

# Detectie van gen amplificaties in NGS data: van lab-data tot klinische rapportage

*PATH project & landelijke KMBP meeting*

Astrid Eijkelenboom, PhD, KMBP

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<b>Disclosure belangen spreker</b>	
(potentiële) Belangenverstrengeling	Geen
Voor bijeenkomst mogelijk relevante relaties met bedrijven	Geen
<ul style="list-style-type: none"><li>• Sponsoring of onderzoeksgeld</li><li>• Honorarium of andere (financiële) vergoeding</li><li>• Aandeelhouder</li><li>• Andere relatie, namelijk ...</li></ul>	Geen

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## Met dank aan:

- Marjolijn Ligtenberg & Ed Schuurin, Bas Tops, Petra Nederlof (PATH WP2)
- Alle KMBP(io) en deelnemers KMBP meeting 29-03-2018
- Alle analisten, bio-informatici, pathologen en anderen betrokken bij validatie en implementatie van op NGS gebaseerde copy nummer analyse in alle centra

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# Manuscript under review

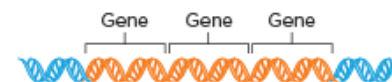
## Recommendations for the Clinical Interpretation and Reporting of Copy Number Gains using gene panel NGS analysis in Routine Diagnostics

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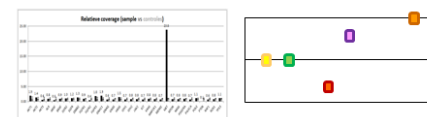
- 1) Department of Pathology, Radboud university medical center, Nijmegen, The Netherlands.
- 2) Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
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- 4) Pathology-DNA, Location Jeroen Bosch Hospital, Den-Bosch, The Netherlands
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- 9) Department of Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
- 10) Department of Pathology, Maastricht University Medical Center, Maastricht, The Netherlands
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- 12) Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
- 13) Department of Human Genetics, Radboud university medical center, Nijmegen, The Netherlands

# Detectie van gen amplificaties in NGS data: van lab-data tot klinische rapportage

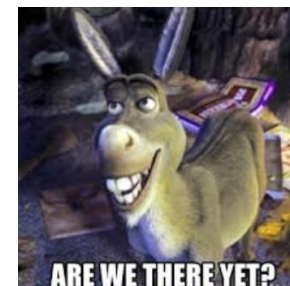
- Wat zijn gen amplificaties (copy number gains)?



- Hoe kun je gen amplificaties detecteren in NGS data?

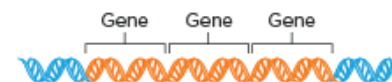


- Vertaling naar routine diagnostiek.

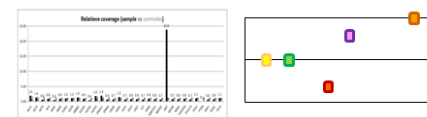


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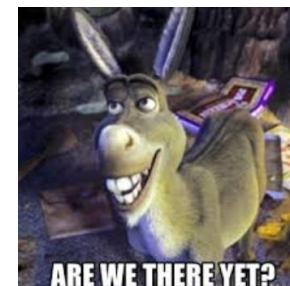
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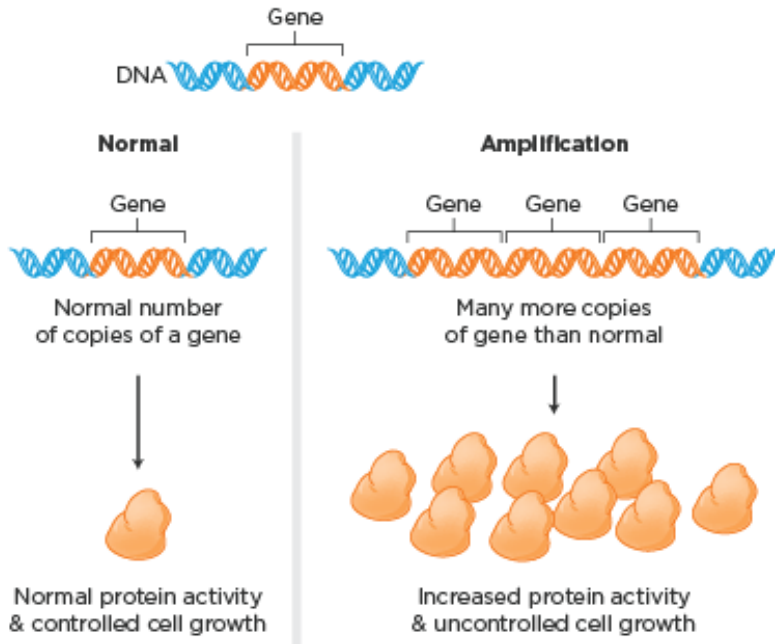
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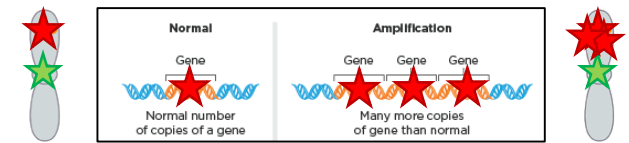
- Vertaling naar routine diagnostiek.



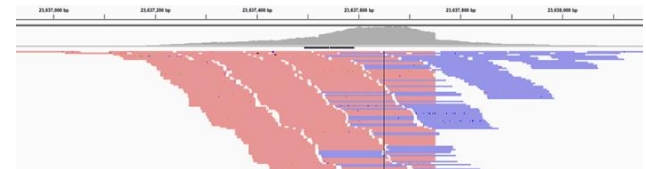
# Gene amplifications



FISH:



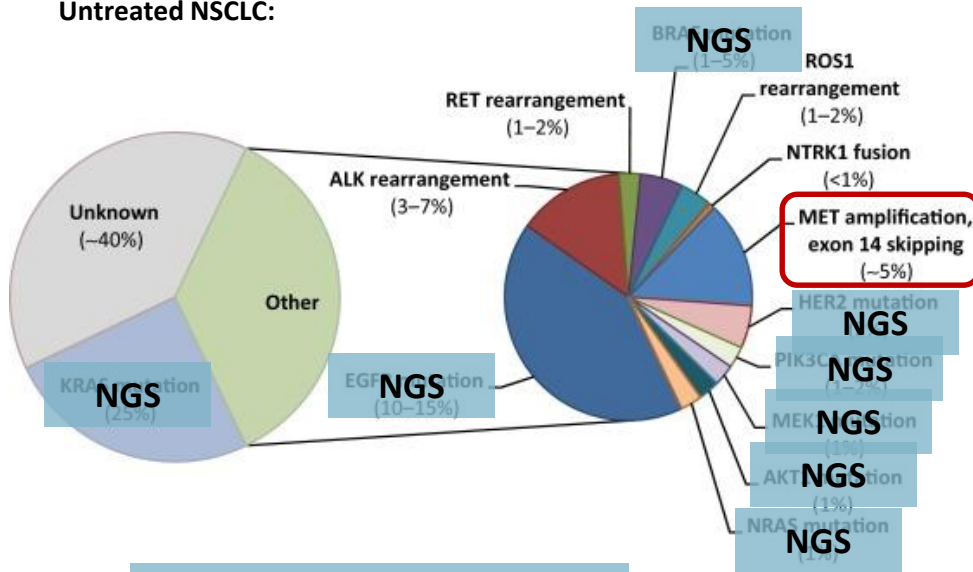
NGS:



<https://lungevity.org/for-patients-caregivers/lung-cancer-101/treatment-options/targeted-therapy>

# Gene amplifications: *just one of many*

Untreated NSCLC:



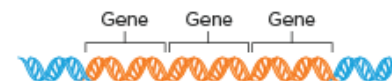
Inhibitors			
EGFR	ALK	ROS	RET
Erlotinib* Gefitinib* Afatinib* Osimertinib* Rociletinib EGF816 ASP8273 HM61713	Crizotinib* Ceritinib* Alectinib* Lorlatinib Brigatinib Ensartinib Entrectinib	Crizotinib* Ceritinib Lorlatinib Cabozantinib Foretinib Entrectinib DS-6051b	Alectinib Cabozantinib Vandetanib Lenvatinib Apatinib Ponatinib Sunitinib Dovitinib
MET	TRK1	HER2	BRAF/MEK
Crizotinib Tivantinib Cabozantinib Foretinib Volitinib Capmatinib MSC2156119J AMG337 AMG208	Entrectinib LOXO-101 DS-6051b	Afatinib Dacomitinib Neratinib Lapatinib Pyrotinib	Vemurafenib Dabrafenib Trametinib Selumetinib

<https://www.cell.com/trends/cancer>

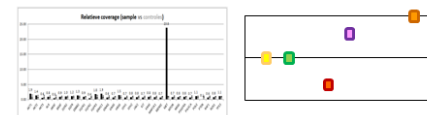


# Detectie van gen amplificaties in NGS data: van lab-data tot klinische rapportage

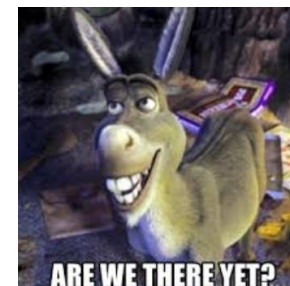
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- Hoe kun je gen amplificaties detecteren in NGS data?



- Vertaling naar routine diagnostiek.



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# Detection of gene amplifications

19 maart 2018: KMBP bijeenkomst in Utrecht

Presentaties van 6 centra over ervaringen CNV detectie middels NGS

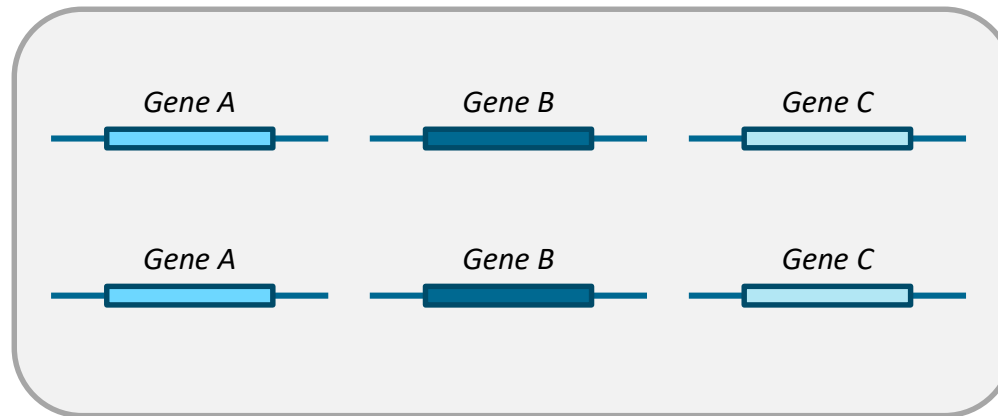
Discussie over:

→ vereisten rapportage (PALGA protocol module)

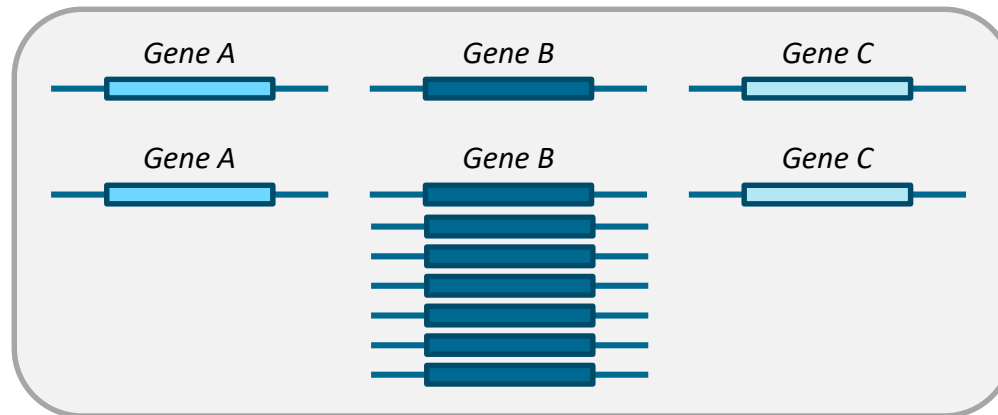
→ NGS vs FISH

# Detection of gene amplifications using NGS

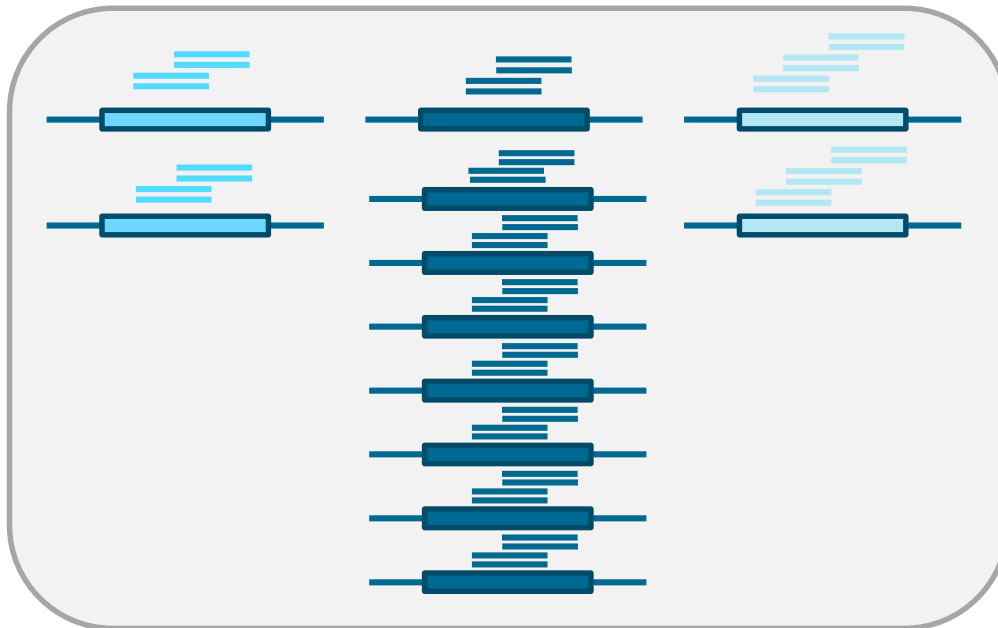
*normal cell*



*tumor cell*



# Detection of gene amplifications using NGS...

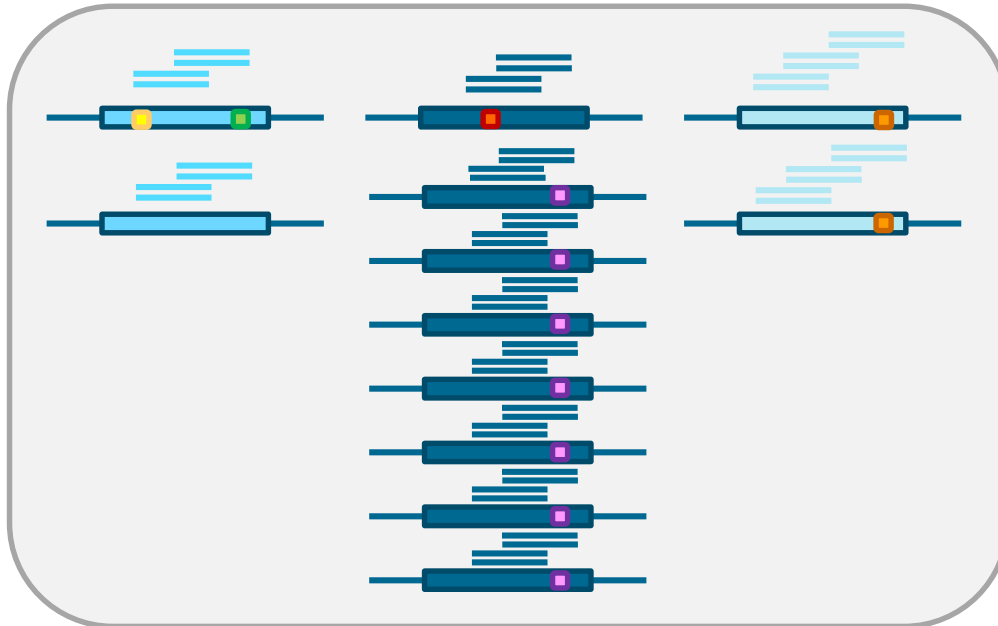


*NGS reads:*

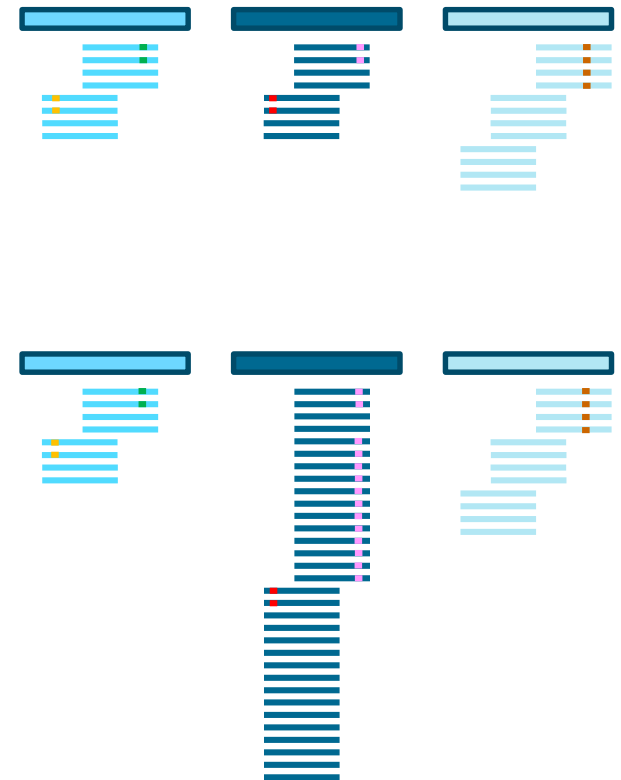


*...by coverage analysis*

# Detection of gene amplifications using NGS...



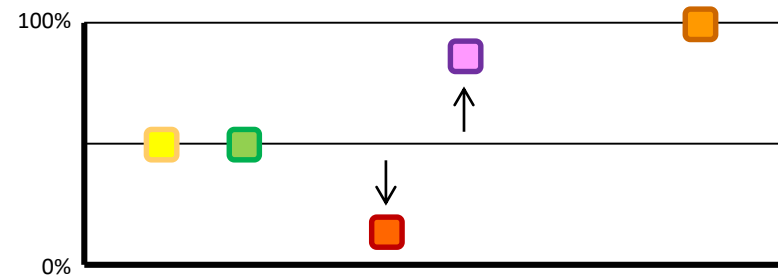
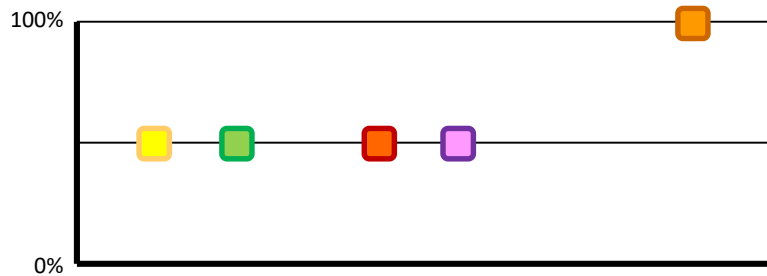
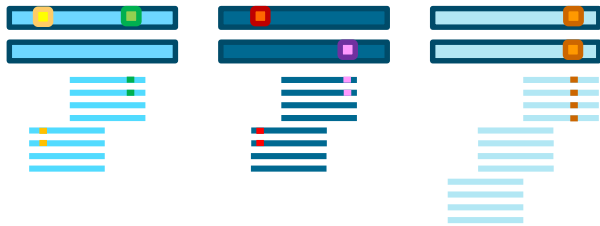
*NGS reads:*



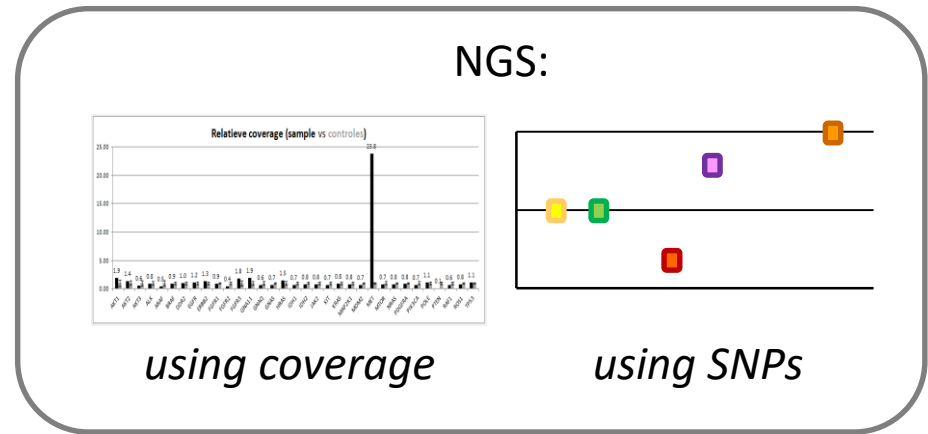
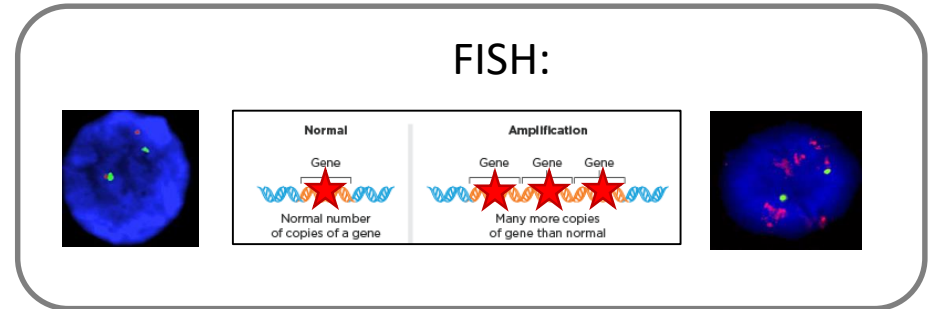
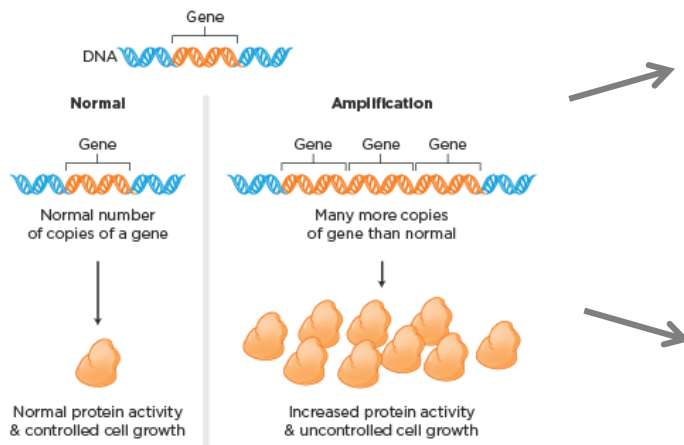
*...by SNP allele frequency*

# Detection of gene amplifications using NGS...

...by SNP allele frequency



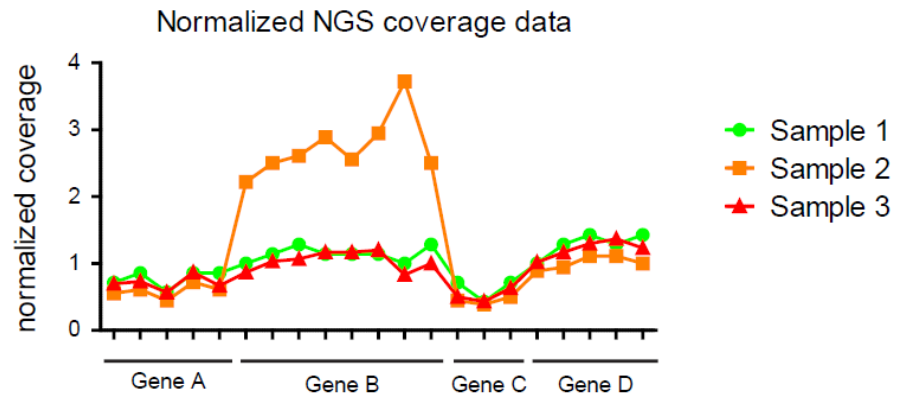
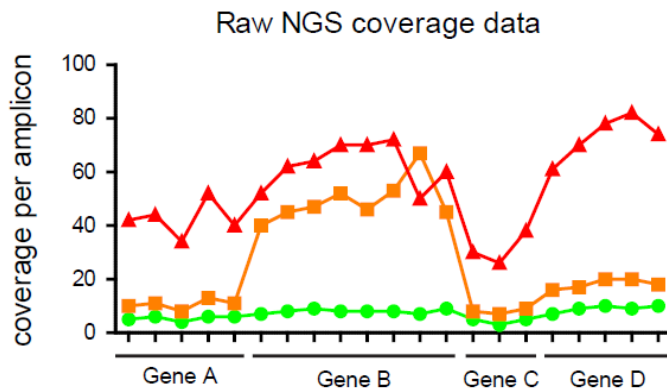
# Detection of gene amplifications



# NGS based detection of gene amplifications

Coverage based detection

## 1) Normalization



*Different approaches can be used for normalization*

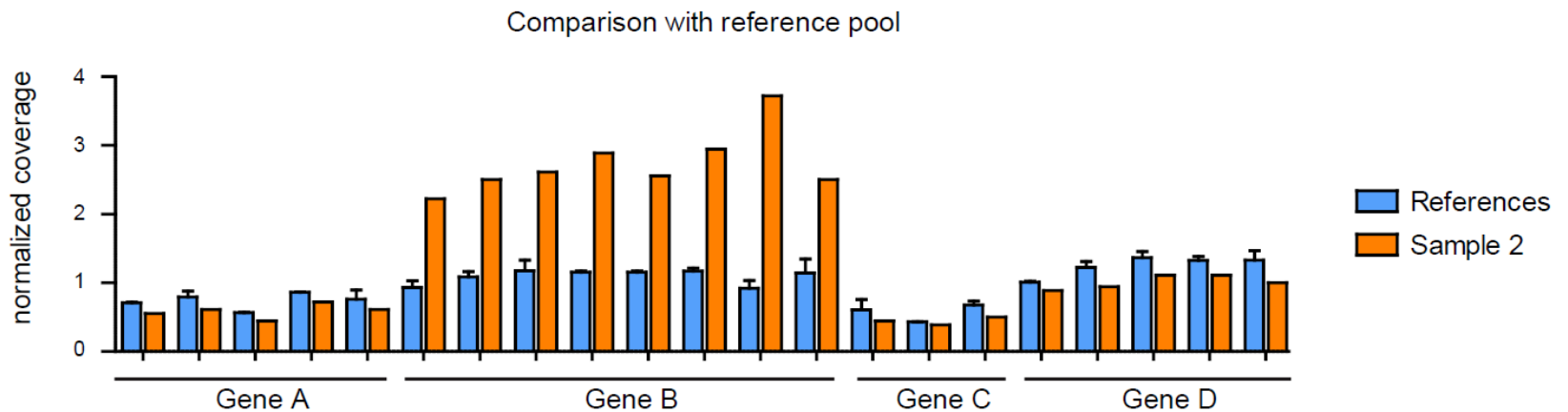


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# NGS based detection of gene amplifications

Coverage based detection

2) Comparison with other samples



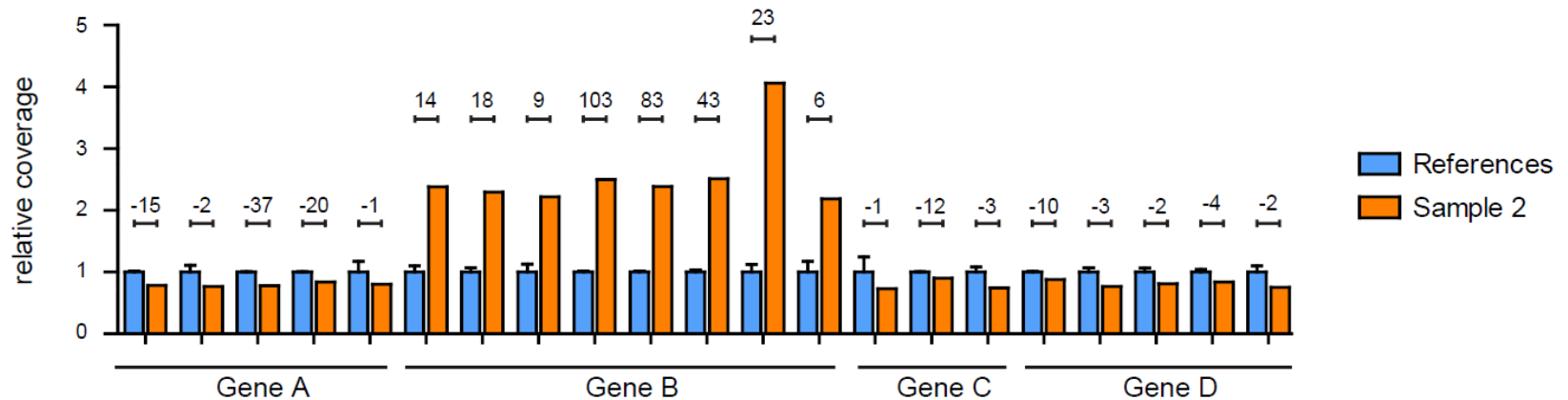
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*Comparison with internal or external reference pool, or mixed approach*

# NGS based detection of gene amplifications

Coverage based detection

3) Relevant quantitative & statistical measures

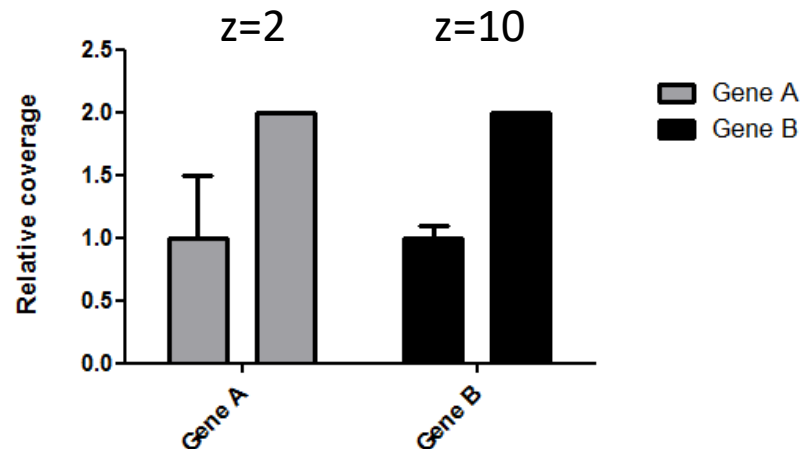


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# NGS based detection of gene amplifications

Coverage based detection

3) Note: statistical measures depend on variation and can differ per gene...



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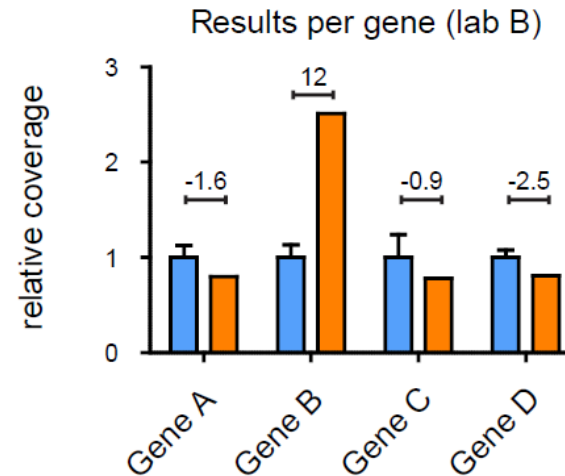
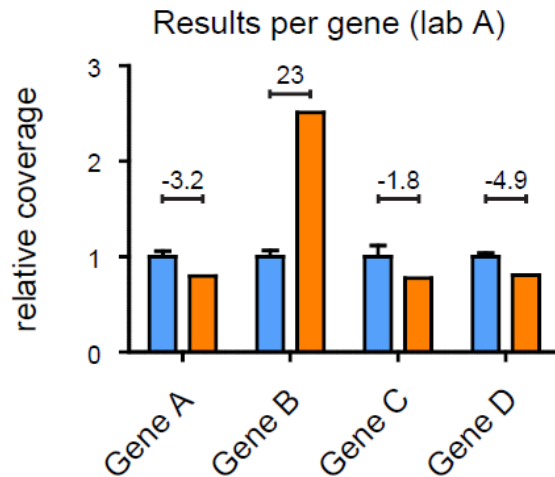
*Z-score: afwijking / stdev → technische waarde, betrouwbaarheid*

*Relatieve score: kwantitatieve weergave van afwijking*

# NGS based detection of gene amplifications

Coverage based detection

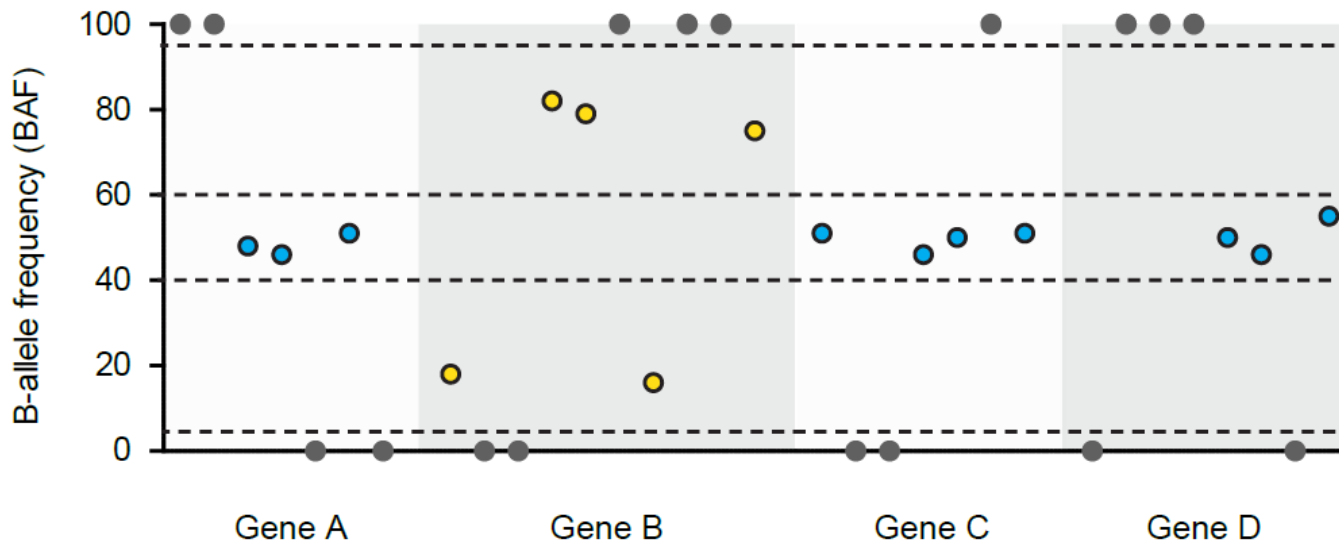
3) Note: statistical measures can be different per gene/batch/lab



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# NGS based detection of gene amplifications

SNP based detection: B allele frequency (BAF)



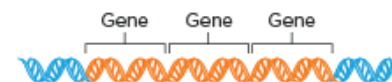
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*Requires sufficient SNPs at/near each gene locus*

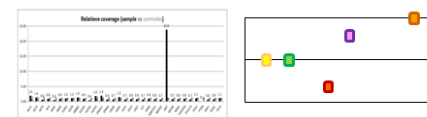
*Does it represent gain or loss? → coverage information required*

# Detectie van gen amplificaties in NGS data: van lab-data tot klinische rapportage

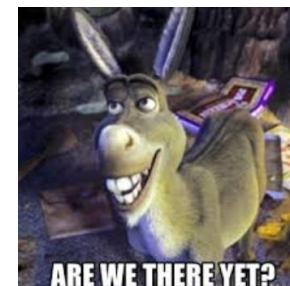
- Wat zijn gen amplificaties (copy number gains)?



- Hoe kun je gen amplificaties detecteren in NGS data?



- Vertaling naar routine diagnostiek.



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# Vertaling naar routine diagnostiek



What about:

- the magnitude of amplification?
- the influence of tumorload?
- analytical sensitivity?
- the clinical report?

---

# Vertaling naar routine diagnostiek



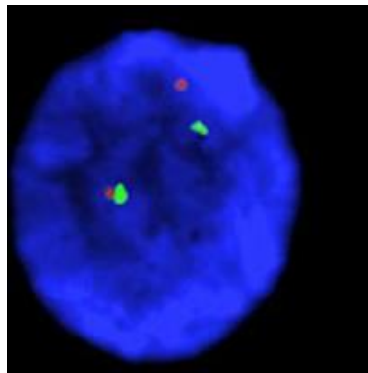
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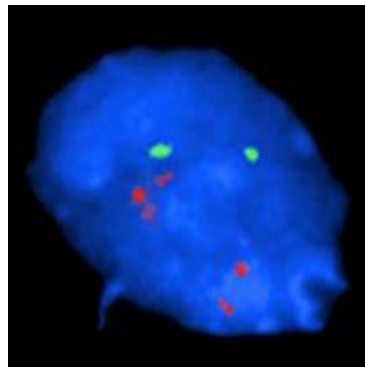


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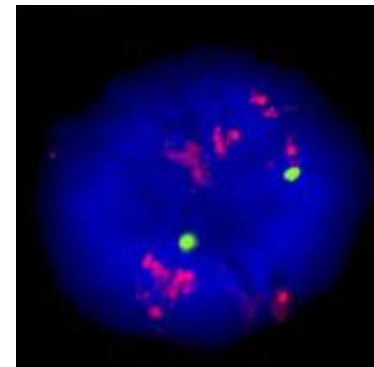
# NGS based detection of gene amplifications is influenced by magnitude of amplification



*normal*



*'low' level*



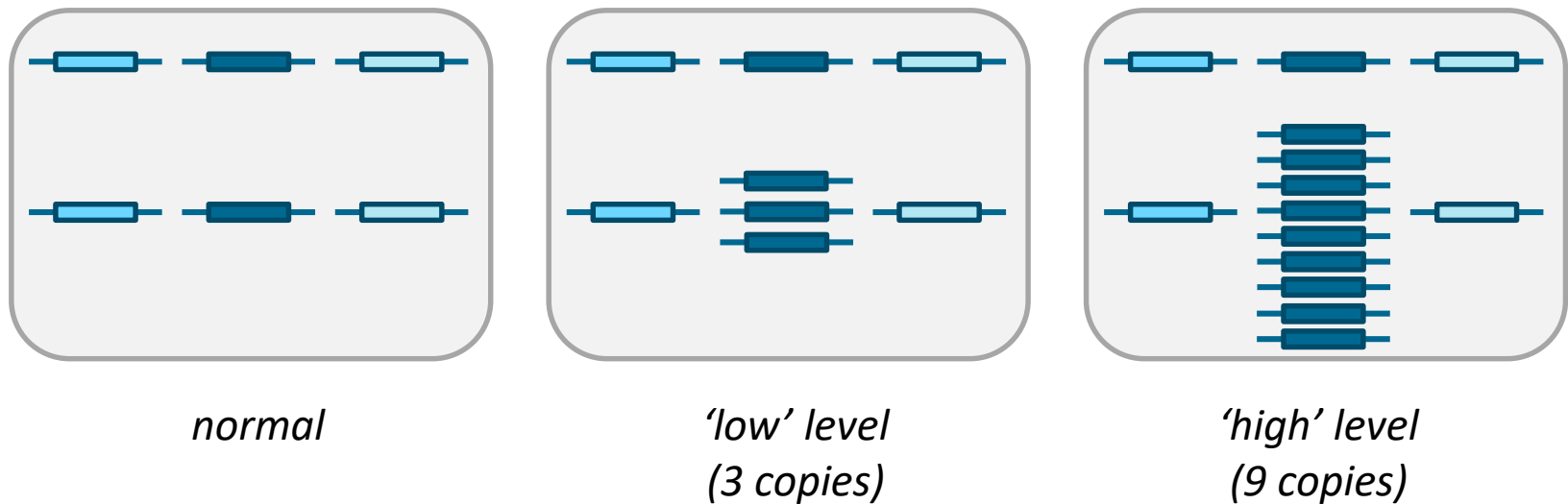
*'high' level*

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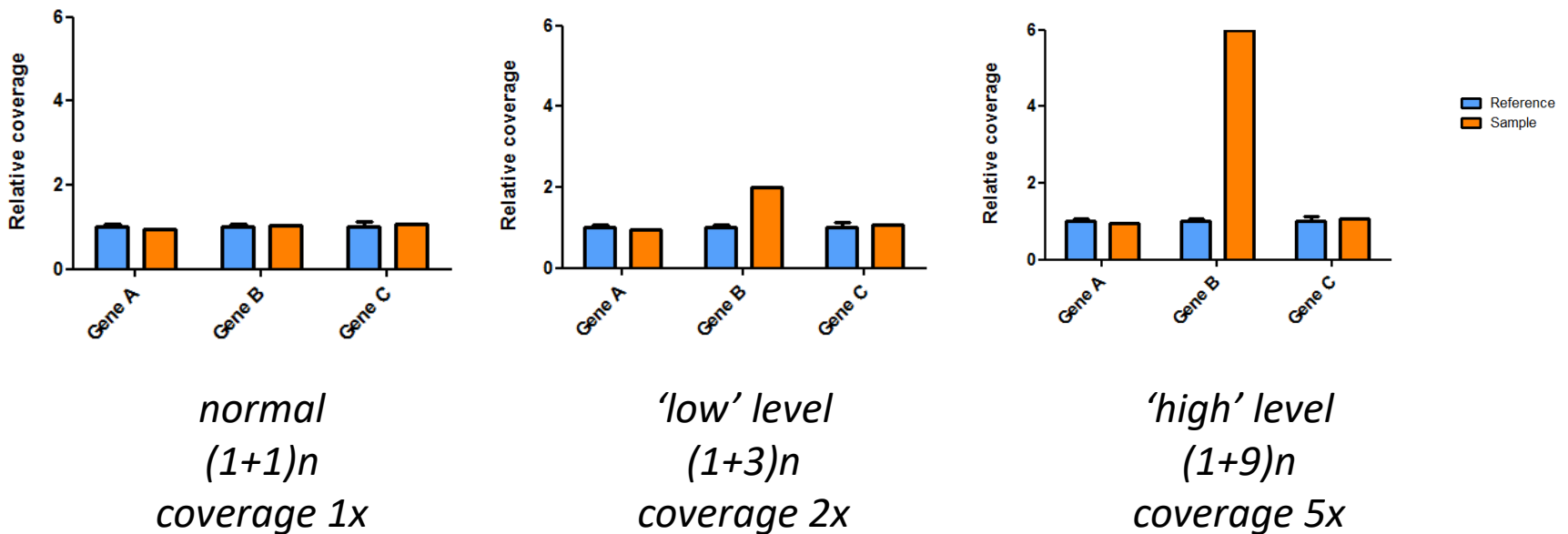
[https://www.researchgate.net/figure/A-picture-showing-the-differences-in-level-of-HER2-neu-gene-amplification-in-tumor\\_fig1\\_23134004](https://www.researchgate.net/figure/A-picture-showing-the-differences-in-level-of-HER2-neu-gene-amplification-in-tumor_fig1_23134004)

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# NGS based detection of gene amplifications is influenced by magnitude of amplification

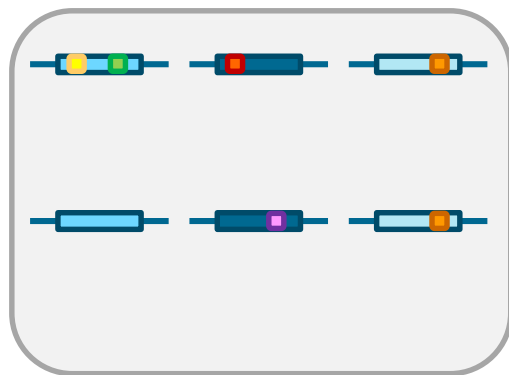


# NGS based detection of gene amplifications is influenced by magnitude of amplification

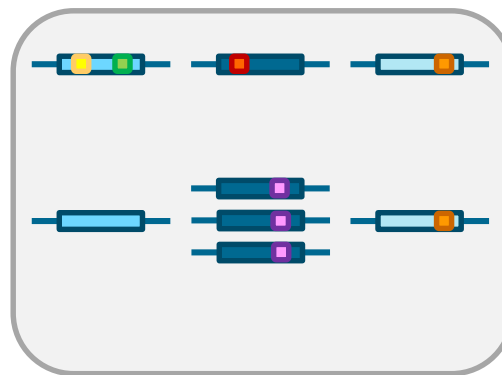


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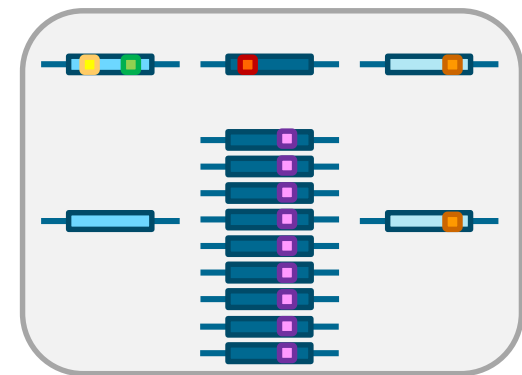
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*normal*



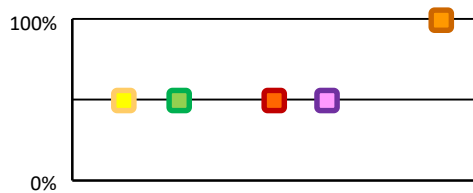
*'low' level  
(3 copies)*



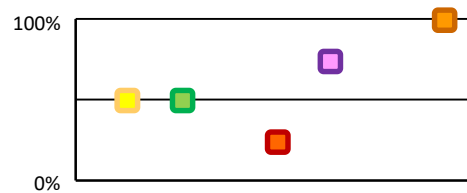
*'high' level  
(9 copies)*

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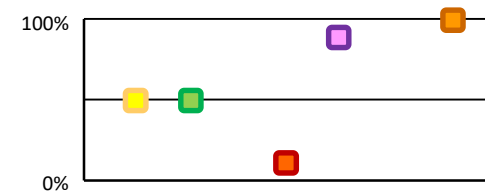
# NGS based detection of gene amplifications is influenced by magnitude of amplification



*normal*  
 $1/2 \rightarrow 50\%$   
 $1/2 \rightarrow 50\%$



*'low' level*  
 $1/4 \rightarrow 25\%$   
 $3/4 \rightarrow 75\%$

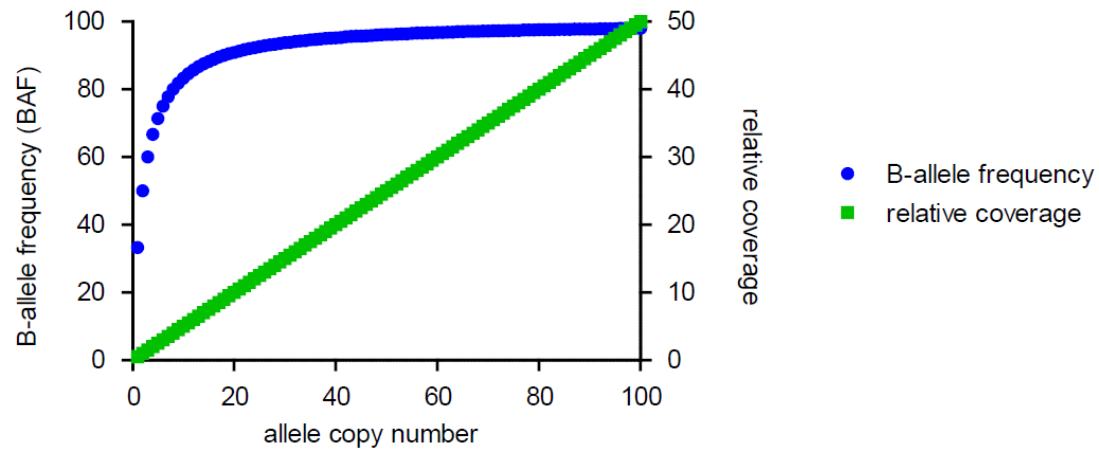


*'high' level*  
 $1/10 \rightarrow 10\%$   
 $9/10 \rightarrow 90\%$

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# NGS based detection of gene amplifications is influenced by magnitude of amplification

Resolution of coverage vs SNP-based detection:



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# Vertaling naar routine diagnostiek

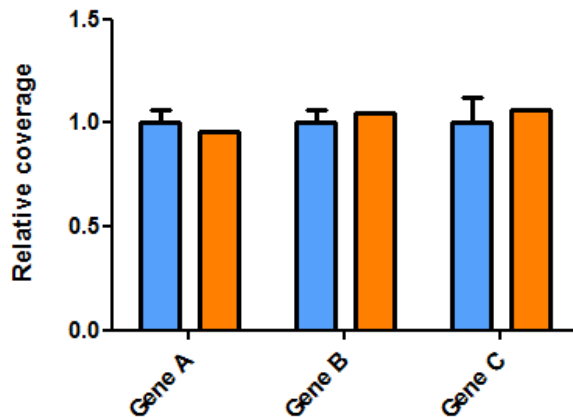


What about:

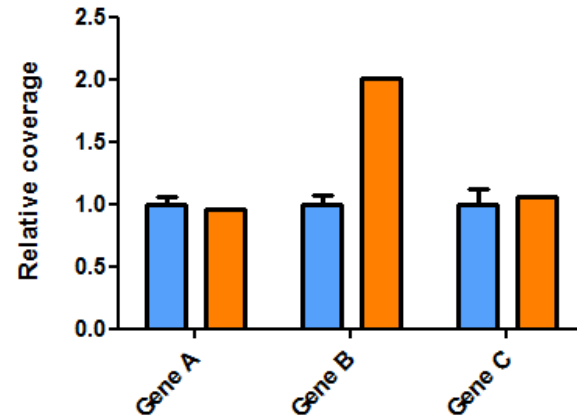
- the magnitude of amplification?
- the influence of tumorload?
- analytical sensitivity?
- the clinical report?

---

# NGS based detection of gene amplifications is influenced by tumorload



$(1+1)n * 100\%$   
→ coverage 1x

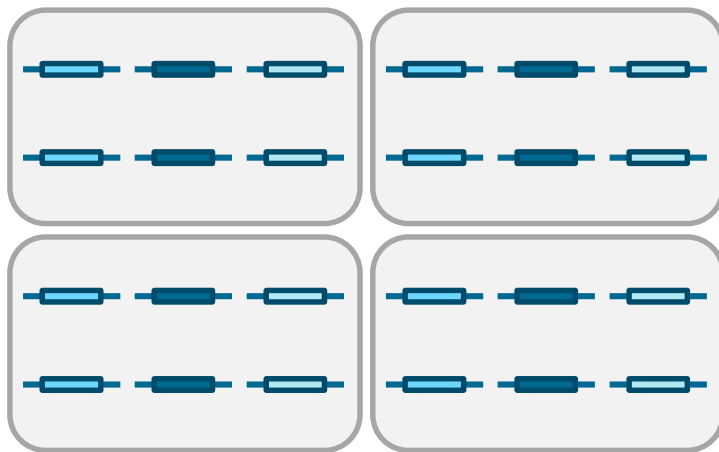


$(1+3)n * 100\%$   
→ coverage 2x

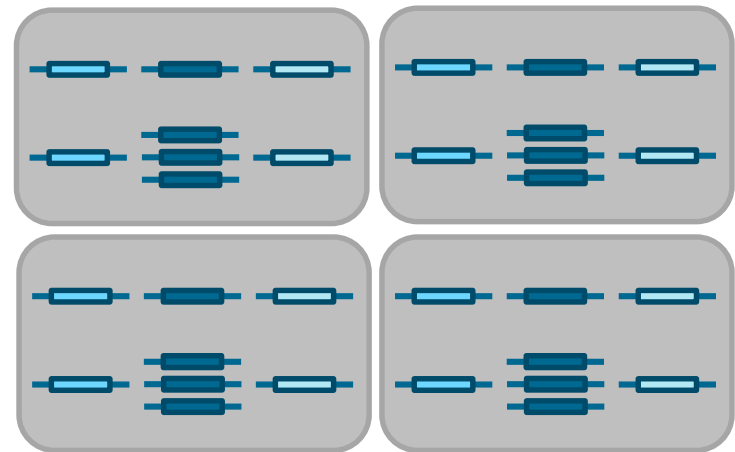


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# NGS based detection of gene amplifications is influenced by tumorload



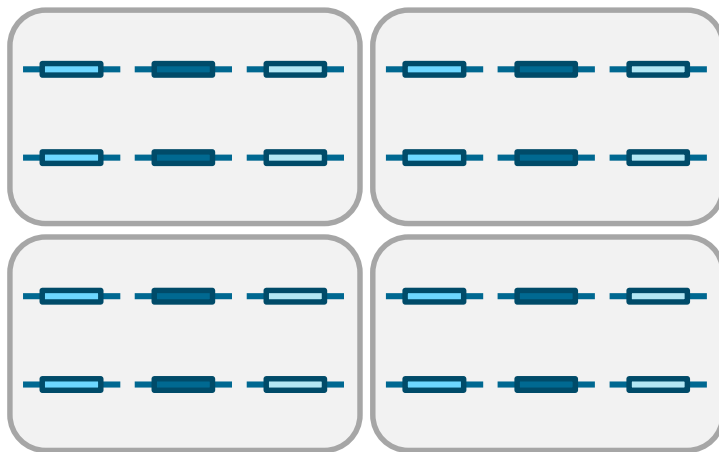
$(1+1)n * 100\%$   
→ coverage 1x



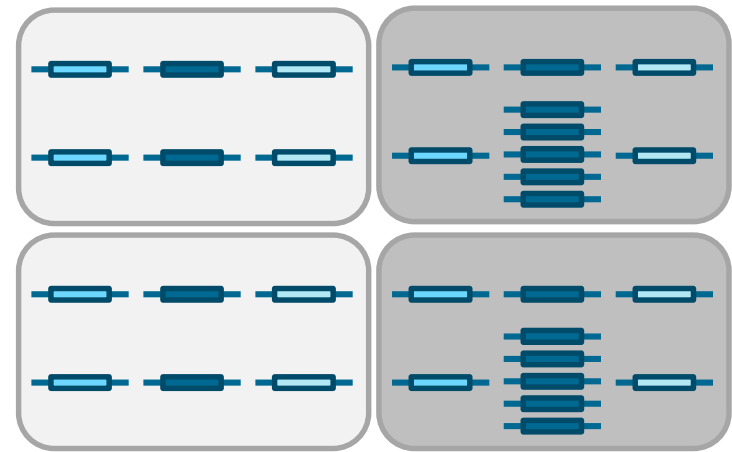
$(1+3)n * 100\%$   
→ coverage 2x

---

# NGS based detection of gene amplifications is influenced by tumorload



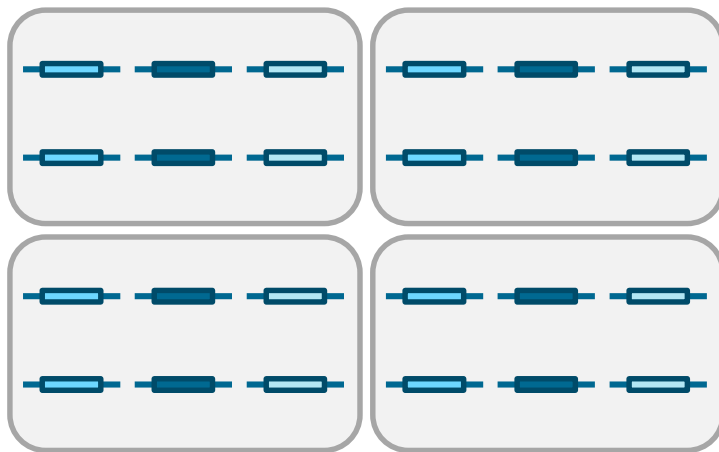
$(1+1)n * 100\%$   
→ coverage 1x



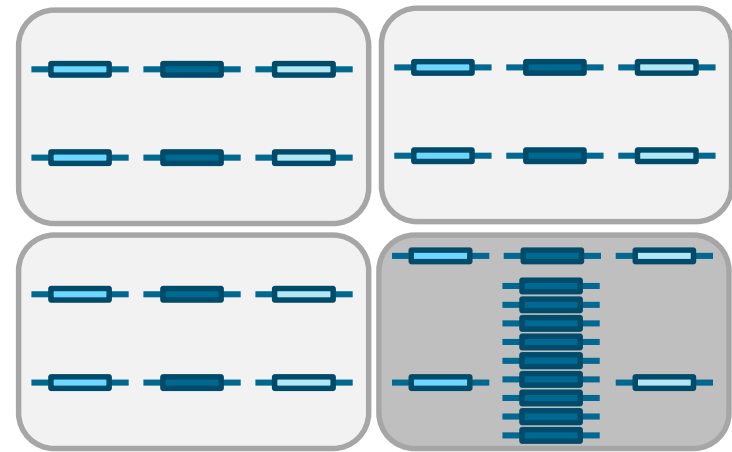
$((1+1)n * 50\%) + ((1+5)n * 50\%)$   
→ coverage 2x

---

# NGS based detection of gene amplifications is influenced by tumorload



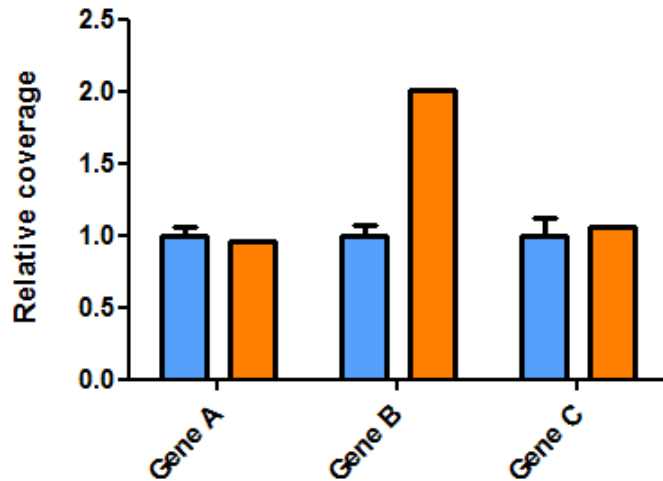
$(1+1)n * 100\%$   
→ coverage 1x



$((1+1)n * 75\%) + ((1+9)n * 25\%)$   
→ coverage 2x

---

# NGS based detection of gene amplifications is influenced by tumorload



*Interpretation of coverage 2x:*

*100% tumorload:  $1+3n$*

*50% tumorload:  $1+5n$*

*25% tumorload:  $1+9n$*

*Does it represent a clinically relevant / targetable gene amplification?*

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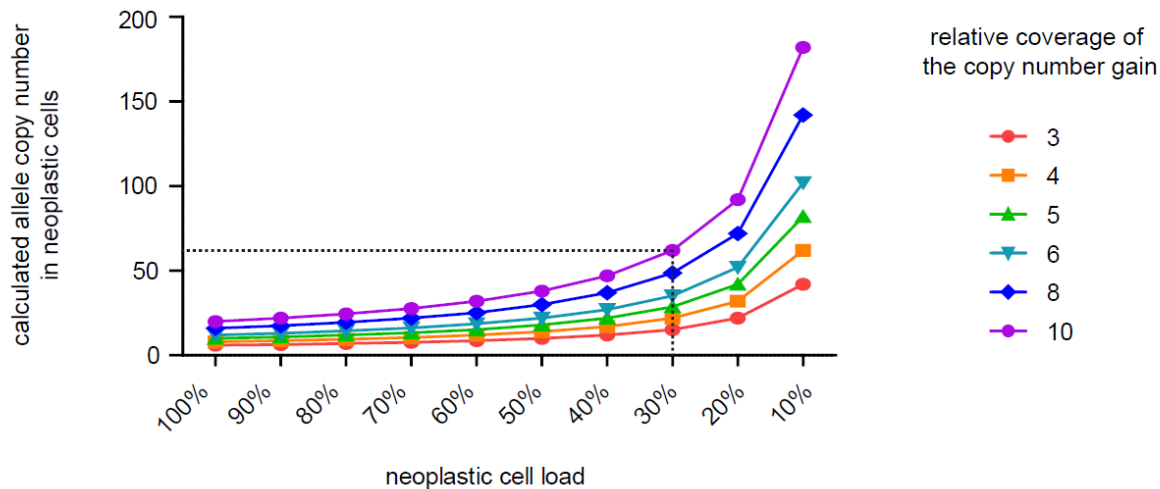
*Note:*

- approximation of tumorload is error prone*
- based on 2n genomes of normal and tumor cells*

# Quantitative interpretation of NGS based detection of gene amplifications

$$\text{estimated allele copy number} = \frac{r \cdot 2 - (1 - t) \cdot 2}{t}$$

r = relative coverage  
t = estimated tumor load (fraction neoplastic cells)



*Clinically relevant? Comparison with FISH required?*

---

# Vertaling naar routine diagnostiek

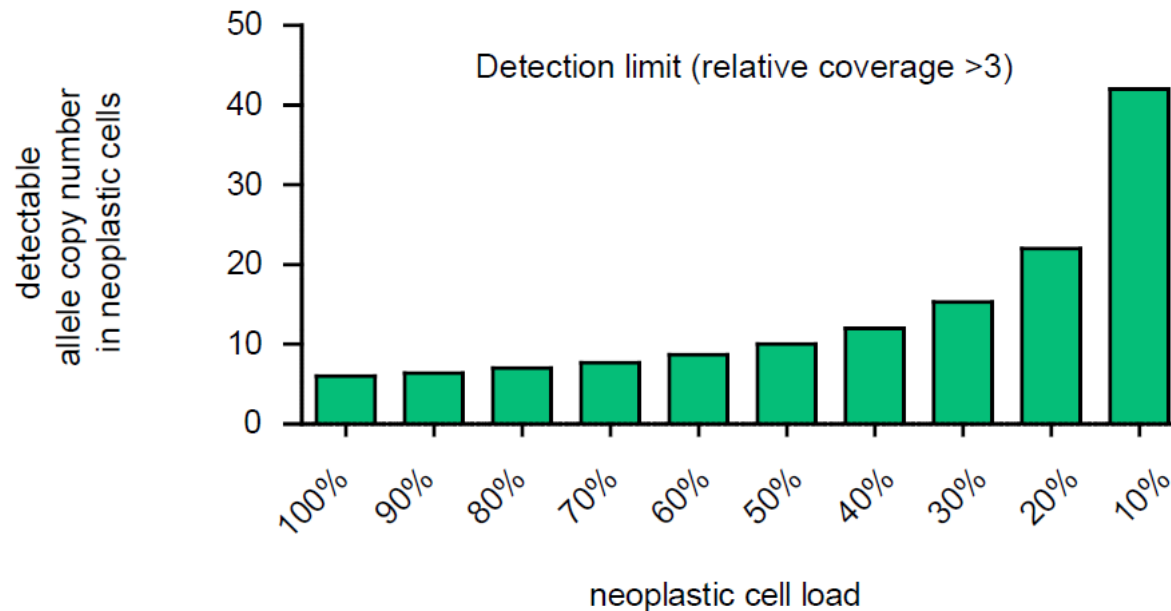


What about:

- the magnitude of amplification?
- the influence of tumorload?
- analytical sensitivity?
- the clinical report?

# Analytical sensitivity

Analytical sensitivity depends on cut-offs and tumorcel%



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# Vertaling naar routine diagnostiek



What about:

- the magnitude of amplification?
- the influence of tumorload?
- analytical sensitivity?
- the clinical report?



---

# NGS based detection of gene amplifications in routine diagnostics – the report

## *Rapporteren:*

- 1) Gen naam / Chromosomale regio
- 2) Type CNV (gen: amplification, deletion, LOH / chromosomale regio: ook (partiële) afwijking, imbalance)
- 3) Kwantitatieve inschatting (vnl bij amplificaties van genen relevant)
  - Relatieve coverage
  - Uitgaande van geschatte tumorcel% komt dit overeen met ongeveer ## kopieën (bij 2n in normaal weefsel).
- 4) Disclaimer: Gevoeligheid van test (bv relatieve coverage >3 wordt gerapporteerd, deleties of laag niveau amplificaties kunnen worden gemist bij een laag tumorcel %)

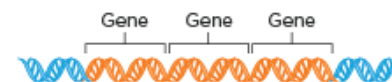
## *Optioneel rapporteren:*

Kwaliteitsscore (z-score o.i.d.). Evt. definitie (rel. coverage/ st.dev normalen) en betekenis beschrijven.

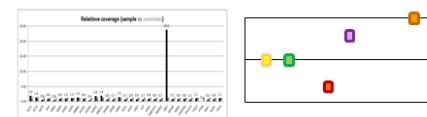
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# Detectie van gen amplificaties in NGS data: van lab-data tot klinische rapportage

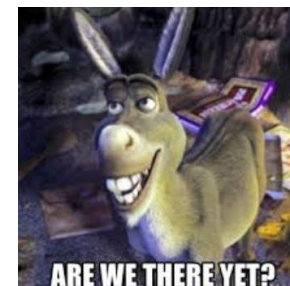
- Wat zijn gen amplificaties (copy number gains)?



- Hoe kun je gen amplificaties detecteren in NGS data?



- Vertaling naar routine diagnostiek.



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# Detectie van gen amplificaties in NGS data: van lab-data tot klinische rapportage

