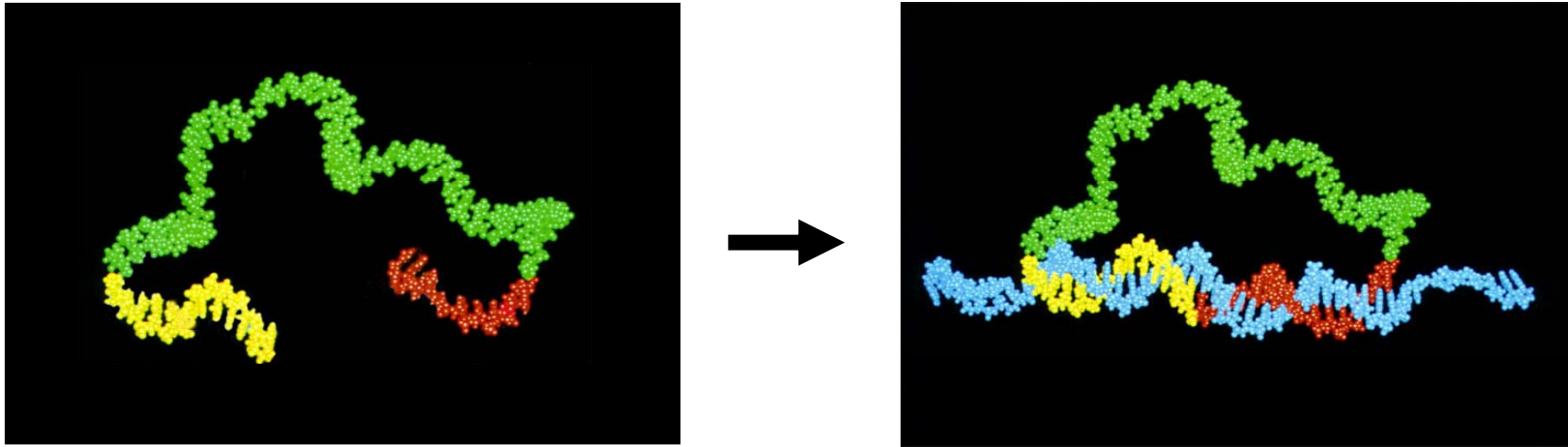# Targeting by probe-based circularisation

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Uppsala University

# Outline

- Padlock probes for multiplex targeted genotyping
- Selector probes for multiplex targeted genome analysis
  - sequencing (clinical sequencing)
  - CNV detection
- Amplified single-molecule detection (digital RCA)
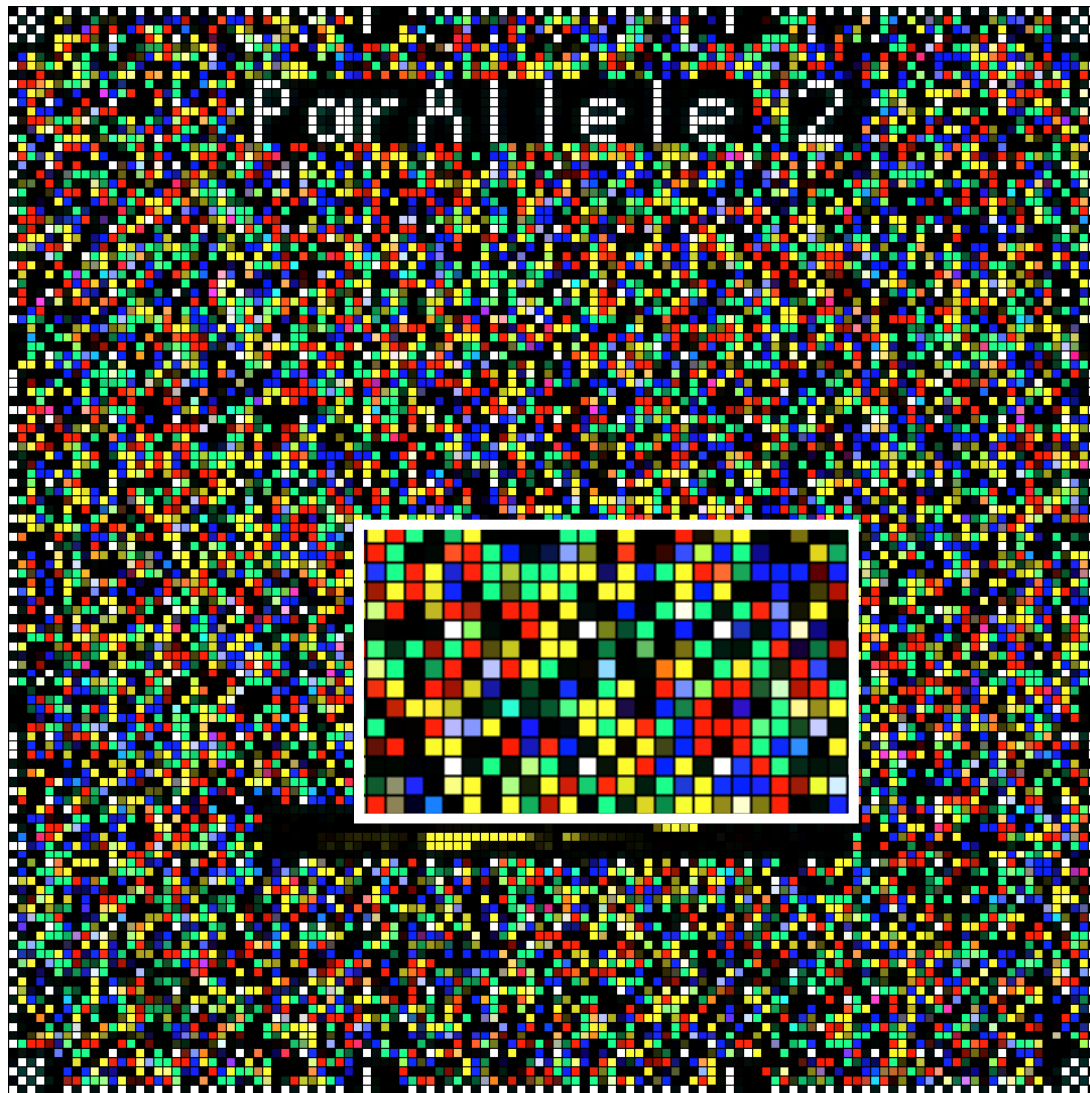- In situ genotyping

# Padlock probes



- Daul-recognition provides specificity
- Robust allele-distinction due to enzymatic ligation using standard reaction conditions
- Probes become topologicaly linked
- Unimolecular

Nilsson, *et al.* (1994) *Science*, **265,** 2085-2088.
Nilsson, *et al.* (1997) *Nature Genetics*, **16,** 252-255.
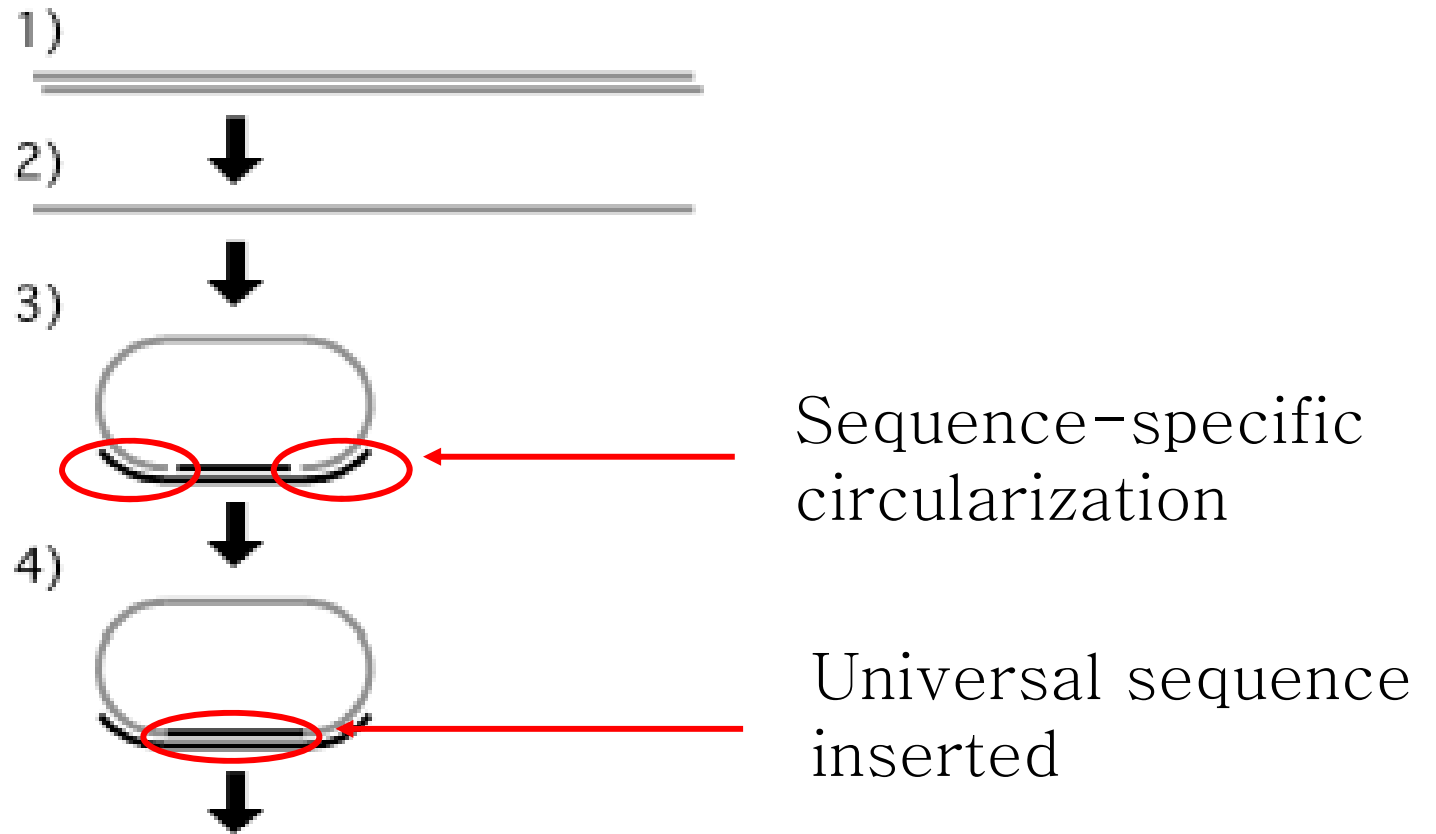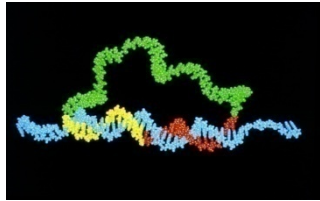Nilsson, *et al.* (2000) *Nature Biotechnol*, **18,** 791-793.

*ParAllele 20K GeneChip®*
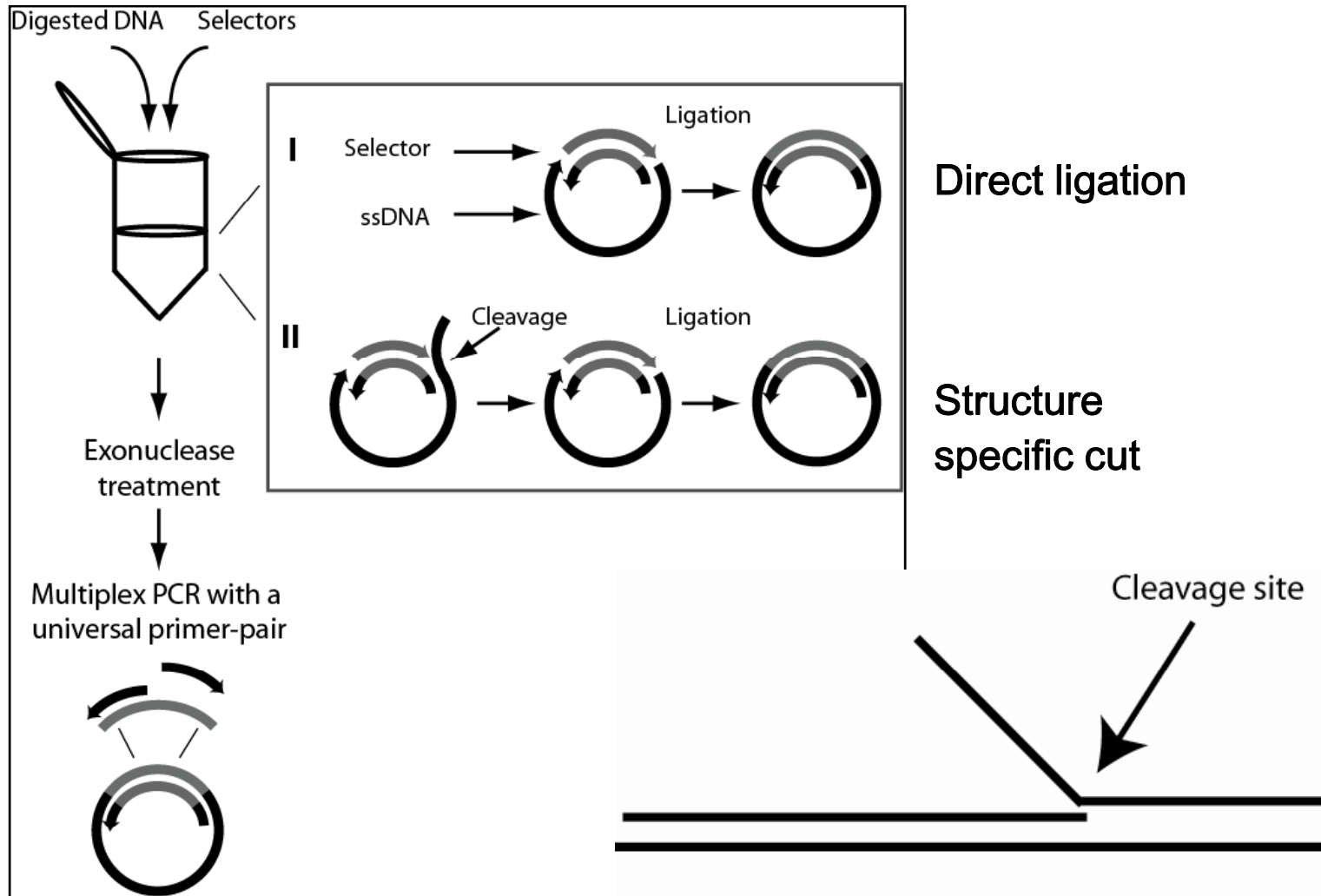
20,000-plex
Genotyping

Affymetrix targeted
genotyping

Hardenbol *et al*,
*Nature Biotechnol* 2003;
Hardenbol *et al*,
*Genome Res* 2005
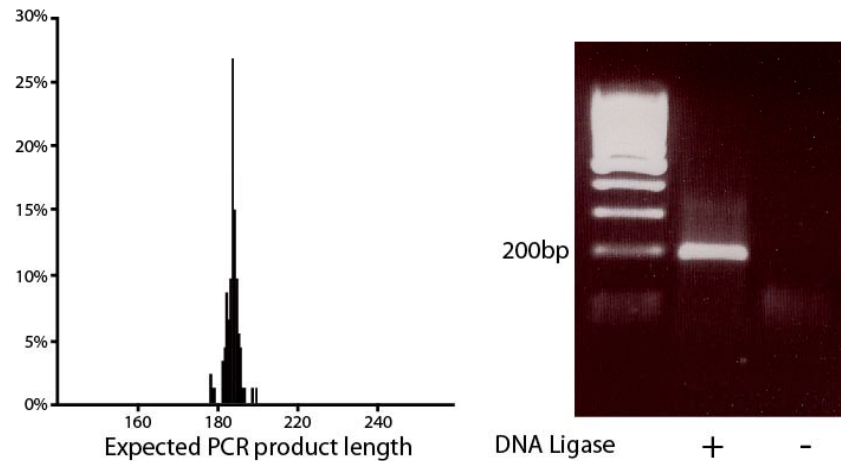
# Targeted genome analysis using selector probes



Sequence-specific circularization

Universal sequence inserted

Circle selection and amplification

# The Selection Process



Digested DNA    Selectors

I    Selector → Ligation
     ssDNA →

**Direct ligation**

II    Cleavage    Ligation

**Structure specific cut**

Exonuclease treatment

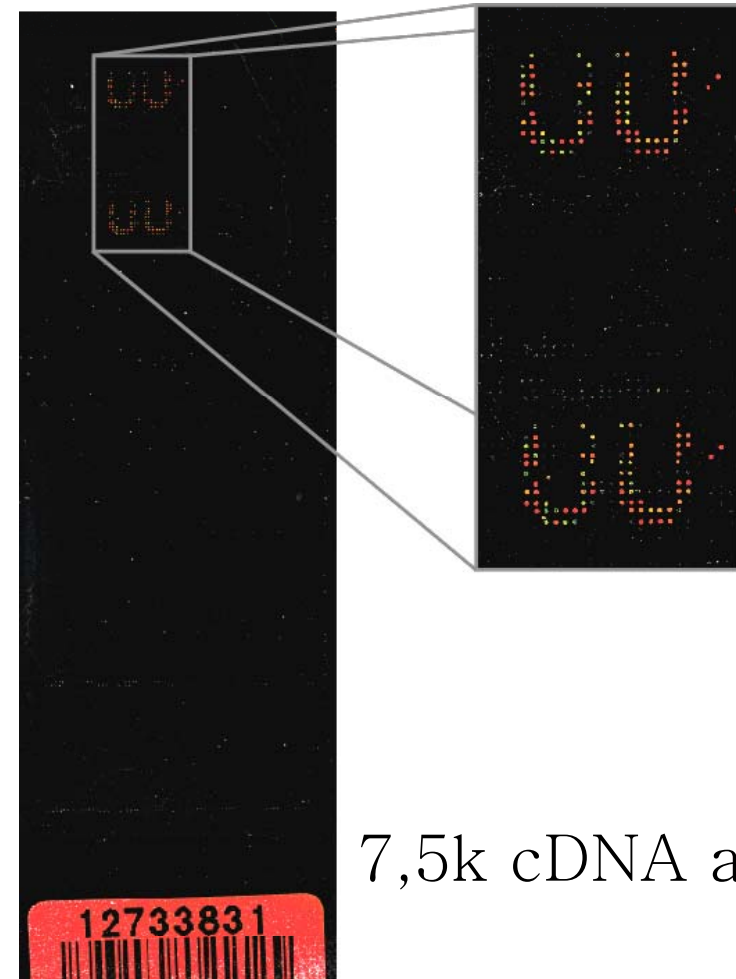Multiplex PCR with a universal primer-pair

Cleavage site

Lyamichev et.al (1993). Science 260, 778-83.

# 96-plex amplification of 96 selected genomic fragments



- 89% efficiency
(96%  <60% GC-content)

7,5k cDNA array

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

# Parallel sequencing of 177 exons in 10 cancer genes



454 sequencing
59.000 reads per exp

90% map to the selected sequence (49 kb) =>
Average enrichment: 600.000

508 probes

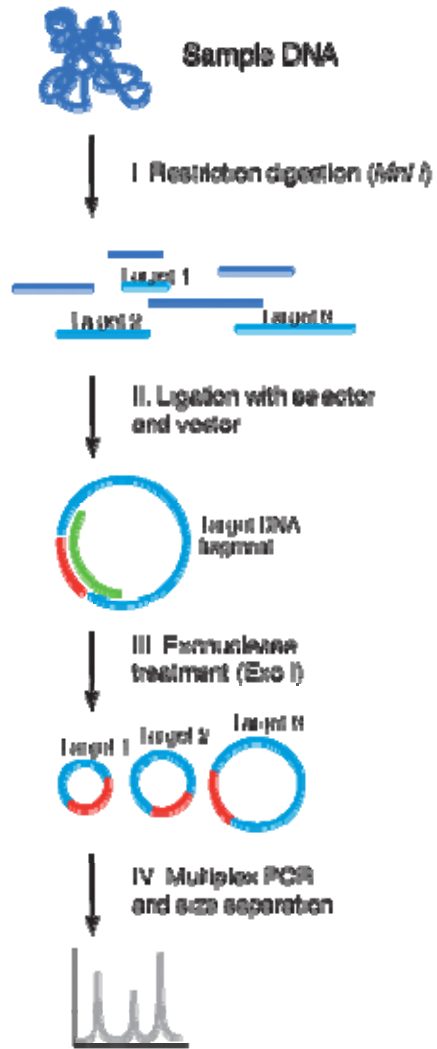Dahl, *et al.* (2007) *PNAS*, **104**, 9387-9392

# Further development

- New probe design
  - reduced cost (4 EUR/probe)
  - More even representation (5-fold range)
  - Compatible with Solexa and SOLiD seq.
- Double the size of selected fragments
- Less input DNA (100 ng)
- Applied to a cancer resequencing project with Tobias Sjöblom at UU.
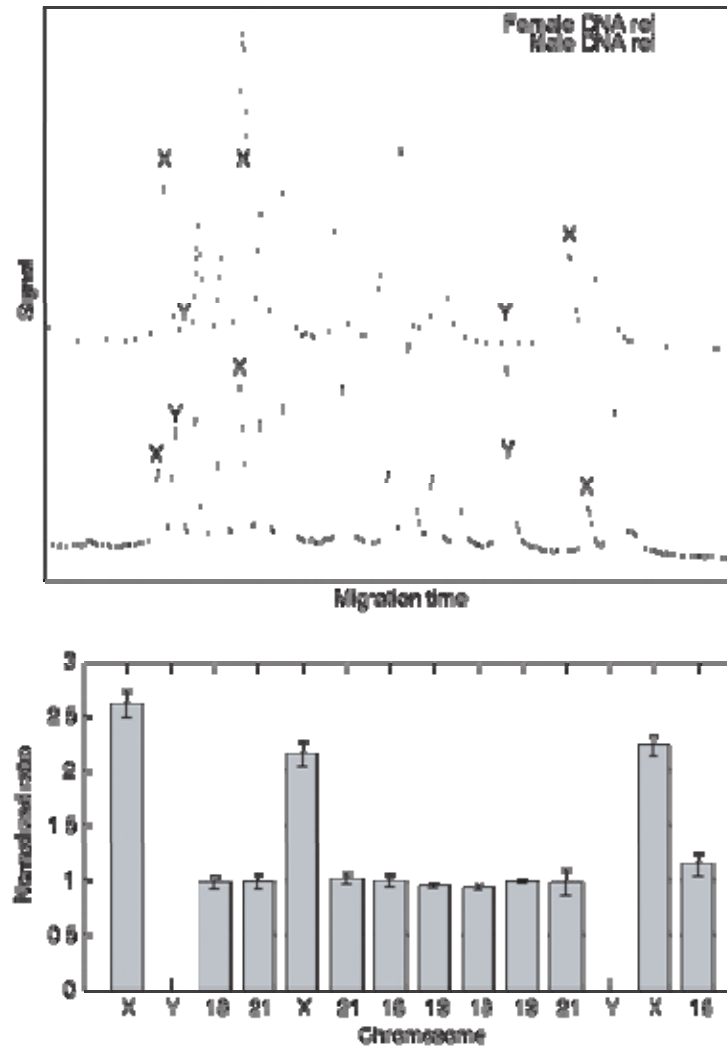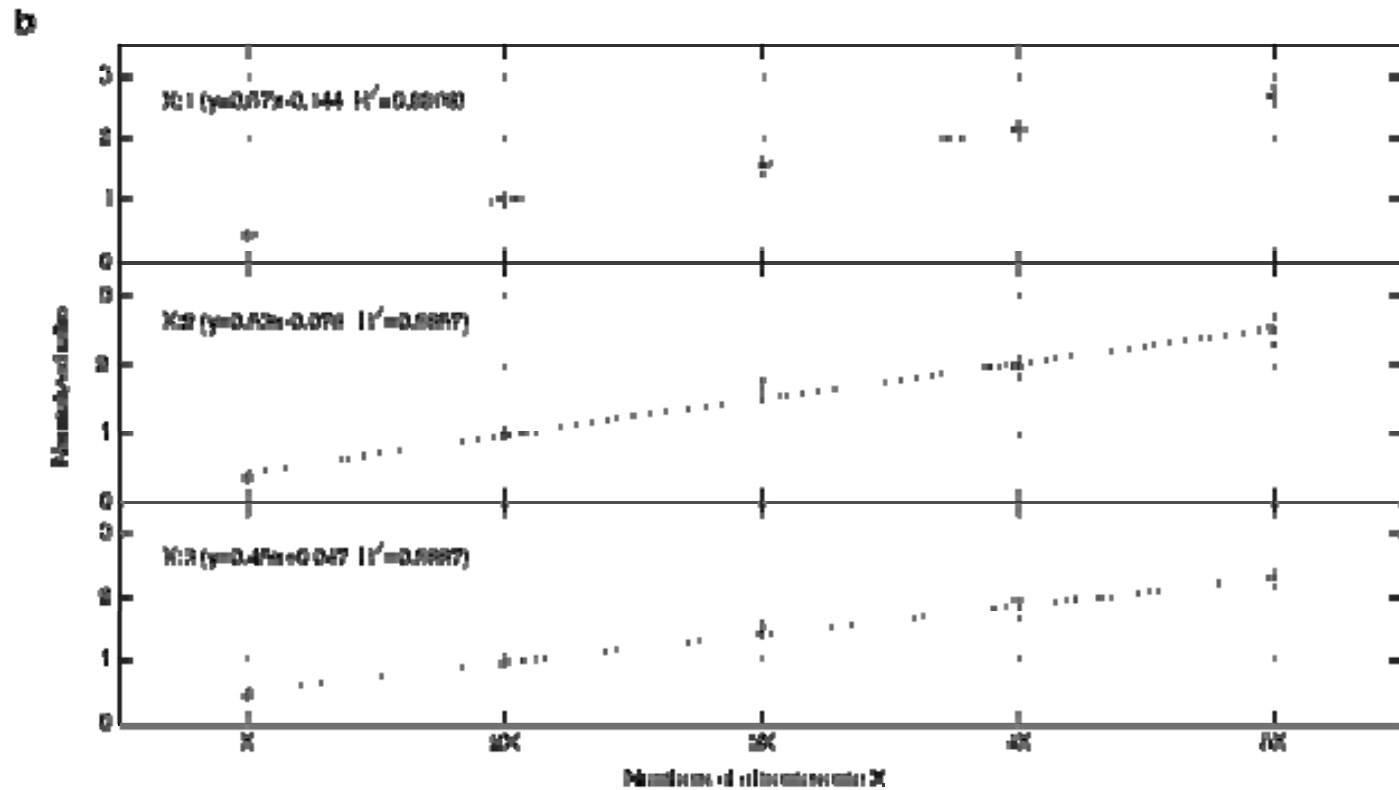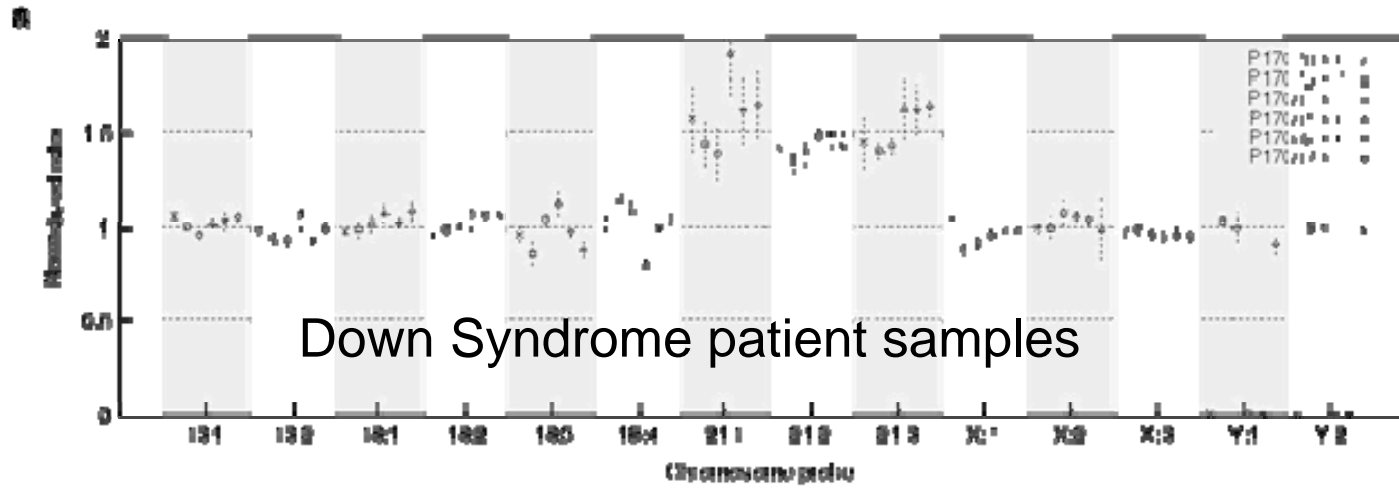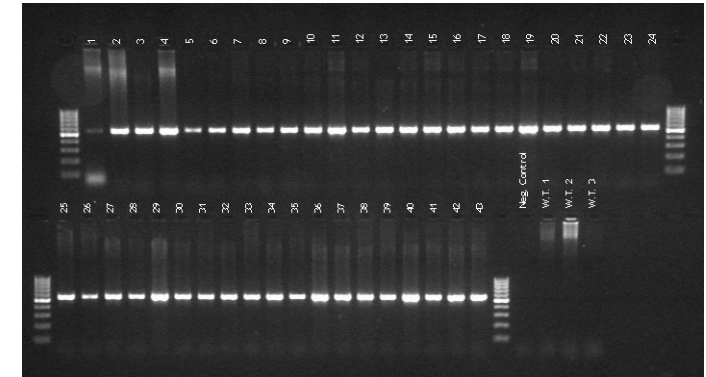- Spun out a company: OLINK GENOMICS

# Copy-number measurements



Isaksson *et al. Nucleic Acids Res.*, 35, e115 (2007)

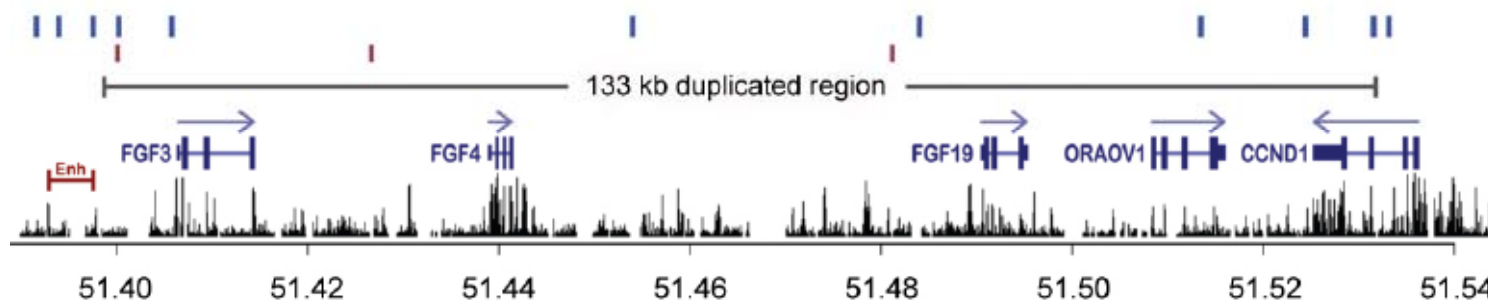# Copy-number measurements



Down Syndrome patient samples

# Sizing a duplication in Rhodesian ridgeback dogs and mapping the break-point



Breakpoint PCR

133 kb duplicated region

FGF3    FGF4    FGF19    ORAOV1    CCND1

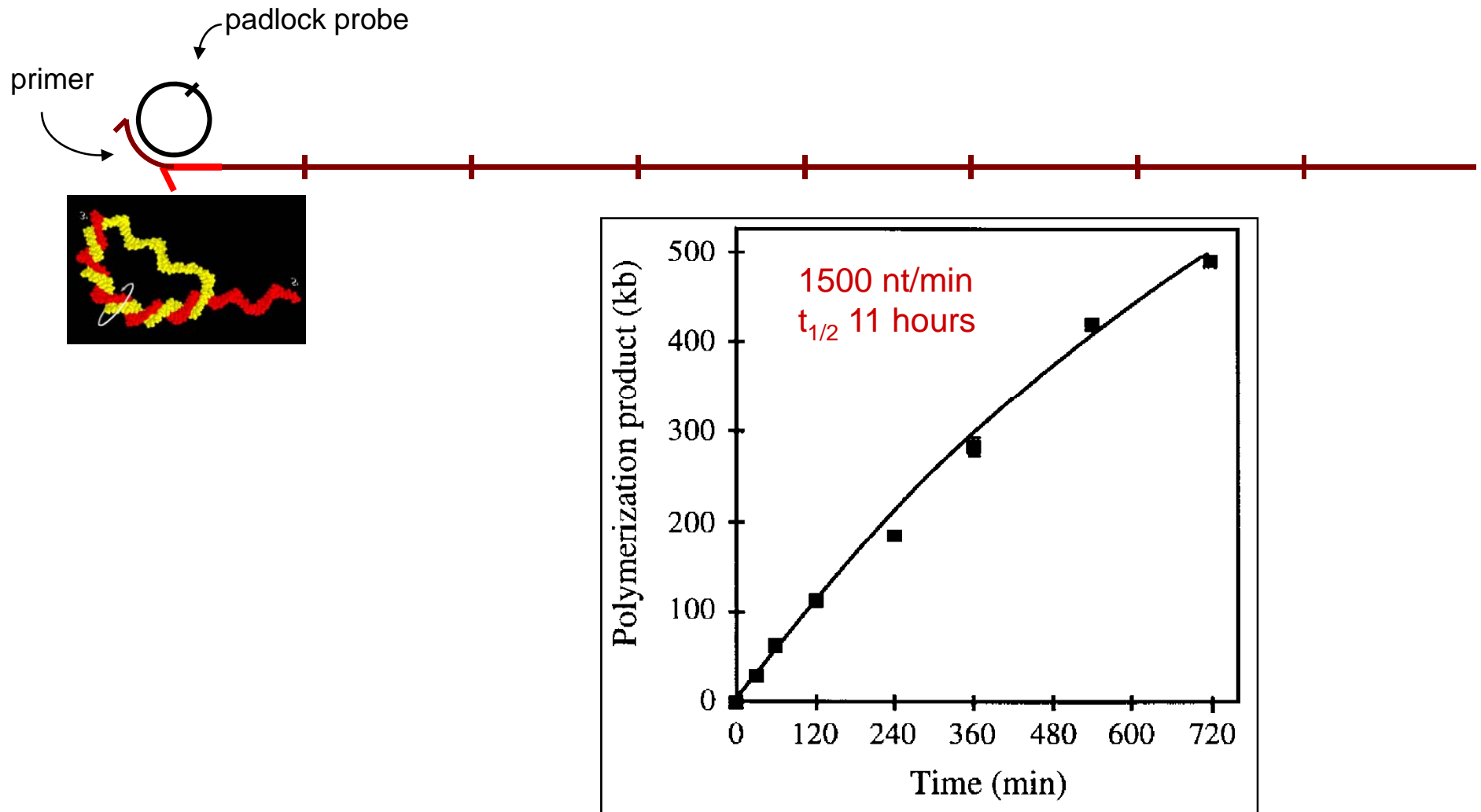Enh
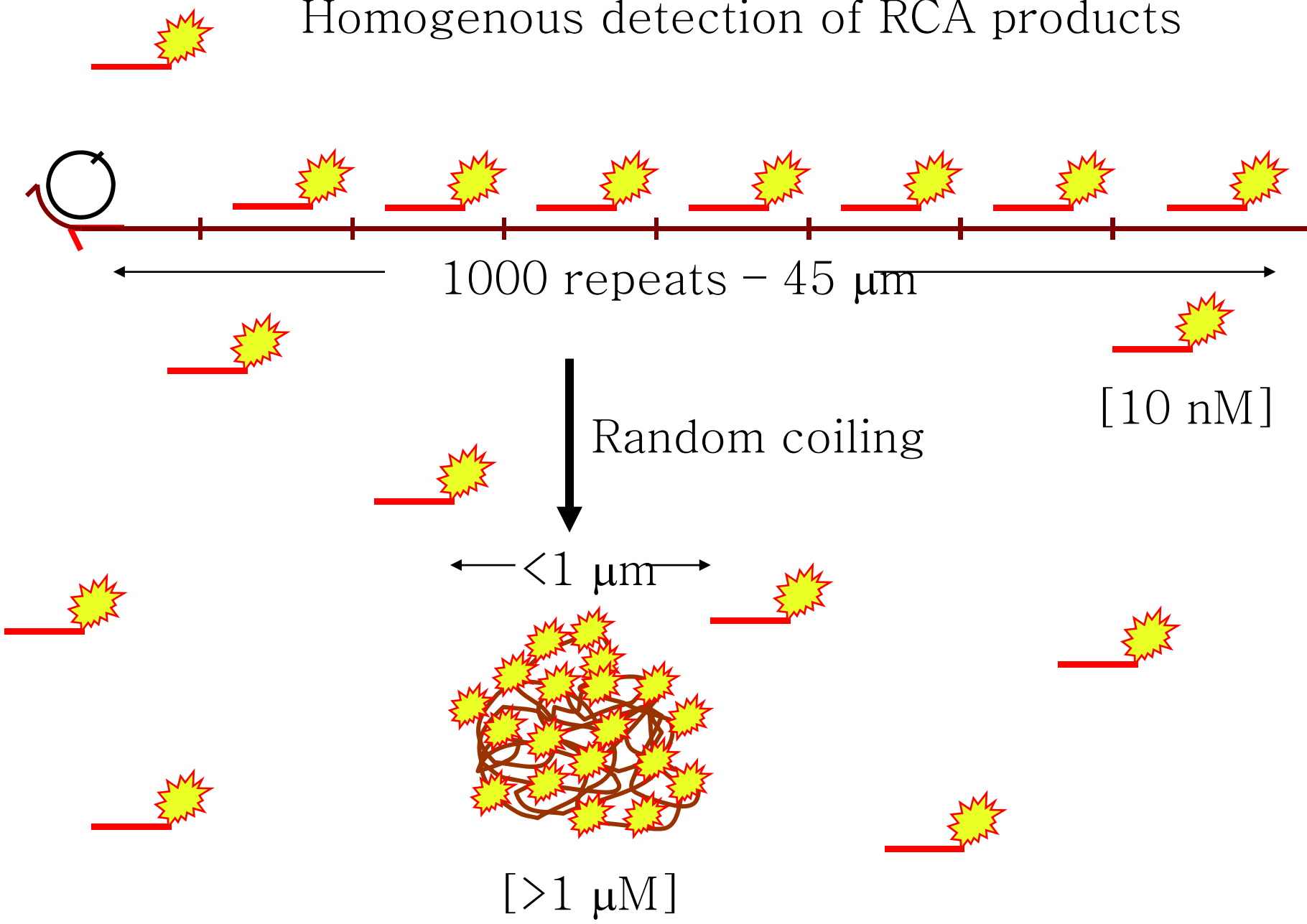
51.40    51.42    51.44    51.46    51.48    51.50    51.52    51.54

Salmon−Hillbertz, *et al.* (2007) *Nature Genet.*, **39**, 1318

# Signal amplification through rolling circle amplification (RCA)

padlock probe

primer



1500 nt/min
$t_{1/2}$ 11 hours

Polymerization product (kb) vs Time (min)

Banér, *et al.* (1998) *Nucleic Acids Res*, **22,** 5073-5078.

Homogenous detection of RCA products

1000 repeats − 45 μm
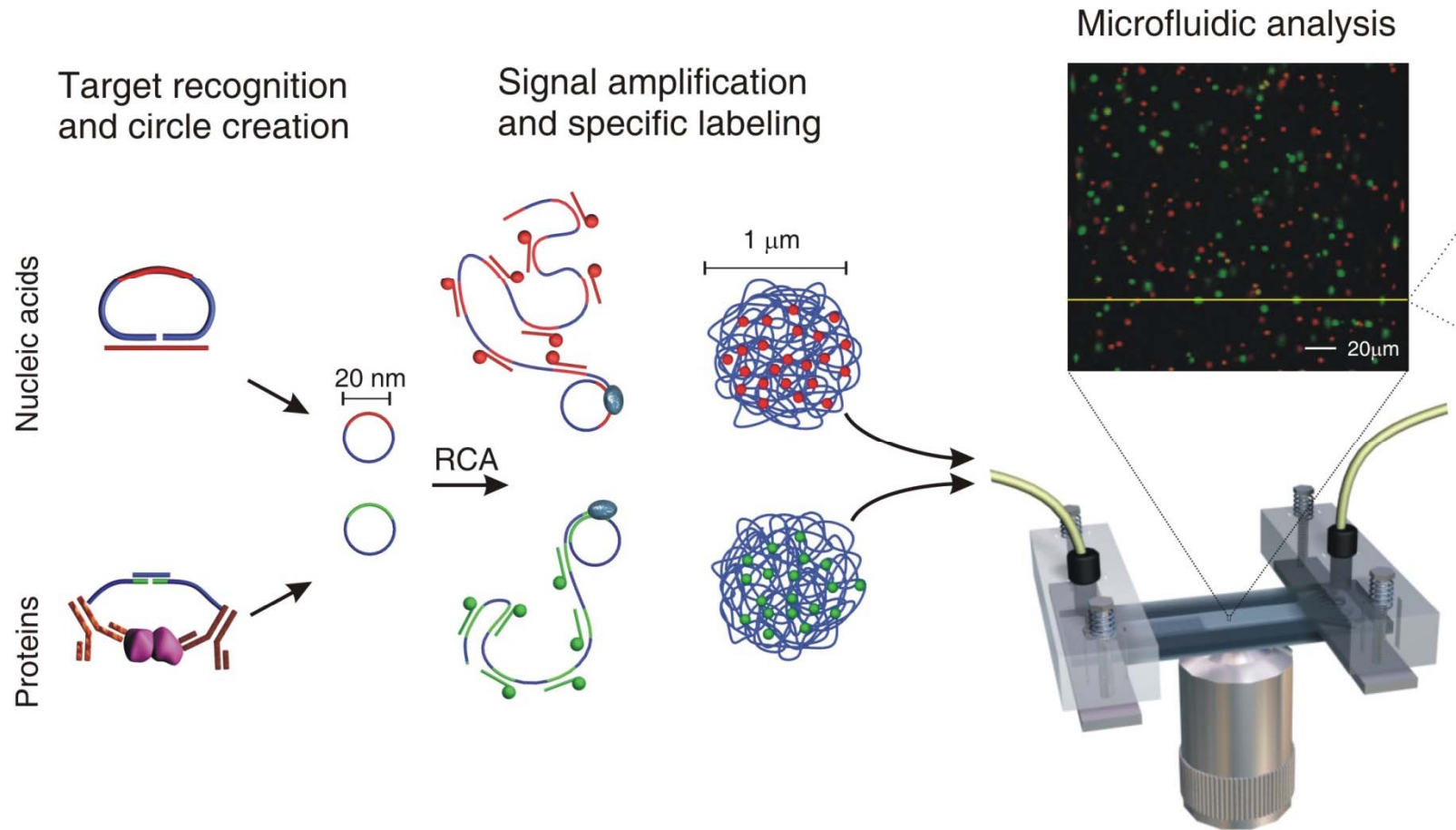
[10 nM]

Random coiling

<1 μm

[>1 μM]

Homogenous detection of RCA products

Blab *et al.* (2004)  *Anal. Chem.,* **76**, 495-8

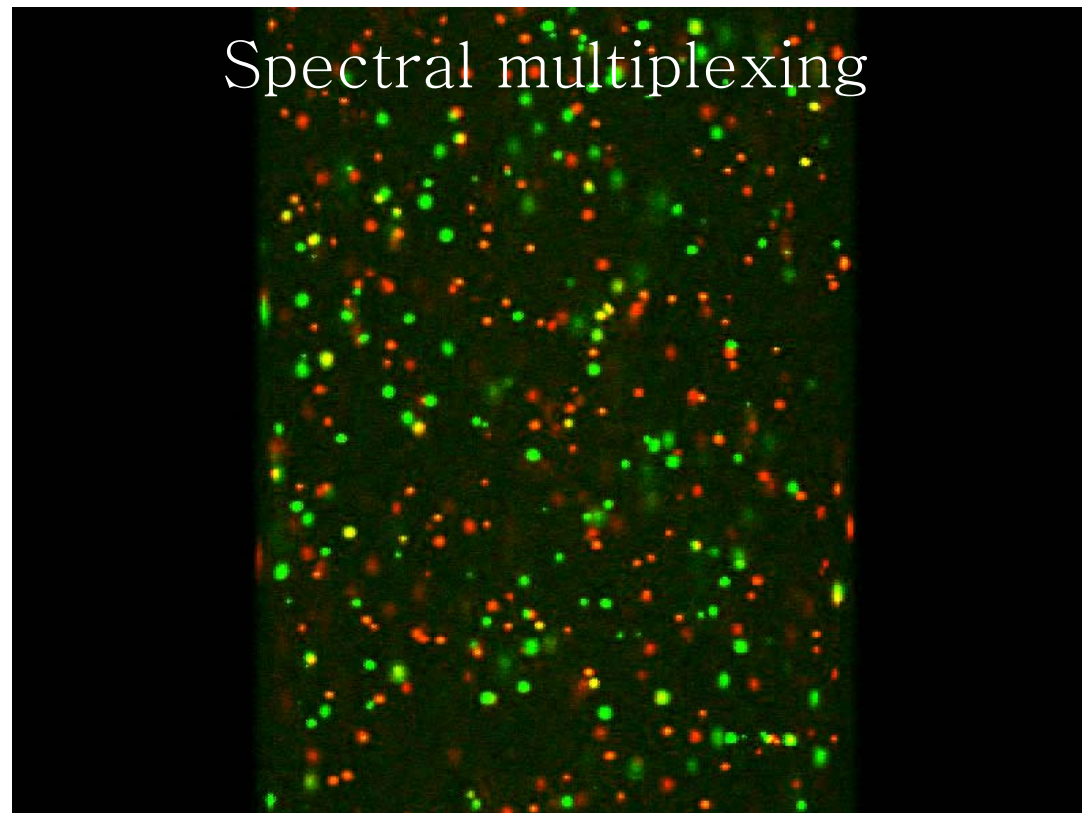# Amplified single-molecule detection (digital RCA)



Jarvius *et al.* (2006) *Nature Methods*, 3, 725-727
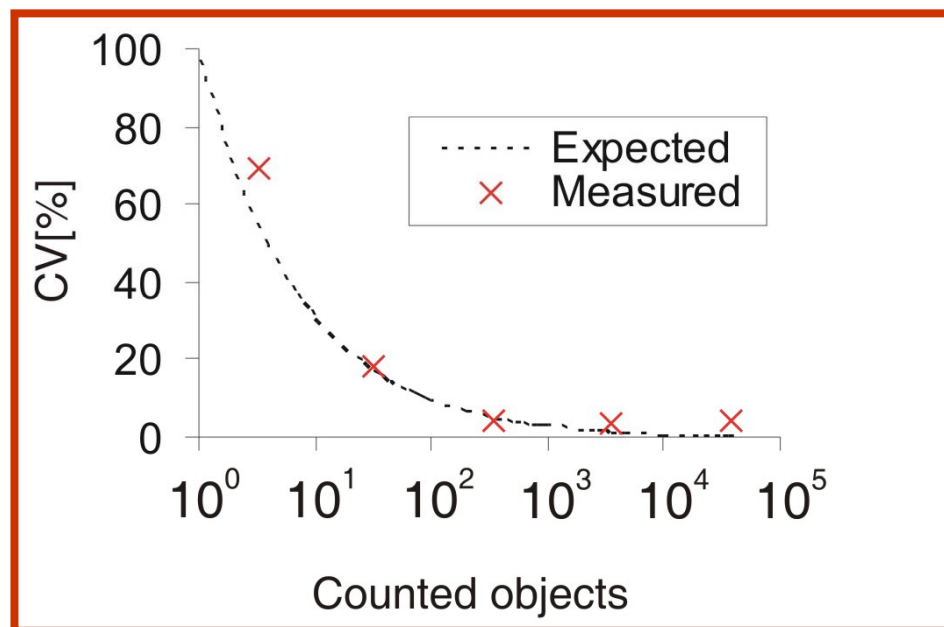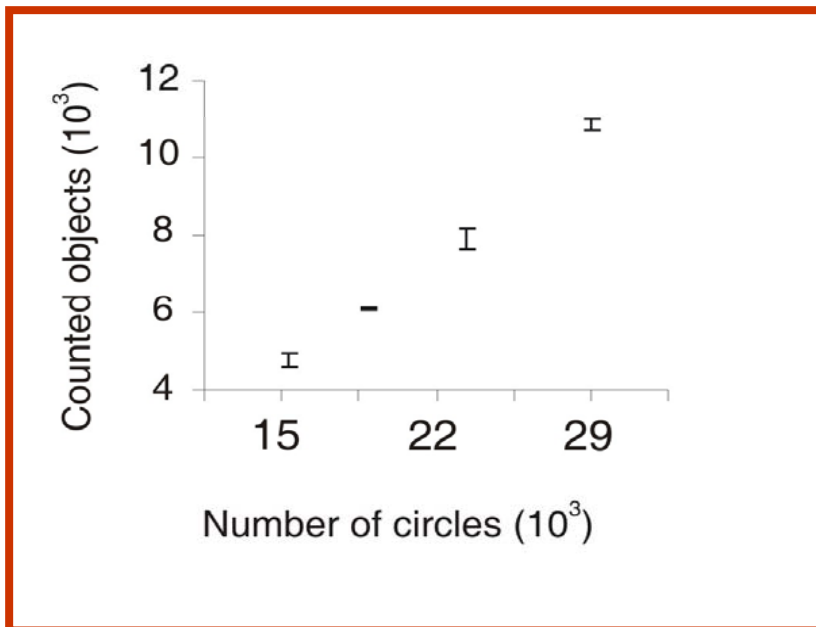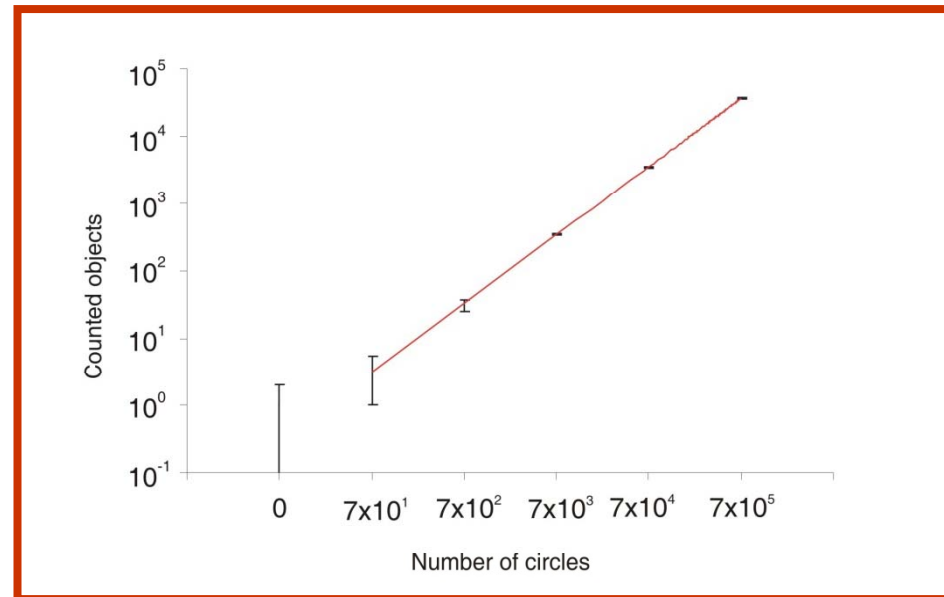
# Amplified single-molecule detection

Advantages:

- Ultimate detection sensitivity
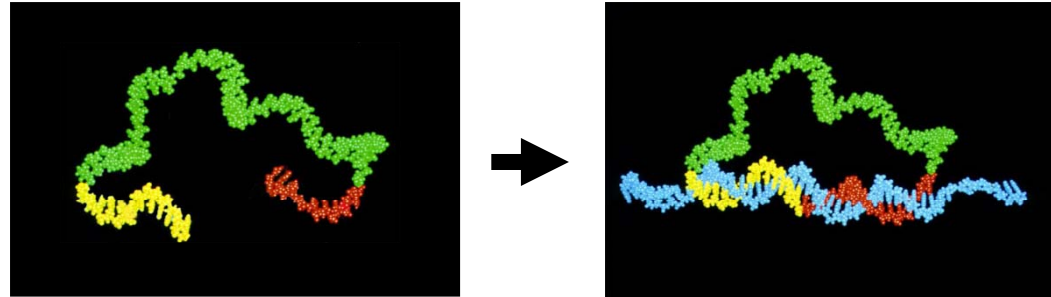- Ultimate quantitative precision



Spectral multiplexing

# Characterization of ASMD key properties

- Dynamic range $10^4$
  - 30 s data acquisition
- High quantitative precision
  - Typically 3 percent
  - Poisson limited (<1000 counts)
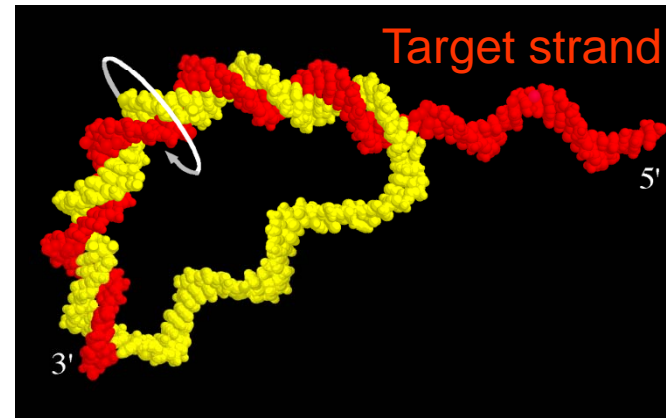- High resolving power

# In situ genotyping

- Padlock probing



- Rolling-circle amplification

# Target-primed RCA



3' exonucleolysis
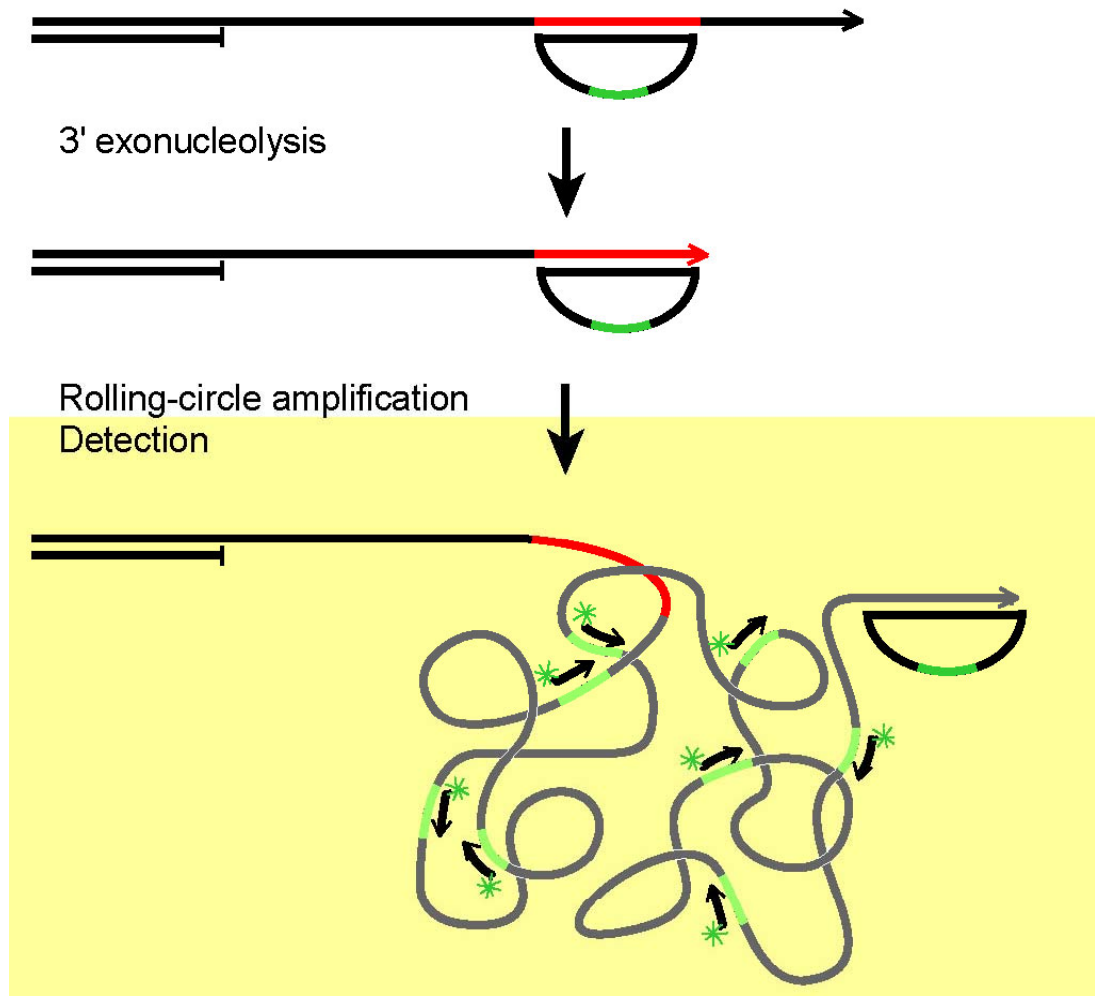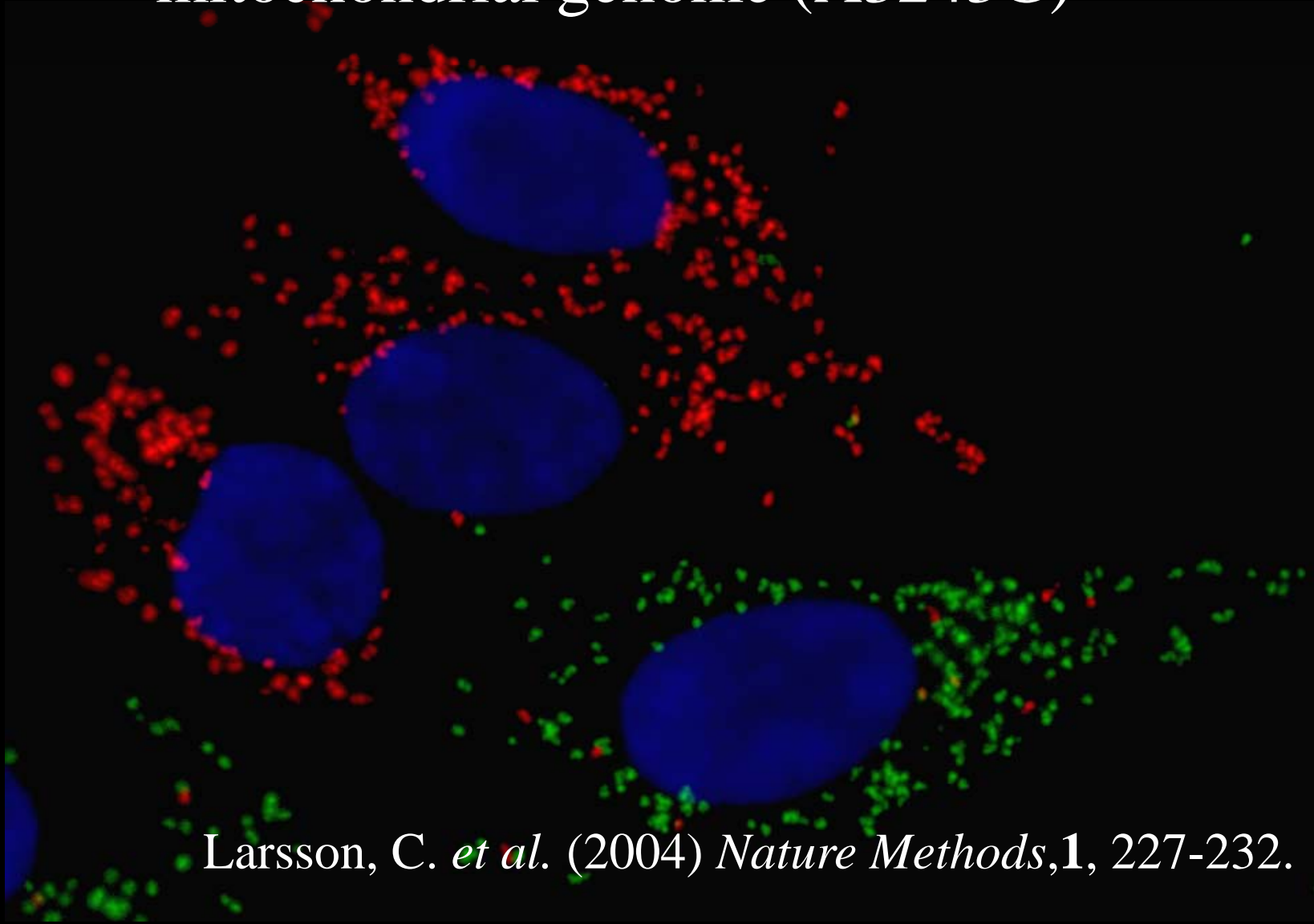
Rolling-circle amplification
Detection

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



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

# Acknowledgements

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