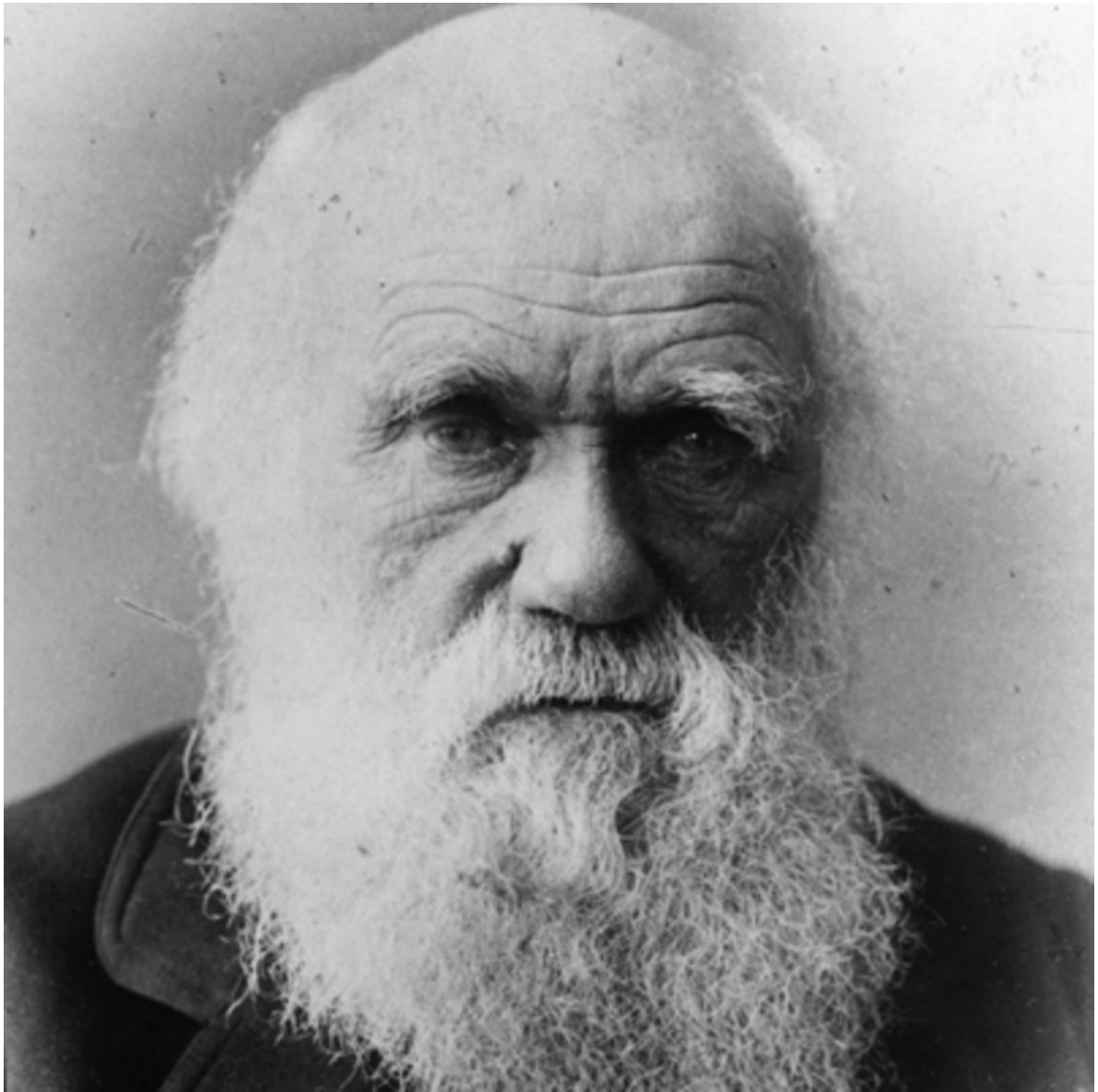
Utveckling av nya molekylärbiologiska metoder

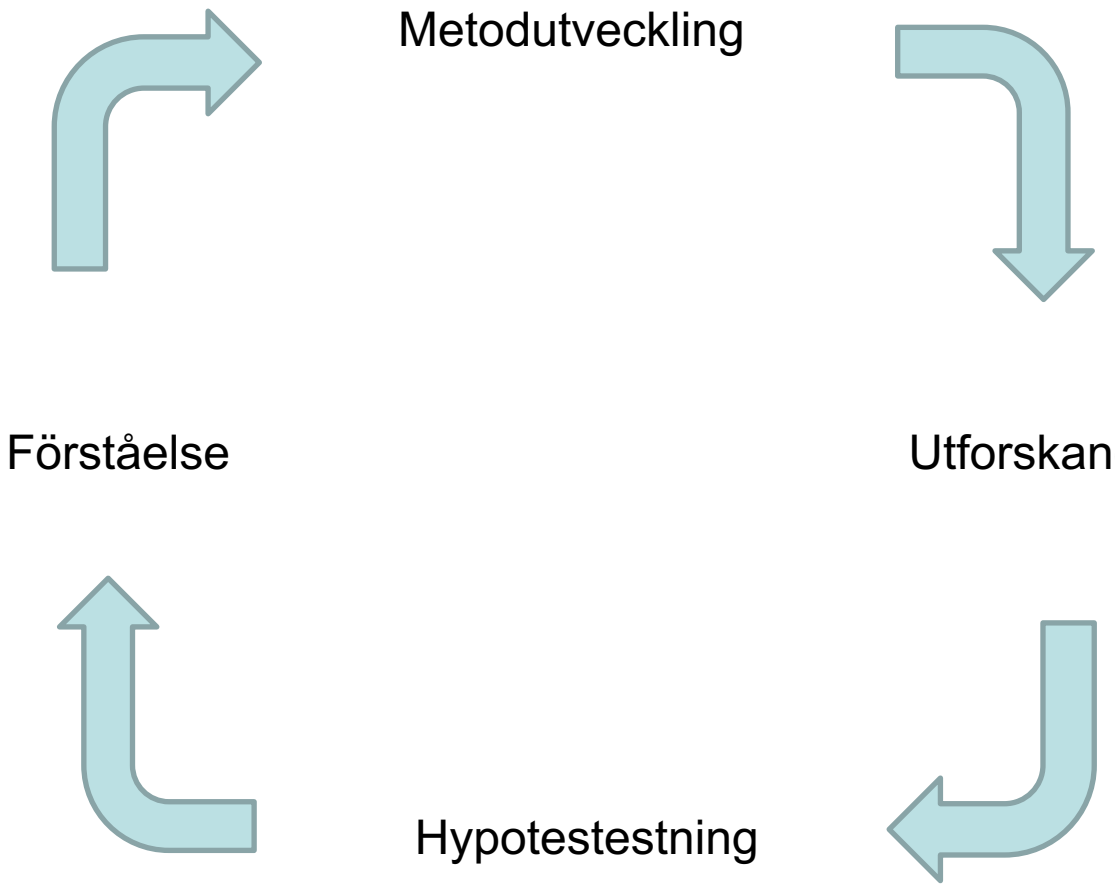
Ola Söderberg, Uppsala University

Vad kan en apotekare arbeta med?

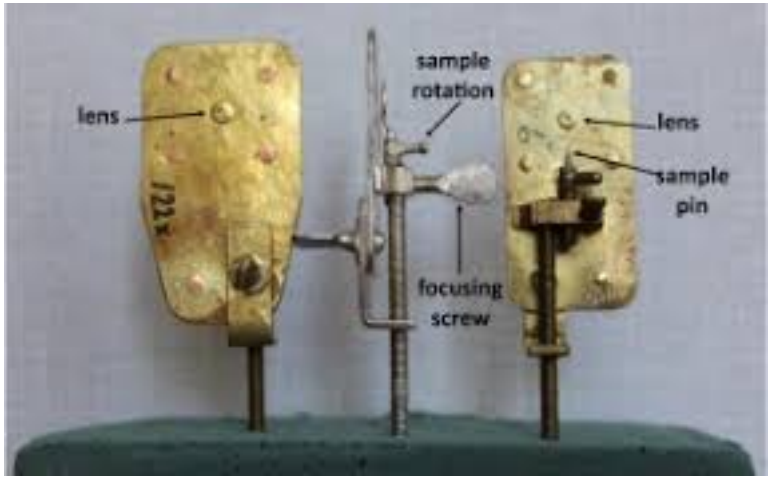
Vad kan ni använda allt ni lärt er till?

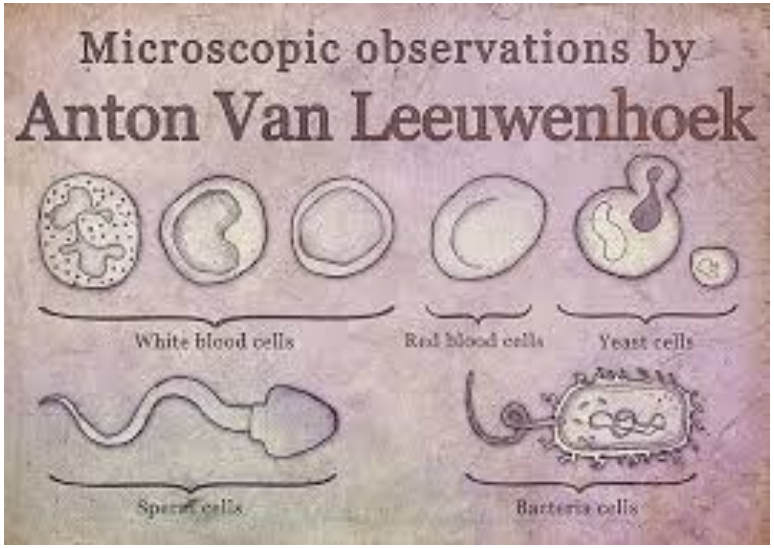
Vad är forskning?

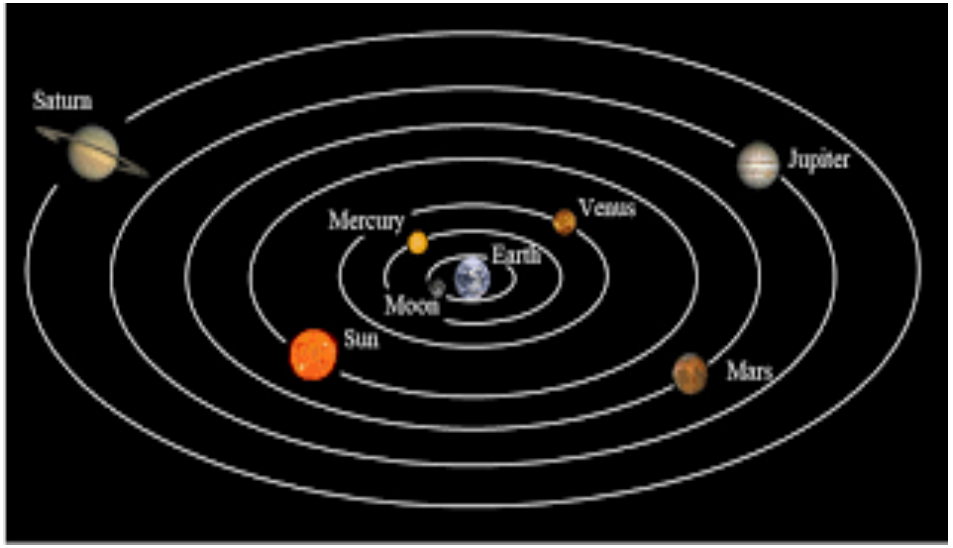



Metodutveckling
Förståelse
Utforskande
Hypotestestning

Metodutvecklig ger nya verktyg och nya möjligheter

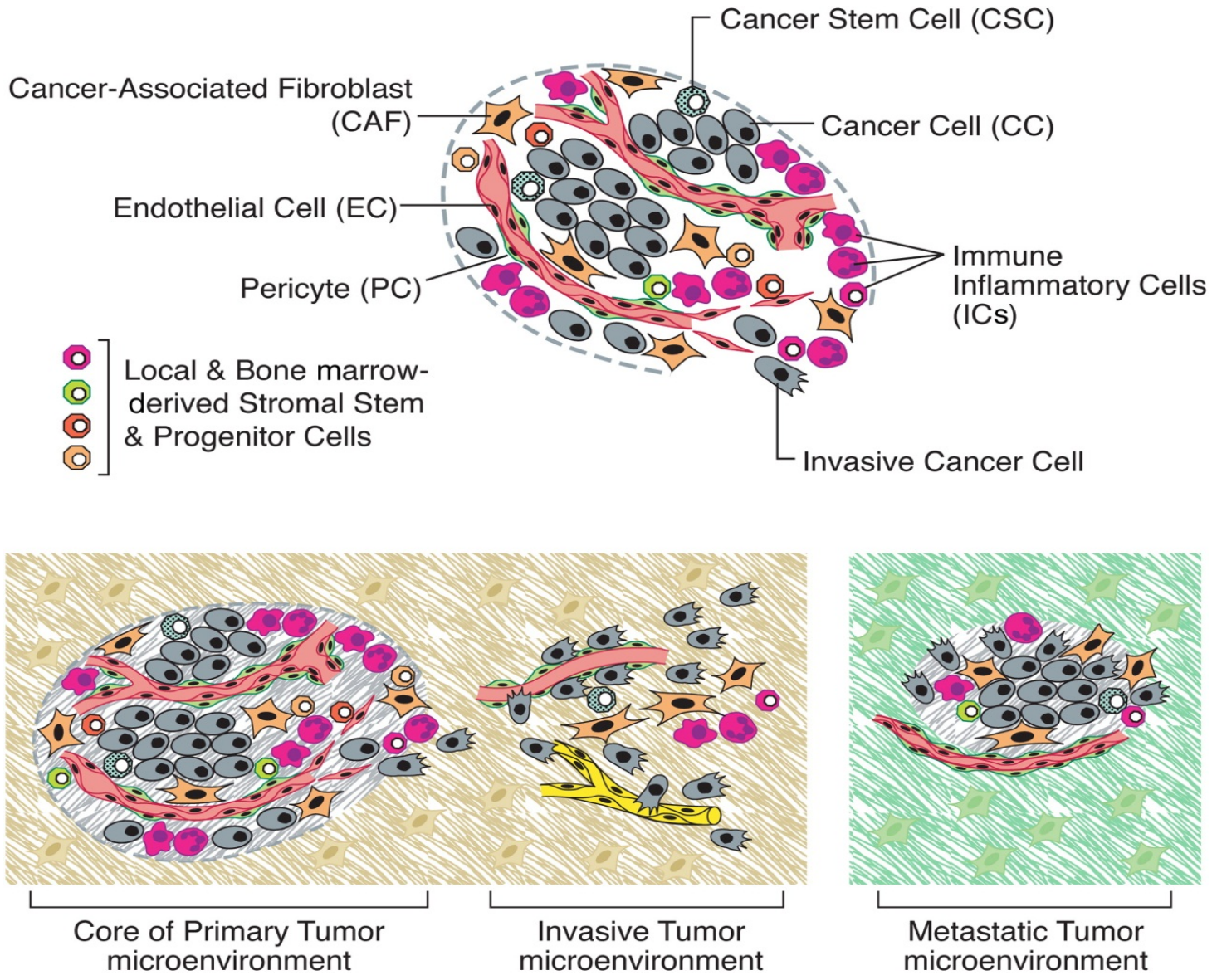






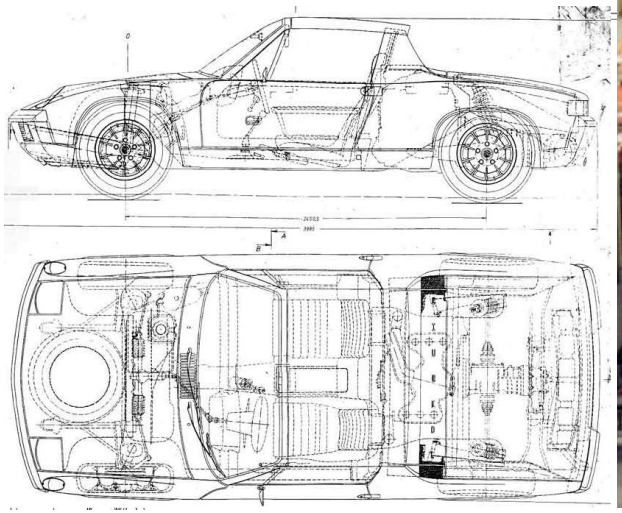
Vad är problemet vi vill lösa?

Vad skulle vi vilja studera?



Hanahan D & Weinberg RA. Cell. 2011; 144(5): 646-674

DNA



RNA

Proteins

Protein complexes

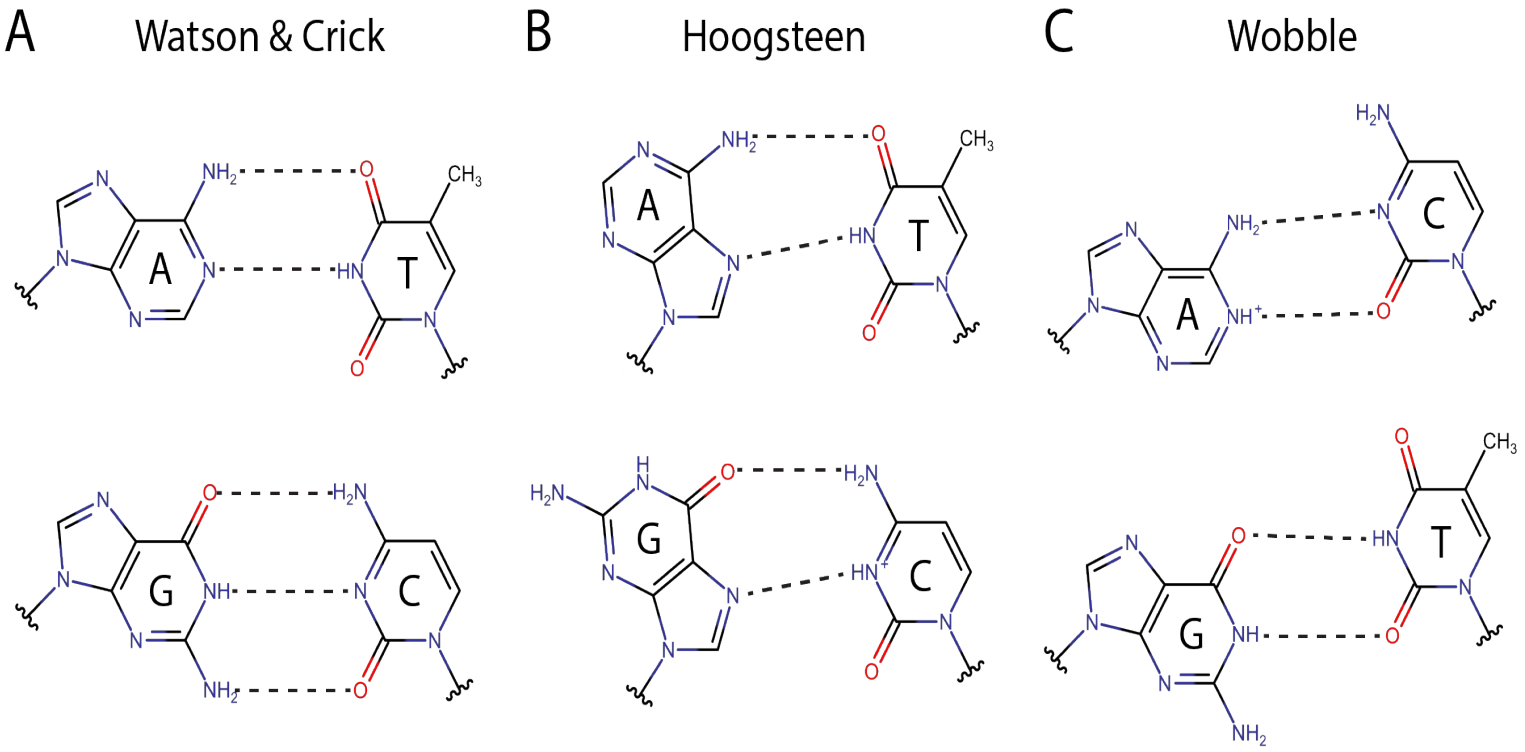
Hur kan man göra en ny metod?

Vi använder komponenter från naturvetenskapen

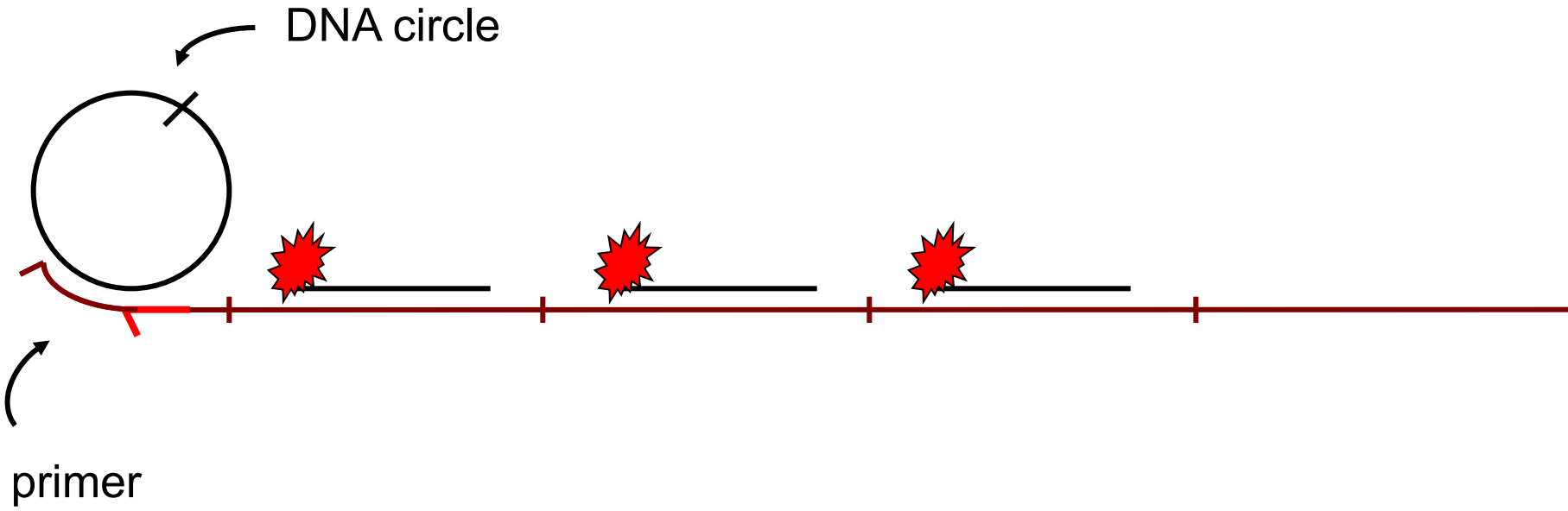
DNA är en enkel och förutsägbar molekyl

En stor repertoir av DNA-modifierande enzym

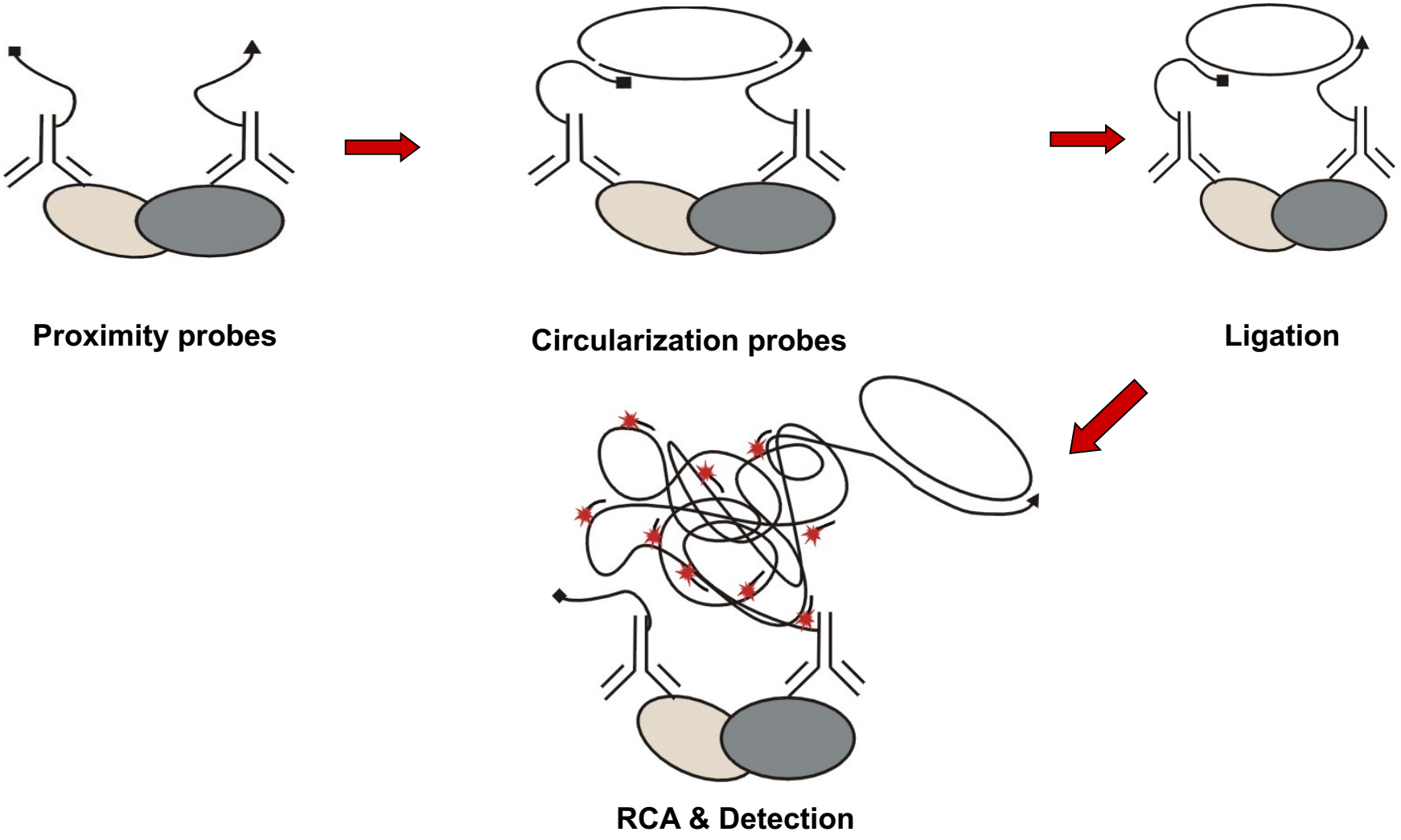
DNA base pairing



Rolling Circle Amplification



in situ Proximity Ligation Assay (*in situ* PLA)

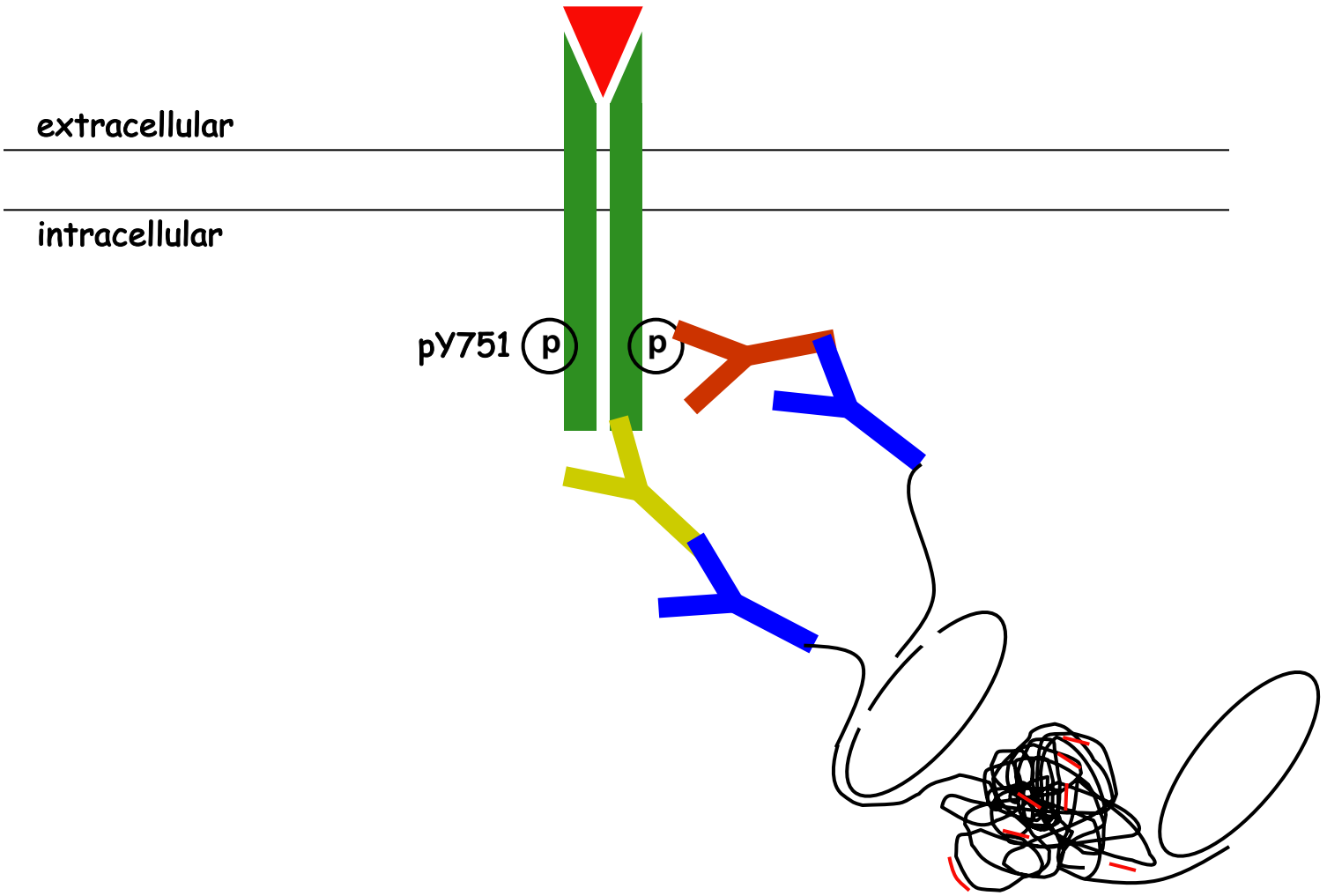



VORTEX GENIE 2
ART

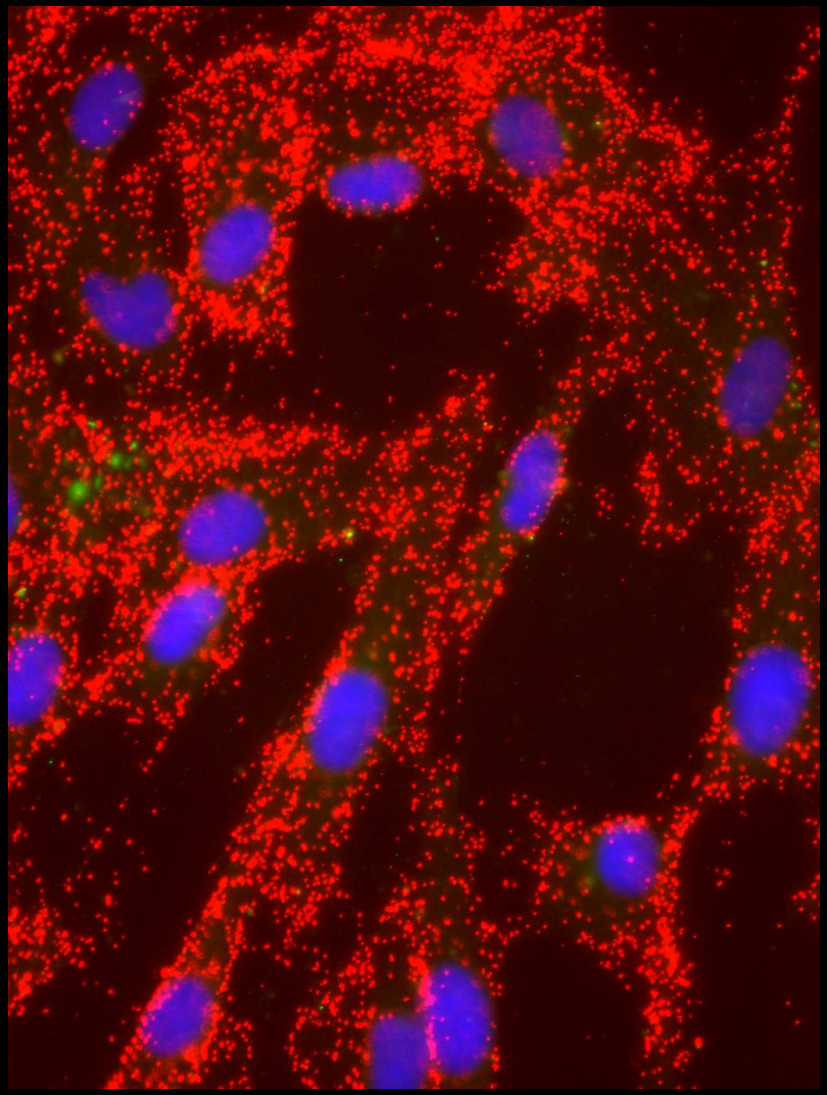
CMYC/MAX interactions

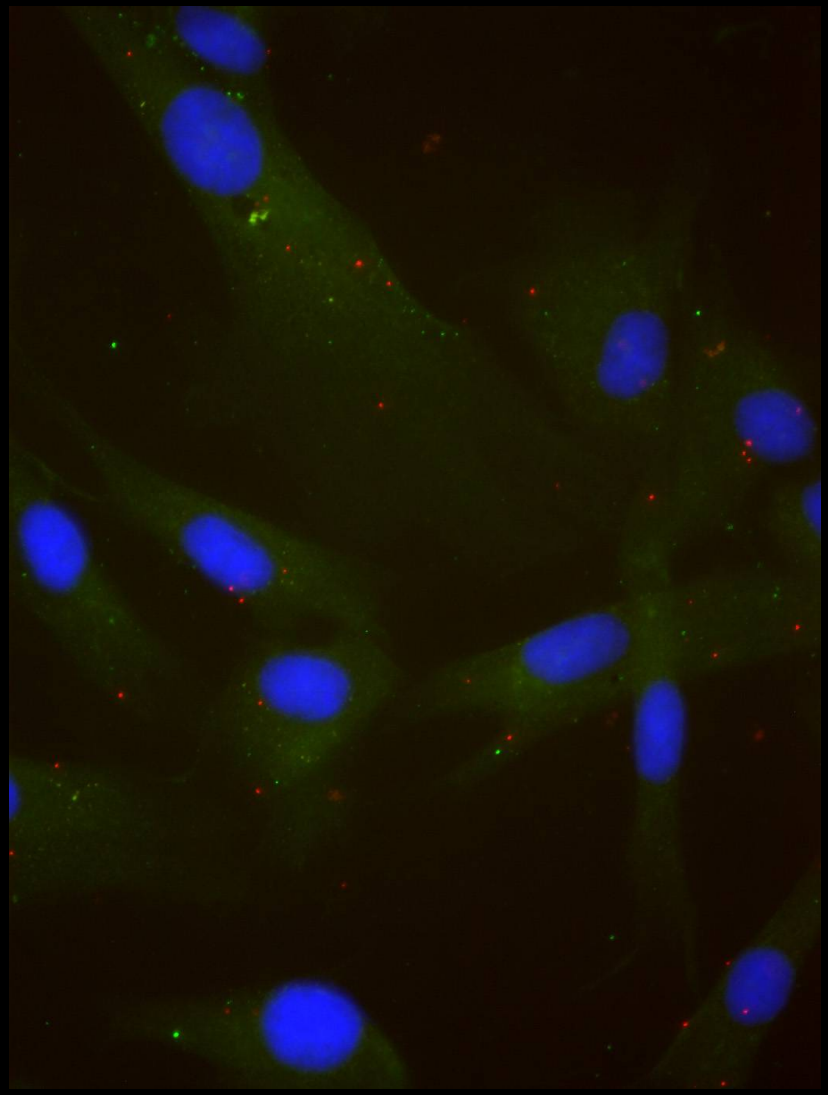
Söderberg *et al*., *Nature Methods* **3**: 995 (2006)

Detection of phosphorylated PDGFRβ (secondary proximity probes)



Detection of phosphorylated PDGFRβ in human Fibroblasts by *in situ* PLA

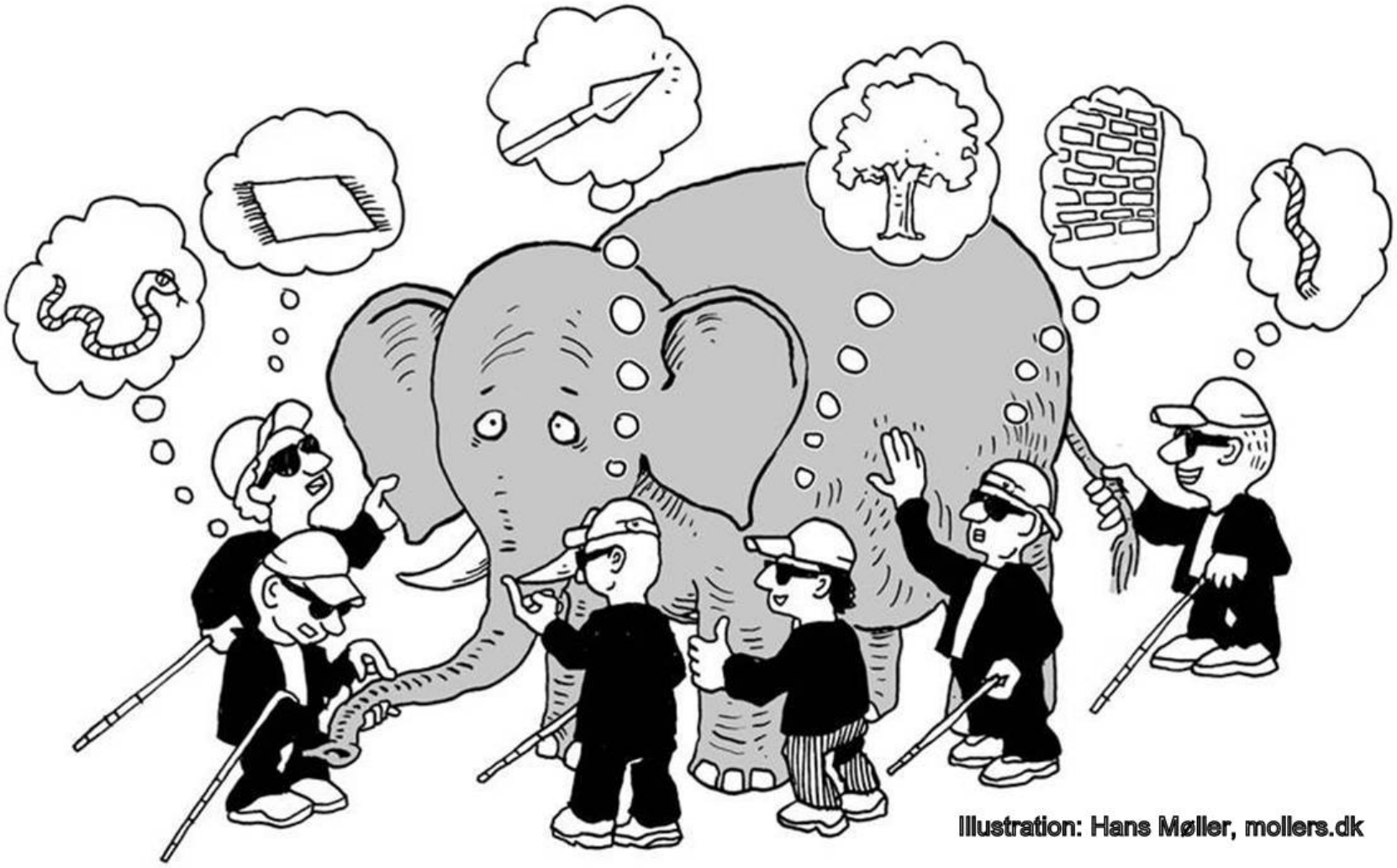




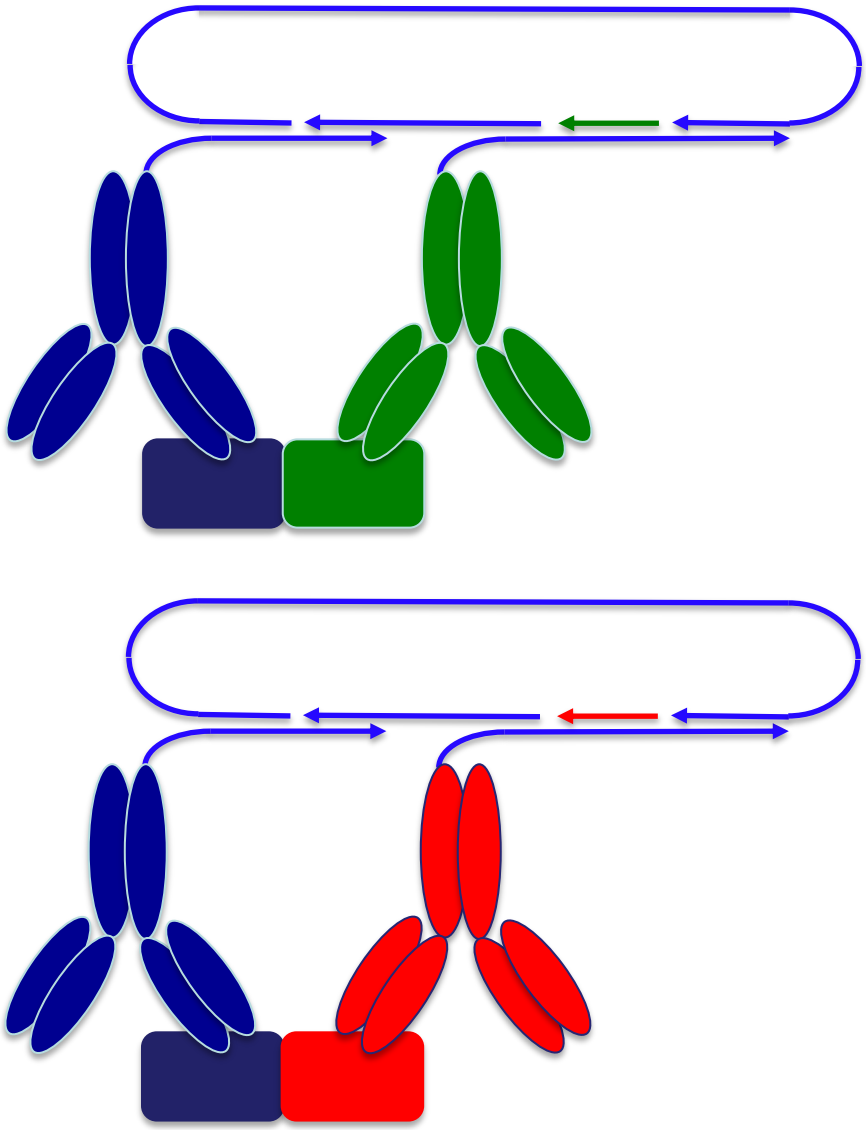
Jarvius, Paulsson, Weibrecht *et al*., *Molecular & Cellular Proteomics*. **6**(9): 1500 (2007)

Räcker det med att detektera en protein interaktion för att förstå biologin?

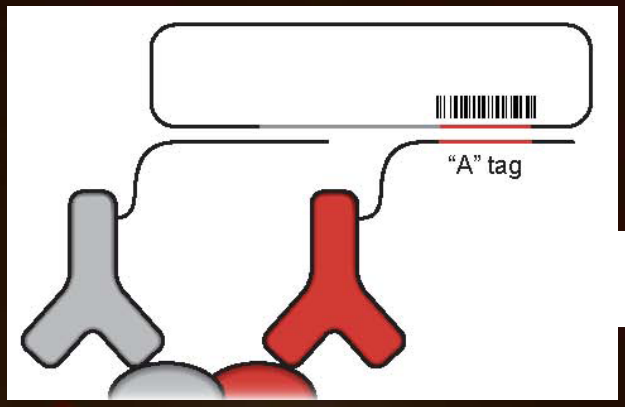
Multiplexing



Multiplex *in situ* PLA



Multiplex *in situ* PLA



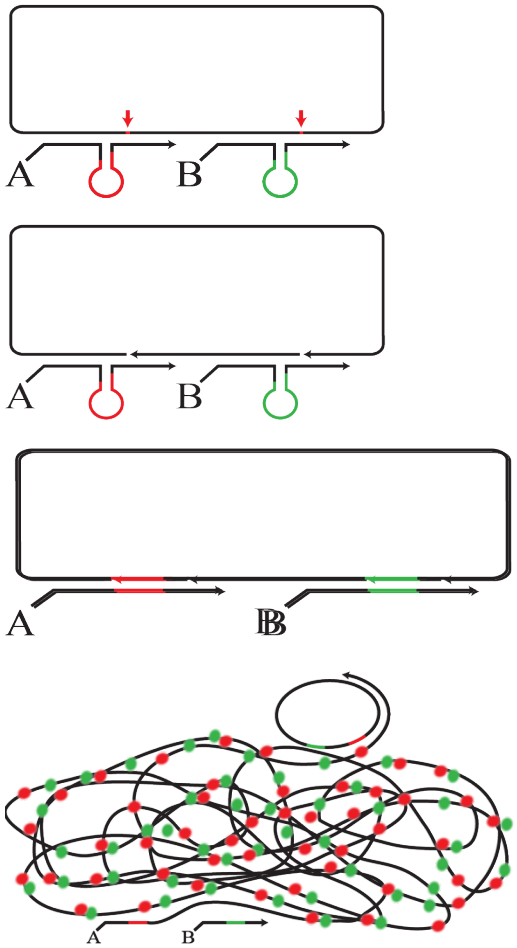
Her2-EGFR
Her2-Her2
Her2-Her3

Leuchowius *et al*., *Mol Cell Proteomics*, **12**(6):1563-71 (2013)

Molecular Boolean analysis - MolBoolean

$$KD = \frac{[A][B]}{[AB]}$$

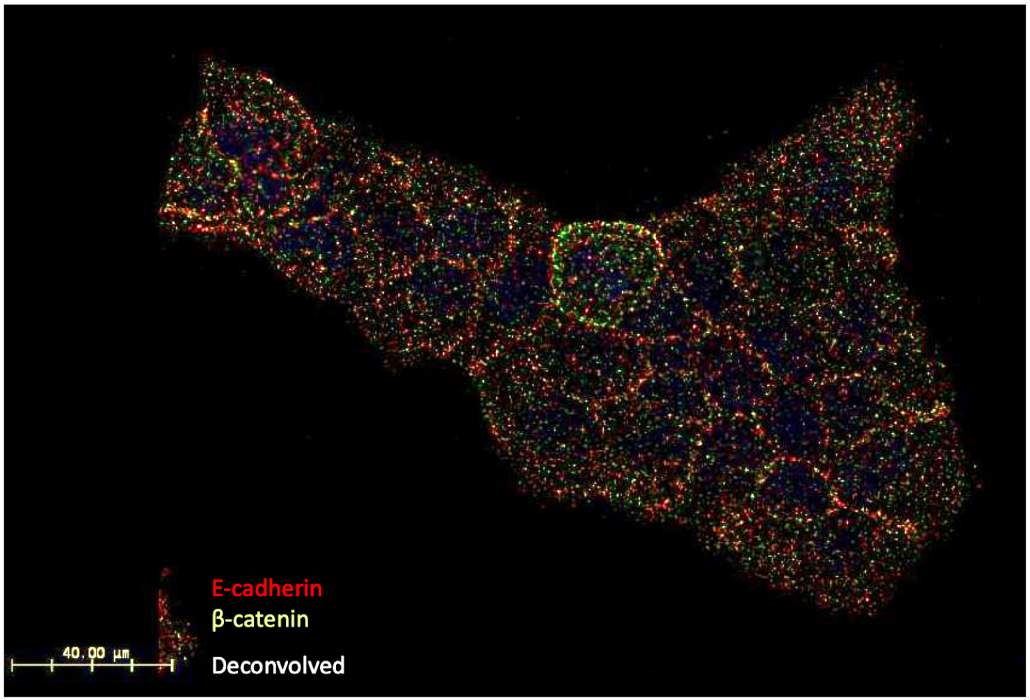
Molboolean design

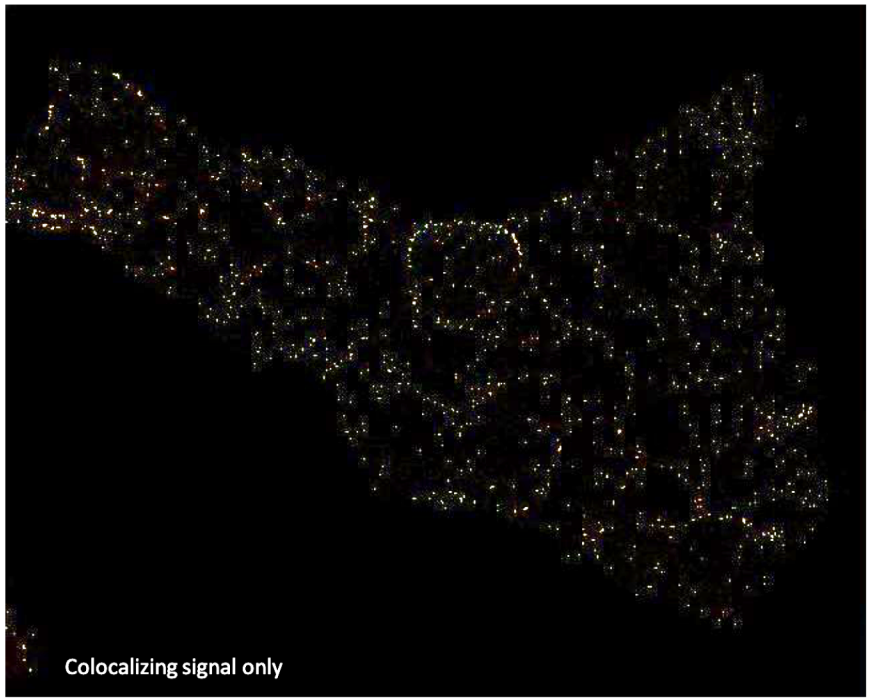


- Probe binding
- Circle hybridization
- Digestion with Nickase
- Tag hybridization and ligation
- Rolling circle amplification
- Detection

Raykova & Kermpatsou *et al*., unpublished

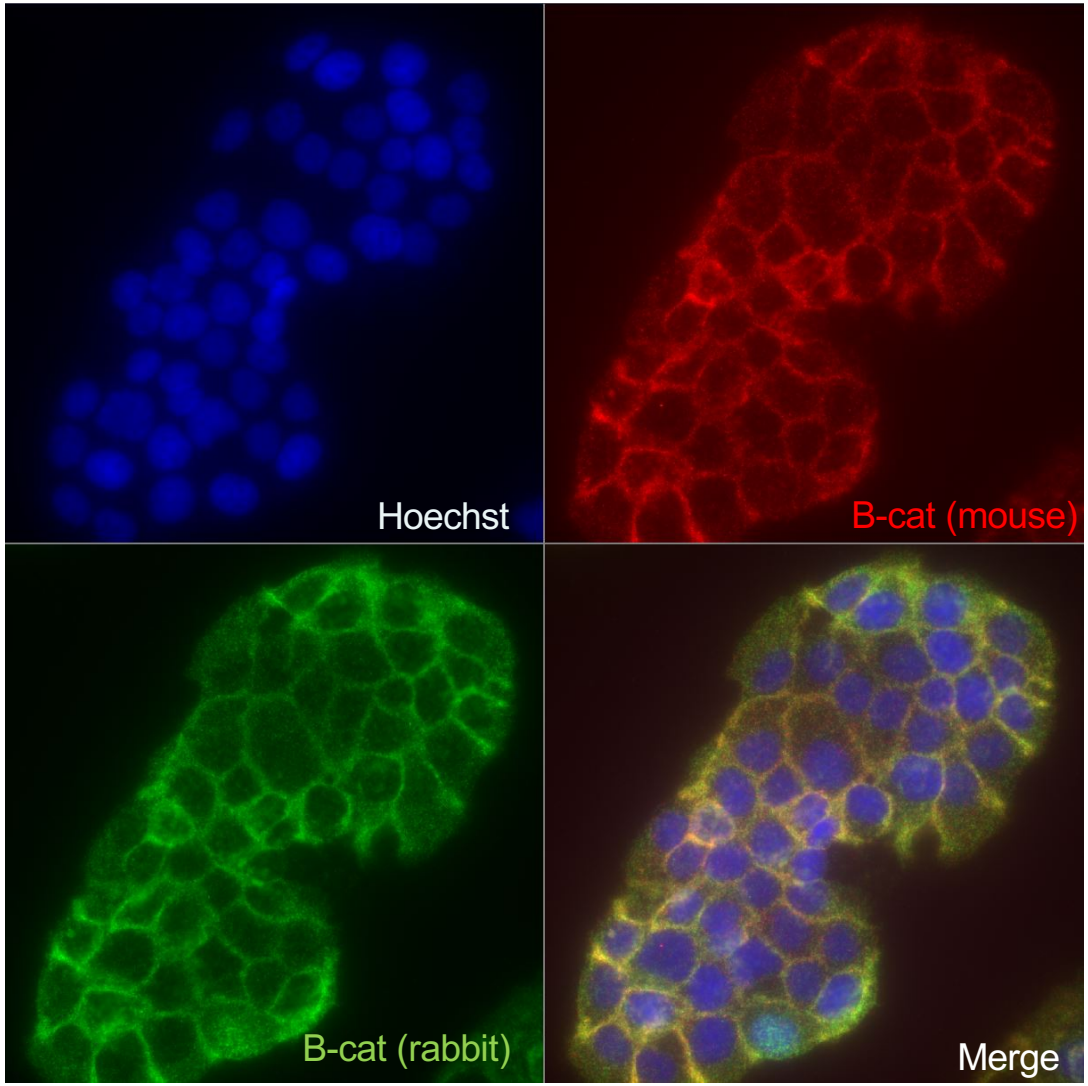
Molboolean analysis of E-Cadherin / β-Catenin

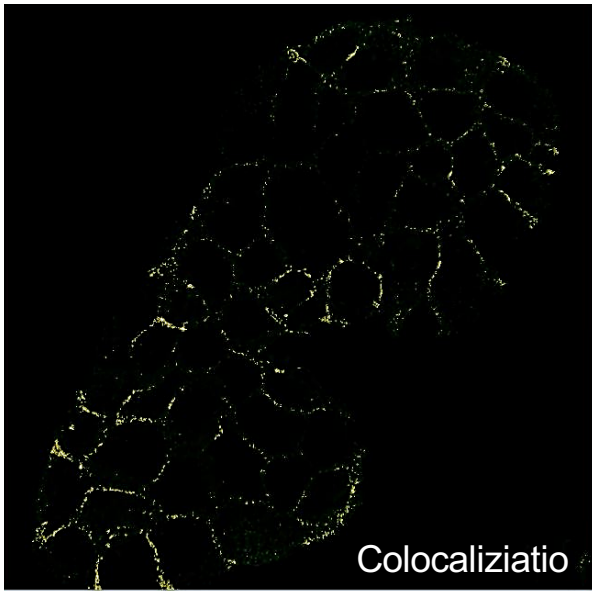




Raykova & Kermpatsou *et al*., unpublished

Pairs of antibodies targeting different epitopes





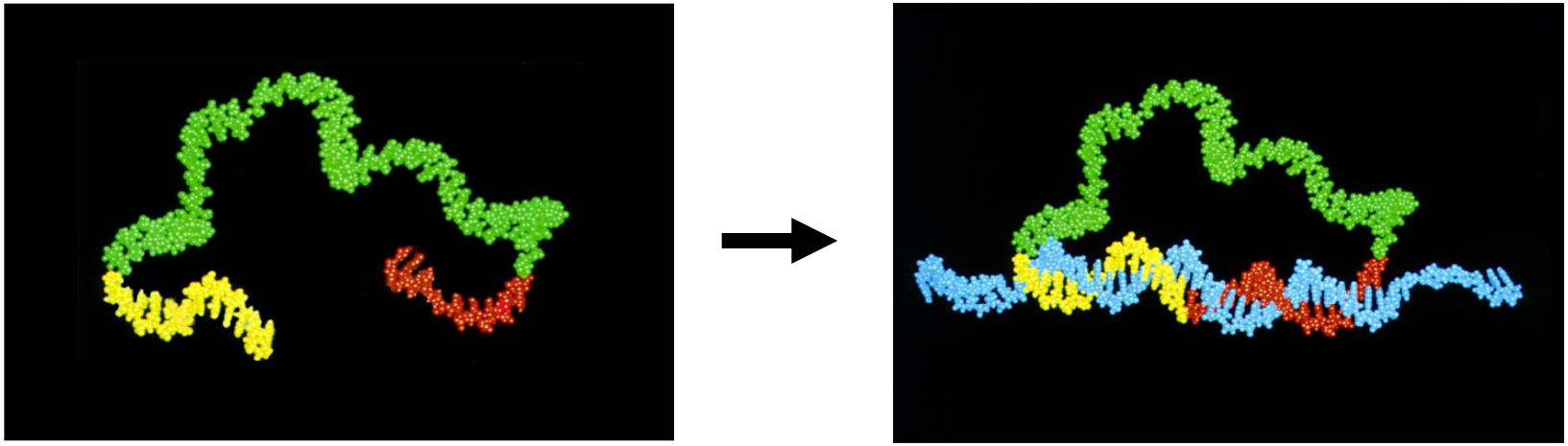
Pearson: 0,604

Raykova & Kermpatsou *et al*., unpublished

Räcker det med att detektera protein interaktioner för att förstå biologin?

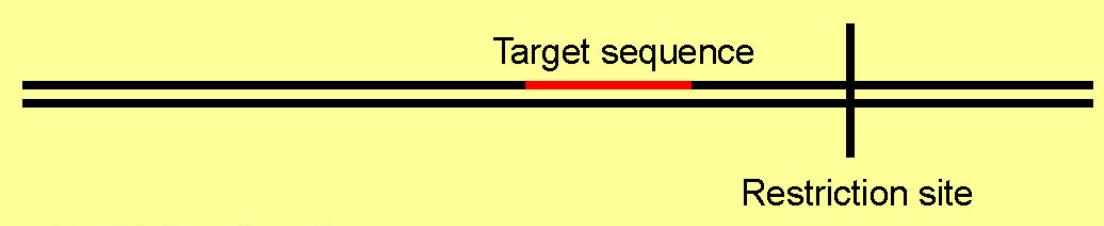
Kan vi kombinera olika slags metoder för att få en djupare förståelse?

Padlock probes

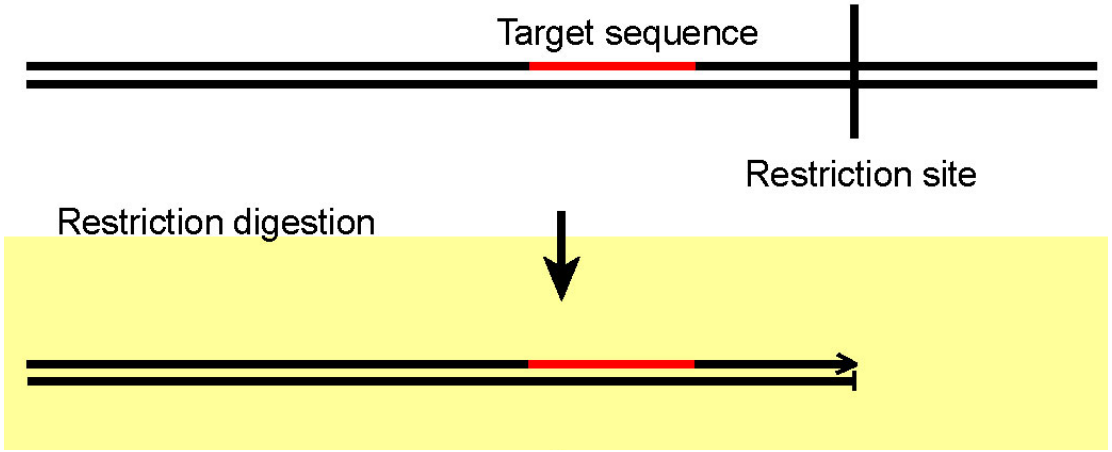


Nilsson *et al.*, *Science* **265:**2085 (1994).
Nilsson *et al.*, *Nature Genetics* **16:**252 (1997).
Nilsson *et al.*, *Nature Biotechnol* **18:**791 (2000).

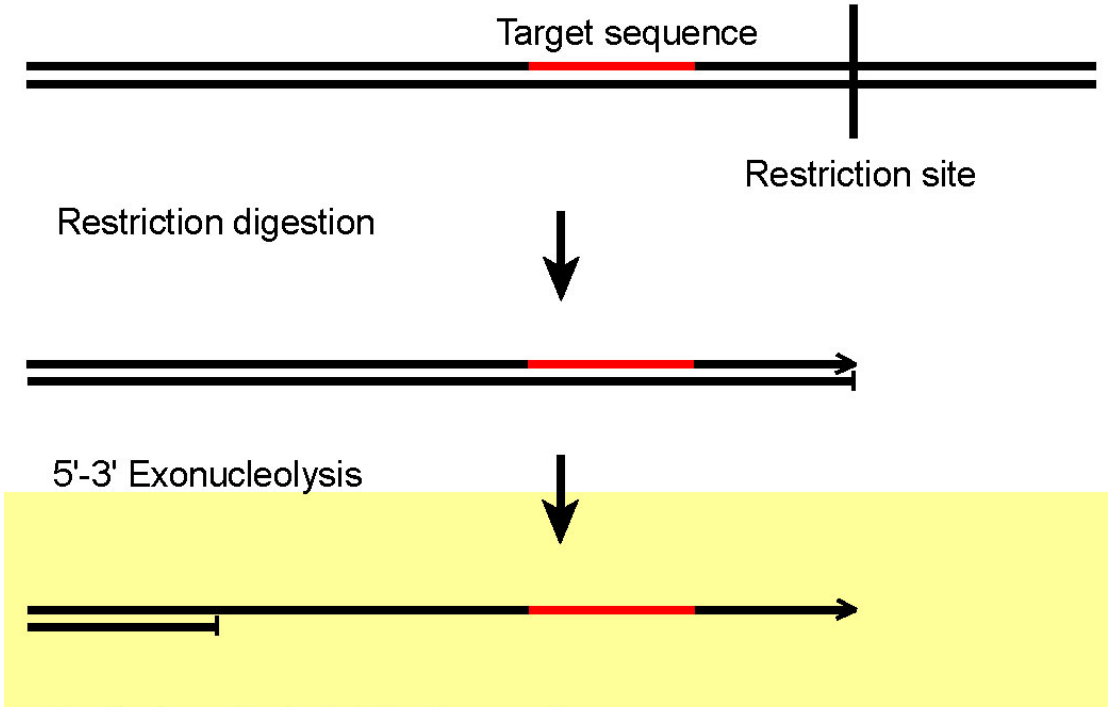
Target preparation – step 1



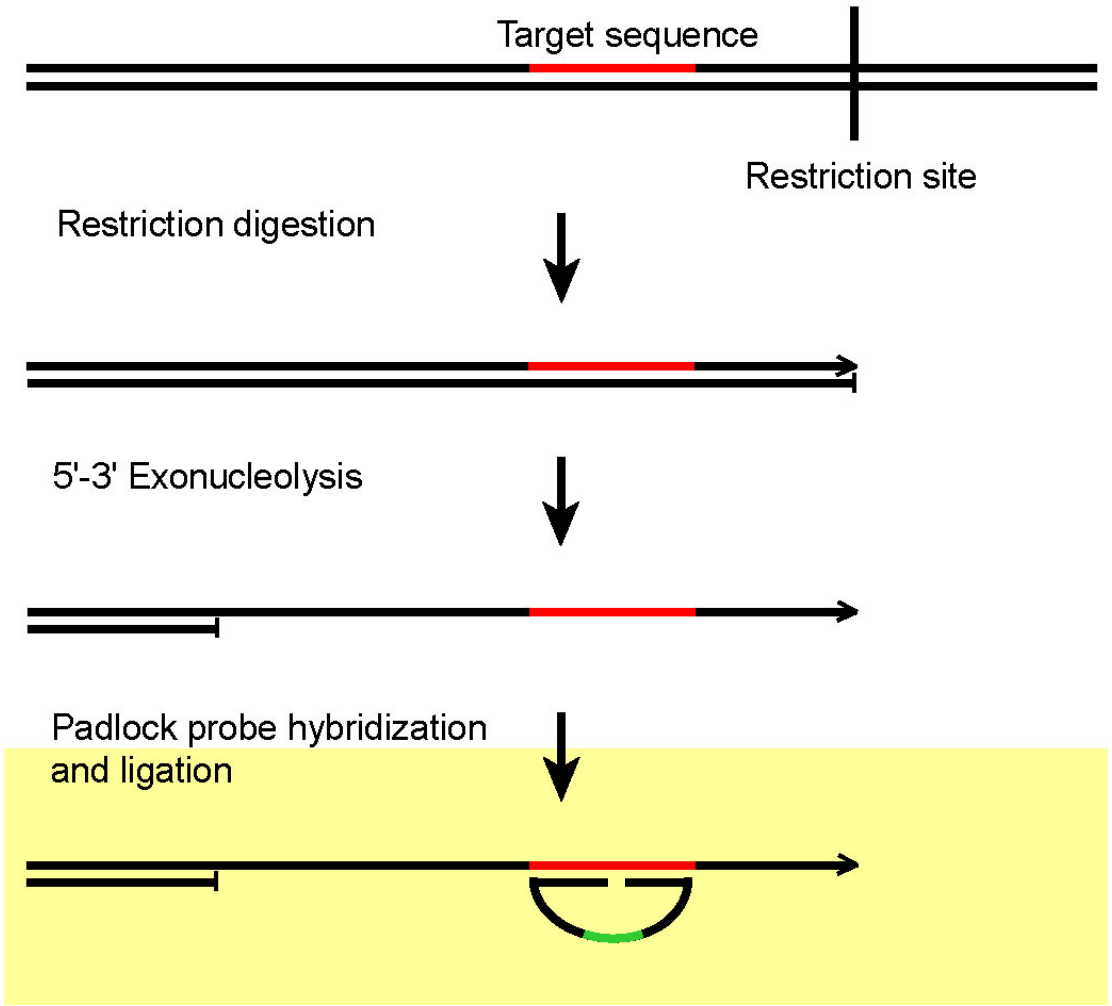
Target preparation – step 2



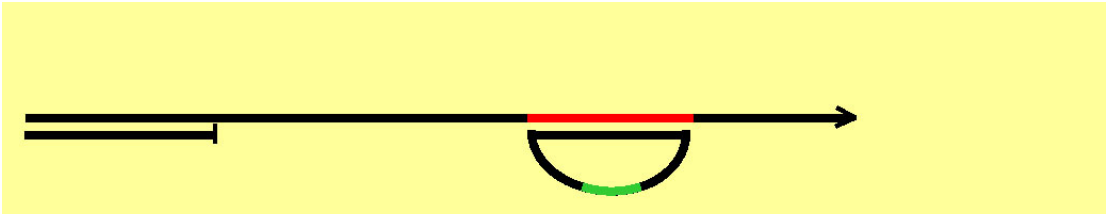
Target preparation – step 3



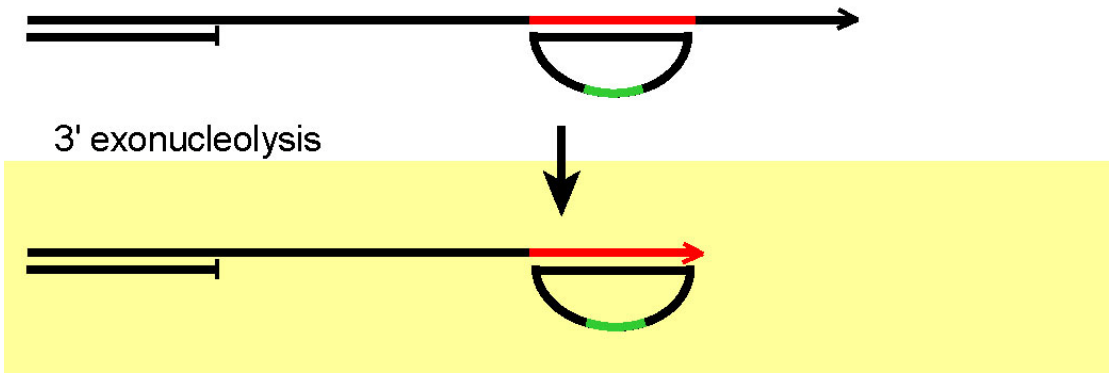
Target recognition by padlock probe



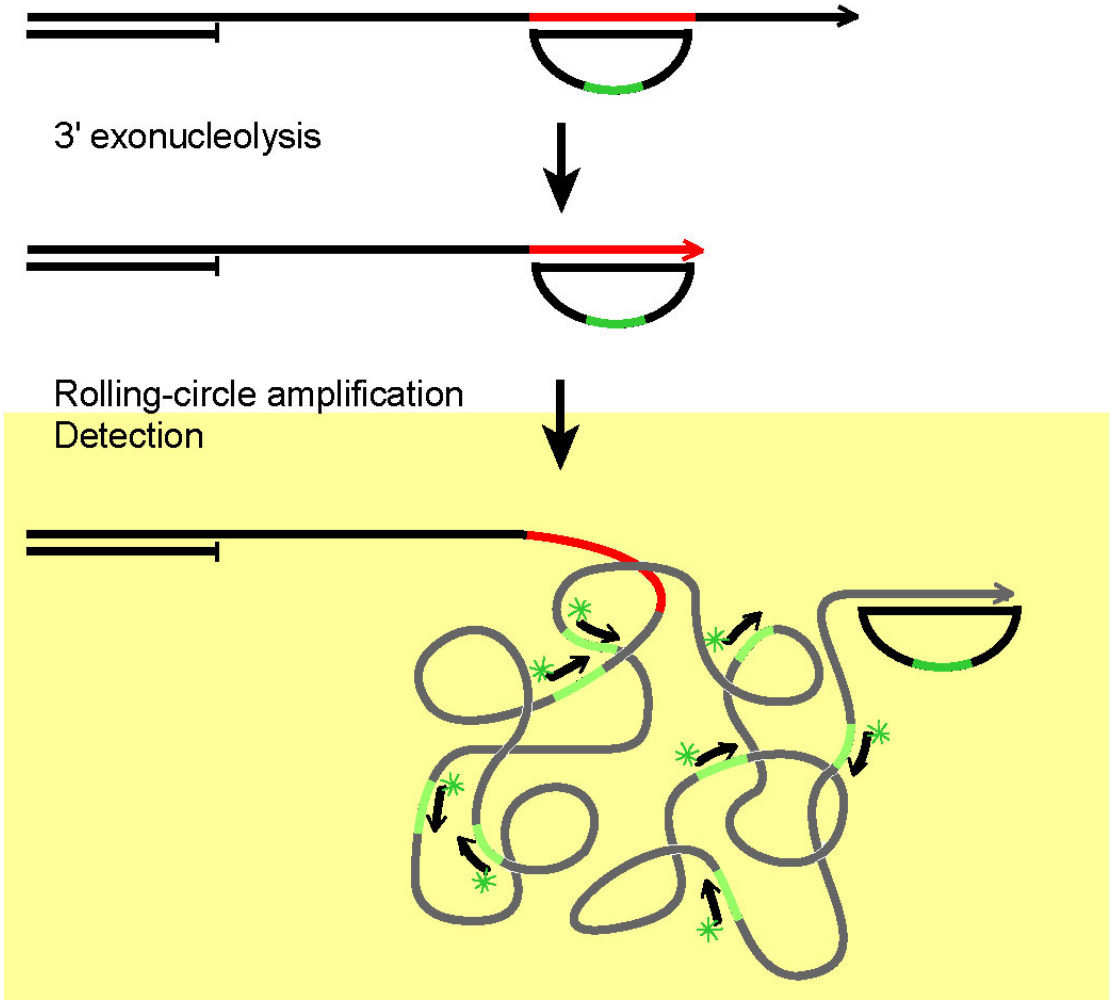
Target-primed RCA



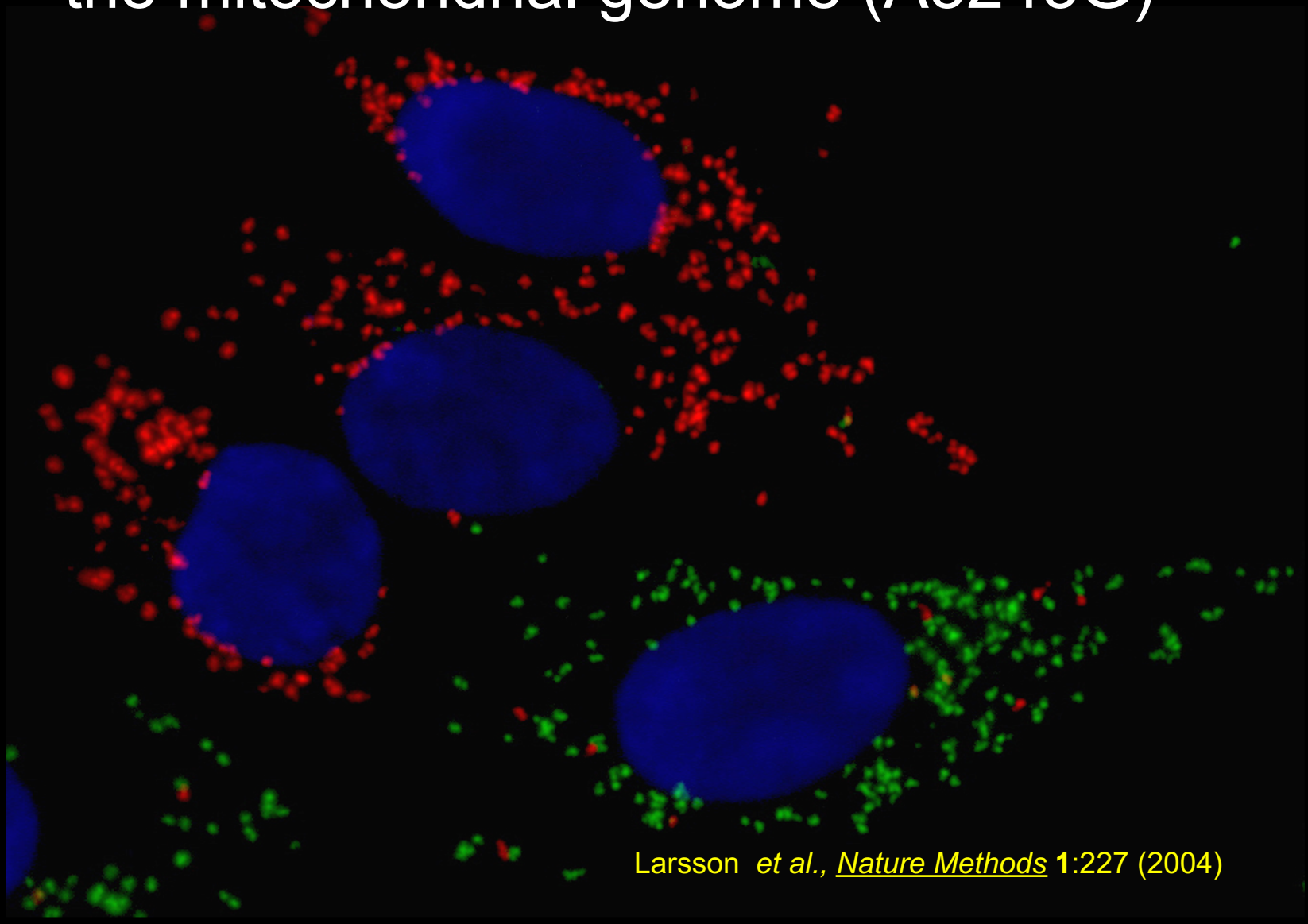
Target-primed RCA



Target-primed RCA

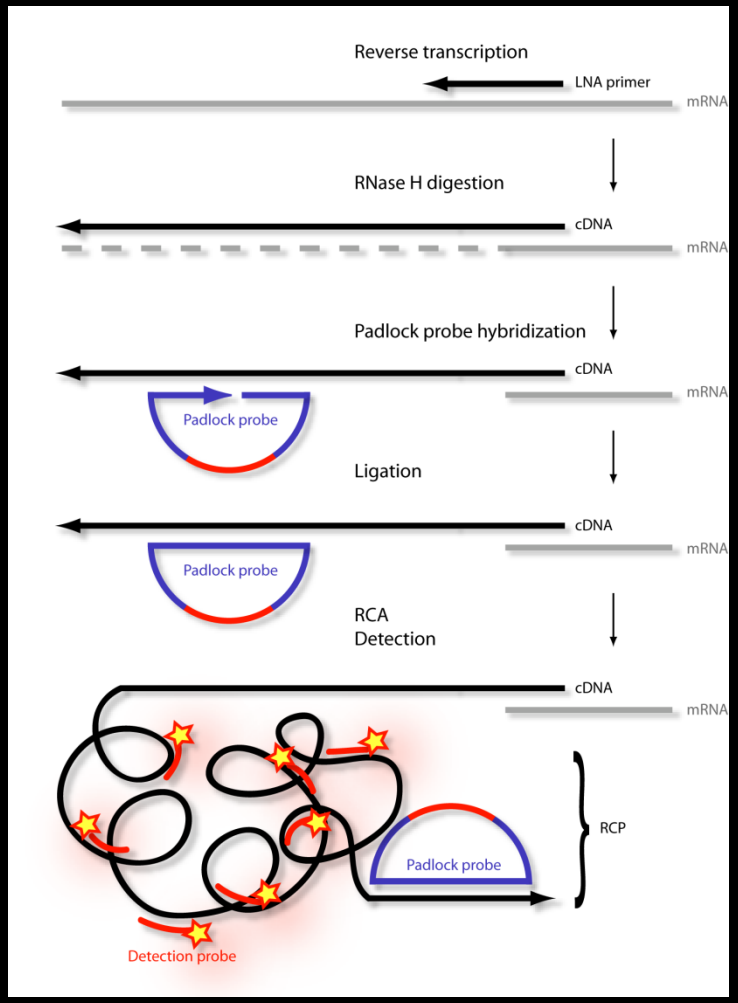


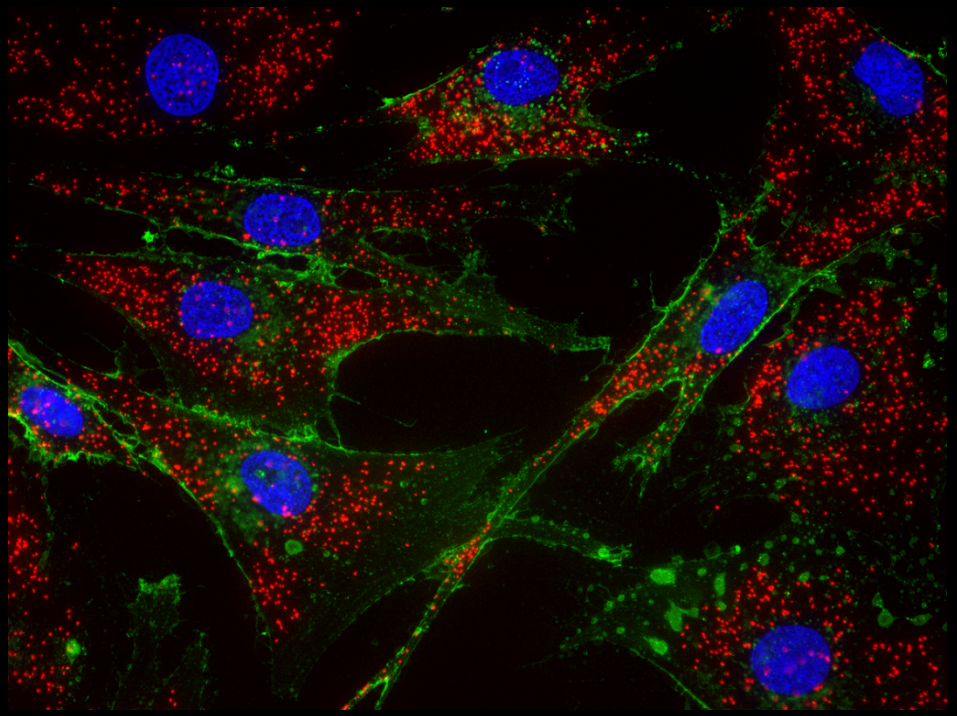
Detection of single nucleotide variation in the mitochondrial genome (A3243G)



Larsson *et al.*, *Nature Methods* **1**:227 (2004)

Detection of single transcripts using padlock probes and RCA



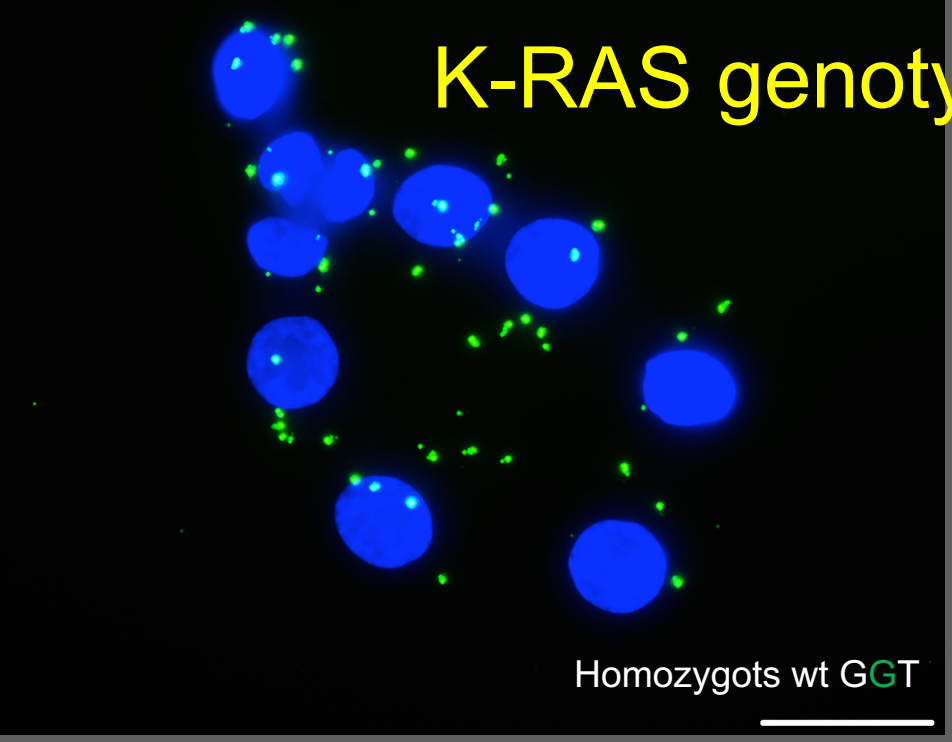


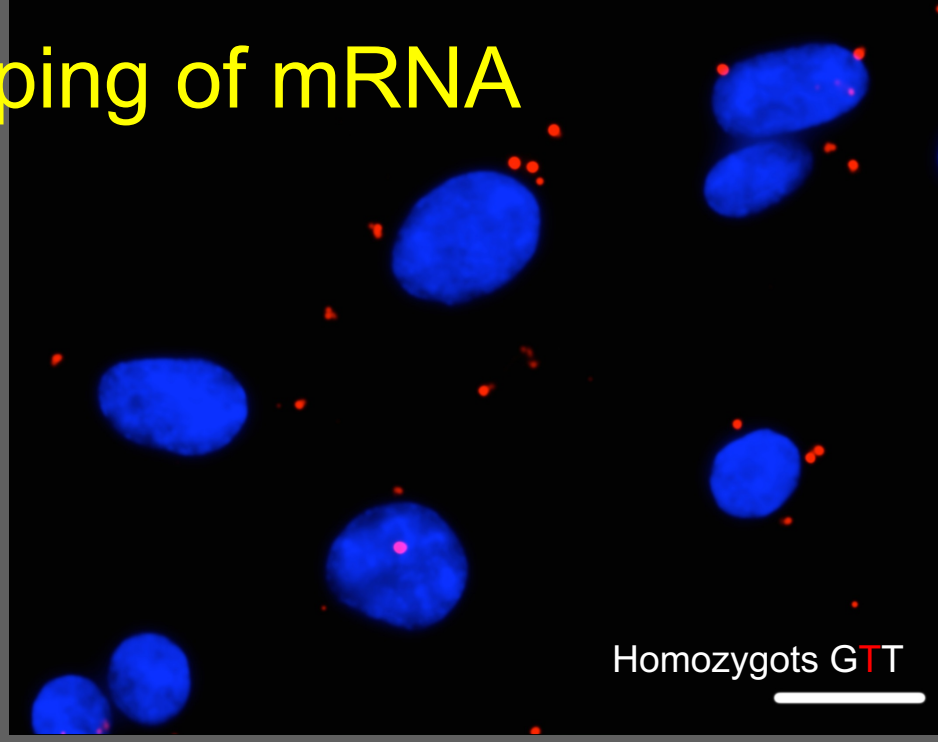
β-actin transcripts
Nuclear staining
Membrane staining

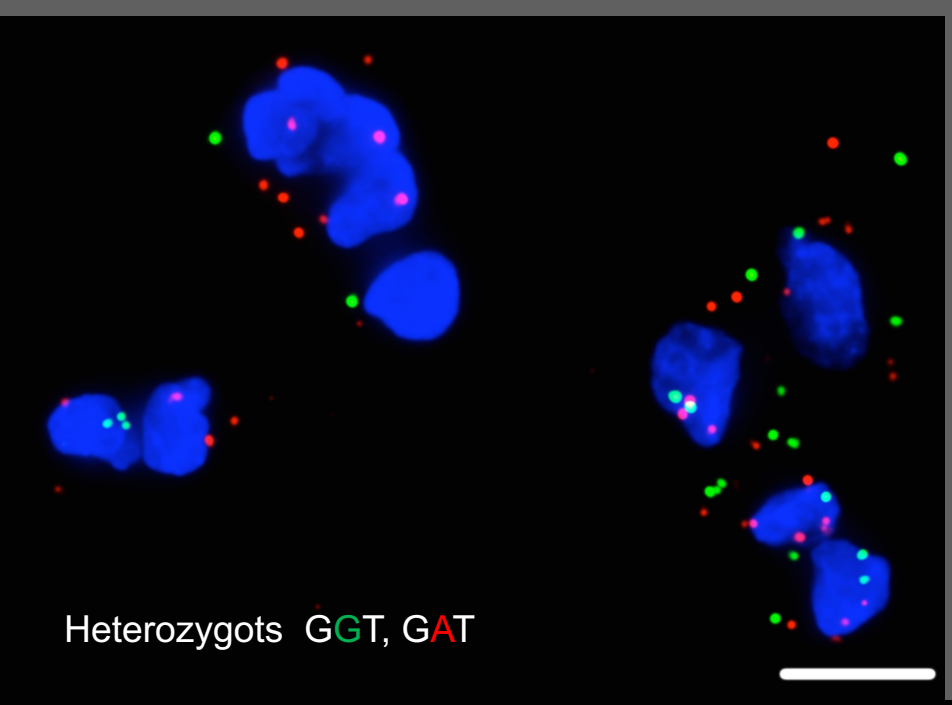
30% detection efficiency

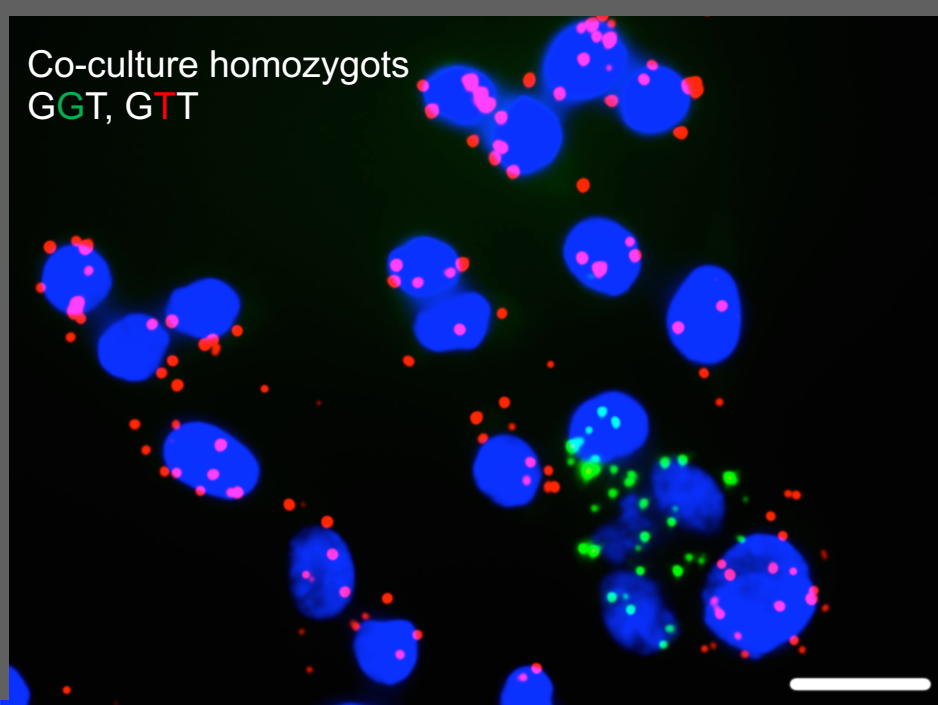
Larsson, C. *et al.* (2010) *Nature Methods*,**7**, 395–397.

K-RAS genotyping of mRNA

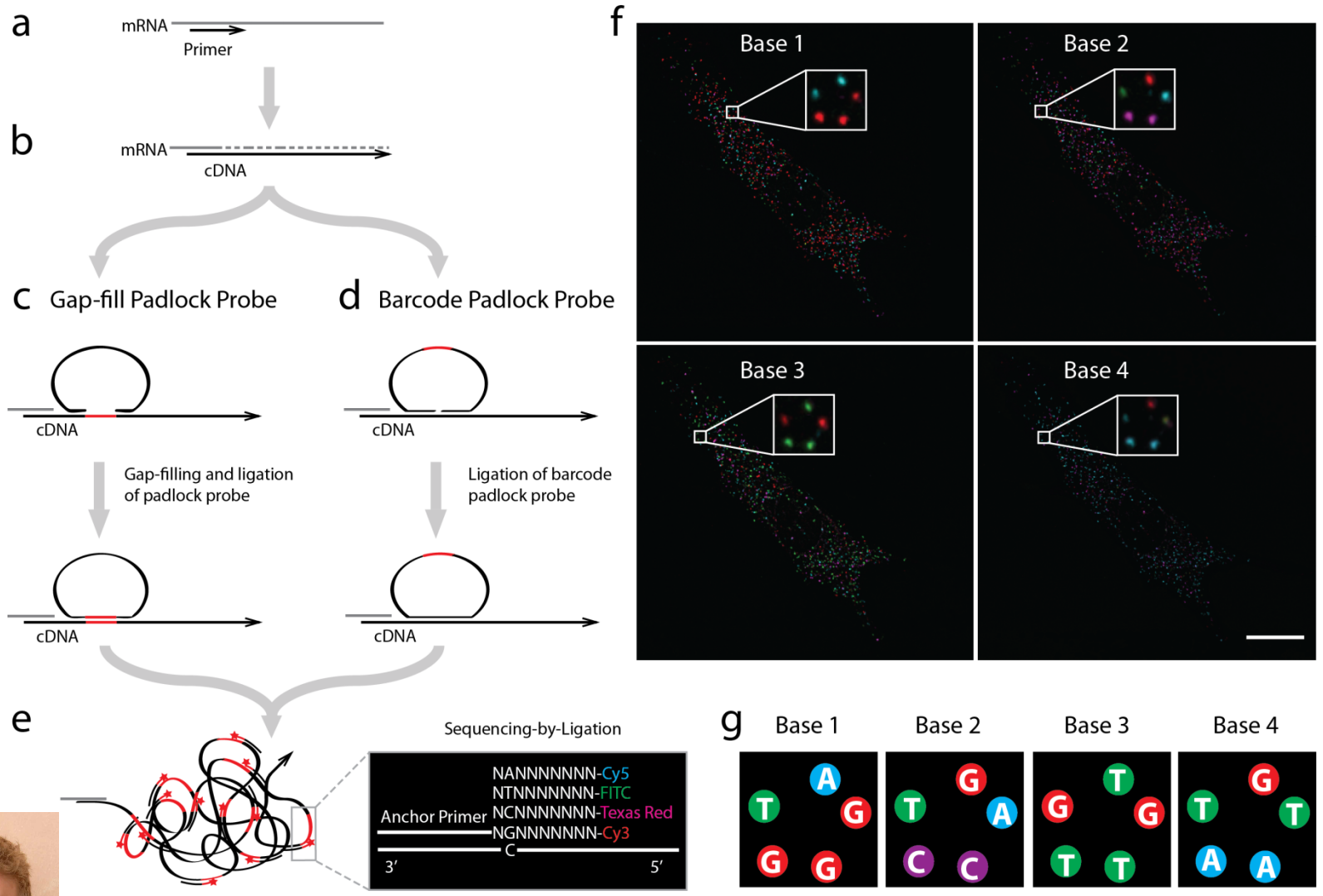






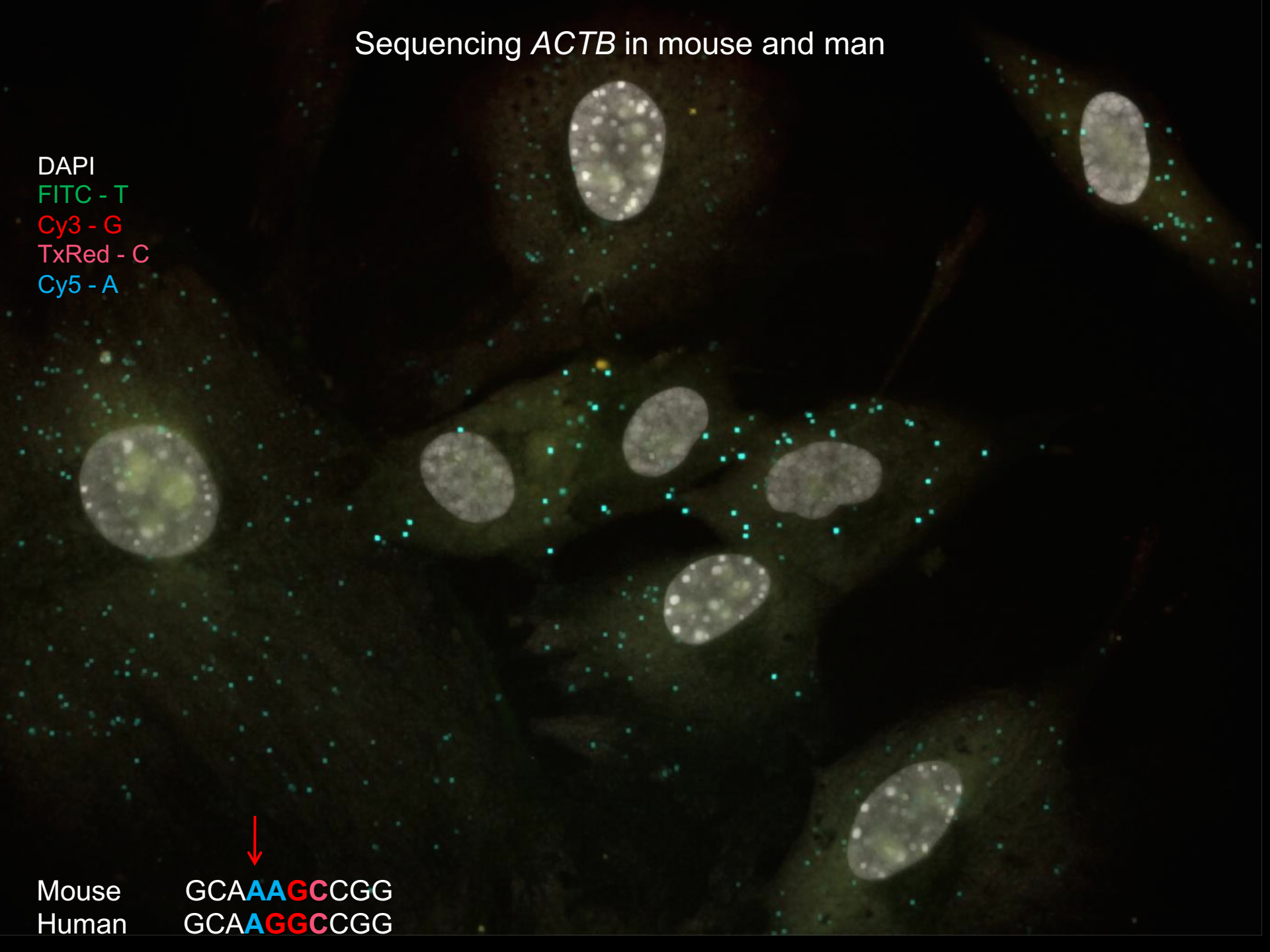


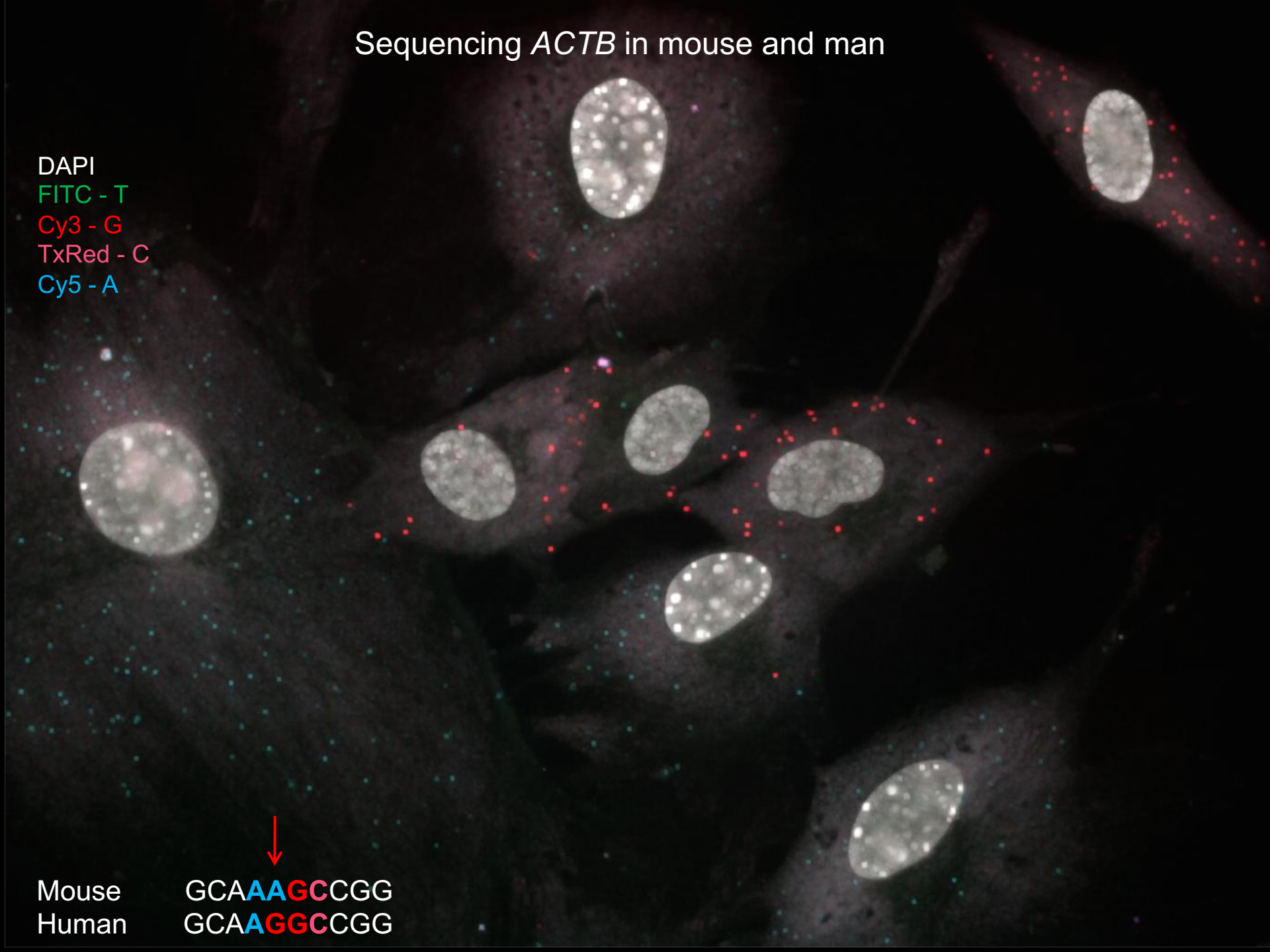
Schematic Illustration of *in situ* Sequencing

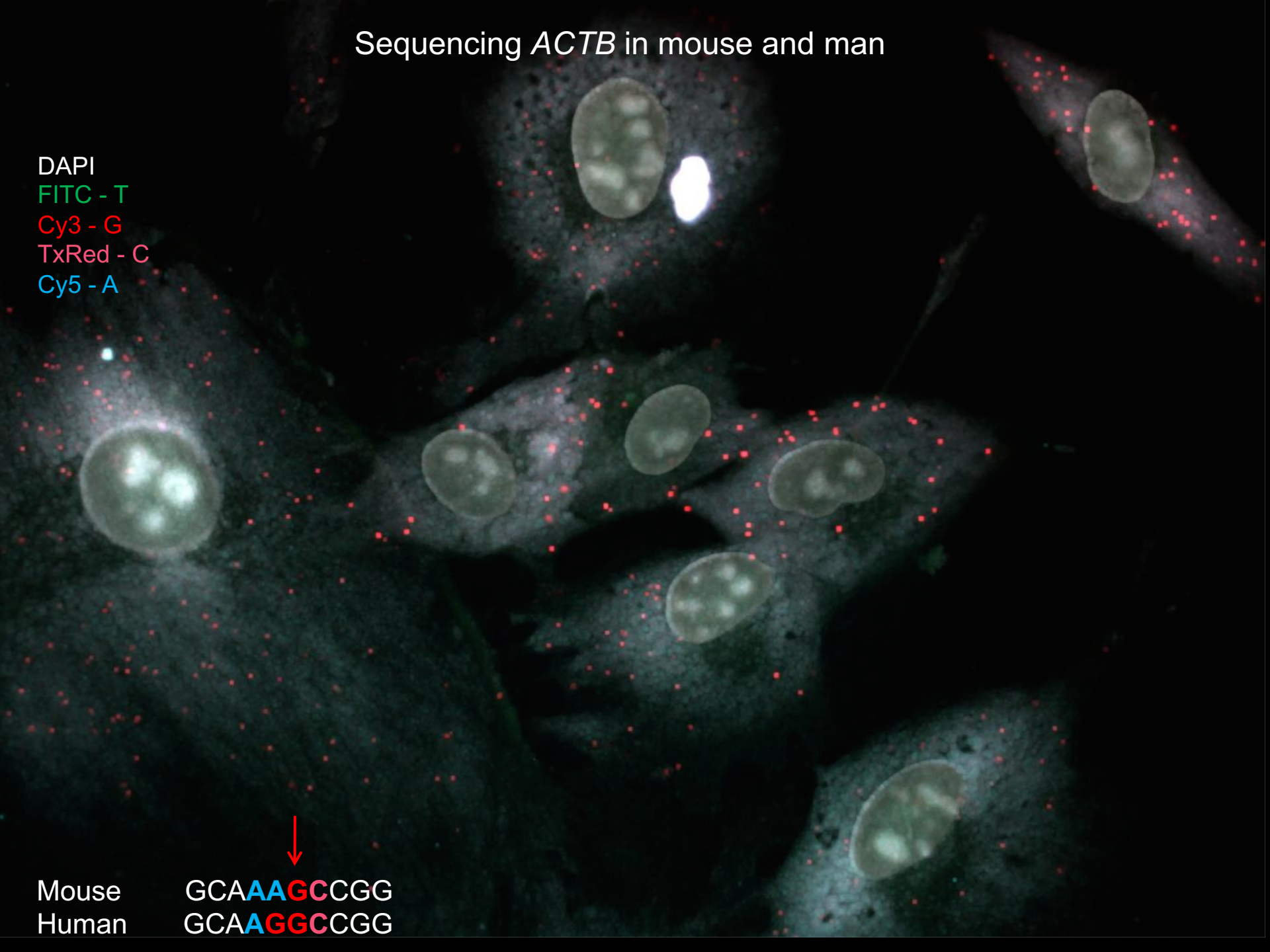


Ke, *et al, Nature Methods*, 10:857 (2013)

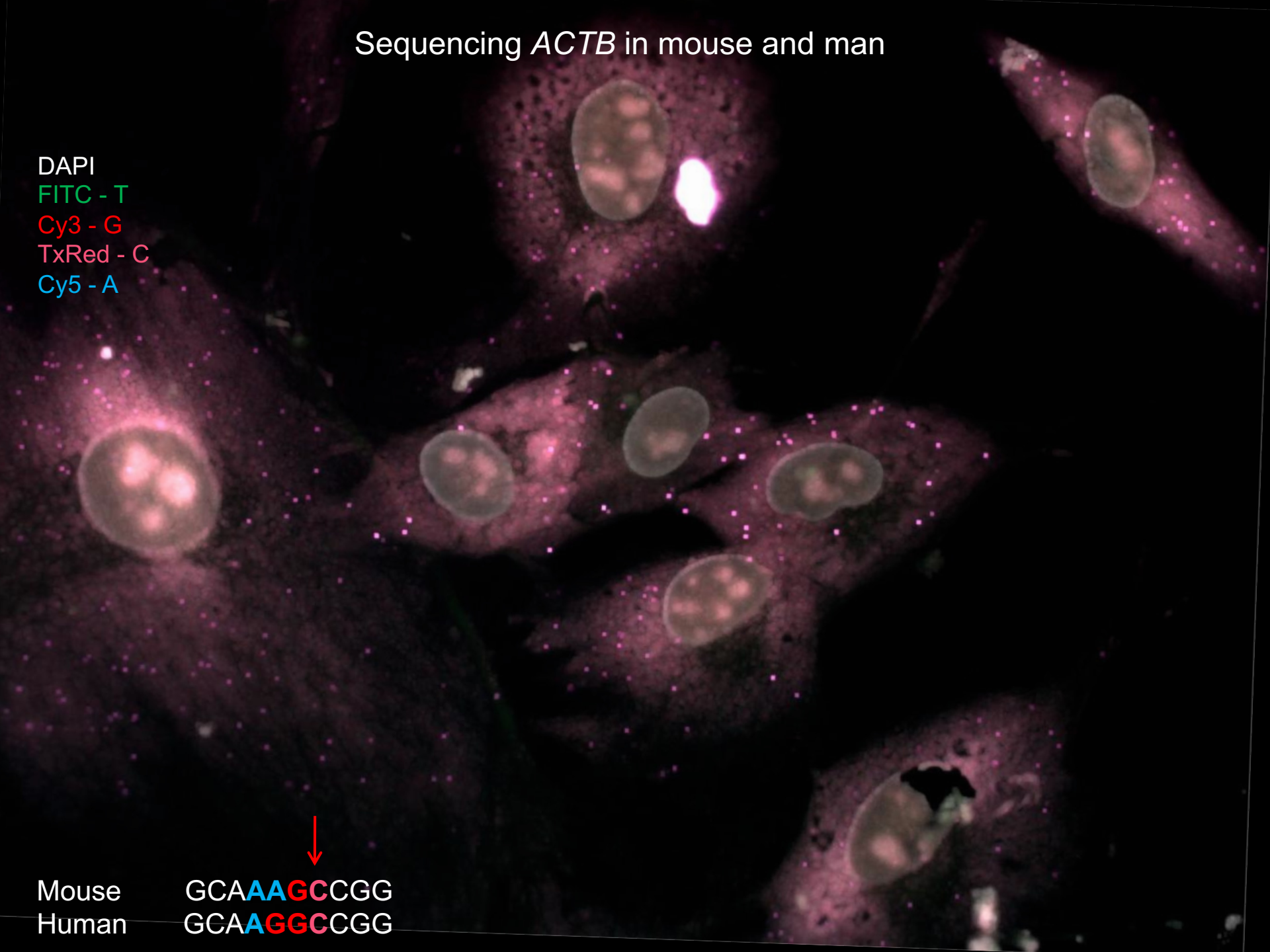
Sequencing *ACTB* in mouse and man



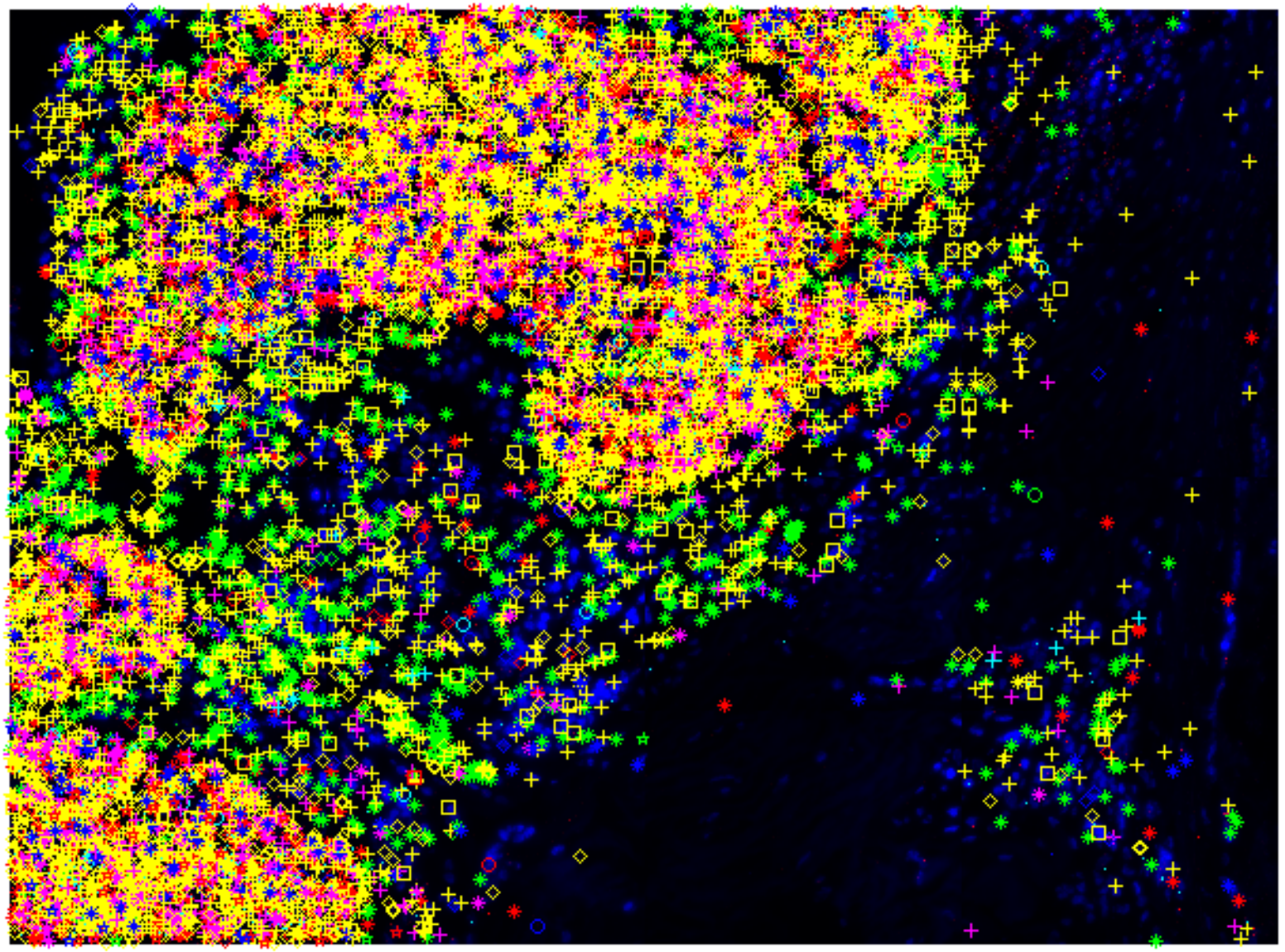

Sequencing *ACTB* in mouse and man
DAPI
FITC - T
Cy3 - G
TxRed - C
Cy5 - A
Mouse GCAAAGCCGG
Human GCAAGGCCGG


Sequencing *ACTB* in mouse and man
DAPI
FITC - T
Cy3 - G
TxRed - C
Cy5 - A
Mouse GCAAAGCCGG
Human GCAAGGCCGG

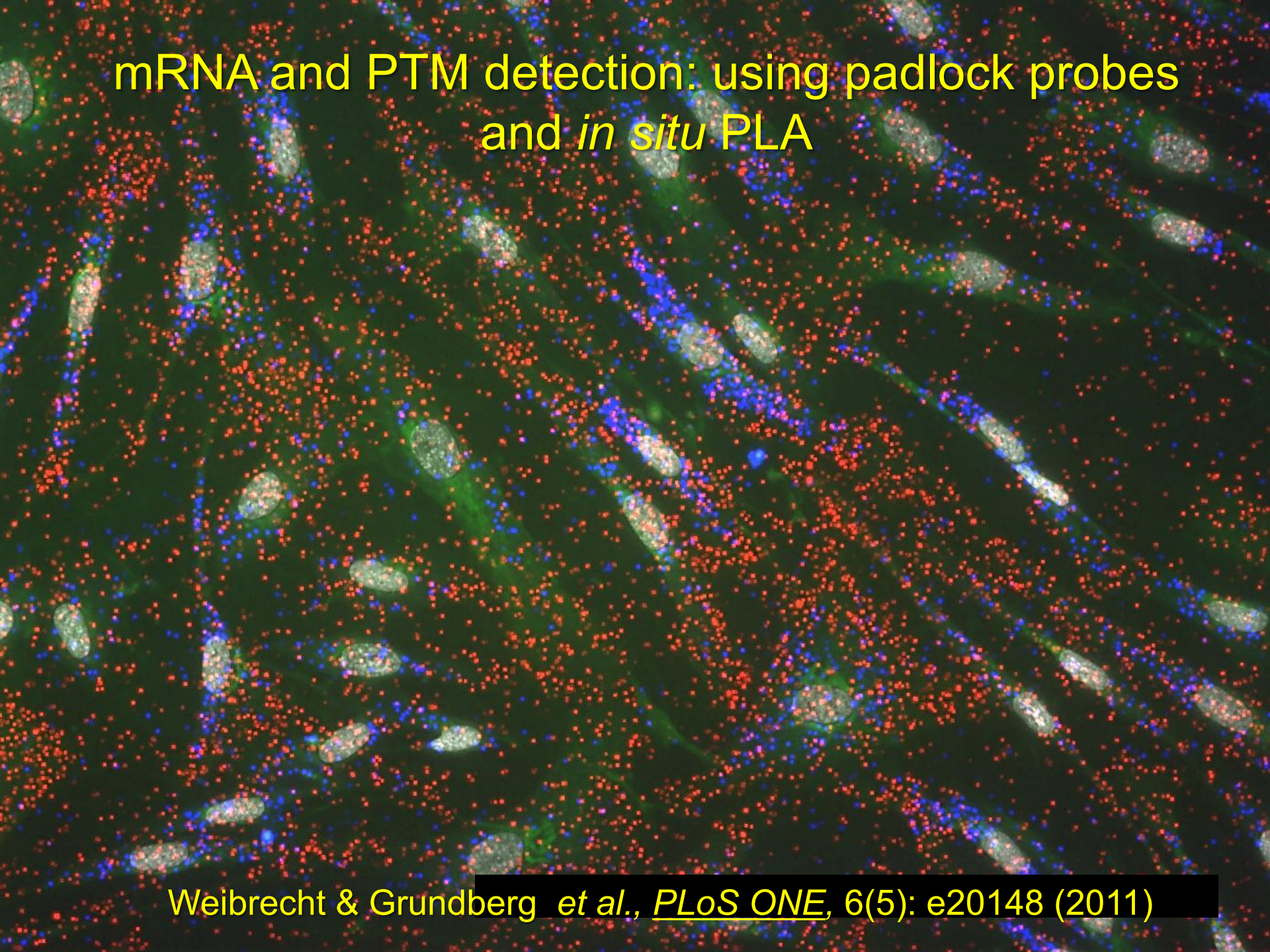
Sequencing *ACTB* in mouse and man



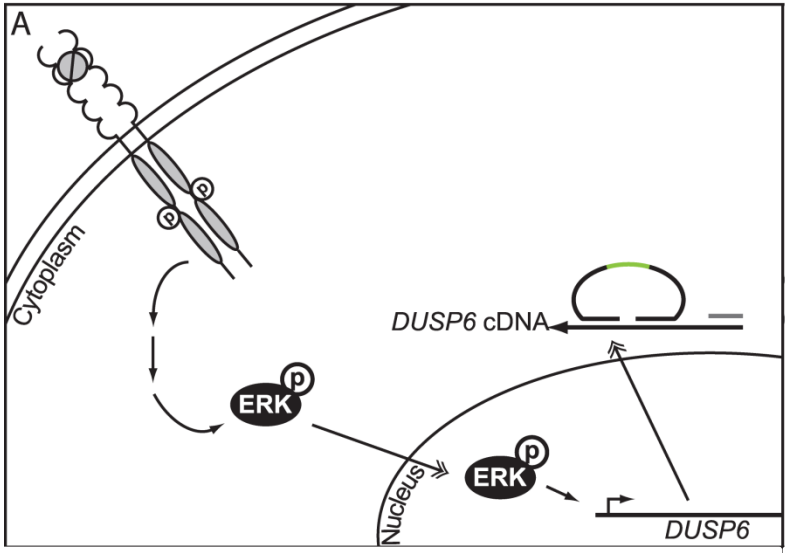
Expression profiling

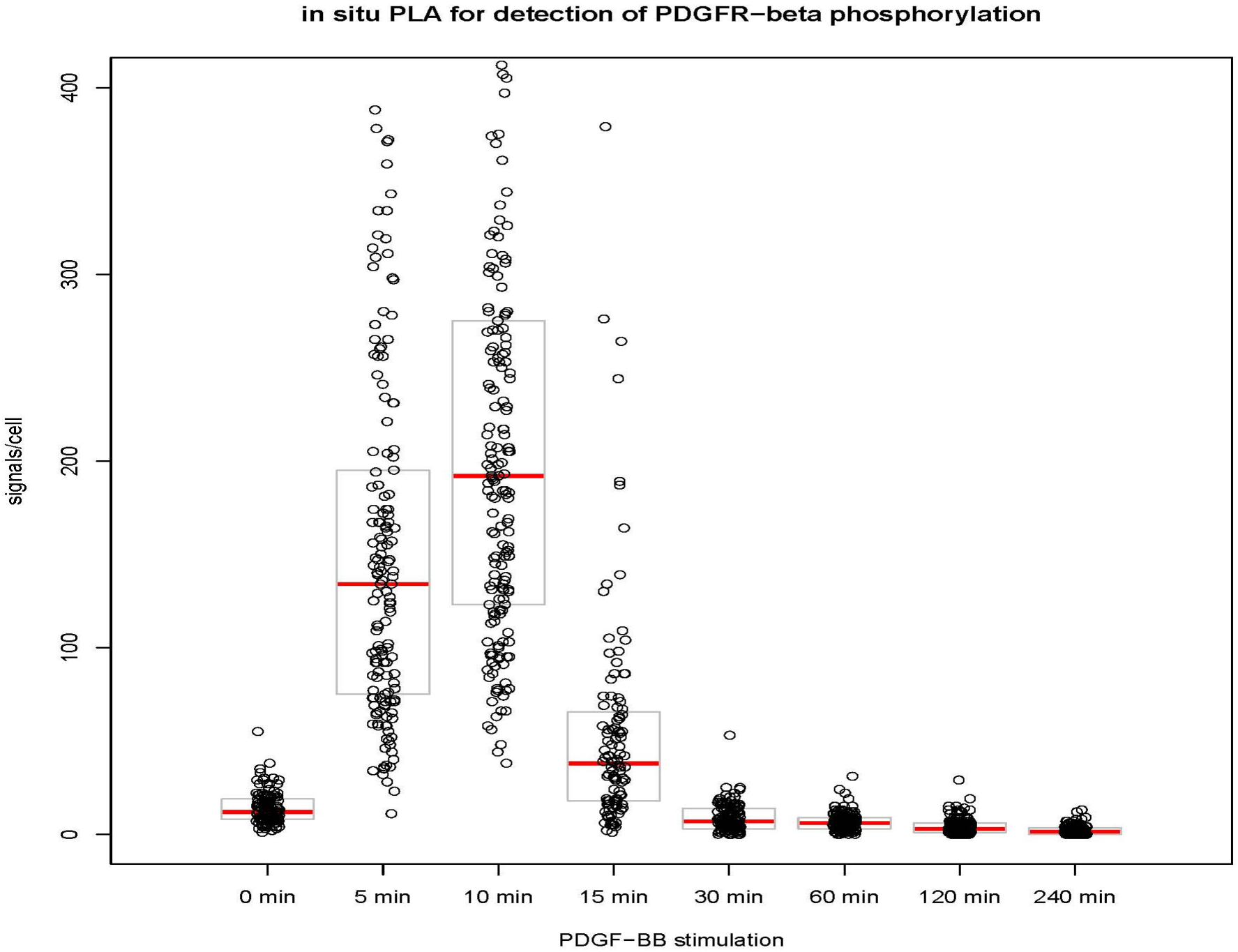


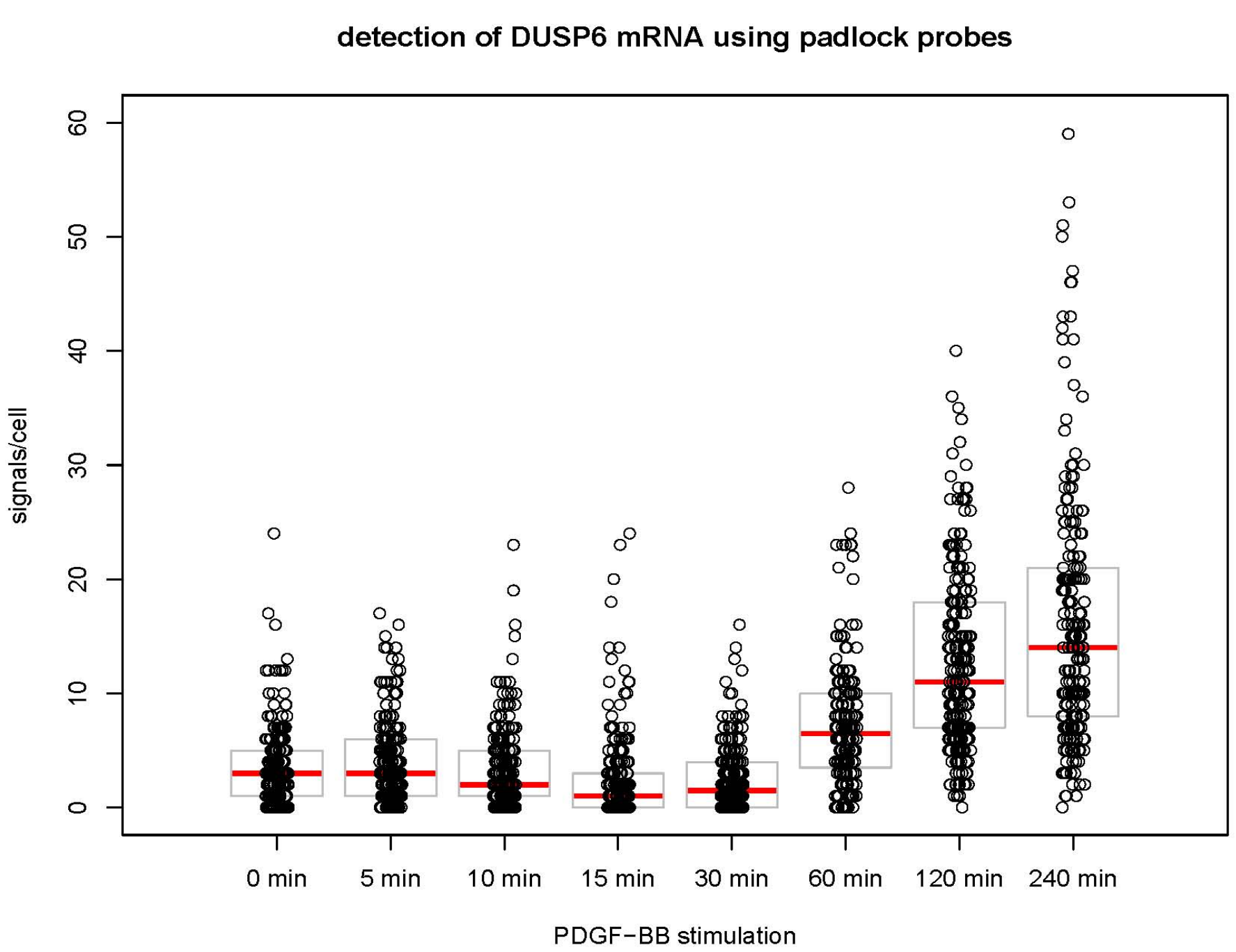
HER2
VIM
KI-67
STK 15
BIRC5
CCNB1
MYBL2
ST-3
CTSL2
ER
BAG1
CD68
BCL2
SCUBE2
ACTB
GAPDH
RPLP0
GUS
TFRC
VEGF
MYC
MET
TP53
EPCAM
CCND1
MUC1
RAC1
HOXB7
ATB1
ETV4


mRNA and PTM detection: using padlock probes and *in situ* PLA
Weibrecht & Grundberg *et al.*, *PLoS ONE*, 6(5): e20148 (2011)

Visualization of signaling activity

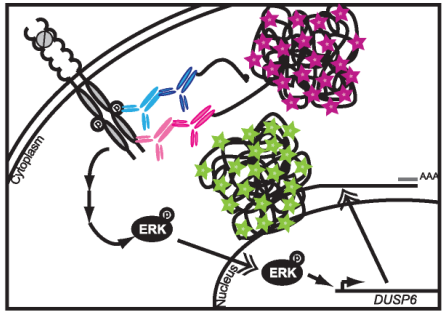



in situ PLA for detection of PDGFR−beta phosphorylation
signals/cell
0
100
200
300
400
0 min
5 min
10 min
15 min
30 min
60 min
120 min
240 min
PDGF−BB stimulation


detection of DUSP6 mRNA using padlock probes
signals/cell
0
10
20
30
40
50
60
0 min
5 min
10 min
15 min
30 min
60 min
120 min
240 min
PDGF−BB stimulation

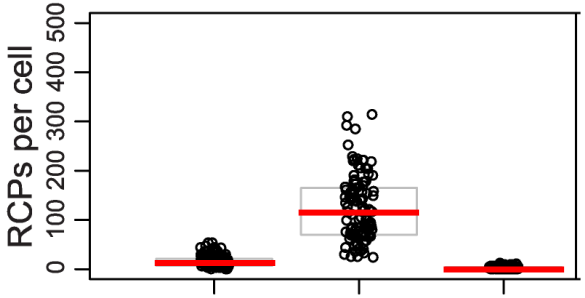
Drug-treatment; proof-of-principle

Bi

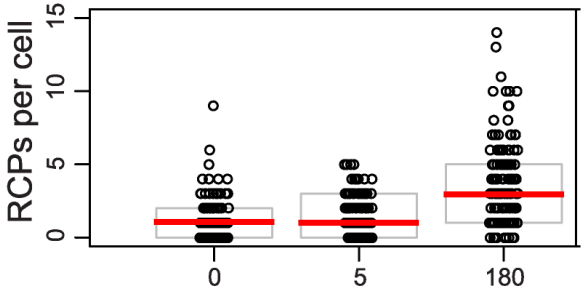


Bii

Simultaneous detection: pPDGFRβ

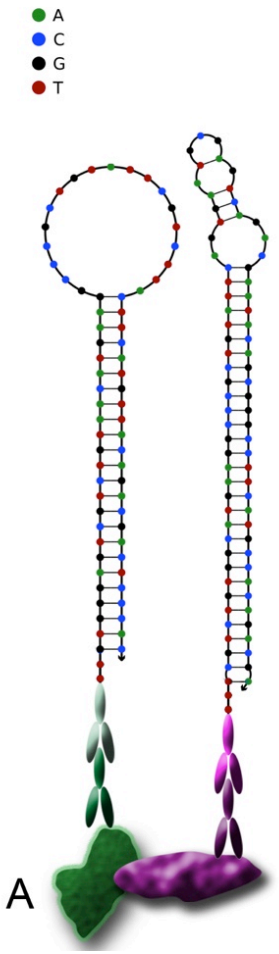


Simultaneous detection: *DUSP6* expression



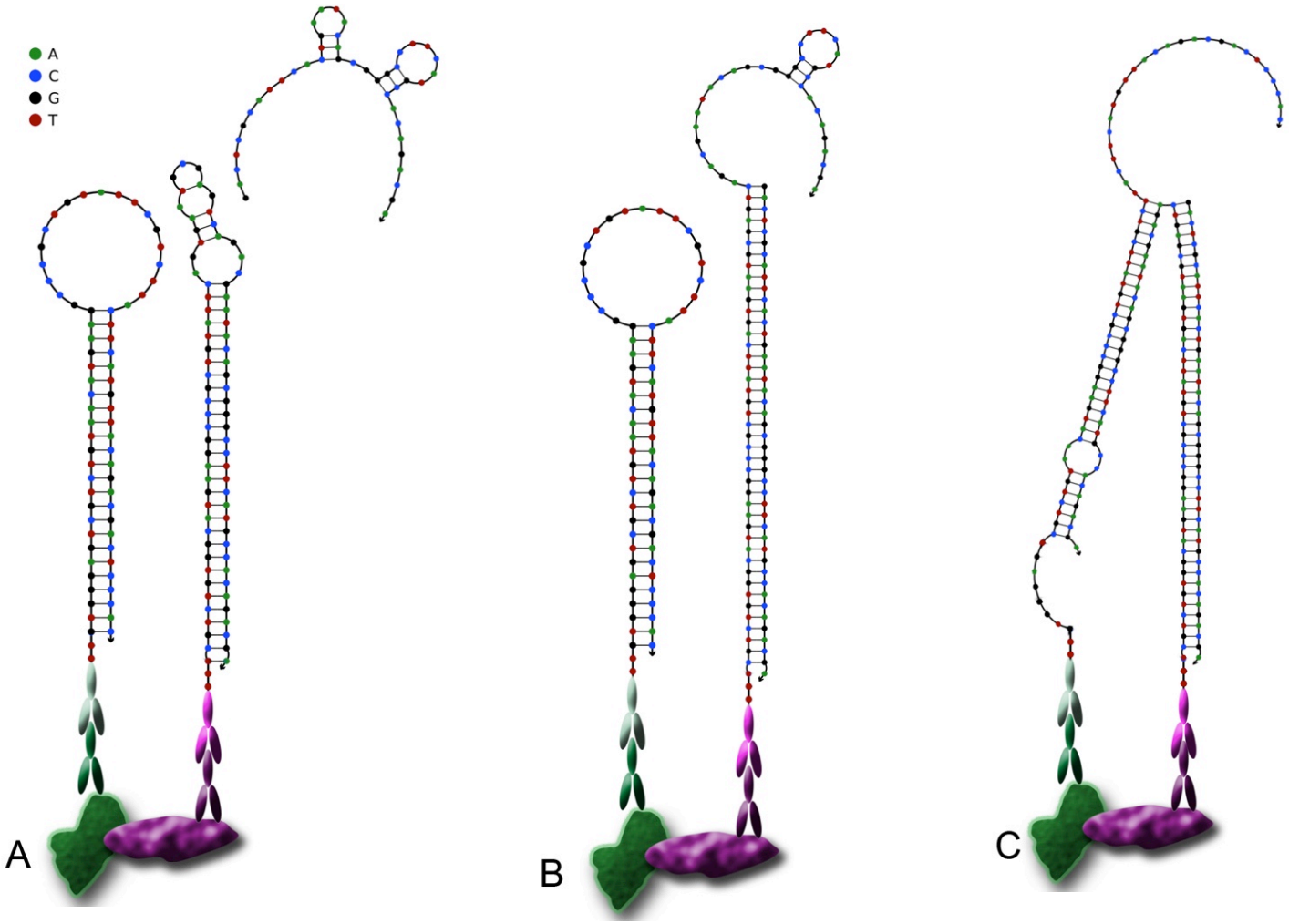
Vad kan vi gör billigare och enklare – så att metoder kan bli tillgängliga för fler?

Proximity dependent initiation of HCR



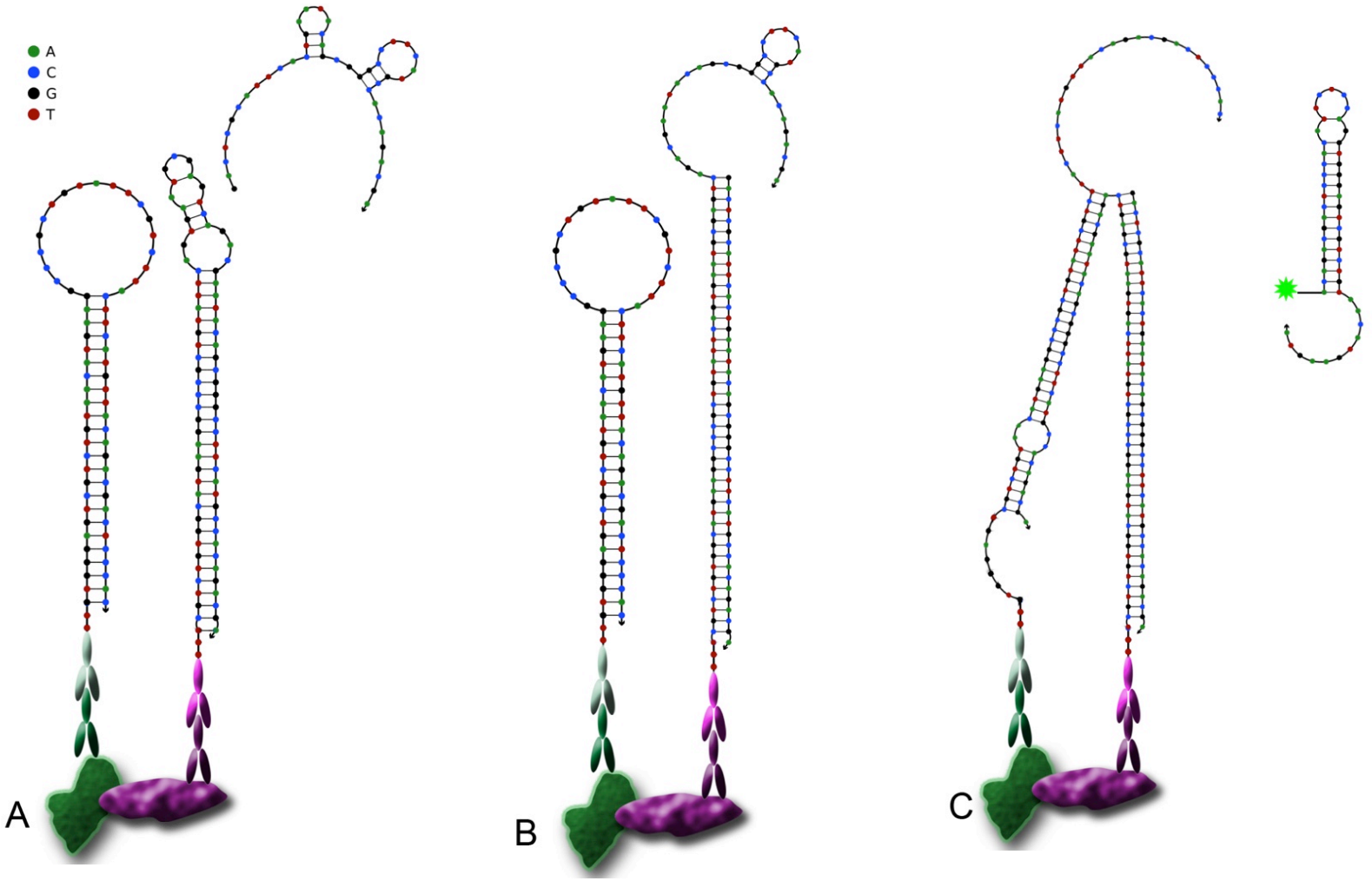
Koos *et a*l., Nature Communications, **6**, 7294 (2015)

Proximity dependent initiation of HCR



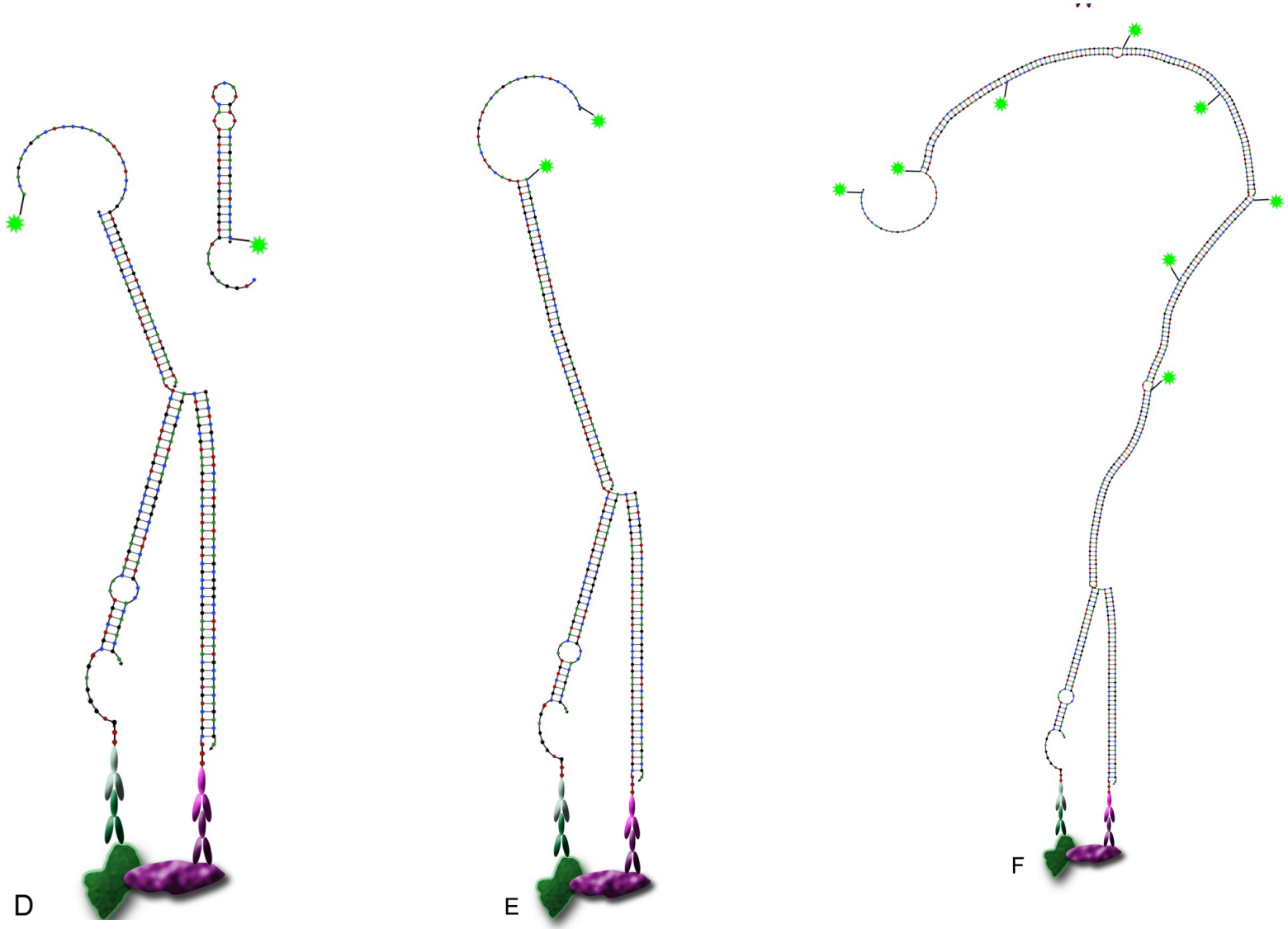
Koos *et al*., Nature Communications, **6**, 7294 (2015)

Proximity dependent initiation of HCR



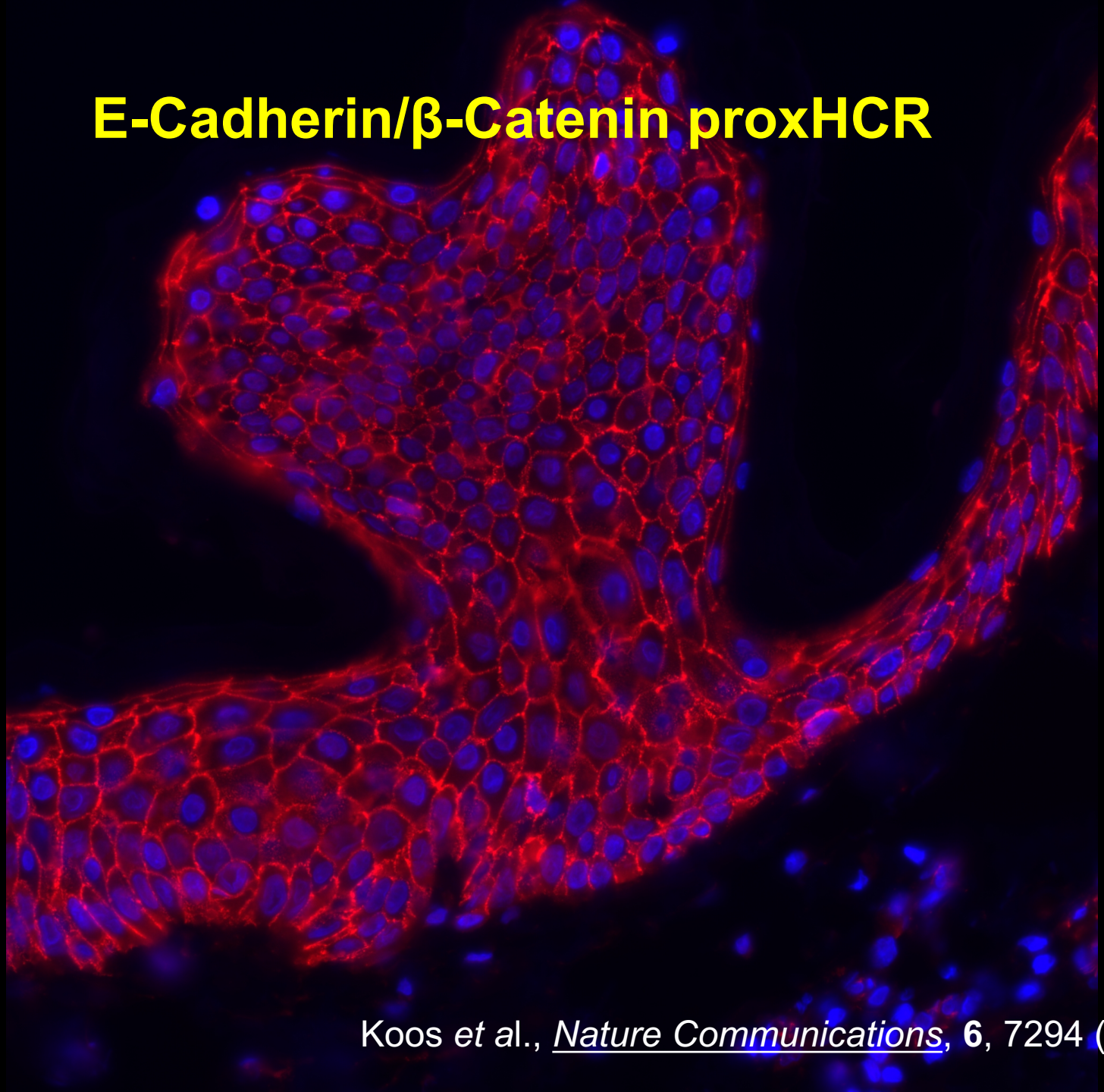
Koos *et a*l., Nature Communications, **6**, 7294 (2015)

Proximity dependent initiation of HCR



Koos *et a*l., Nature Communications, **6**, 7294 (2015)

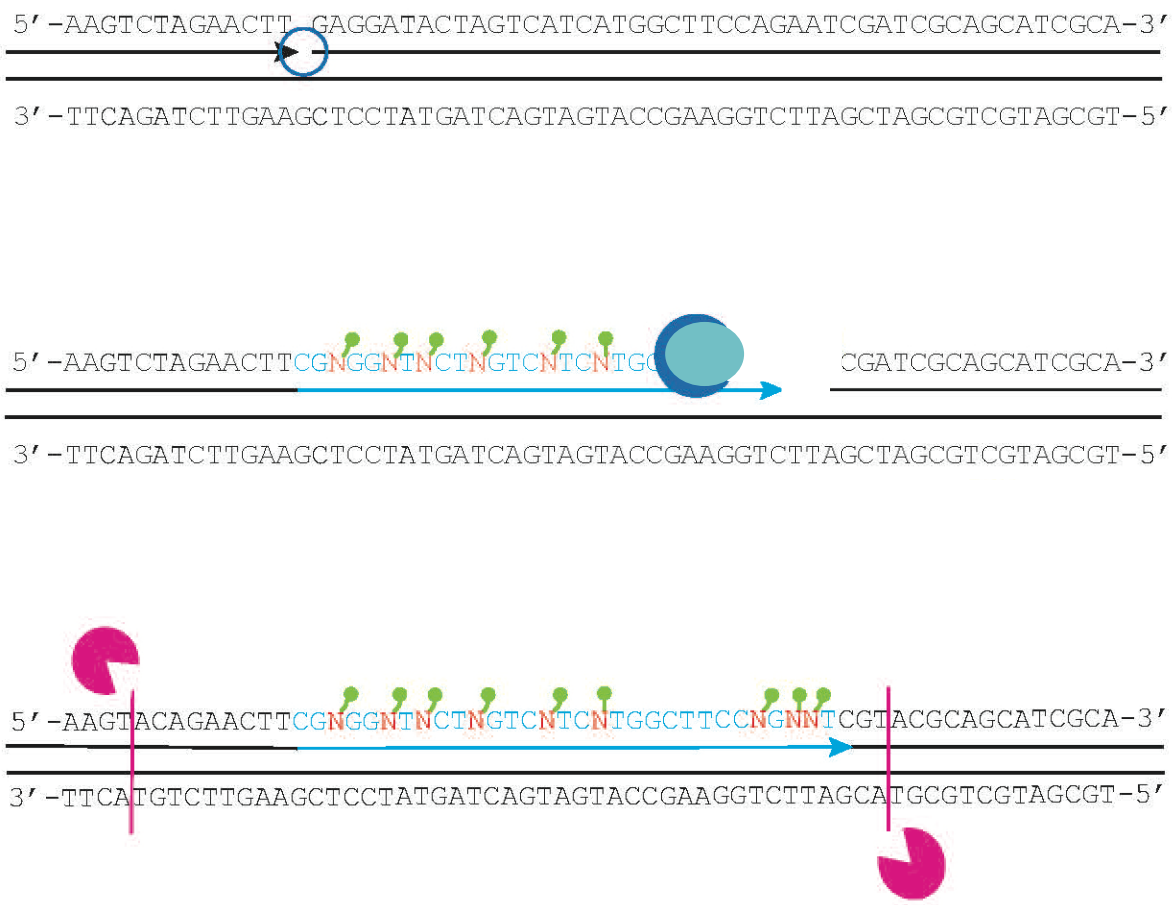
E-Cadherin/β-Catenin proxHCR



Koos *et al*., *Nature Communications*, **6**, 7294 (2015)

Och nu något helt annorlunda

Identifying DNA nicks



Wenson *et al*., unpublished

What do you want of a DNA polymerase?

✅ it would need to be highly error prone (possibility to use only 3 nucleotides)

✅ it woul

✅ it wou

reading)

Sloppymerase™

-

✅ it would have to have 5´to 3´exonuclease activity to remove the strand in front of the nick while it is synthesizing a new strand

✅ tolerate biotinylated nucleotides

Sloppymerase™ is highly error-prone

Oligo	GGATCCGGCCAAGCTTCGAGCTGAATTCTGCAGTACATTAA
- dCTP	
1. F	GGATAAGGAGAAGGTTTGAGTTGAATTGTGTAGTATATTAA
2. F	GGATTGGGGTGAATTTTGG----------AGTATATTAA
3. F	GGATGAAGTGAA-TTTTGA----------AGTATATTAA
4. F	GGATTAGGGG----------TGAAG--TAGTATTAATTAA
5. F	GGATAGGGT-AAG-----AGTAGTAG------TAGTATTAA
6. F	GGATTTGAG----------GTGAATTTTGAAGTAAATTAG
7. F	GGATAAGG----------GTGAAATTTGAAGTATATTAA
8. F	GGATAAGGTTAAG-AGAGAGATGAATTATGAAGTATATTAA
9. F	GGATTTGGGAAAGTTTAGAGGTGAATTTTGTAGTATATTAA
10.F	GGATGAGGGG----------TGAATTATGGAGT-TATTAA
11.F	GGATTGGGGAAAGGTTTGAGATGAATTTTGGAGTATATTAA
12.F	GGATTTGGTGAAGTA----------AA
dNTP	
13.F	GGATCCGGCCAAGCTTCAAGCTGAATTCTGCAGTACATTAA
14.R	GGATCCG----AGCGTCGAGCTGAATTCTGCAGTCCATTAT
15.F	GGATCCGGCCAAGCTTCGAGCTGAATCCTGCAGTACATTAA
16.F	GGATCCGGCCAAGCTTCAAGCTGAATTCTGCAGTACATTAA
17.R	GGATCCGGCCAAGCTTCGAGCTGAATTCTGCAGTACATTAG
18.R	GGATCCGGCCAAGCTTCGAGCTGAATTCTGCAGTACATTAA
19.R	GGATCCGGCCAAGTTTCGAGCTGAATTCTGCAGTACATTAA
20.F	GGATCCGGCCAAGCTTCGAGCTGAATTCTGCAGTACATTAA

Sloppymerase™
- what else fun can you do with it?

Expressing Sloppymerase™ in bacteria
Can you speed up evolution of recombinant proteins?

Expressing Sloppymerase™ in mamalian cells
Can you use it as a model system for cancer – increasing mutation rate in defined cell populations?

Suggested reading

Analysis of protein interactions in situ by proximity ligation assays
Koos B, Andersson L, Clausson CM, Grannas K, Klaesson A, Cane G, Söderberg O.
Curr Top Microbiol Immunol. 2014, 377:111-26

Proximity ligation assays: a recent addition to the proteomics toolbox
Weibrecht I, Leuchowius KJ, Clausson CM, Conze T, Jarvius M, Howell WM, Kamali-Moghaddam M, Söderberg O.
Expert Rev Proteomics. 2010, 7(3):401-9

Next generation Pathology – surveillance of tumor microecology
Koos B, Kamali-Moghaddam M, David L, Sobrinho-Simões M, Dimberg A, Nilsson M, Wählby C, Söderberg O.
J Mol Biol. 2015, 427(11): 2013-2022

Let there be light!
Raykova D, Koos B, Asplund A, Gelléri M, Ivarsson Y, Danielsson UH & Söderberg O
Proteomes, 2016, **4**(4): 36


TÄNKA RÄTT ÄR STORT
MEN TÄNKA FRITT ÄR STÖRRE