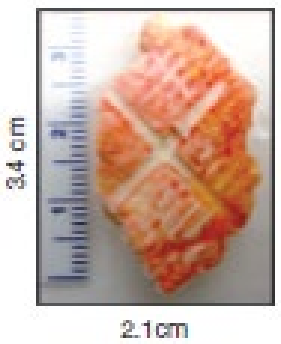
Predictive factors

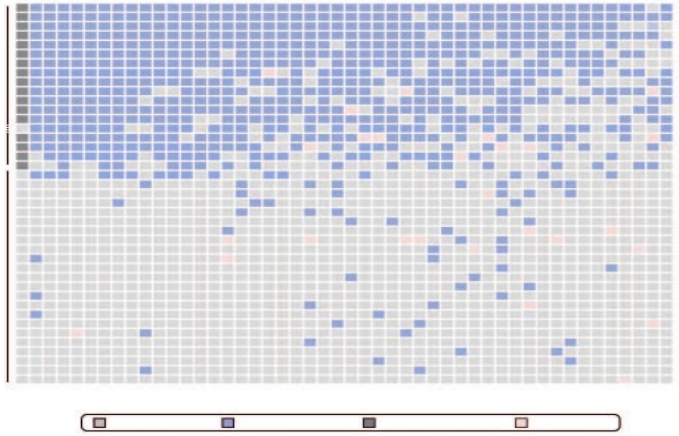
Prognostic factors

Tumor/Microenvironment

Tumor heterogeneity in breast cancer occurs at single cell level

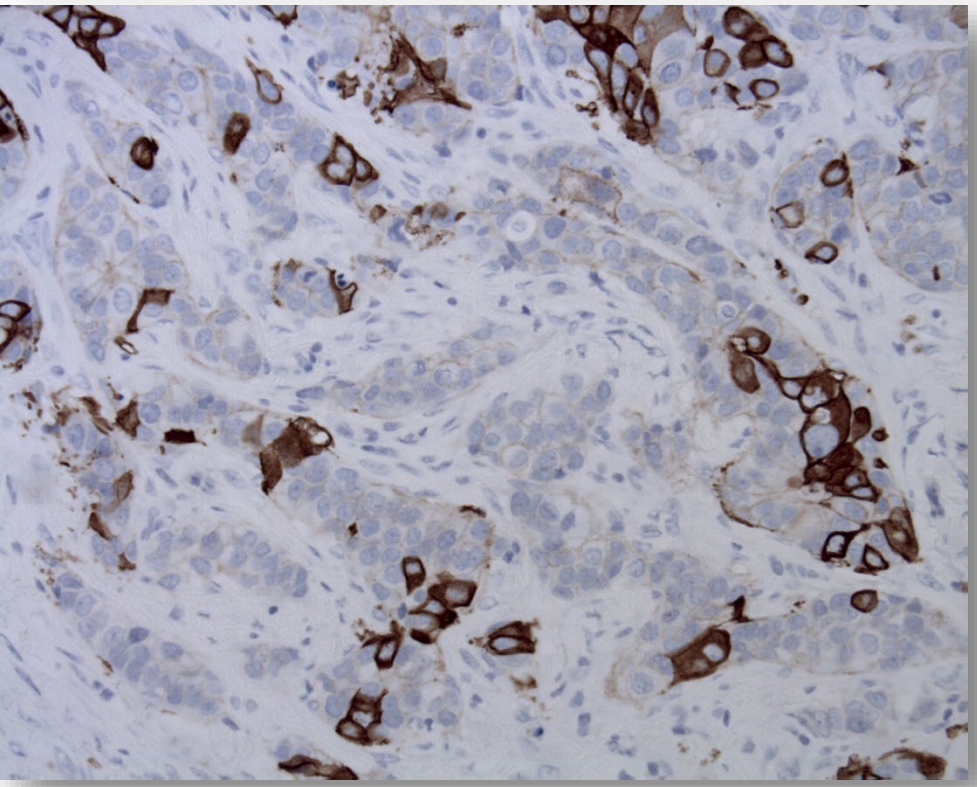


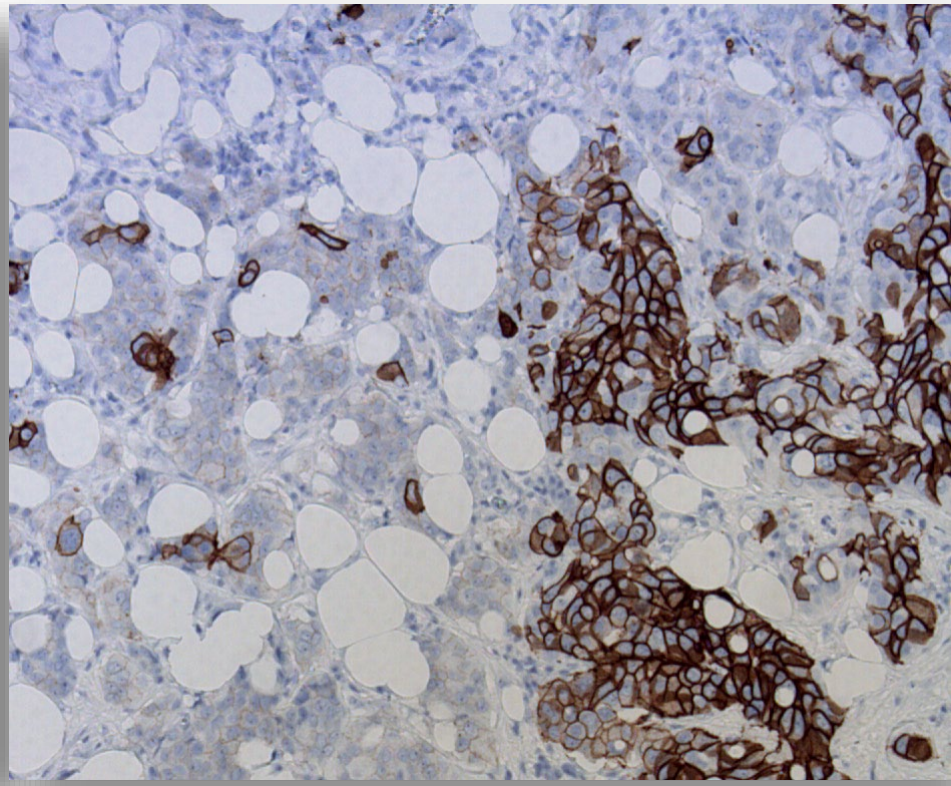




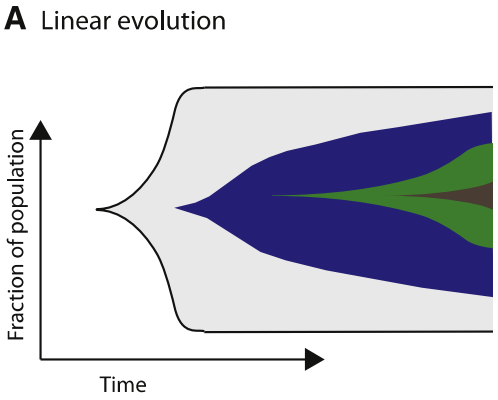
Wang, Nature, 2014

Intratumoral heterogeneity at the bedside: HER2



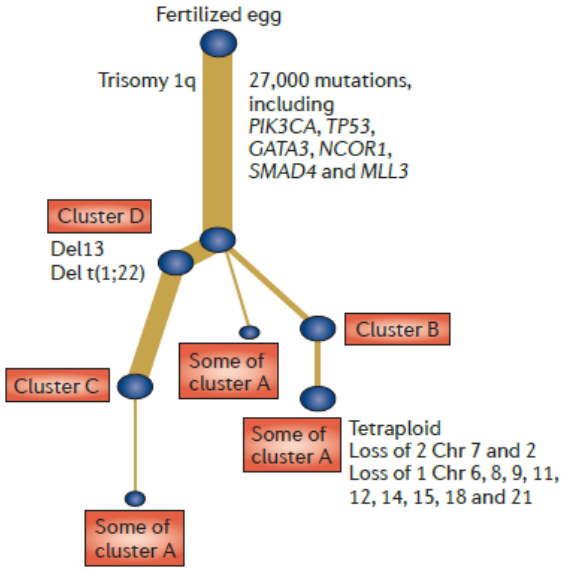


From the parent tumor to its progeny



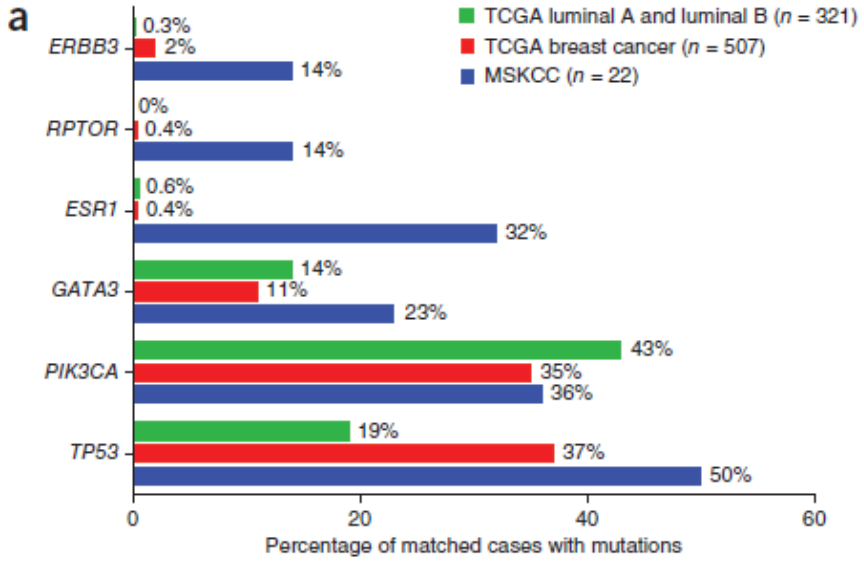
McGranahan & Swanton, Cancer Cell, 2015

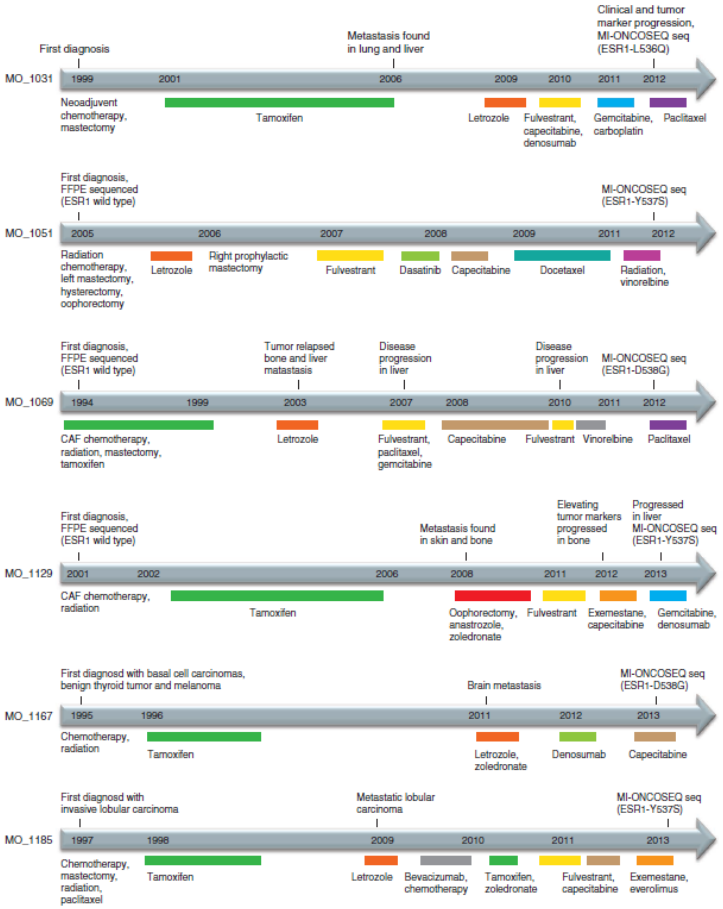
Darwin C, On The Origin of Species, 1859



Yates & Campbell, Nat Rev Genet, 2015

Activating ESR1 mutations lead to hormone therapy resistance





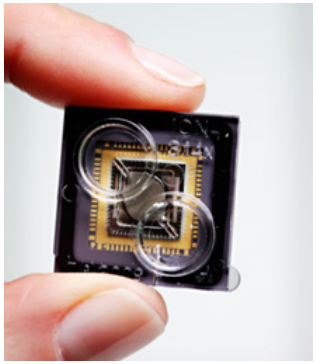
Toy, Nat Genet, 2013

Robinson, Nat Genet, 2013

NGS, it's all about procedures
Which platform?

	Preparazione libreria	*Preparazione templato*	*Sequenziamento*
Solexa Genome Analyzer (Illumina)	Frammentazione random	Amplificazione clonale in fase solida (*Bridge Amplification)*	Terminazione ciclica reversibile, a 4 colori
454 (Roche)	Frammentazione random	Amplificazione clonale mediante PCR in emulsione	Pirosequenziamento
SOLiD (Life/APG)	Frammentazione random	Amplificazione clonale mediante PCR in emulsione	Mediante ligazione
HeliScope (Helicos BioSciences)	Frammentazione random	Templato a singola molecola	Terminazione ciclica reversibile, a 1 colore
SMRT (Pacific Bioscences)	Frammentazione random	Templato a singola molecola	Real-time sequencing

Ion Chef Systems



Ion torrent pgm

Ion S5™ System

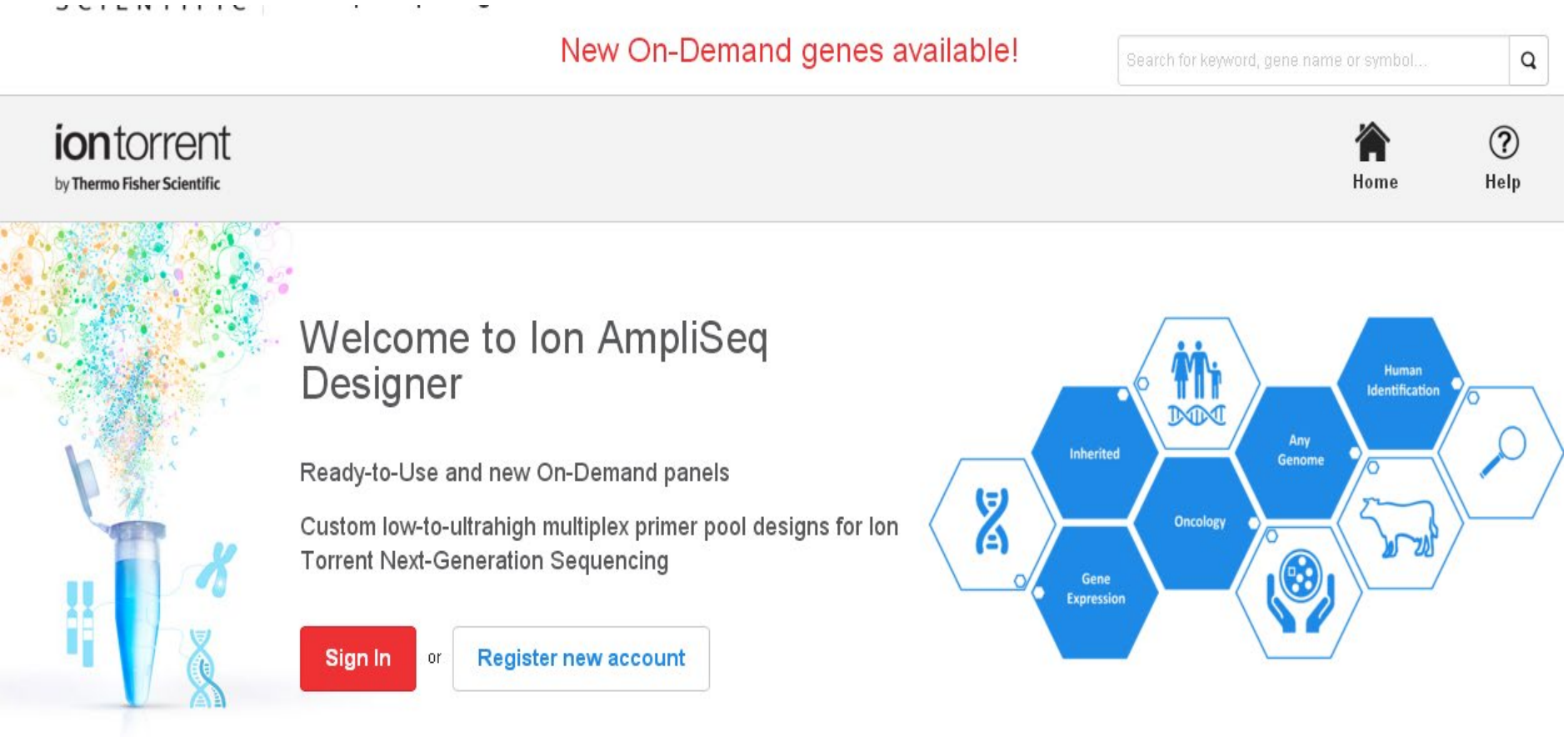
Ion AmpliSeq Cancer Hotspot Panel

- Requires only 10 ng of FFPE or higher-quality gDNA, yields library in ~3.5 hours
- Panel targets >2,800 COSMIC mutations in 50 cancer-associated genes
- A single tube of primers for 207 amplicons (avg length= 154 bp)
- Samples can be barcoded and library-prep automated for multiplexing

The 50 targeted 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*	

Tackling complexity: customized panels



Crossing the border: is it a germinal or somatic business?

Ion AmpliSeq *BRCA1* and *BRCA2* Panel	
Targets	Coding regions of *BRCA1* and *BRCA2* genes
Average amplicon length	200 bp
Primer pools	167 pairs of primers in three primer pair pools
Input DNA	30 ng*
Amplicon coverage	• 100% of all targeted coding exons and exon–intron boundaries • Expanded target regions—additional coverage 10–20 bases beyond the targeted coding exon and exon–intron boundaries • Sequence coverage redundancy with overlapping amplicons across exons • High-fidelity primers
Verification	Verified by two laboratories on 65 samples with known mutations, including homopolymer variants with 7 and 9 bases; these samples were previously detected using capillary electrophoresis, and verification on the Ion PGM™ System yielded 100% sensitivity**
Multiplexing recommended	8 samples on an Ion 316™ Chip

The bigger the better?

Ion AmpliSeq™ Comprehensive Cancer Panel target gene list

The Ion AmpliSeq™ Comprehensive Cancer Panel targets the exons of 409 tumor suppressor genes and oncogenes frequently cited and frequently mutated. Strategically designed to interrogate coding DNA sequences and splice variants across multiple gene families simultaneously, our pathway-based gene selection profiles the mutational spectrum in cancer driver genes and drug targets along with sig apoptosis genes, DNA repair genes, transcri inflammatory response genes, and growth f single assay. Additionally, this panel targets targeted in the focused Ion AmpliSeq™ Cance genes are indicated in dark grey cells.

ABL1	AURKA	BMPR1A	CDK4	CTNNB1	EPHB4	FANCD2
ABL2	AURKB	BRAF	CDK6	CYLD	EPHB6	FANCF
ACVR2A	AURKC	BRD3	CDK8	CYP2C19	ERBB2	FANCG
ADAMTS20	AXL	BRIP1	CDKN2A	CYP2D6	ERBB3	FAS
AFF1	BAI3	BTK	CDKN2B	DAXX	ERBB4	FBXW7
AFF3	BAP1	BUB1B	CDKN2C	DCC	ERCC1	FGFR1
AKAP9	BCL10	CARD11	CEBPA	DDB2	ERCC2	FGFR2
AKT1	BCL11A	CASC5	CHEK1	DDIT3	ERCC3	FGFR3
AKT2	BCL11B	CBL	CHEK2	DDR2	ERCC4	FGFR4
AKT3	BCL2	CCND1	CIC	DEK	ERCC5	FH
ALK	BCL2L1	CCND2	CKS1B	DICER1	ERG	FLCN
APC	BCL2L2	CCNE1	CMPK1	DNMT3A	ESR1	FLI1
AR	BCL3	CD79A	COL1A1	DPYD	ETS1	FLT1
ARID1A	BCL6	CD79B	CRBN	DST	ETV1	FLT3
ARID2	BCL9	CDC73	CREB1	EGFR	ETV4	FLT4
ARNT	BCR	CDH1	CREBBP	EML4	EXT1	FN1
ASXL1	BIRC2	CDH11	CRKL	EP300	EXT2	FOXL2
ATF1	BIRC3	CDH2	CRTC1	EP400	EZH2	FOXO1
ATM	BIRC5	CDH20	CSF1R	EPHA3	FAM123B	FOXO3
ATR	BLM	CDH5	CSMD3	EPHA7	FANCA	FOXP1
ATRX	BLNK	CDK12	CTNNA1	EPHB1	FANCC	FOXP4

IDH1	KRAS	MLH1	NFKB2	PIK3C2B	RARA	SOCS1	TOP1
IDH2	LAMP1	MLL	NIN	PIK3CA	RB1	SOX11	TP53
IGF1R	LCK	MLL2	NKX2-1	PIK3CB	RECQL4	SOX2	TPR
IGF2	LIFR	MLL3	NLRP1	PIK3CD	REL	SRC	TRIM24
IGF2R	LPHN3	MLLT10	NOTCH1	PIK3CG	RET	SSX1	TRIM33
IKBKB	POT1	MMP2	NOTCH2	PIK3R1	RHOH	STK11	TRIP11
IKBKE	LPP	MN1	NOTCH4	PIK3R2	RNASEL	STK36	TRRAP
IKZF1	LRP1B	MPL	NPM1	PIM1	RNF2	SUFU	TSC1
IL2	LTF	MRE11A	NRAS	PKHD1	RNF213	SYK	TSC2
IL21R	LTK	MSH2	NSD1	PLAG1	ROS1	SYNE1	TSHR
IL6ST	MAF	MSH6	NTRK1	PLCG1	RPS6KA2	TAF1	UBR5
IL7R	MAFB	MTOR	NTRK3	PLEKHG5	RRM1	TAF1L	UGT1A1
ING4	MAGEA1	MTR	NUMA1	PML	RUNX1	TAL1	USP9X
IRF4	MASI1	MTRR	NUP214	PMS1	RUNX1T1	TBX22	VHL
IRS2	MALT1	MUC1	NUP98	PMS2	SAMD9	TCF12	WAS
ITGA10	MAML2	MUTYH	PAK3	POU5F1	SBDS	TCF3	WHSC1
ITGA9	MAP2K1	MYB	PALB2	PPARG	SDHA	TCF7L1	WRN
ITGB2	MAP2K2	MYC	PARP1	PPP2R1A	SDHB	TCF7L2	WT1
ITGB3	MAP2K4	MYCL1	PAX3	PRDM1	SDHC	TCL1A	XPA
JAK1	MAP3K7	MYCN	PAX5	PRKAR1A	SDHD	TET1	XPC
JAK2	MAPK1	MYD88	PAX7	PRKDC	SEPT9	TET2	XPO1
JAK3	MAPK8	MYH11	PAX8	PSIP1	SETD2	TFE3	XRCC2
JUN	MARK1	MYH9	PBRM1	PTCH1	SF3B1	TGFBR2	ZNF384
KAT6A	MARK4	NBN	PBX1	PTEN	SGK1	TGM7	ZNF521
KAT6B	MBD1	NCOA1	PDE4DIP	PTGS2	SH2D1A	THBS1	
KDM5C	MCL1	NCOA2	PDGFB	PTPN11	SMAD2	TIMP3	
KDM6A	MDM2	NCOA4	PDGFRA	PTPRD	SMAD4	TLR4	
KDR	MDM4	NF1	PDGFRB	PTPRT	SMARCA4	TLX1	
KEAP1	MEN1	NF2	PER1	RAD50	SMARCB1	TNFAIP3	
KIT	MET	NFE2L2	PGAP3	RAF1	SMO	TNFRSF14	
KLF6	MITF	NFKB1	PHOX2B	RALGDS	SMUG1	TNK2	

Hot topics

- Centralizing vs. distributing
- Social and political issues
- Personnel training
- Partnership with universities and companies
- Advisory board
- Relationship with pre-clinical and research units
- Personalized approach
- Communication

Lung cancer workflow

FFPE material of primary tumor/metastasis

NGS with the Hot spot cancer panel

EGFR
KRAS
BRAF
STK11
ALK
PTEN
Others…

FISH for ALK and ROS
IHC for PD1

Recurrence after target treatment

Re-biopsy →

NGS /Hot spot
T790M not well covered
High coverage

Liquid biopsy →

Easy® EGFR RT PCR
CE IVD test

Updating MD training in a brand new world

- Pathology post-graduate course: five years
- Autopsy, surgical pathology, cytology, IHC interpretation, grading, staging, intraop exams

- Same as above, plus:

-Molecular labs

-Molecular MTD

-Oncology/pharmacology

-Bioinformatics

-Stages abroad

-Ethics

-Psychology