



Target enrichment using selector probes

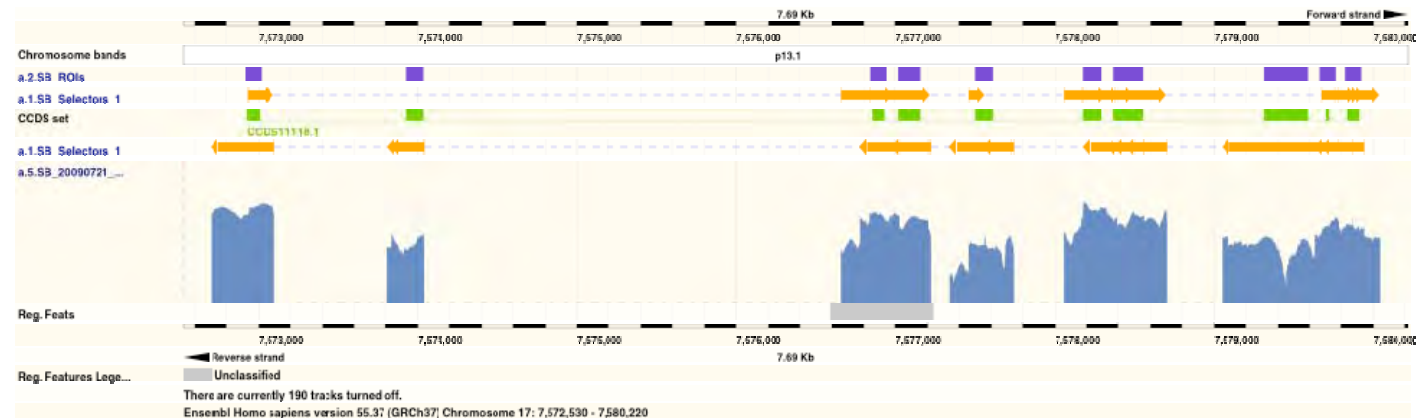
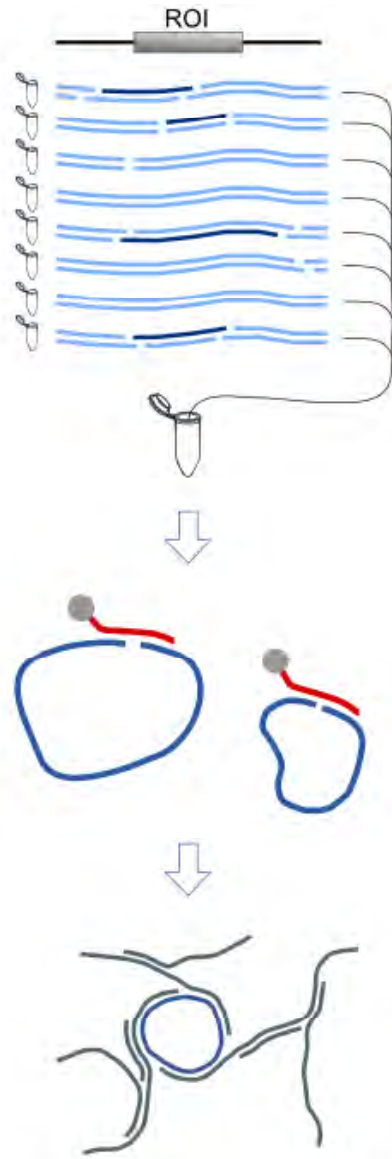
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Uppsala University

Target enrichment using selector probes

- Even with radically improved sequencing throughput, targeting will remain important when deep sequencing is required, for example:
- to find somatic mutations in genetically heterogeneous tumor samples
- and probably for diagnostics.

The selector technique



Dahl, *et al.* (2005) *Nucleic Acids Res.* **33**, e71

Dahl, *et al.* (2007) *Proc. Natl. Acad. Sci. USA* **104**, 9387-9392

Johansson, *et al.*, submitted

Gene panel for somatic mutation detection

AKT1

AKT2

AKT3

APC

ATM

BRAF

CCND1

CCNE1

CDKN2A

CTNNB1

EGFR

FBXW7

GNAS

HER2

HER3

HER4

IDH1

KRAS

MET

MRE11A

NF1

TP53

PIK3CA

PTEN

SMAD2

SMAD3

SMAD4

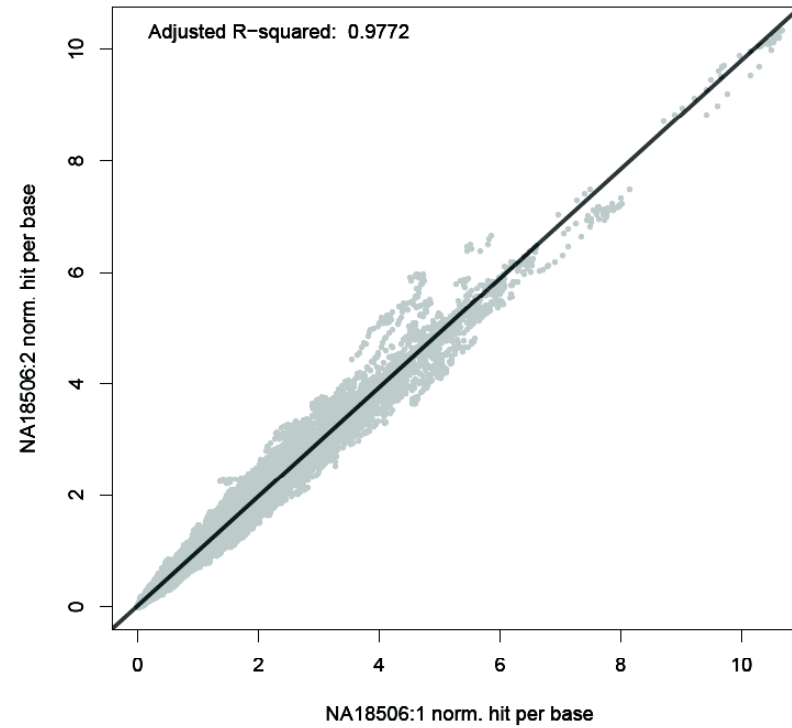
STK11

501 exons

222 kb amplified region

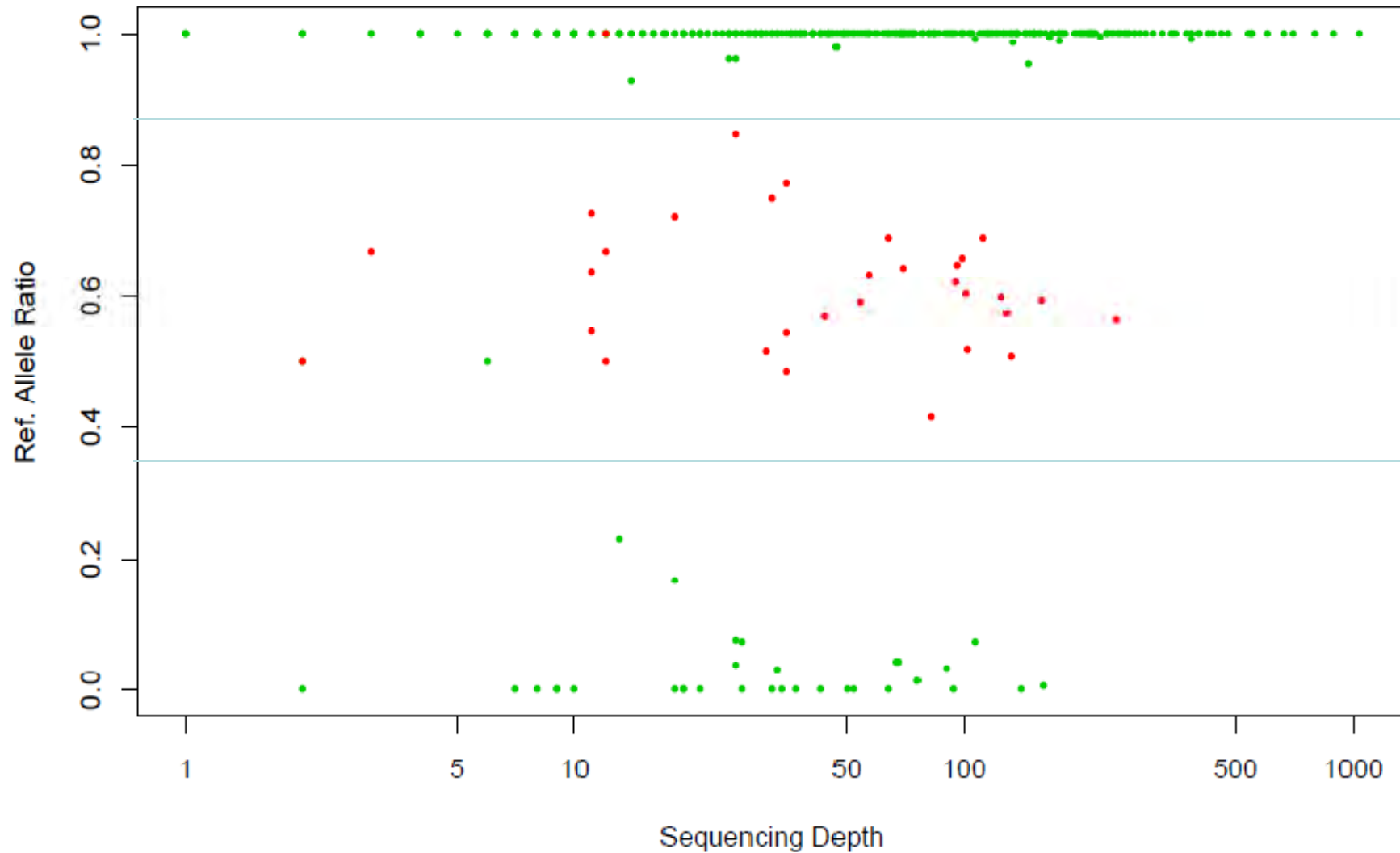
Bar-coded SOLiD3 sequencing

Reproducible coverage



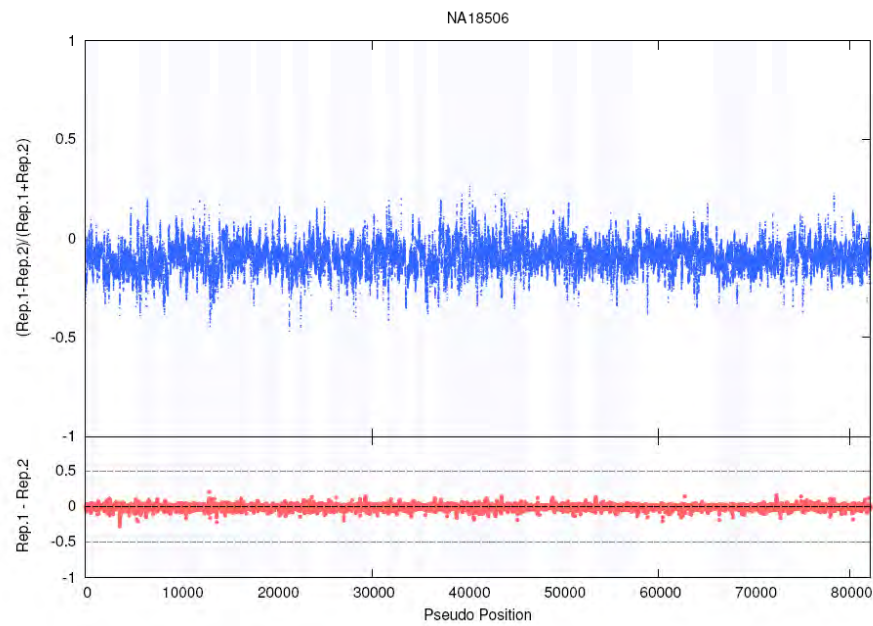
Concordance with HapMap SNPs

NA18506 Ampregion

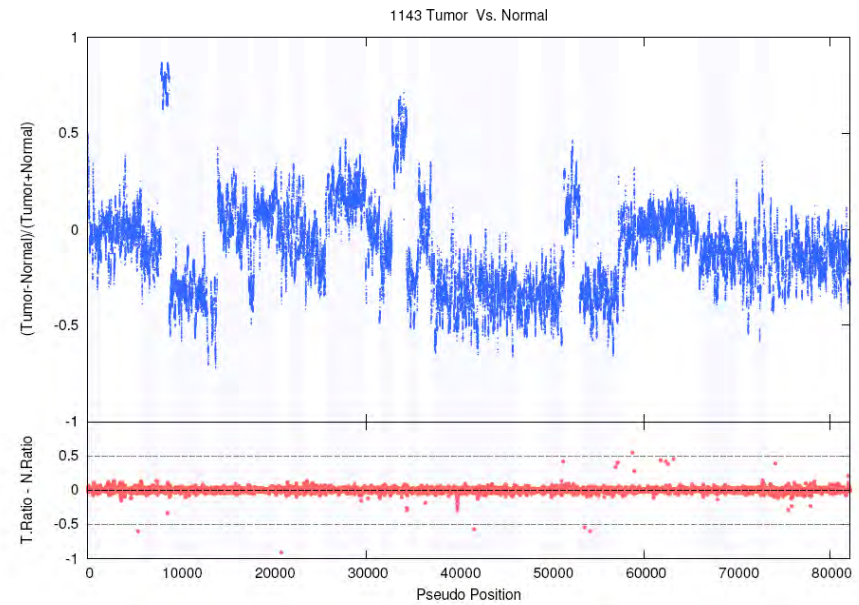


Somatic mutation detection

Rep1/rep2



Tumor/normal



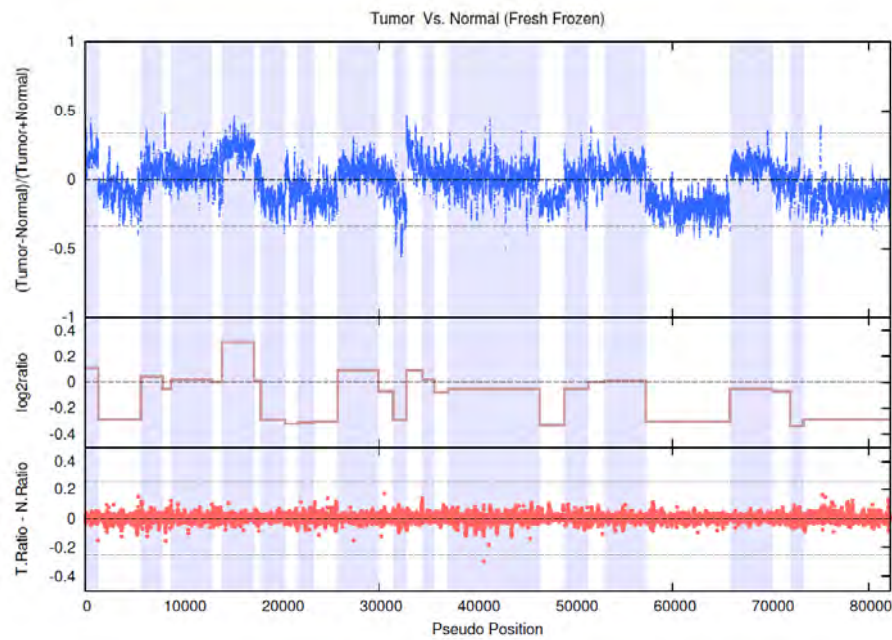
Exon1-----Exon 501

Somatic mutations

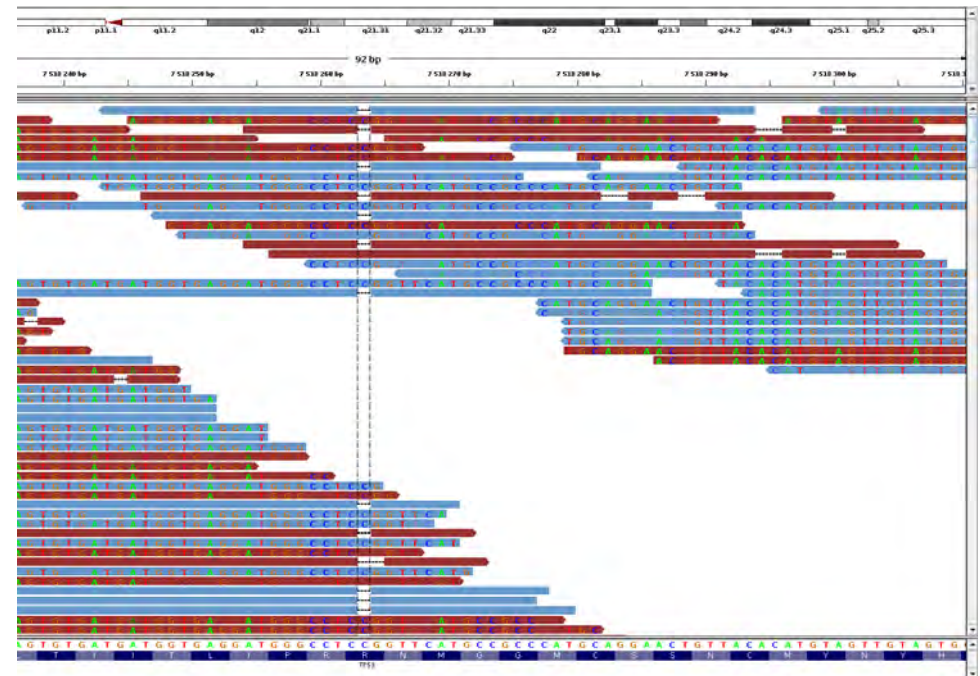
Chr# (hg18)	Pos. (hg18)	Gene	Ref. Seq	Ratio	Normal				Ratio	Tumor				T.Ratio-N.Ratio	Call
					A	G	C	T		A	G	C	T		
NC_000005,8	112190753	APC	T	0,45	0	0	68	56	1	0	0	0	119	0,55	LOH, CNN
NC_000005,8	112203669	APC	G	0,56	95	121	1	0	1	0	280	1	0	0,44	LOH, CNN
NC_000005,8	112204224	APC	G	0,58	214	292	0	0	1	1	587	0	0	0,42	LOH, CNN
NC_000005,8	112204458	APC	T	0,6	0	63	0	96	0,99	0	0	2	175	0,38	LOH, CNN
NC_000005,8	112205070	APC	G	0,54	81	95	1	0	1	0	205	1	0	0,46	LOH, CNN
NC_000007,12	116126908	MET	C	0,57	0	2	128	95	0,03	0	0	2	72	0,54	LOH
NC_000007,12	116127498	MET	A	0,69	83	38	0	0	0,09	4	42	0	0	0,6	LOH
NC_000007,12	116223004	MET	C	0,66	0	0	71	37	1	0	0	64	0	0,34	LOH
NC_000007,12	116223258	MET	G	0,58	138	196	5	1	0,98	1	141	1	1	0,4	LOH
NC_000007,12	116223333	MET	G	0,59	132	192	3	0	0,99	0	134	1	0	0,41	LOH
NC_000011,8	69172091	CCND1	G	0,42	128	96	3	0	0,09	932	92	6	2	0,33	LOH, AMP
NC_000011,8	93865568	MRE11A	C	0,58	0	1	36	25	1	0	0	46	0	0,42	LOH
NC_000011,8	107644504	ATM	T	1	0	0	0	24	0,58	3	2	0	7	0,42	?
NC_000011,8	107668697	ATM	C	0,62	0	0	202	123	0,05	0	2	6	109	0,57	LOH
NC_000017,9	7518263	TP53	C	1	0	0	24	0	0,09	0	1	2	20	0,91	C->T
NC_000017,9	26532901	NF1	G	0,61	26	40	0	0	1	0	50	0	0	0,39	LOH
NC_000017,9	35137563	ERBB2	C	0,73	0	10	27	0	0,13	0	33	5	0	0,6	LOH

Analysis of fresh-frozen clinical lung cancer samples

Tumor vs normal

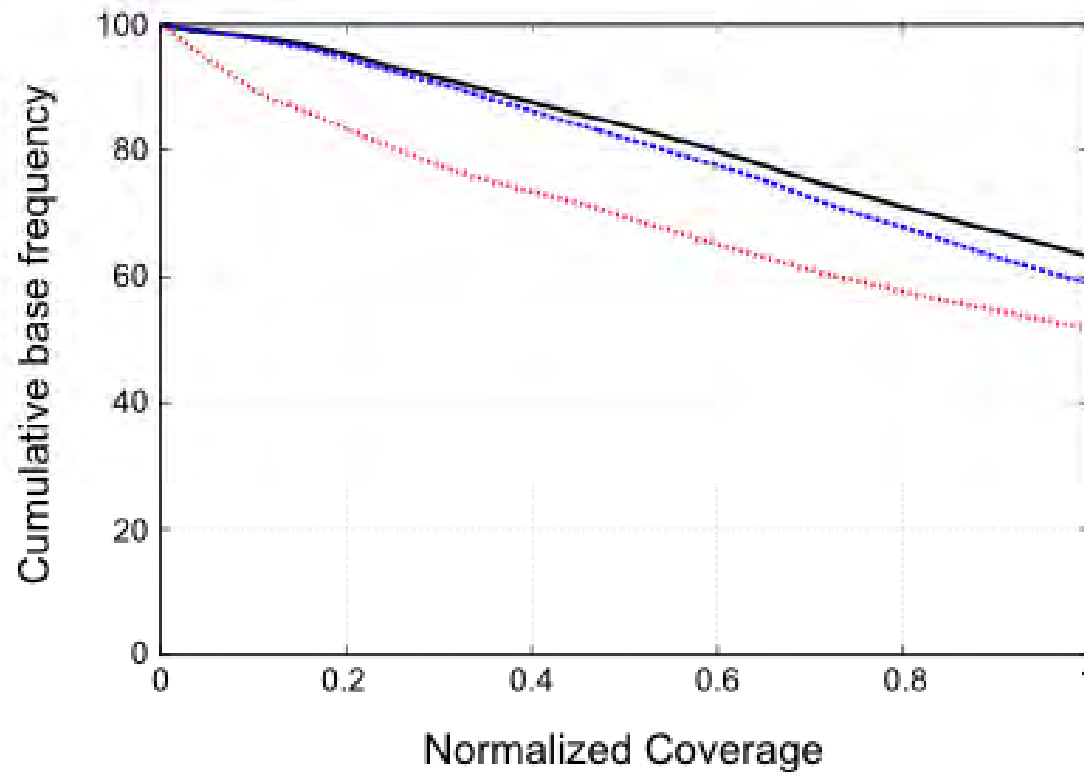


Detection of TP53 indel

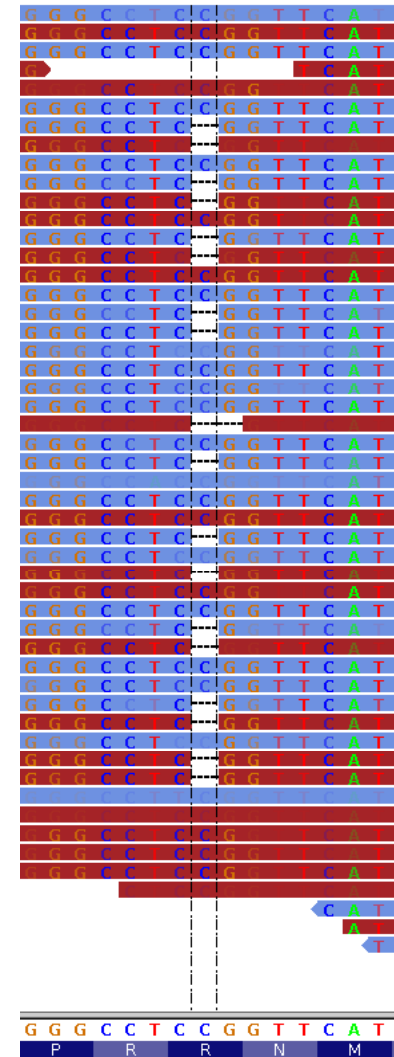


↑
delC

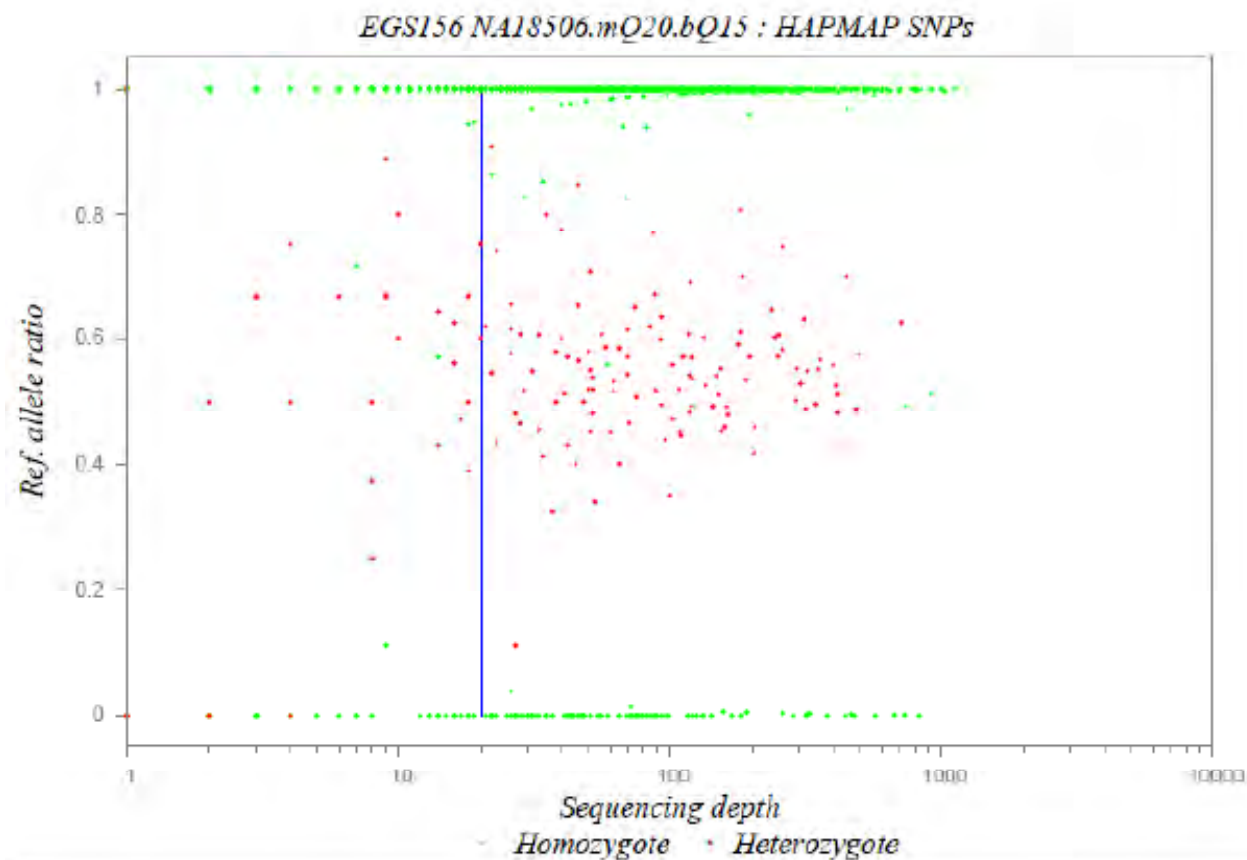
Comparison cells, fresh tumor, and FFPE



Cell line
Fresh frozen
FFPE



Sequencing 156 genes mutated in primary immuno deficiency



-1,6 Mbp target
-Bar-coded Illumina sequencing

FP7 Eurogenescan project:
Edvard Smith, Karolinska Institute
GATC

Next generation target amplification

- Selector Technology
 - Advantages
 - Specific, virtually no off target reads (94% specificity)
 - Low bias – high coverage at low cost (98% coverage at 10x over-sequencing)
 - Quick & Convenient – Single tube/Well
 - No expensive instrumentation

Olink Genomics Reagent kit



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Step 1/4. Enter Target Genes Or Transcripts

Specify the genes to design a Selector Probe library for. In the next step, you will be asked to select between different data sources that define sets of coordinates for each gene.

Enter a single gene name (symbol) or GeneId on each line, or upload a file containing a list of genes names or GeneIds. You can mix gene names and GeneIds.

[Show example](#)

Enter data: Or upload:

```
672  
BRCA1  
CC0S11459.1  
NM_007295
```

Design report



Design report



Design name: Test

General information

Design name: Test
Design by: Olink Genomics Selector Design Web Application
Designed by: Mr Patrik Friksor
Organization: Olink Genomics
Date: 2010-05-12

Design statistics

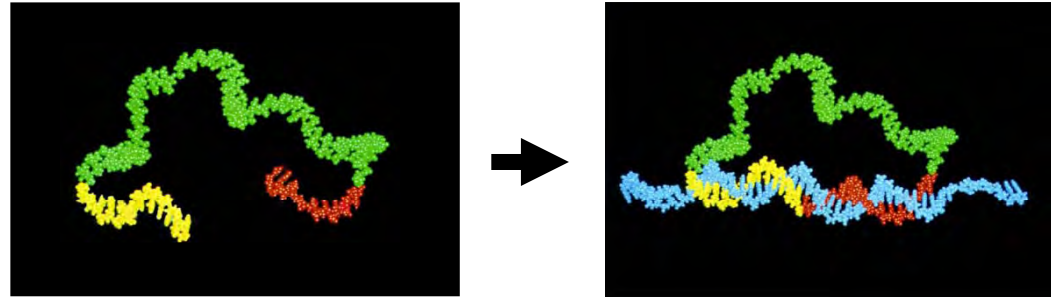
Number of bp covered: 13090
Number of targets: 23
Target region size: 6601
Target covered: 6538 (99.05%)
Average amplicon % on target: 66.89 %

Exons coverage

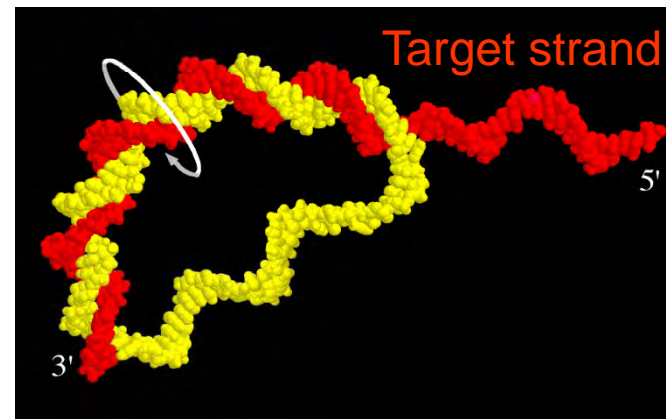
NM	100.00 %
672	100.00 %
CCDS11459.1	96.85 %
BRCA1	98.98 %

In situ genotyping

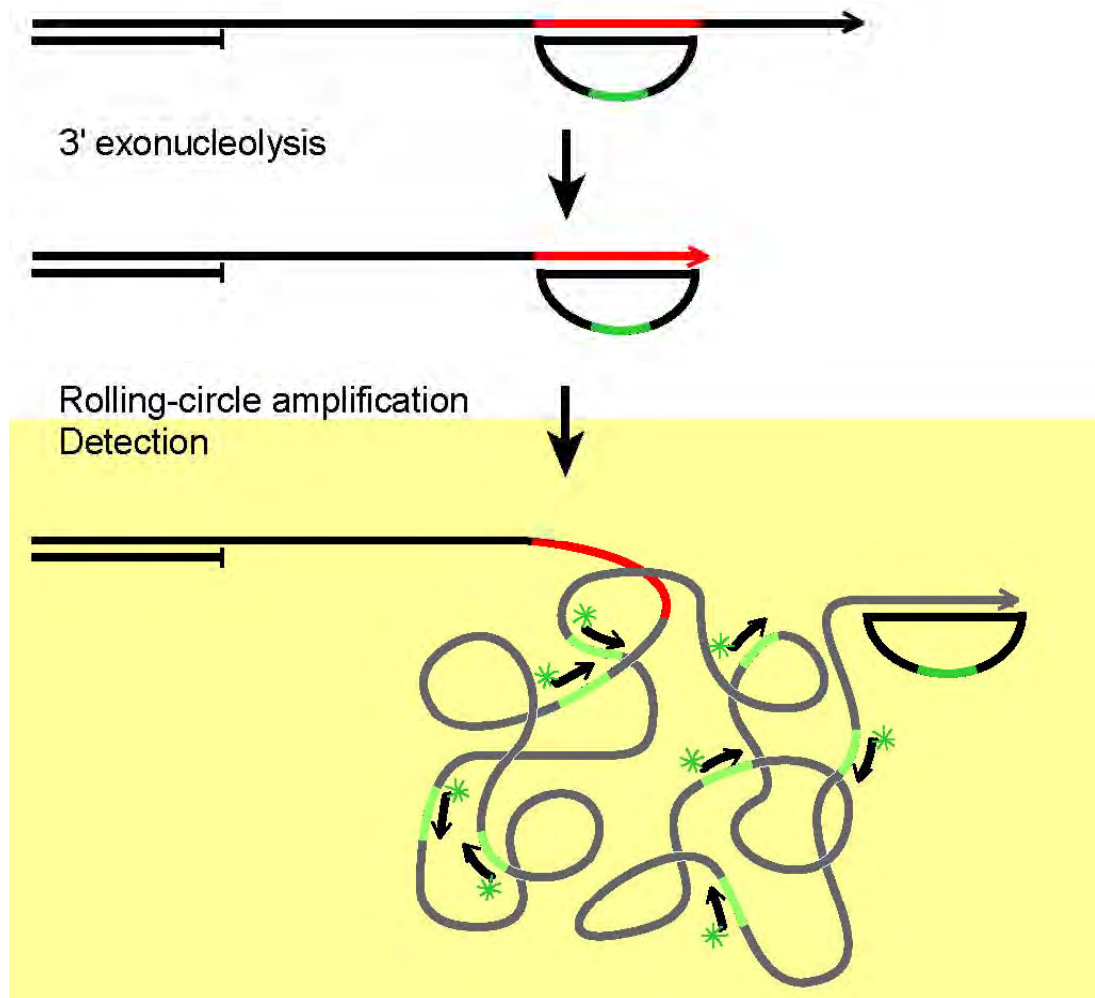
- Padlock probing



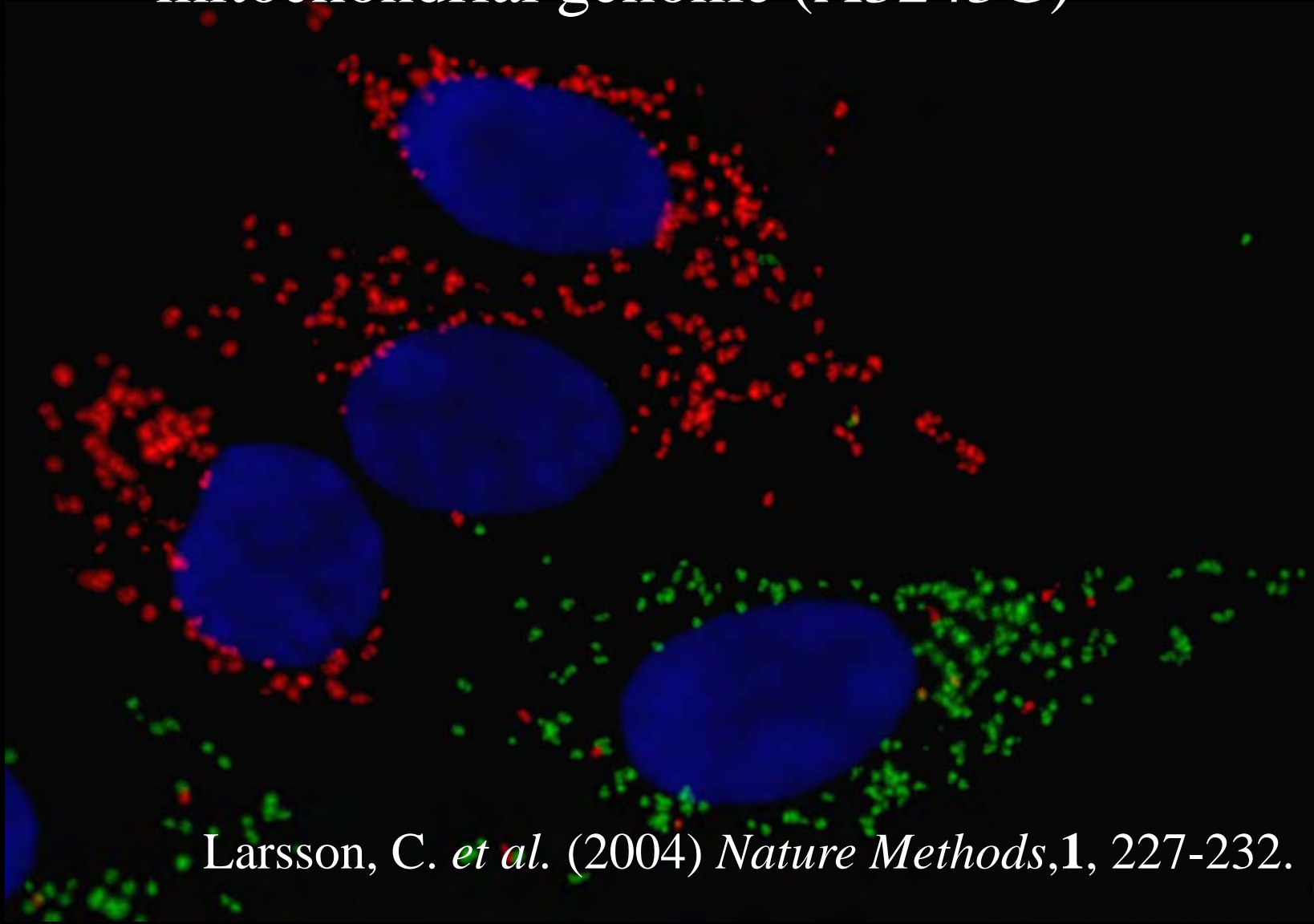
- Rolling-circle amplification



Target-primed RCA

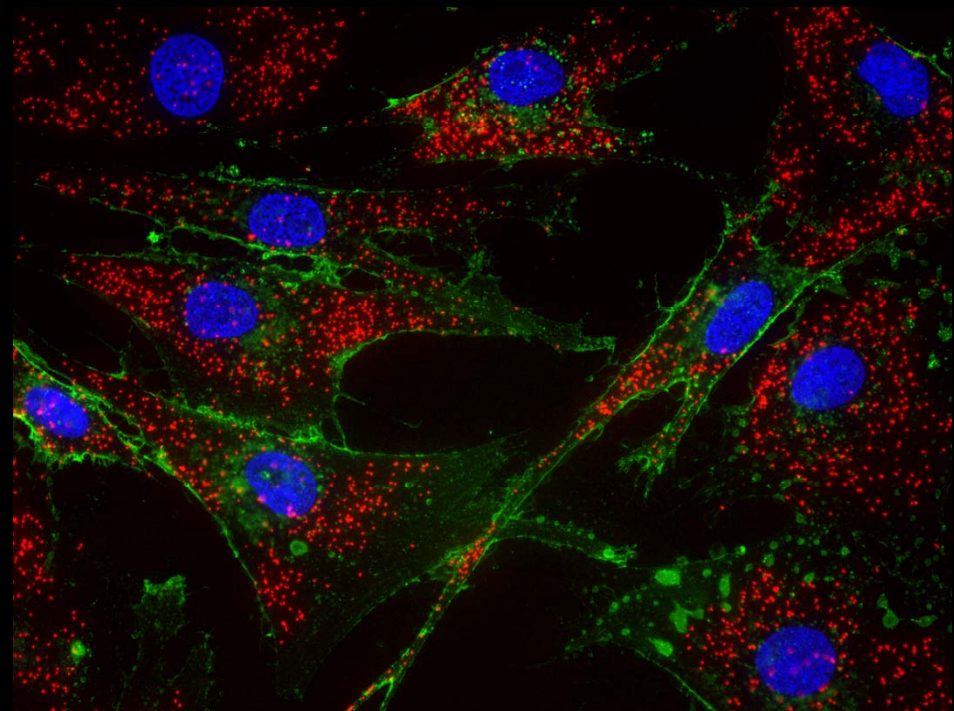
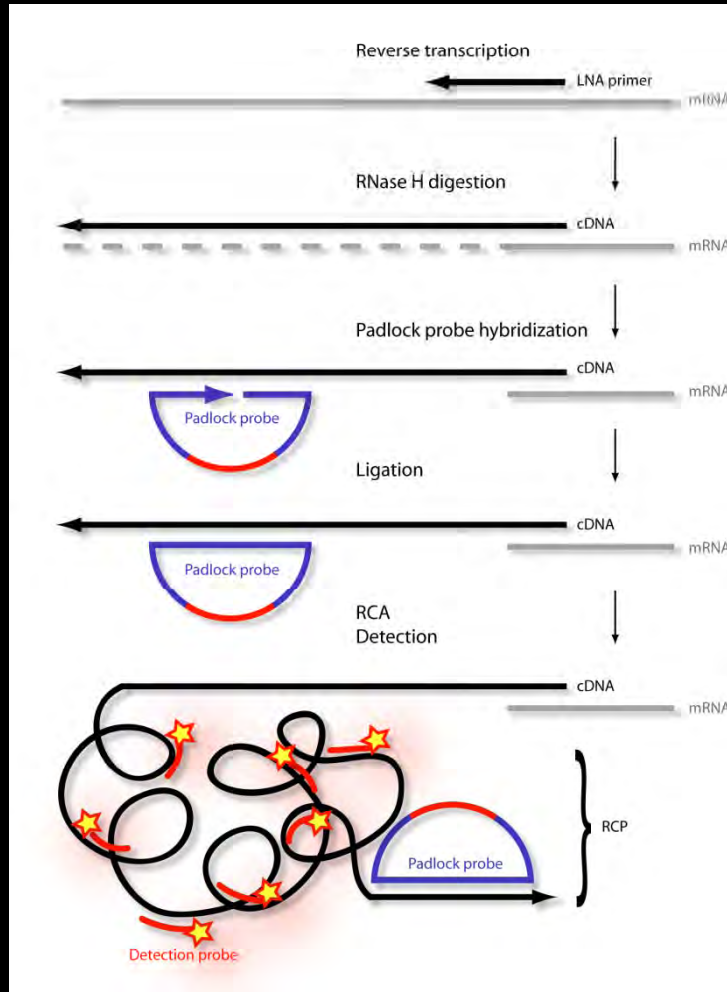


Detection of single nucleotide variation in the mitochondrial genome (A3243G)



Larsson, C. *et al.* (2004) *Nature Methods*, **1**, 227-232.

Detection of single transcripts using padlock probes and RCA

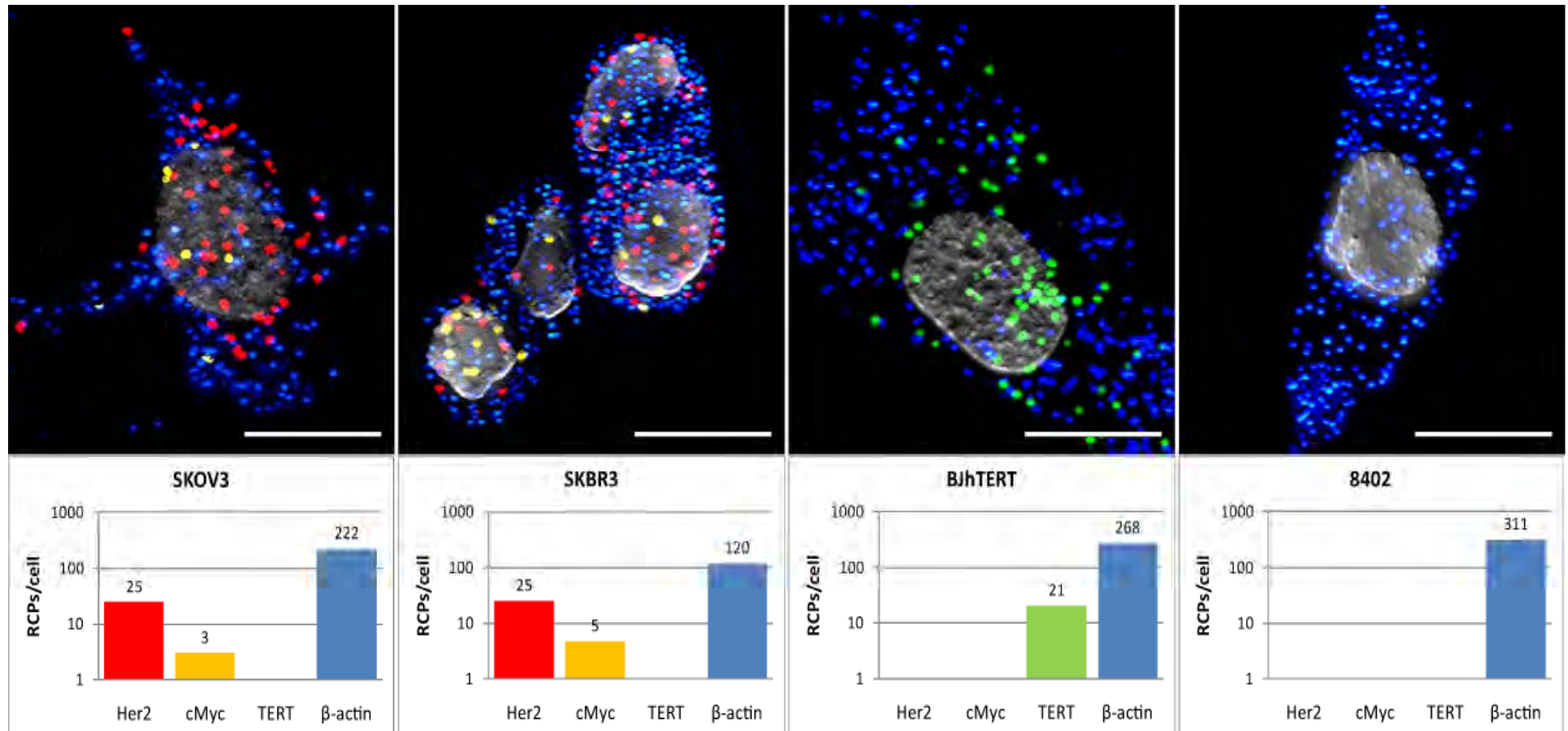


β -actin transcripts
Nuclear staining
Membrane staining

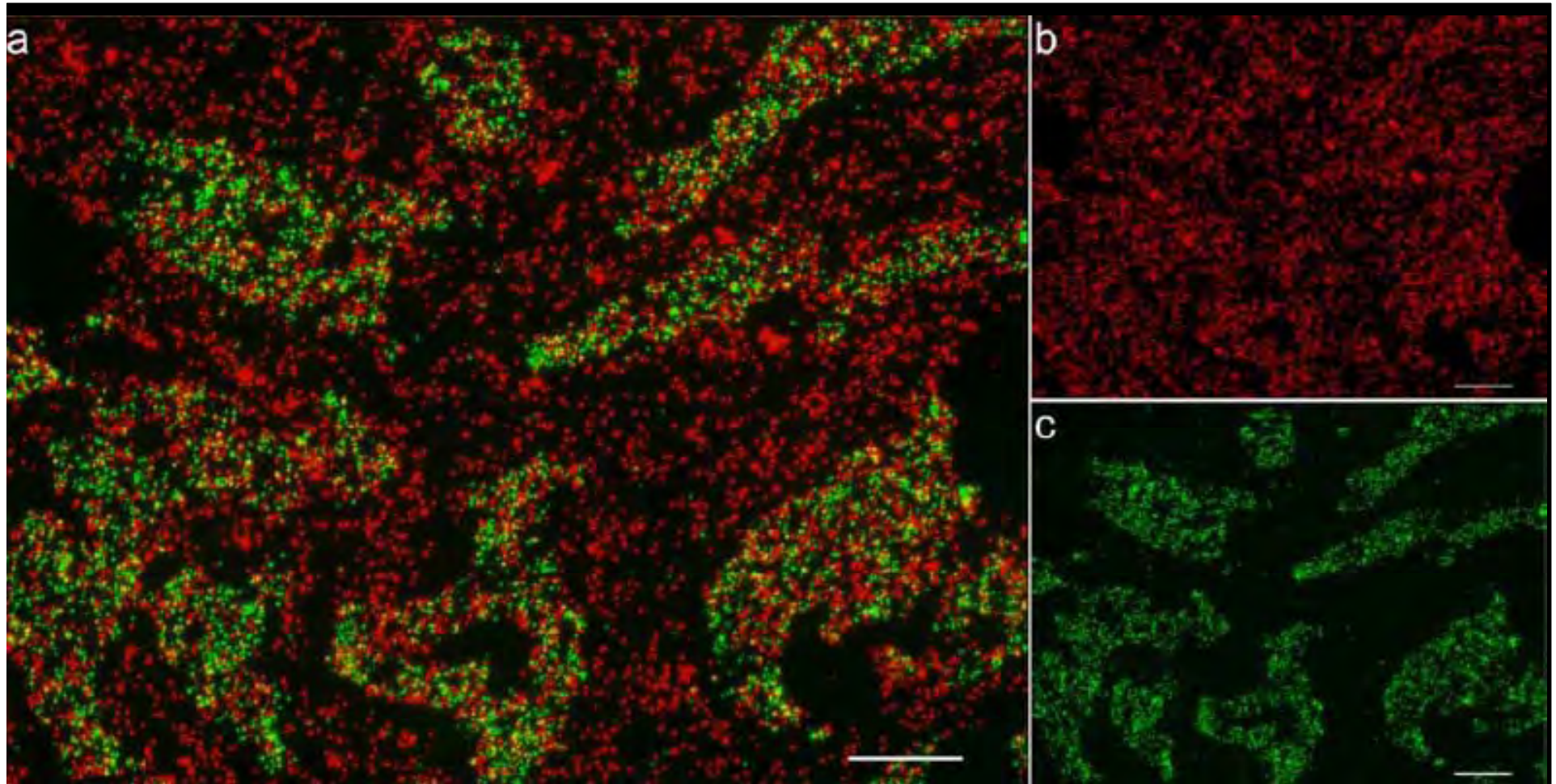
30% detection efficiency

Larsson, C. *et al.* (2010) *Nature Methods*, 7, 395–397.

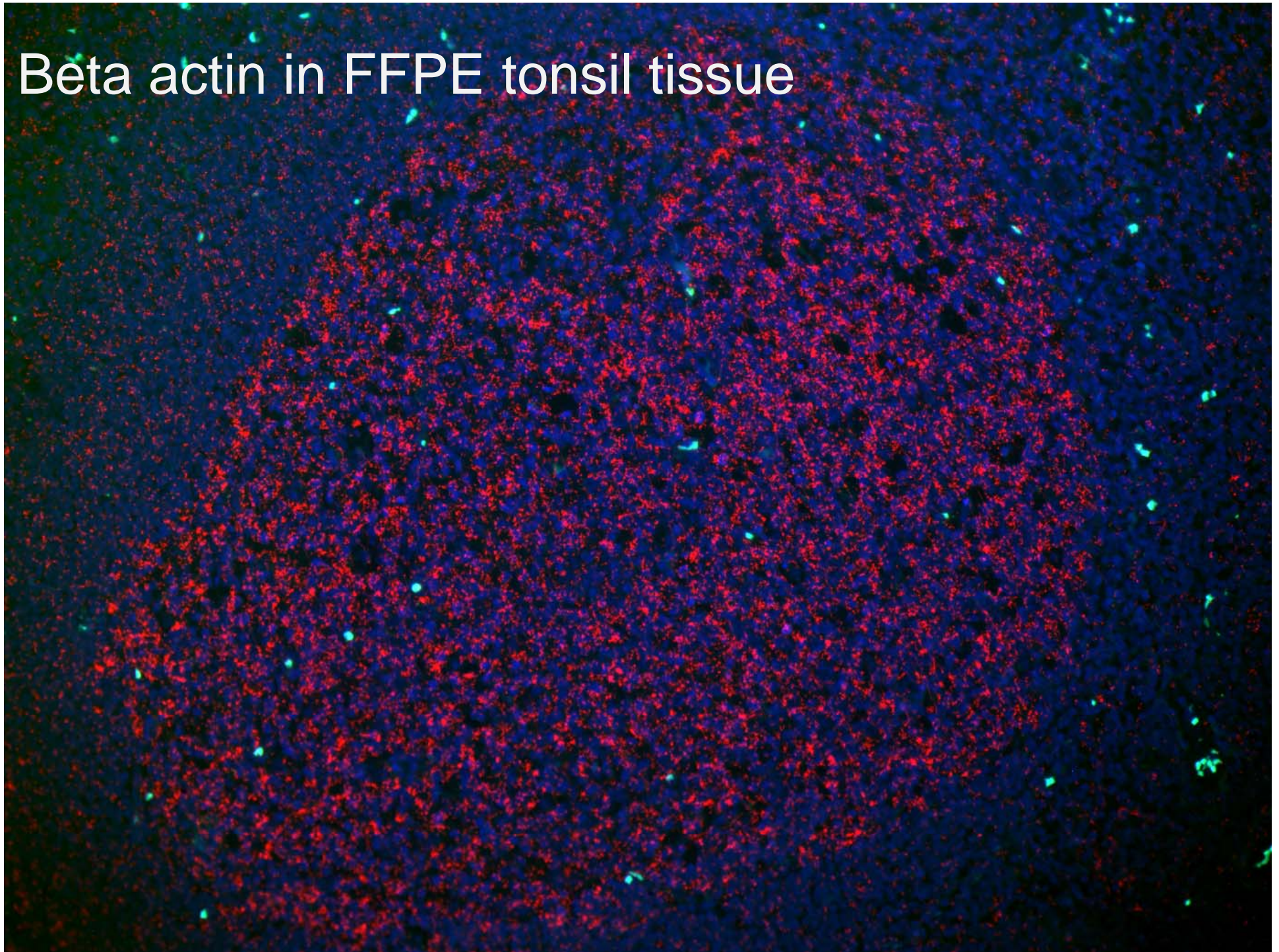
Multiplex detection of the cancer related transcripts **Her2**, **cMyc** and **TERT** + **β -actin** in different cell lines



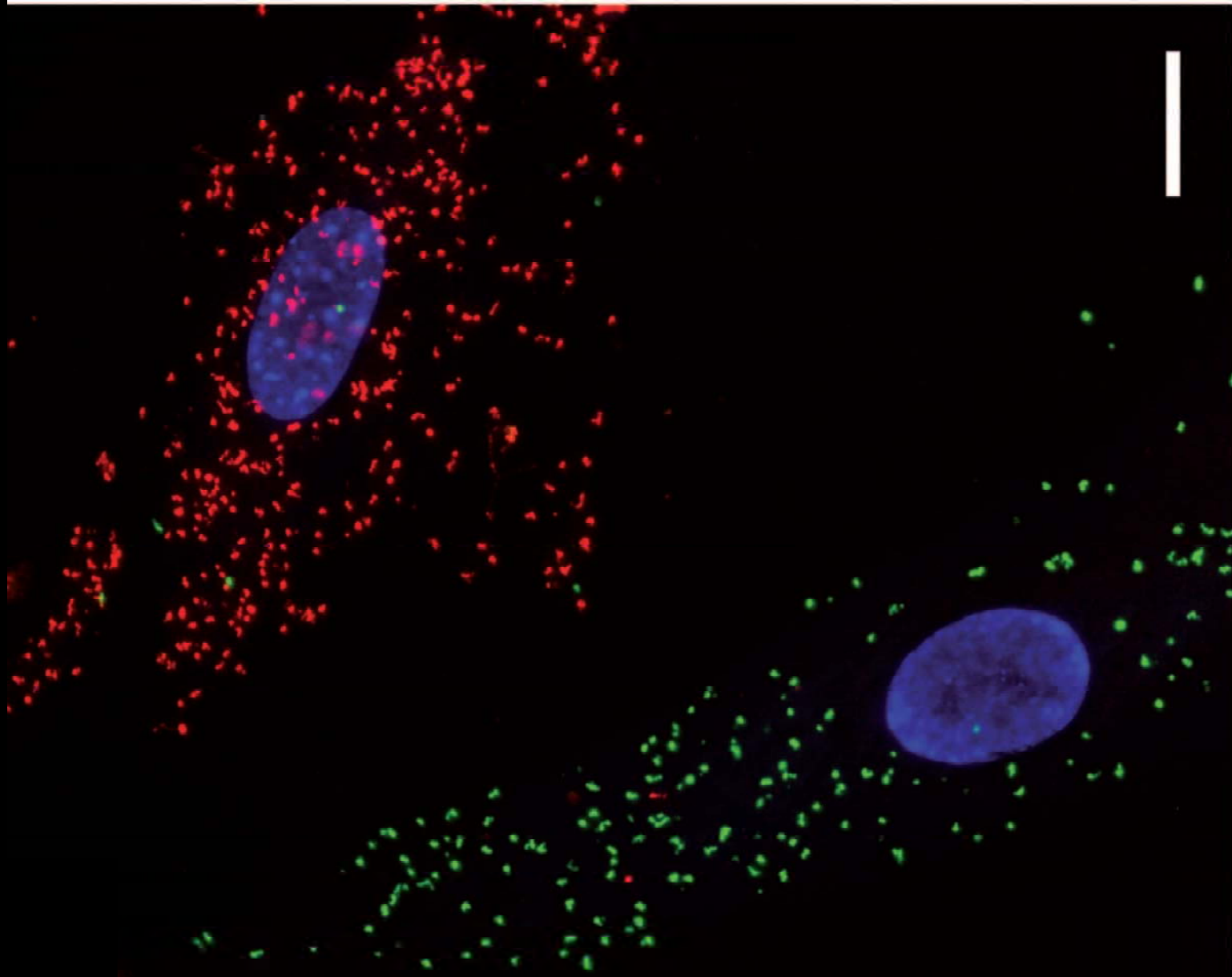
Detection of Her2 transcripts in Her2+ breast cancer tissue



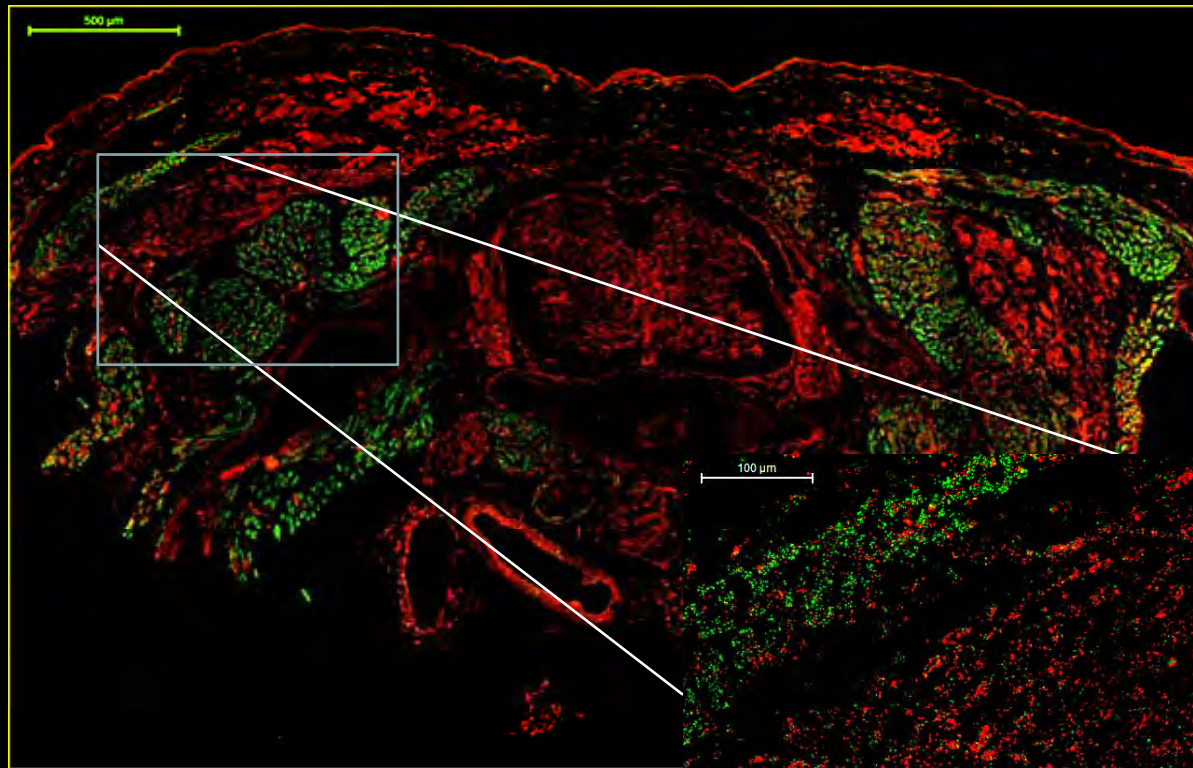
Beta actin in FFPE tonsil tissue



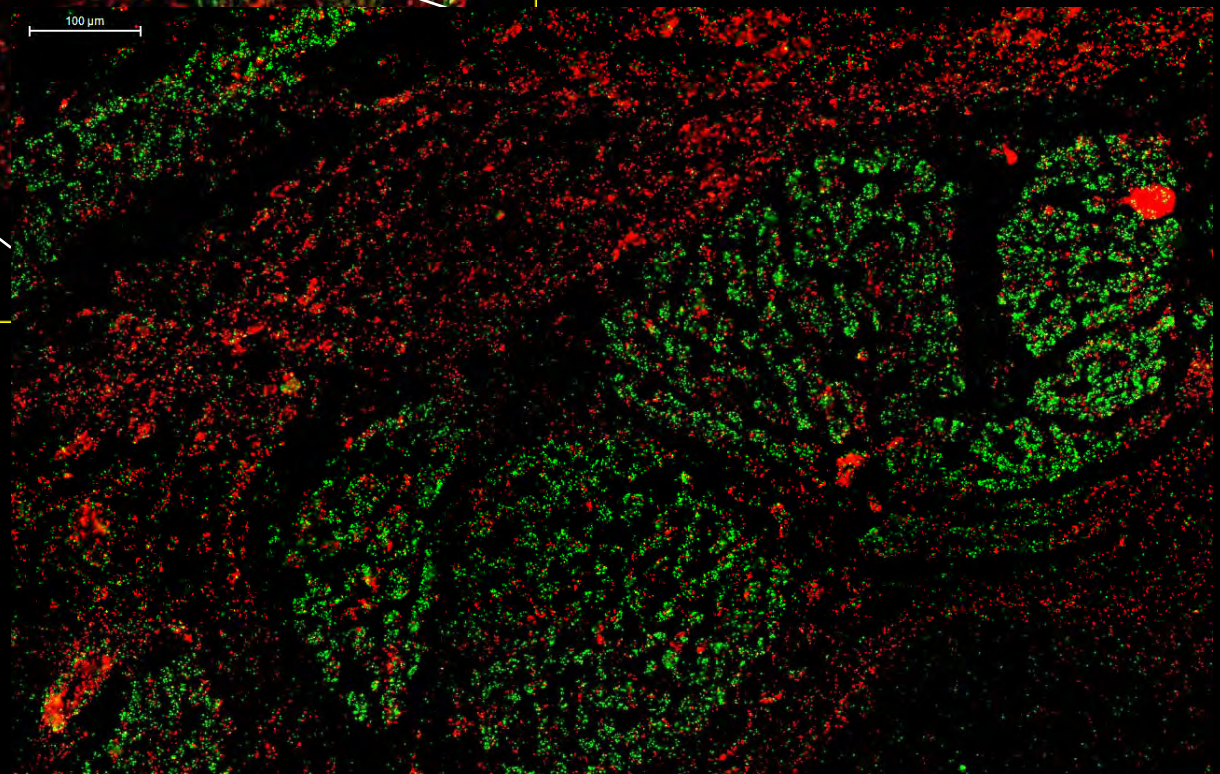
In situ genotyping of transcripts



In situ detection of transcripts in tissue



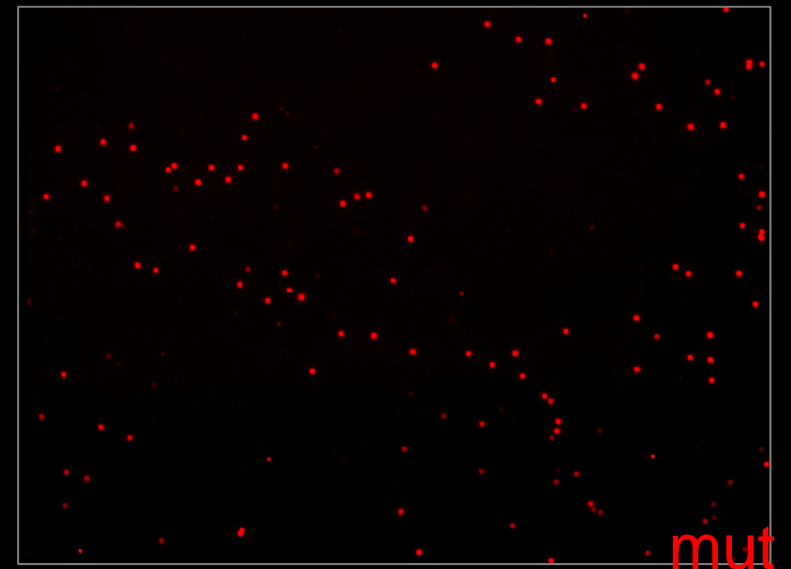
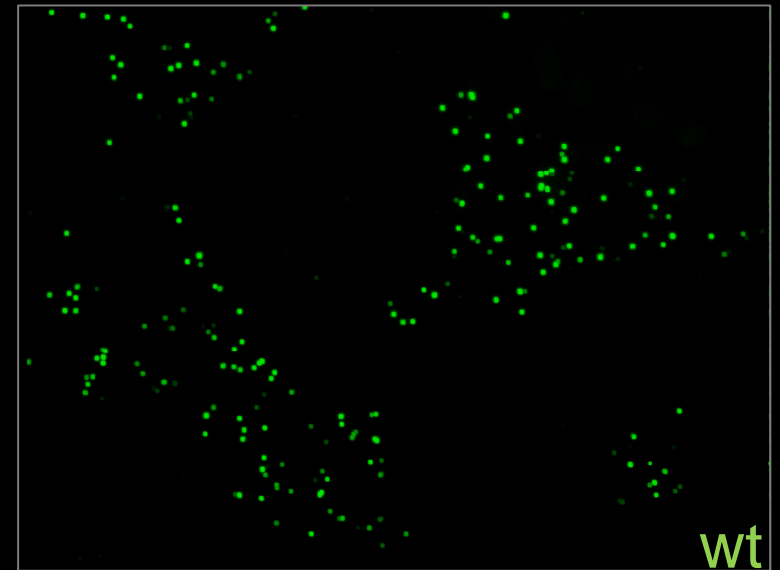
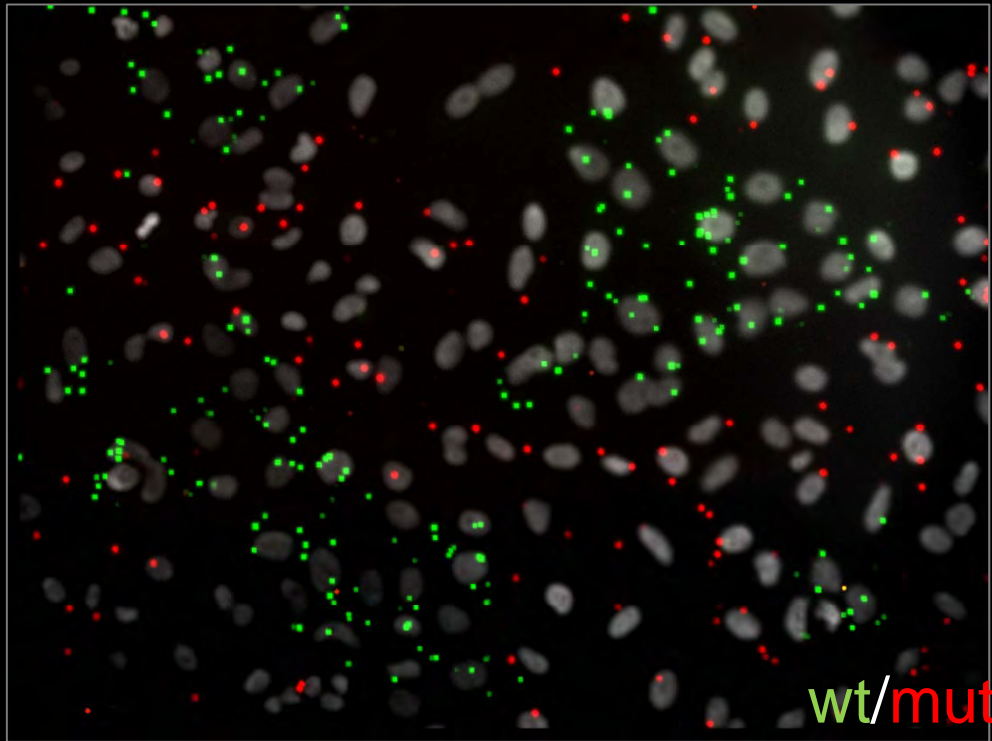
Mouse embryonic tissue, detection of α - and β -actin



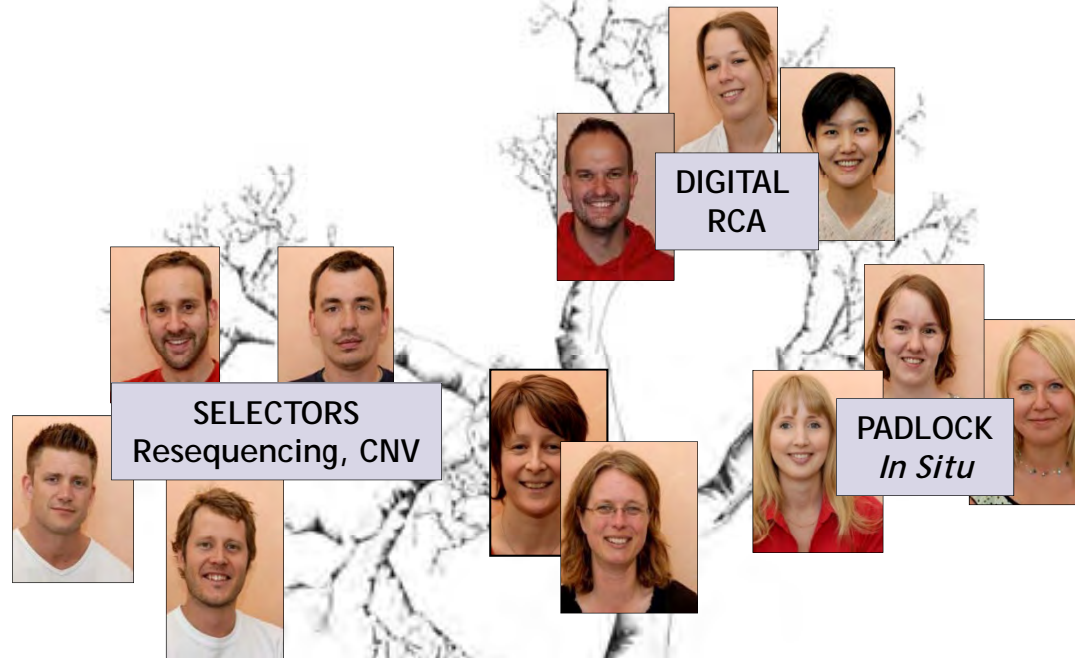
α -actins: skeletal muscle tissues

β -actins: most cell types as components of the cytoskeleton

Detection of a codon 12 *KRAS* point mutation



Molecular Diagnostics



The Wallenberg, Gustafsson, and Fernström foundations



Acknowledgements



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Yuki Tanaka
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Jenny Göransson
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