DBCG's 30 års jubilæumsmøde

PRÆDIKTIVE GENETISKE FAKTORER METODER

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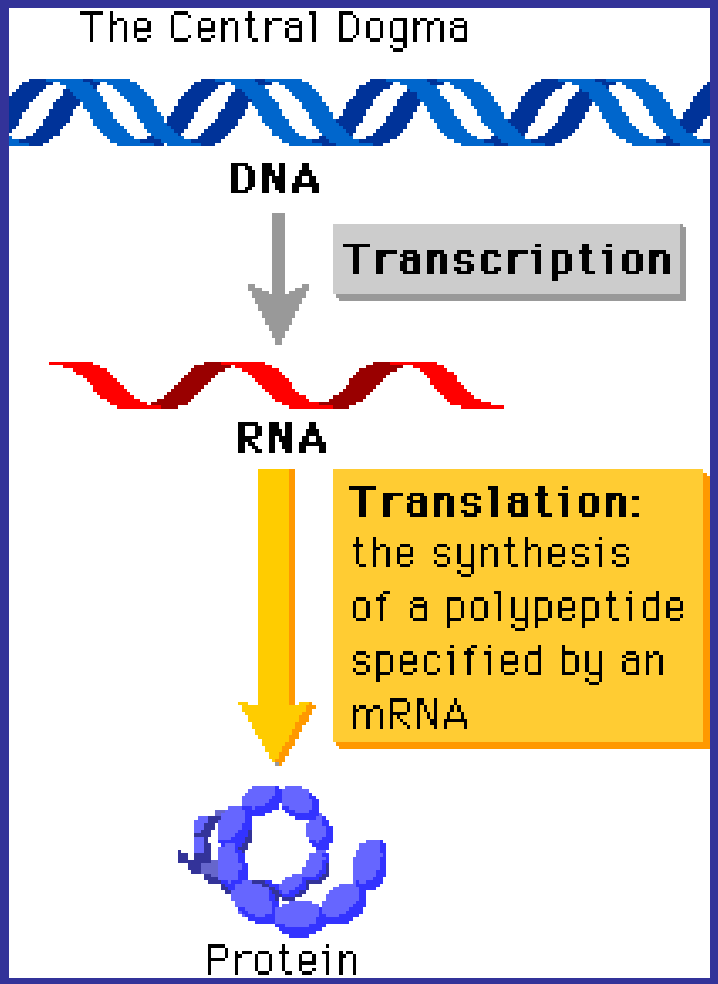
The Central Dogma
DNA
Transcription
RNA
Translation:
the synthesis
of a polypeptide
specified by an
mRNA
Protein

>gi|6552298|ref|NM_007294.1| Homo sapiens breast cancer 1, early onset (BRCA1), transcript variant BRCA1a, mRNA

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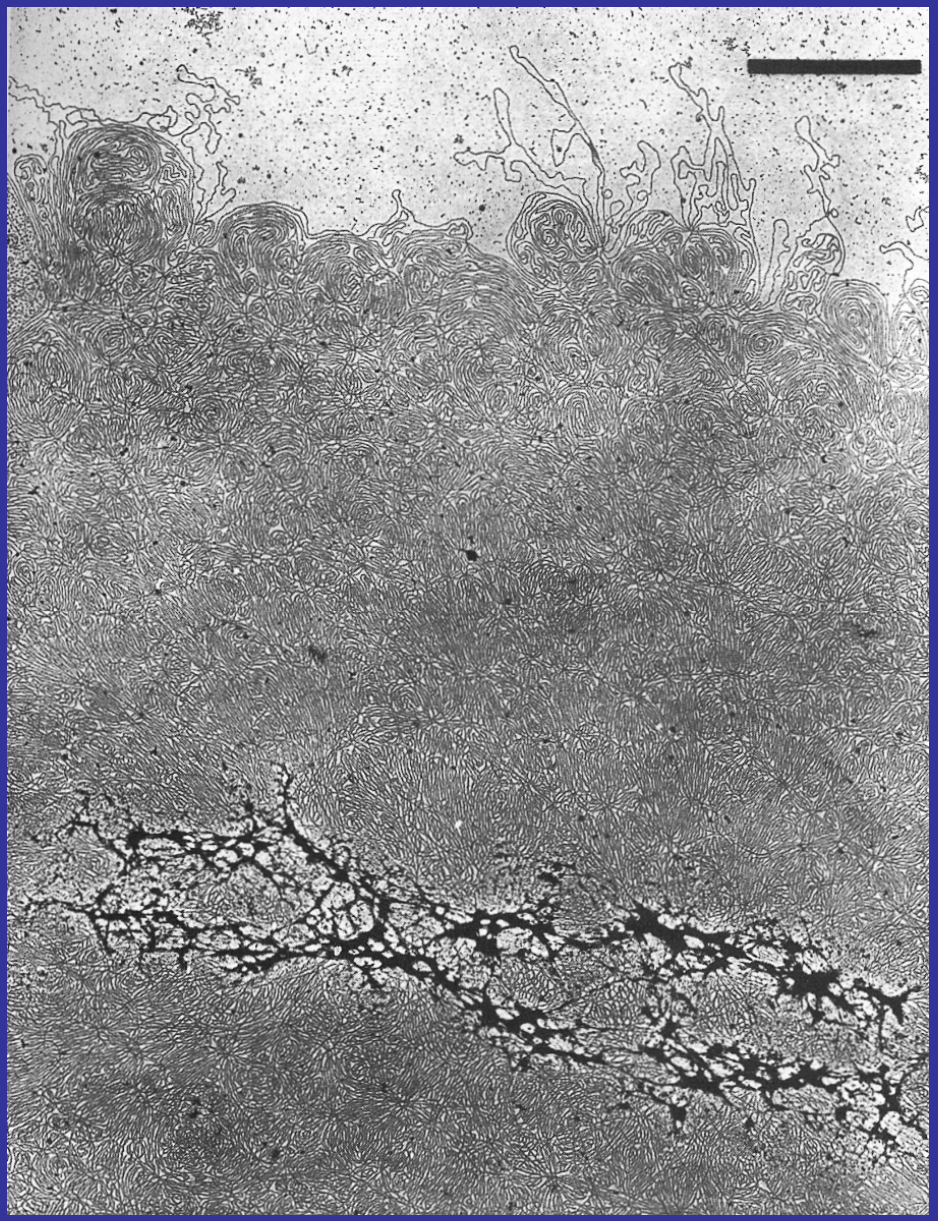


GENOME SIZE AND VARIATION

Size:

3×10^9 base pairs

30,000 genes

Variation:

0.1 – 0.2 % of basepairs polymorphic (SNPs)

higher number of rare variants

Analyse af DNA variation

Germline (blodprøve):

SNP analyse (enkelt/globalt (array-baseret, >500.000)) – hyppig variation

medfødt variation i sygdomsrisiko, behandlingsrespons eller prognose

DNA sekvens analyse – sjælden variation

medfødt variation i sygdomsrisiko, behandlingsrespons eller prognose

DNA kopi-antal-variation (CNV)

Analyse af DNA variation

Somatisk (tumor):

DNA sekvens analyse

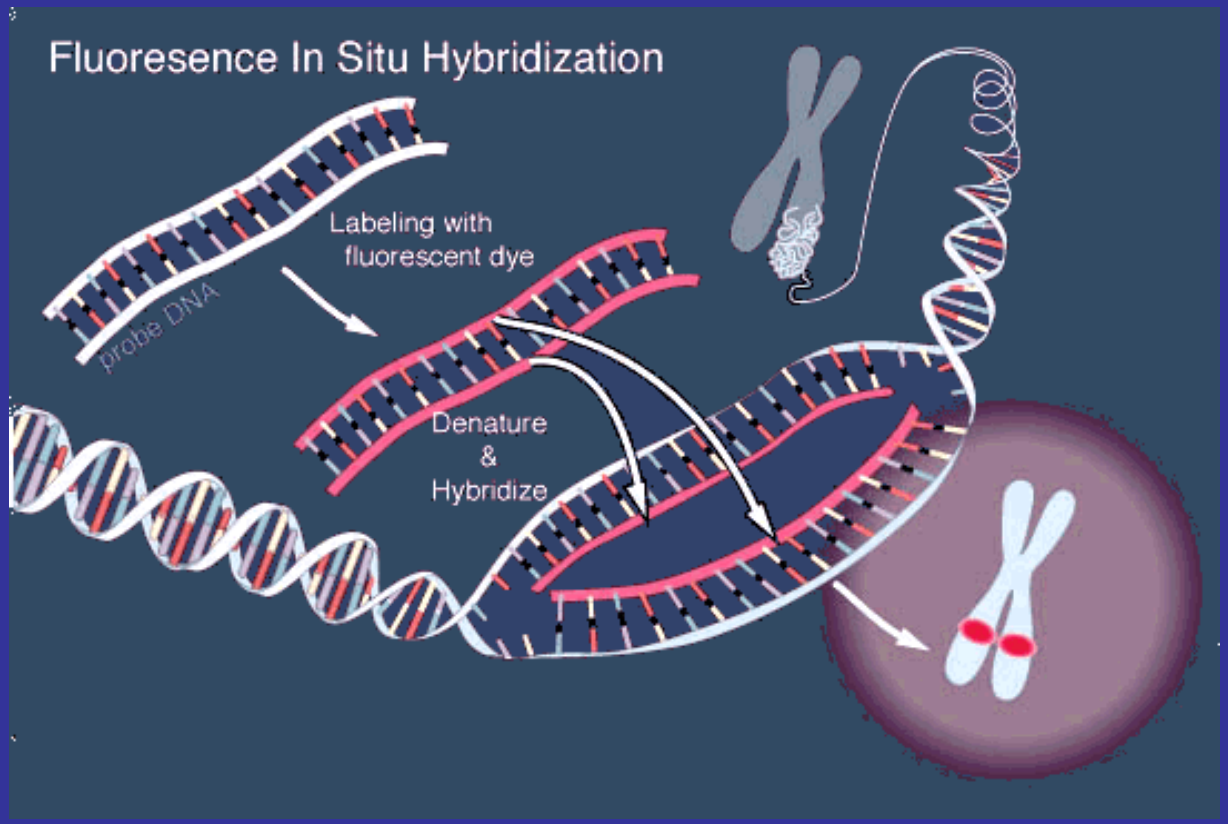
variation i behandlingsrespons eller prognose

DNA kopi-antal-variation (CNV)

effekt af somatisk gen-amplificering eller gen-deletion på behandlingsrespons eller prognose

FISH
RT-PCR (real-time PCR)
qRT-PCR (kvantitativ real-time PCR)
arrayCGH

Fluorescence *in situ* hybridization FISH



Analyse af DNA variation

Somatisk (tumor):

DNA sekvens analyse

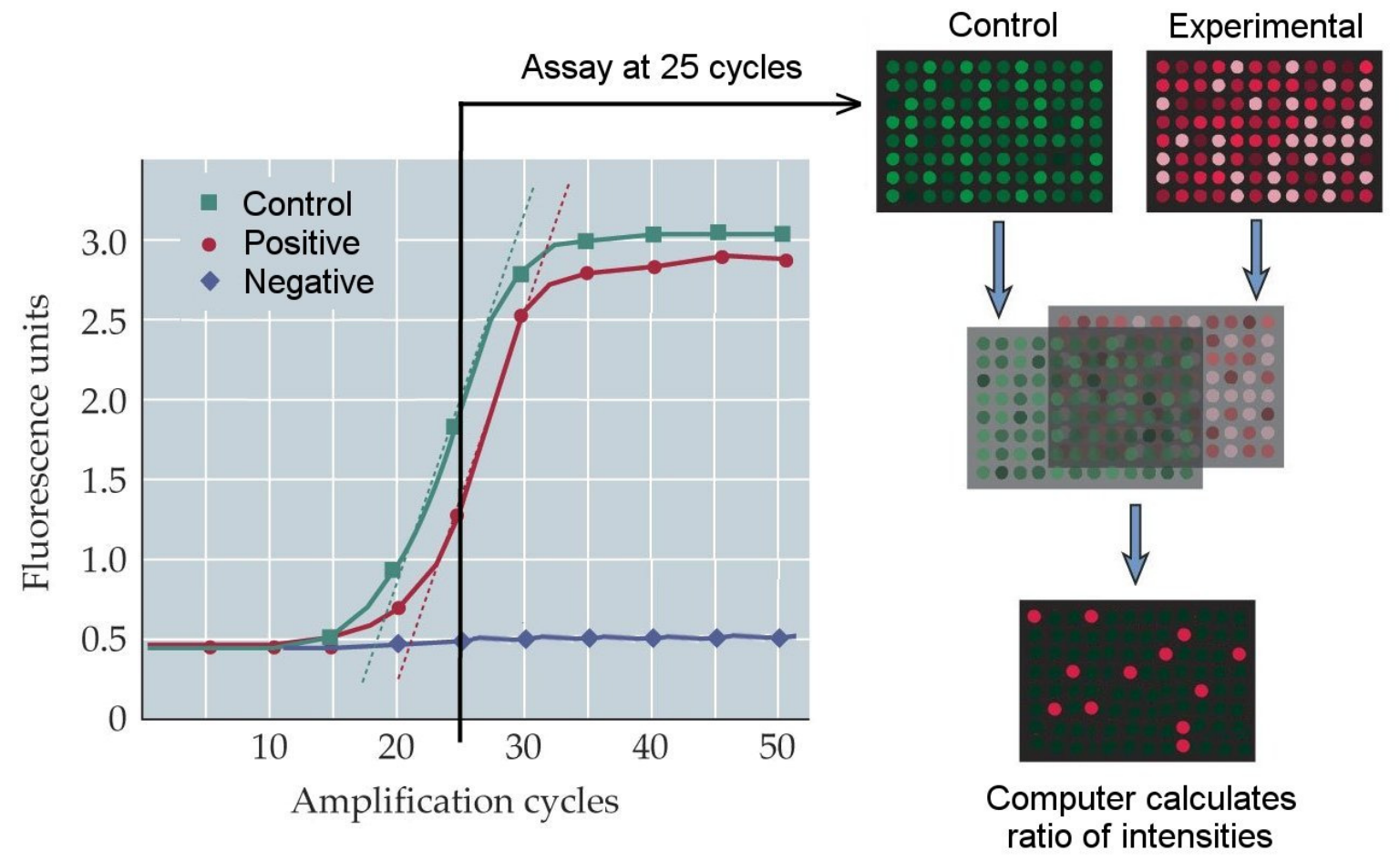
variation i behandlingsrespons eller prognose

DNA kopi-antal-variation (CNV)

effekt af somatisk gen-amplificering eller gen-deletion på behandlingsrespons eller prognose

FISH
RT-PCR (real-time PCR)
qRT-PCR (kvantitativ real-time PCR)
arrayCGH

RT-PCR


Assay at 25 cycles
Control
Positive
Negative
Fluorescence units
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
Amplification cycles
Control
Experimental
Computer calculates
ratio of intensities

Analyse af DNA variation

Somatisk (tumor):

DNA sekvens analyse

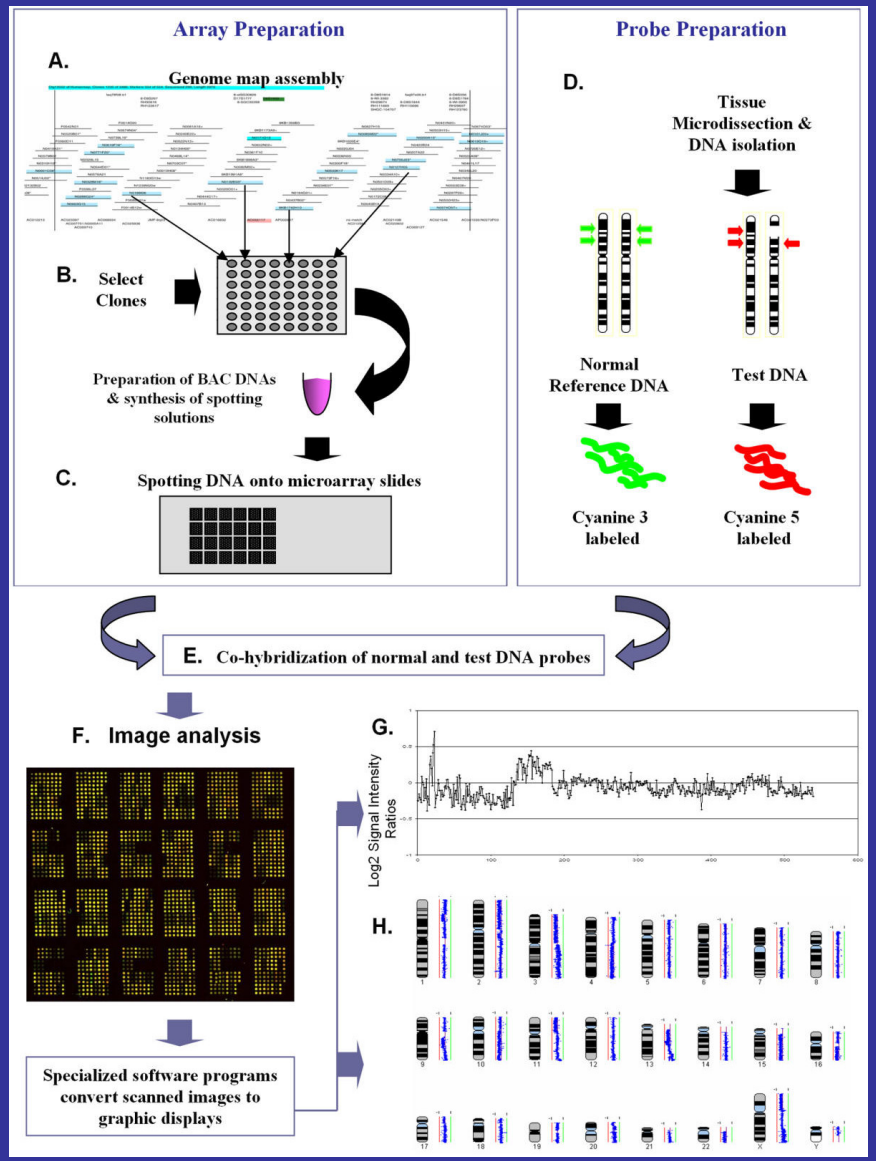
variation i behandlingsrespons eller prognose

DNA kopi-antal-variation (CNV)

effekt af somatisk gen-amplificering eller gen-deletion på behandlingsrespons eller prognose

FISH
RT-PCR (real-time PCR)
qRT-PCR (kvantitativ real-time PCR)
arrayCGH

arrayCGH – comparative genome hybridization



by M. Haemakers

RNA analyse (tumor) – gen-ekspression:

Kvantitative målinger af mRNA (30-40.000) +/- splicevarianter

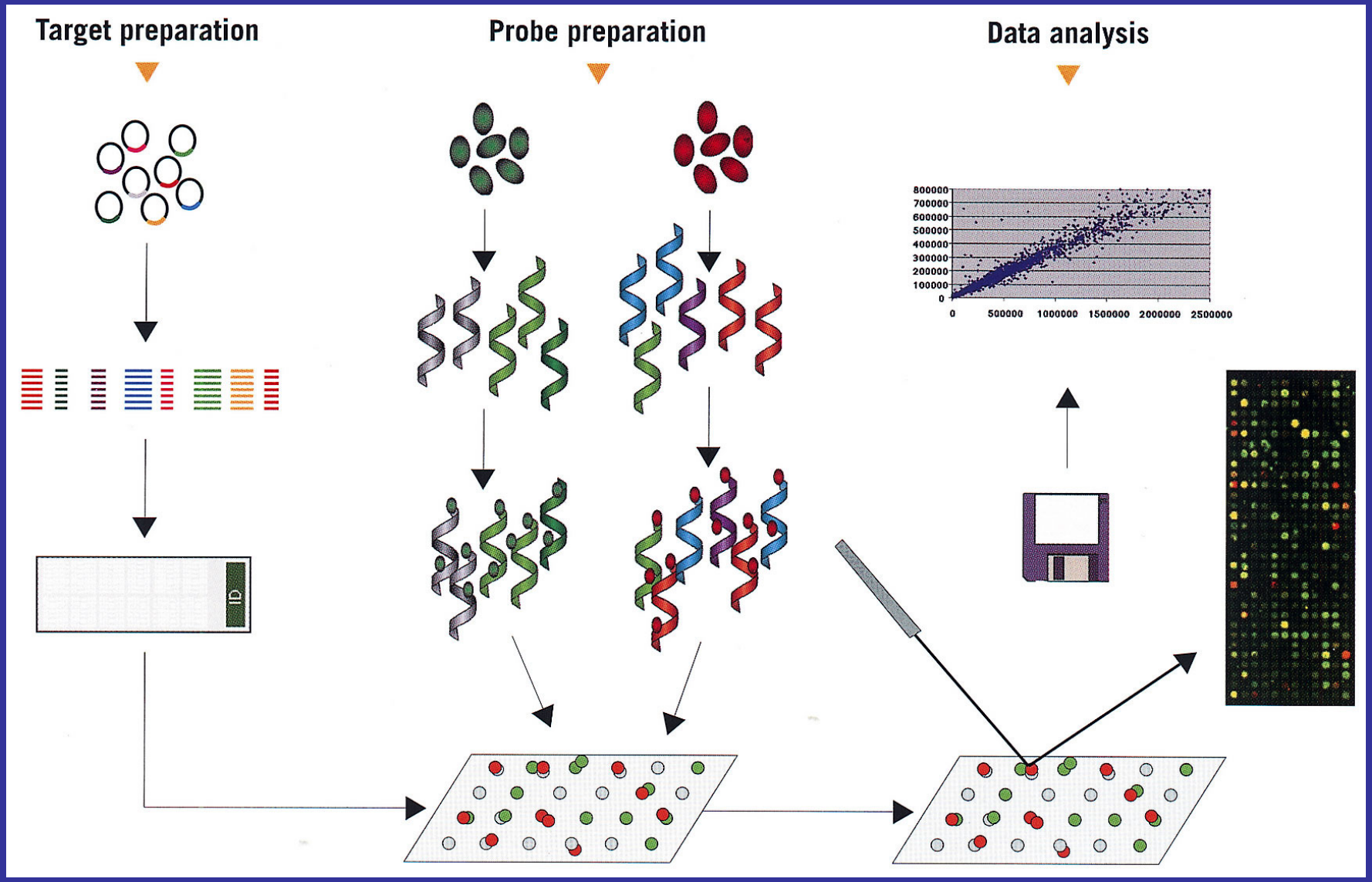
Single gen: QRT-PCR, kvantitativ reverse transcriptase PCR

Oligogen: Low density arrays (LDA), PCR-baseret

Global: Microarrays

Kvantitative målinger af microRNA (<2000)

Formål:
Aktivitet af enkelte gener eller grupper af gener
med effekt på / association til behandlingsrespons og prognose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Probe preparation
Data analysis
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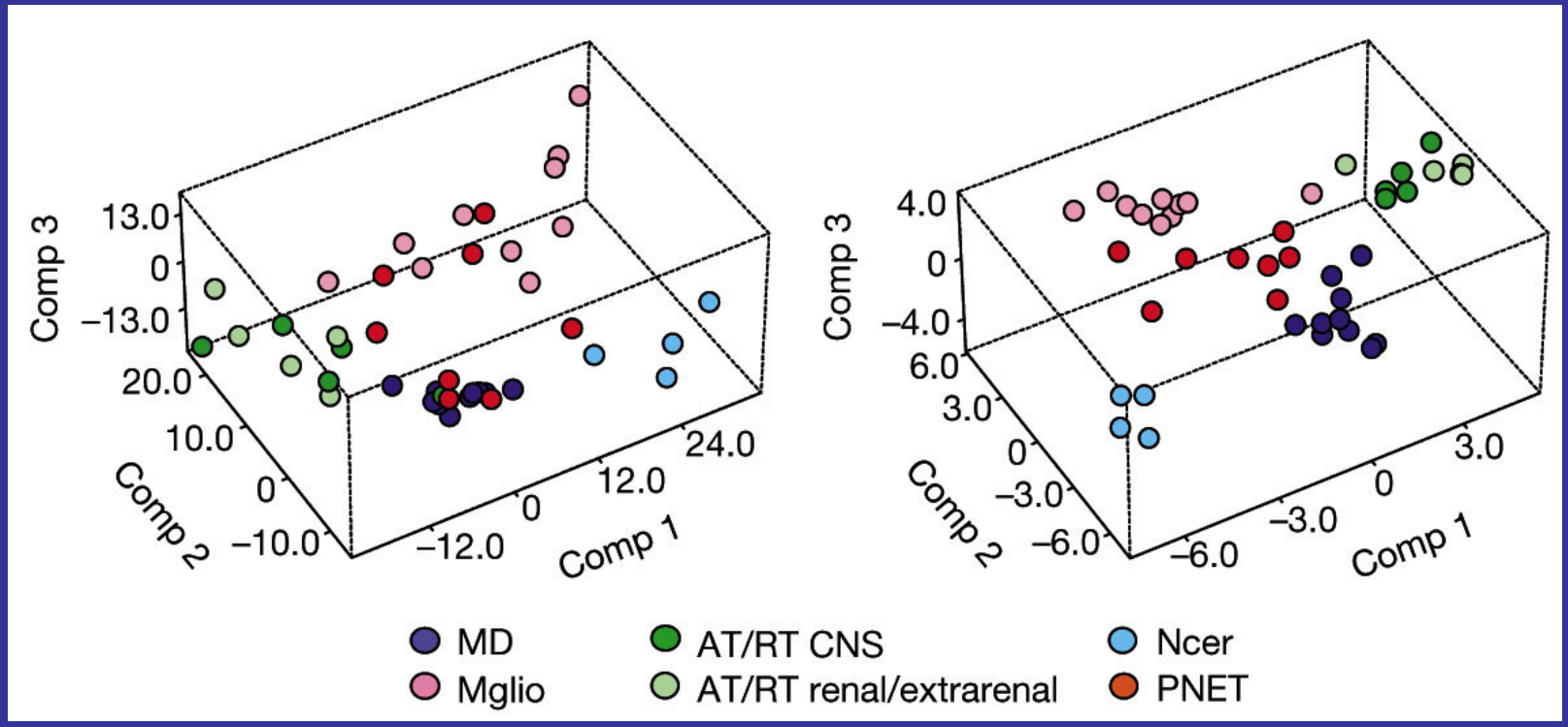
700000
600000
500000
400000
300000
200000
100000
0
0
500000
1000000
1500000
2000000
2500000



From Pomeroy et al
Nature 2002

Identifikation af klinisk relevante profiler

Udvælgelse af gen-sæt (feature selection)
Definition af regel/prædiktor

Validering:
Trænings-sæt – test-sæt
Leave-one-out