FISH *HER2*:

Perquè em costa comptar senyals?

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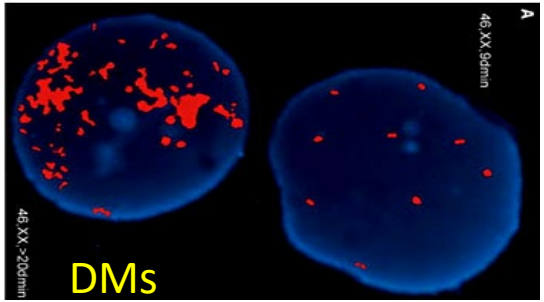
UAB Universitat Autònoma de Barcelona

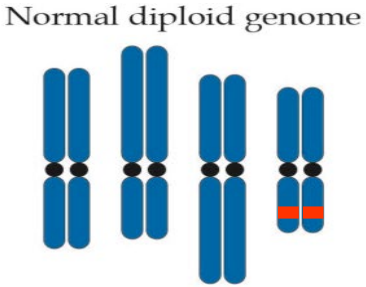
Salut/

Germans Trias i Pujol Hospital

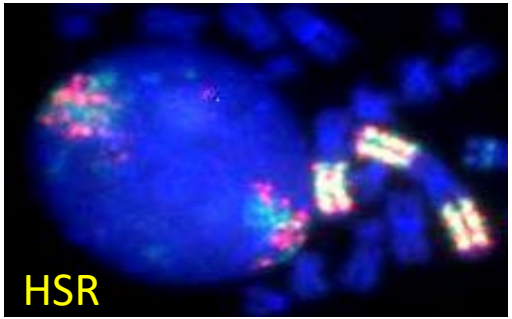
Amplificació: Increment en el nombre de còpies d'una **regió cromosòmica concreta**

Double minutes (DMs)

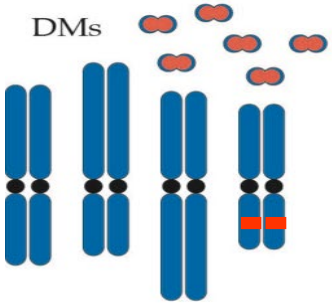


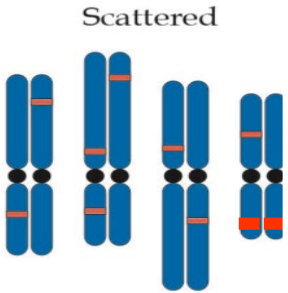


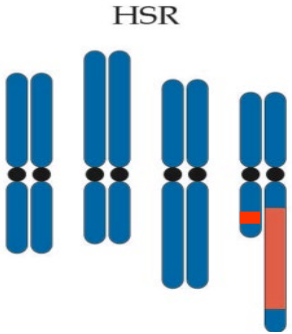
Homogeneous staining region (HSR)



EGFR
MYC
MDM2

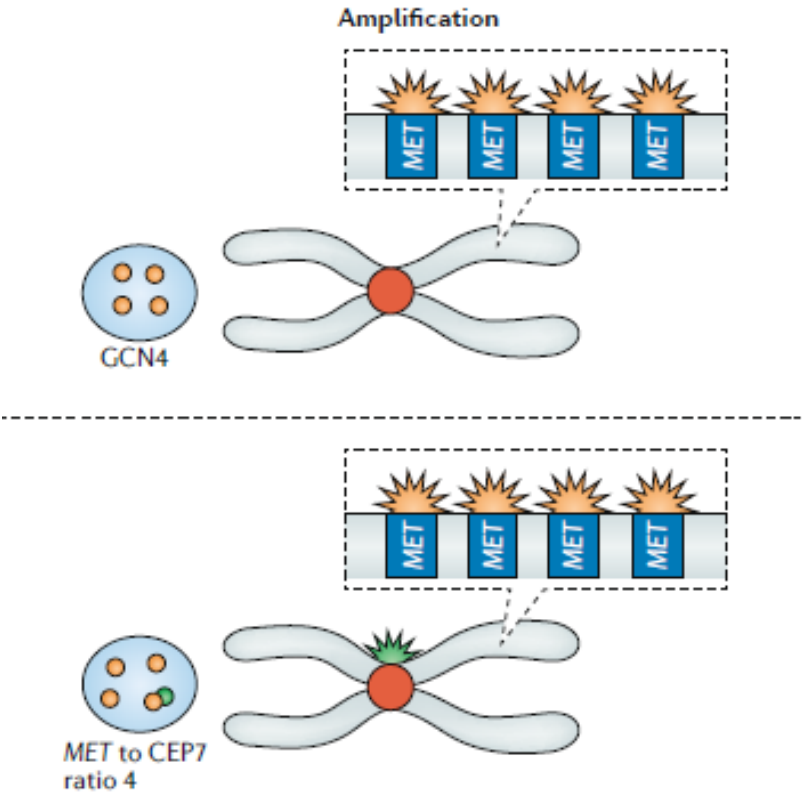






HER2
NMYC

http://cdn.intechopen.com/pdfs/42311/InTech-Model_systems_facilitating_an_understanding_of_mechanisms_for_oncogene_amplification.pdf



GCN: nombre de còpies gen/cèl·lula

Ratio: nº còpies gen/cromosoma (centròmer)

Guo.*Nat Reviews Clin Oncol* 2020

Criteris d'amplificació: **gen** específic i **tumor** específic

TABLE 1. *Criteria for HER2 positivity by immunohistochemistry and FISH in different tumor types*

	Breast (ASCO/CAP 2018) (29)	Gastric (ASCO/CAP 2016) (30)	Colorectal (HERACLES trial) (31)	Endometrial serous (Fader et al clinical trial) (24,25)
HER2 IHC 3+	> 10% circumferential, strong, complete	≥ 10%, strong complete, or basolateral/lateral	≥ 50% strong complete, or basolateral/ lateral	> 30% strong complete, or basolateral/ lateral
HER2 FISH amplification	*HER2*/CEP17 ratio ≥ 2.0 and HER2 signal ≥ 4.0/nucleus OR ratio < 2.0 and HER2 signal ≥ 6.0/nucleus (if IHC score 2+ or 3+)	*HER2*/CEP17 ratio ≥ 2.0 OR ratio < 2.0 and HER2 signal > 6.0 /nucleus	*HER2*/CEP17 ratio ≥ 2.0 in ≥ 50% of cells	*HER2*/CEP17 ratio ≥ 2.0

Buza. *Int J Gynecol Pathol* 2020

Evolució de les guies d'interpretació en càncer de mama

Test platform		2007	2013	2018	FDA	
IHC	3+[a]	>30%	>10%	>10%	>10%	% pos. tumor cells
	2+[b]	circular	incomplete	circular	circular	Membrane staining
ISH (dual color)	positive	Ratio >2.2 or >6.0	Ratio ≥2.0 Ratio <2.0 & CN ≥6.0	ISH Group 1	Ratio ≥2.0	HER2-CN/CEP-17 HER2-CN
	borderline	Ratio 1.8-2.2 or CN 4-6	Ratio <2.0 and CN 4- <6	ISH Group 2-4*	n.d.	*further work-up consider IHC
	negative	Ratio <1.8 or CN <4.0	Ratio <2.0 and CN <4	ISH Group 5	Ratio <2.0	HER2-CN/CEP-17 HER2-CN
IHC-ISH-Histology	Discordancy	n.d.	consider histological tumor types		n.d.	

Rüschoff et al. *Pathologe* 2021

AMPLIFICAT

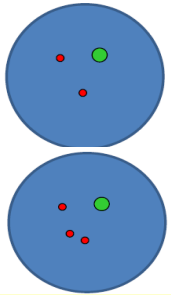
NO AMPLIFICAT

Rüschoff et al. *Pathologe* 2021

2018

IHC Score	ISH	Ratio ≥ 2 .0		Ratio < 2 .0
		CN ≥ 4	CN < 4	CN < 4
		ISH Group 1	ISH Group 2	ISH Group 5
0		POSITIVE	NEG + comment	NEGATIVE
1		POSITIVE	NEG + comment	NEGATIVE
2		POSITIVE	NEG + comment	NEGATIVE
3		POSITIVE	POSITIVE	POSITIVE

AMPLIFICAT



NO AMPLIFICAT

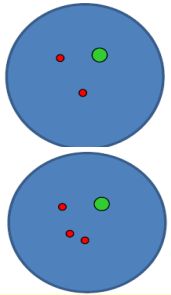
MONOSÒMIC

Rüschoff et al. *Pathologe* 2021

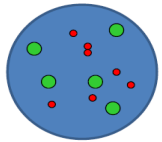
2018

IHC Score	ISH	Ratio ≥ 2 .0		Ratio < 2 .0		
		CN ≥ 4	CN < 4	CN ≥ 6		CN < 4
		ISH Group 1	ISH Group 2	ISH Group 3		ISH Group 5
0		POSITIVE	NEG + comment	NEG + comment		NEGATIVE
1		POSITIVE	NEG + comment	NEG + comment		NEGATIVE
2		POSITIVE	NEG + comment	POSITIVE		NEGATIVE
3		POSITIVE	POSITIVE	POSITIVE		POSITIVE

AMPLIFICAT



MONOSÒMIC



COAMPLIFICAT

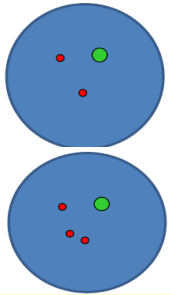
NO AMPLIFICAT

Rüschoff et al. *Pathologe* 2021

2018

IHC Score	ISH	Ratio ≥ 2 .0		Ratio < 2 .0		
		CN ≥ 4	CN < 4	CN ≥ 6	CN ≥ 4 to <6	CN < 4
		ISH Group 1	ISH Group 2	ISH Group 3	ISH Group 4	ISH Group 5
	0	POSITIVE	NEG + comment	NEG + comment	NEGATIVE	NEGATIVE
	1	POSITIVE	NEG + comment	NEG + comment	NEG + comment	NEGATIVE
	2	POSITIVE	NEG + comment	POSITIVE	NEG + comment	NEGATIVE
	3	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE

AMPLIFICAT



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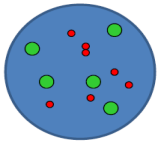

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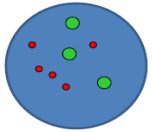


NO AMPLIFICAT

NO AMPLIFICAT

Rüschoff et al. *Pathologe* 2021

***HER2* IQFISH pharmDx™**
(Dako Omnis)

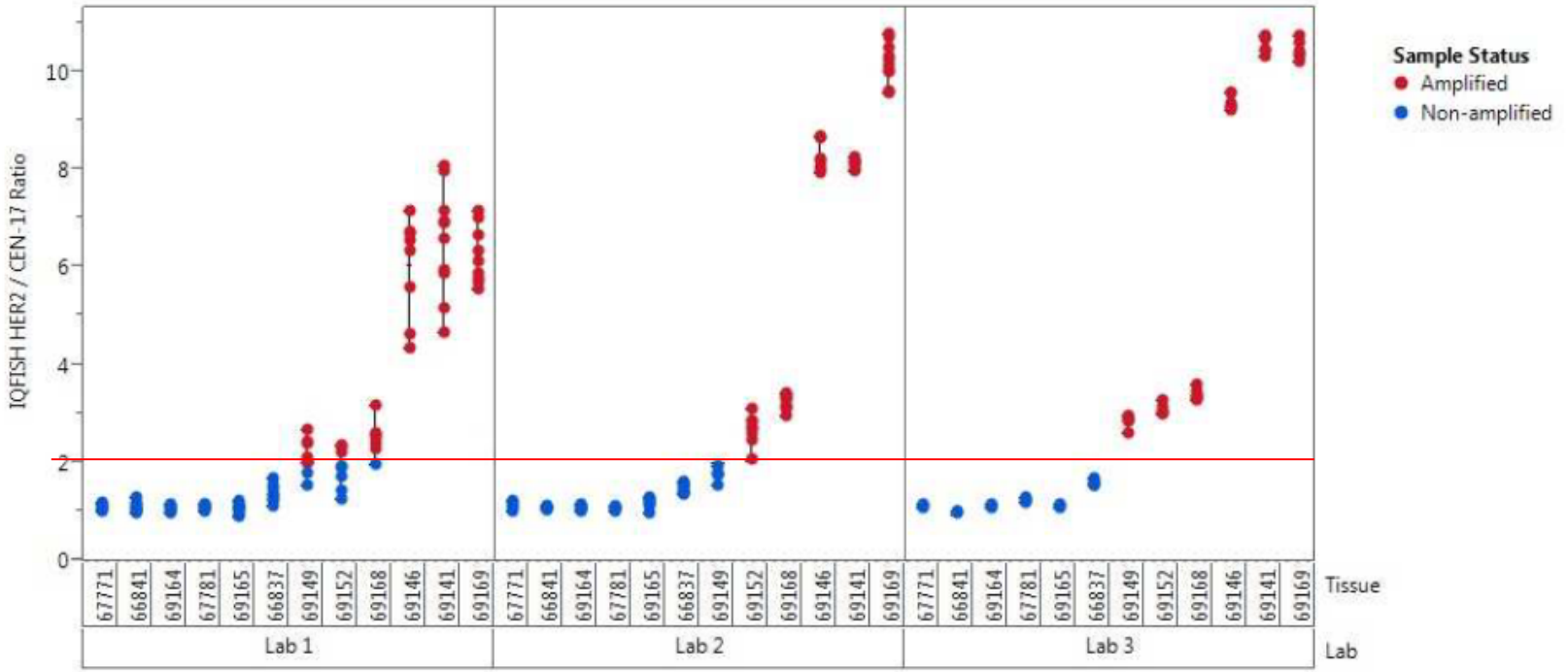
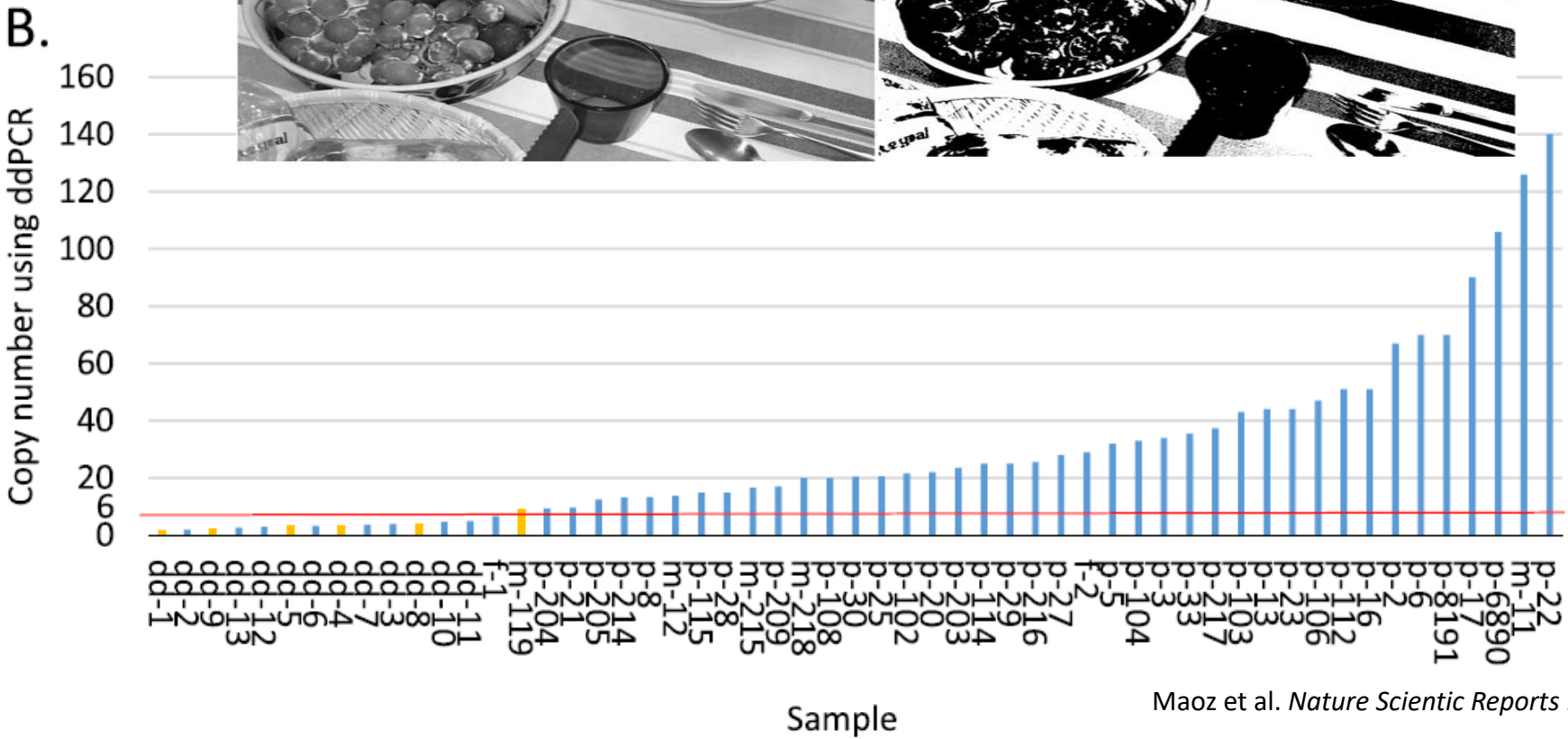


Figura 9. Gráfico en el que se representa la variabilidad de las proporciones *HER2*/CEN-17 en unidades no transformadas obtenidas en el estudio de reproducibilidad día a día y centro a centro de *HER2* IQFISH pharmDx™ (Dako Omnis) en muestras tisulares de cáncer gástrico.

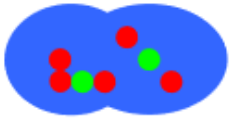
Gown AM.
Modern Pathol 2008



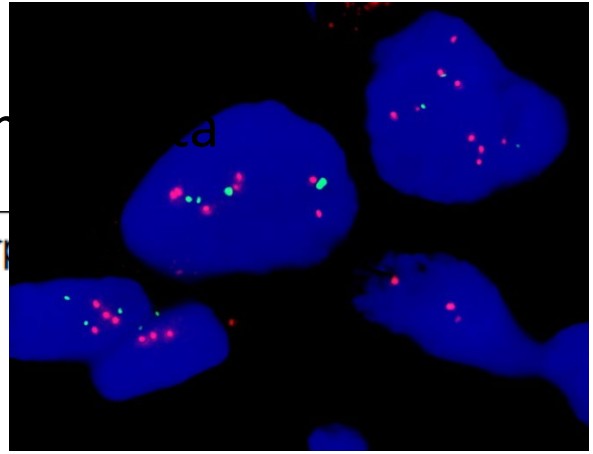
Maoz et al. *Nature Scientic Reports* 2019

Ji et al. *PLOS ONE* 2015

- Tumors amb poc citoplasma i cel·lularitat n[illegible]ca



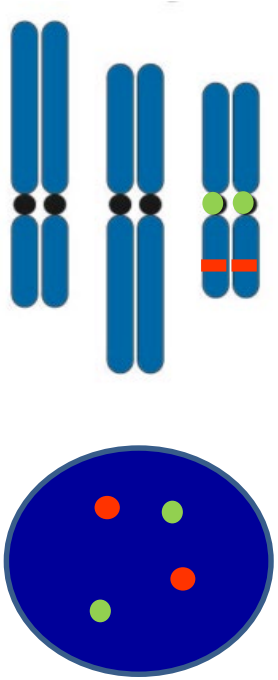
No contar. Los núcleos se superp[illegible]
de los núcleos son visibles.



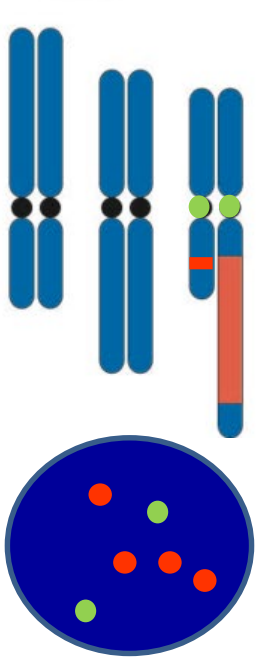
- Tumors amb poc citoplasma i cel·lularitat molt alta

- Estroma abundant i fibrós, nuclis atrapats... (ex: lobelars)

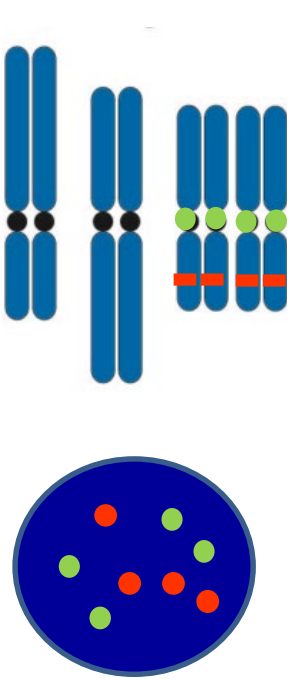
Disòmic
No amplificat



Disòmic
Amplificat

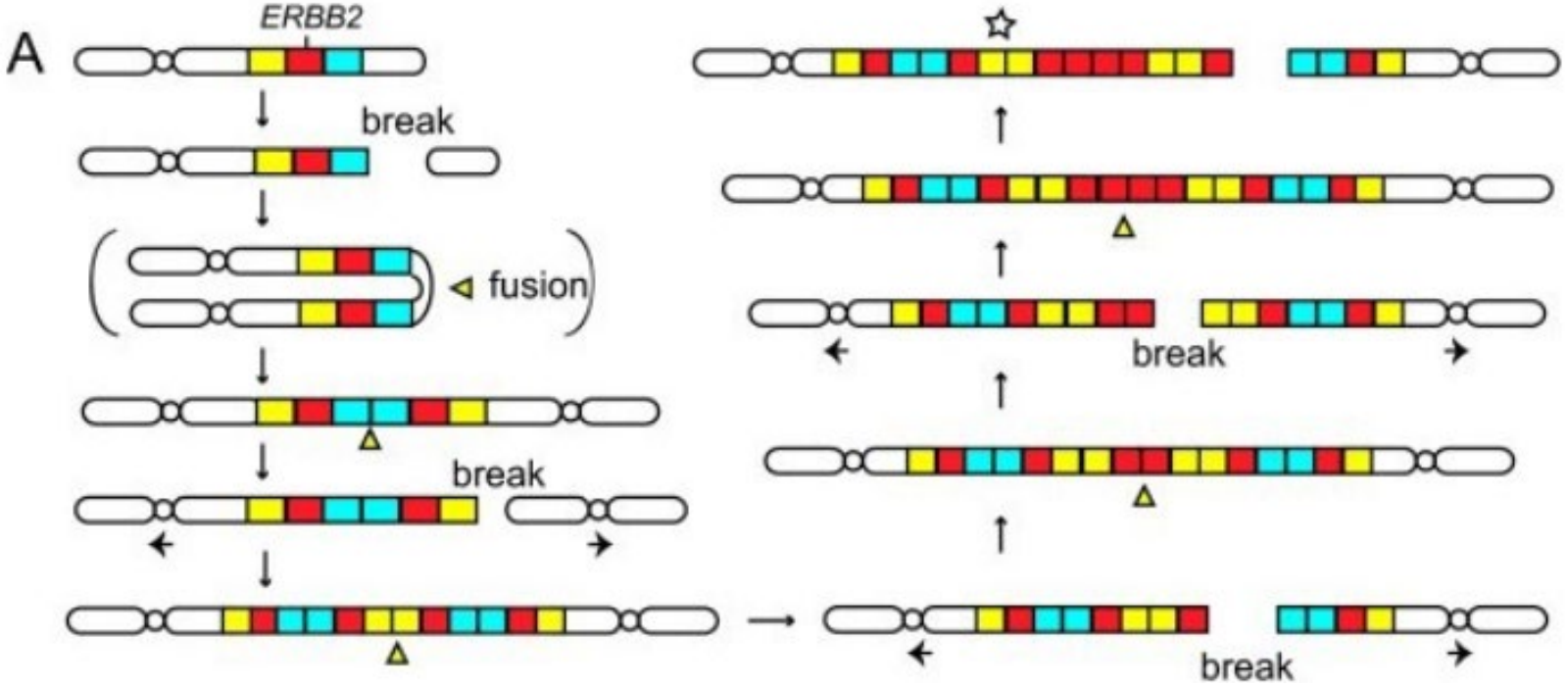


Polisòmic
No amplificat

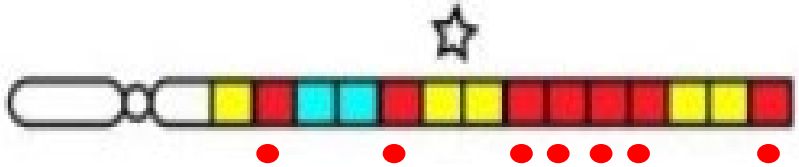


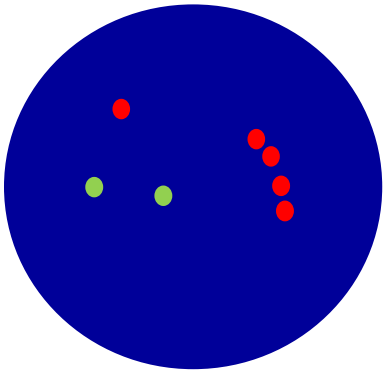
GCN; ratio gen/cromosoma

Break-fusion-break (BFB) cycles



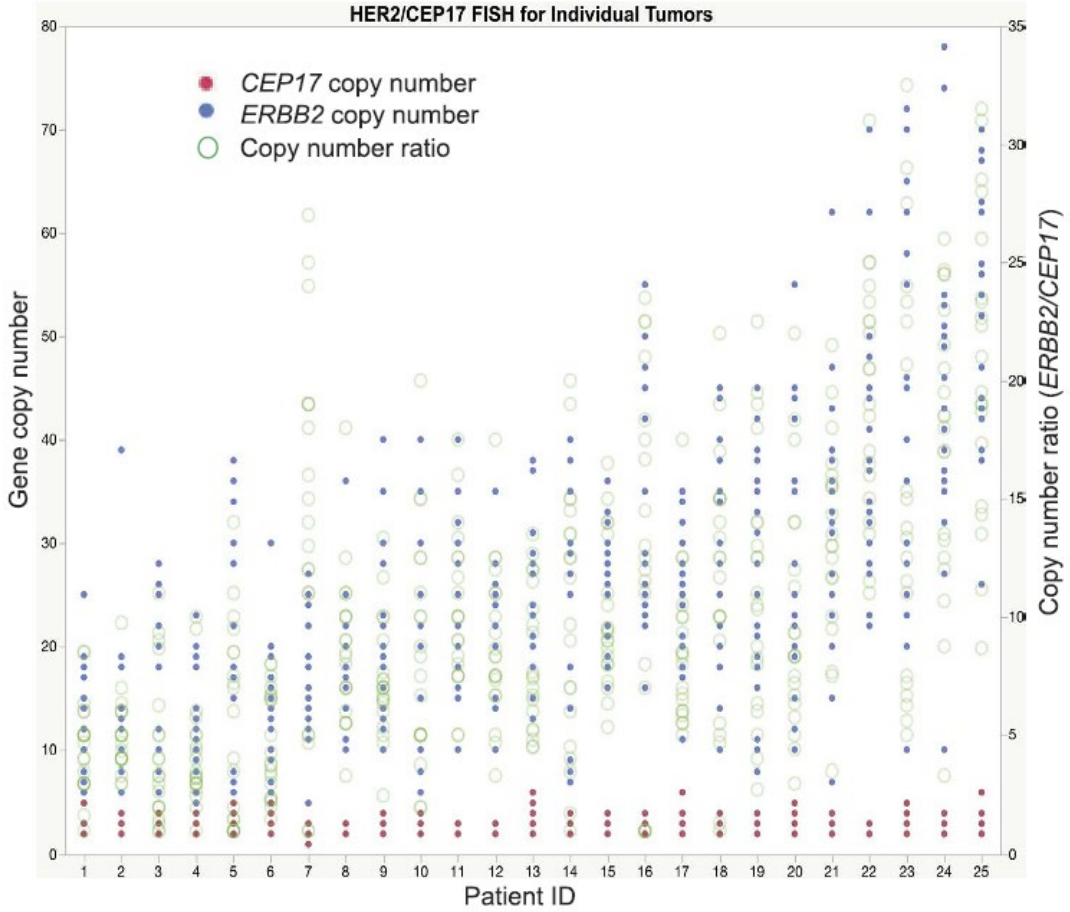
Marotta et al. *Breast Cancer Res* 2012





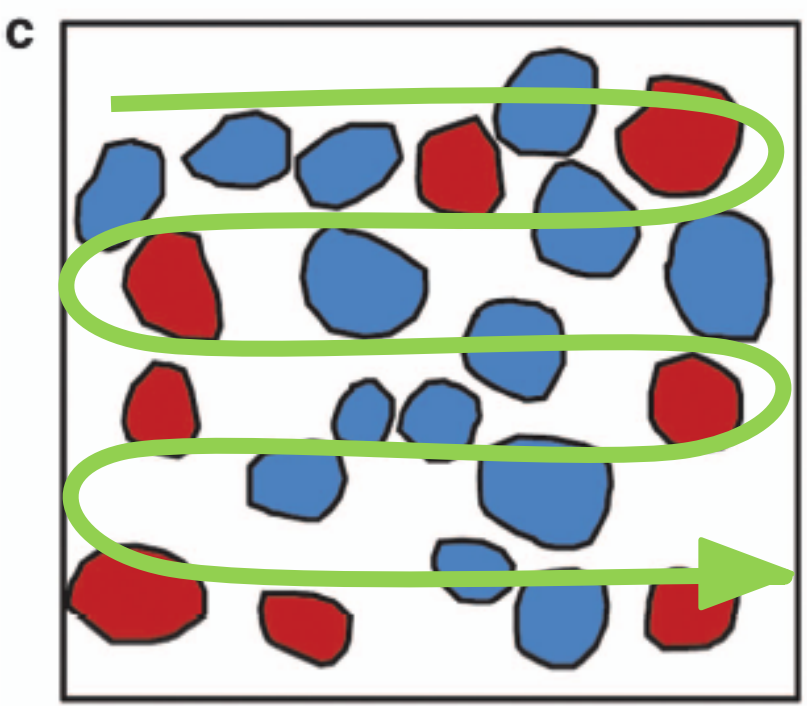
***HER2* IQFISH pharmDx™**
(Dako Omnis)
N.º de catálogo GM333

Contar como 2 señales verdes y 3 señales rojas. Dos señales que sean del mismo tamaño y estén separadas por una distancia igual o inferior al diámetro de una señal se cuentan como una sola señal.

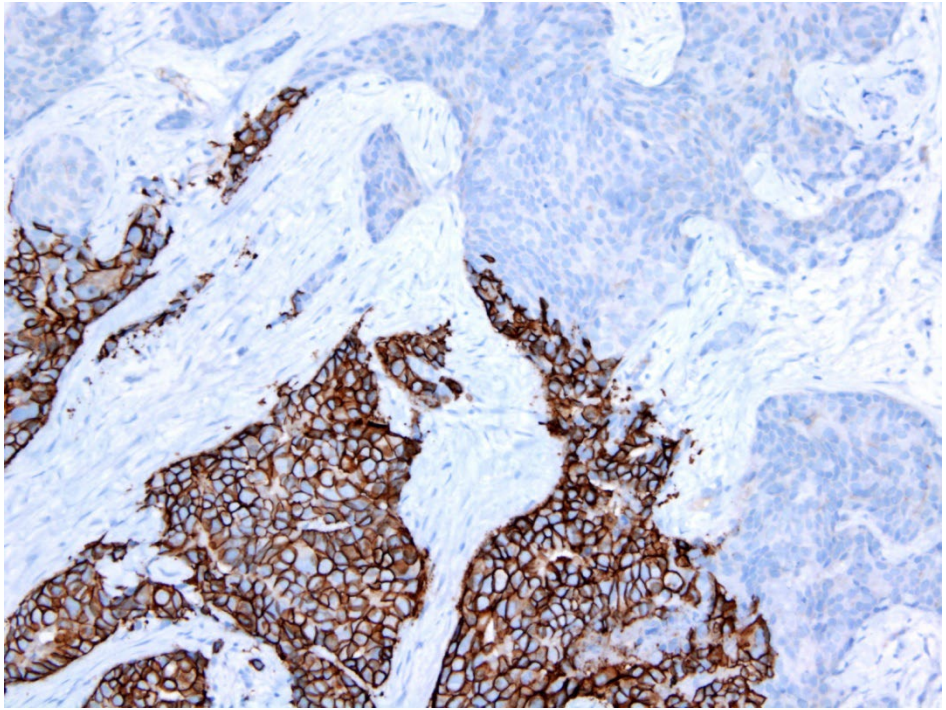


El propi mecanisme d'amplificació comporta heterogeneitat

Marotta et al. *Nature Scientific Reports* 2017

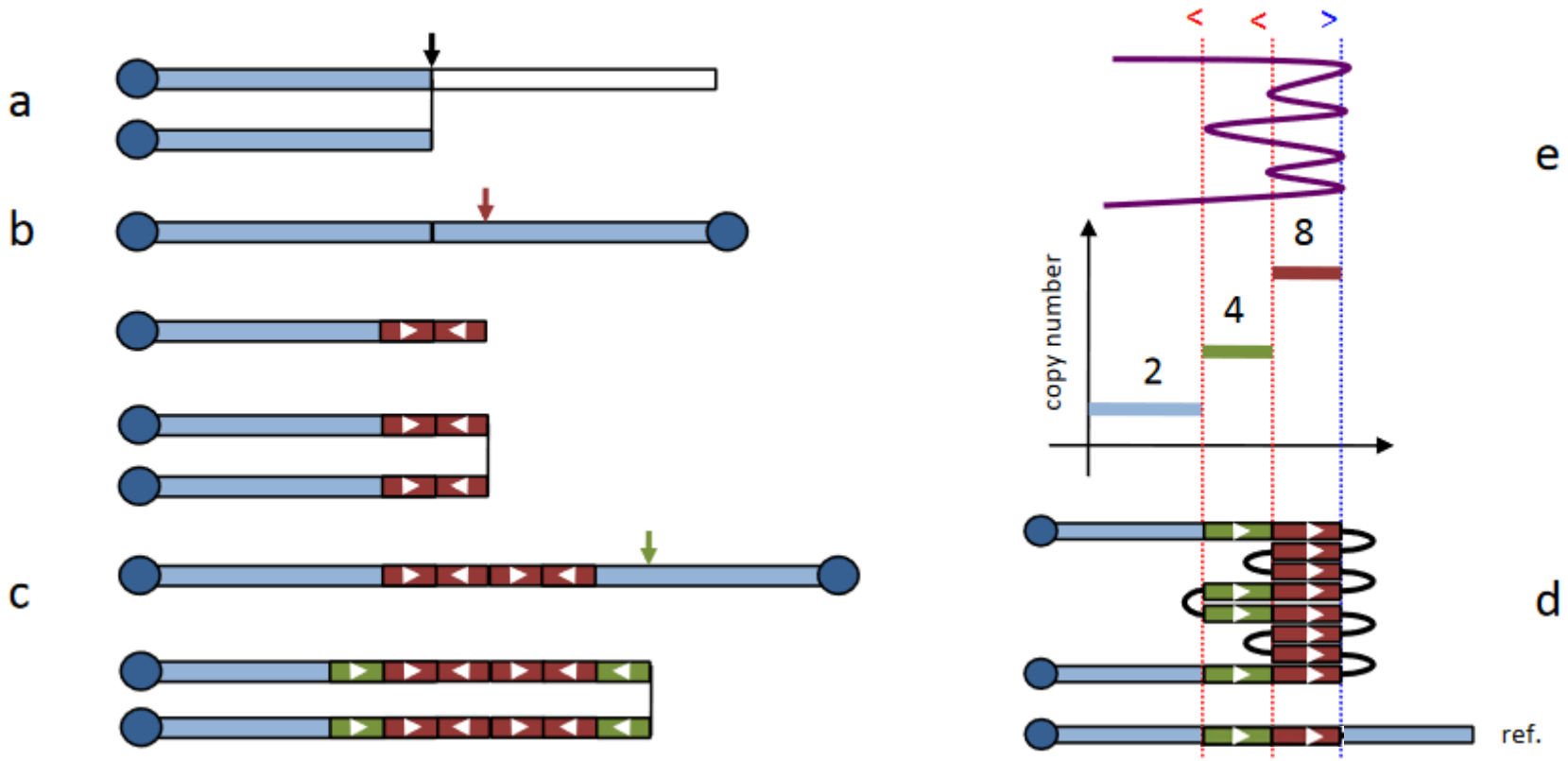


Genetically heterogeneous
for *HER2* amplification
(scattered amplified cells)

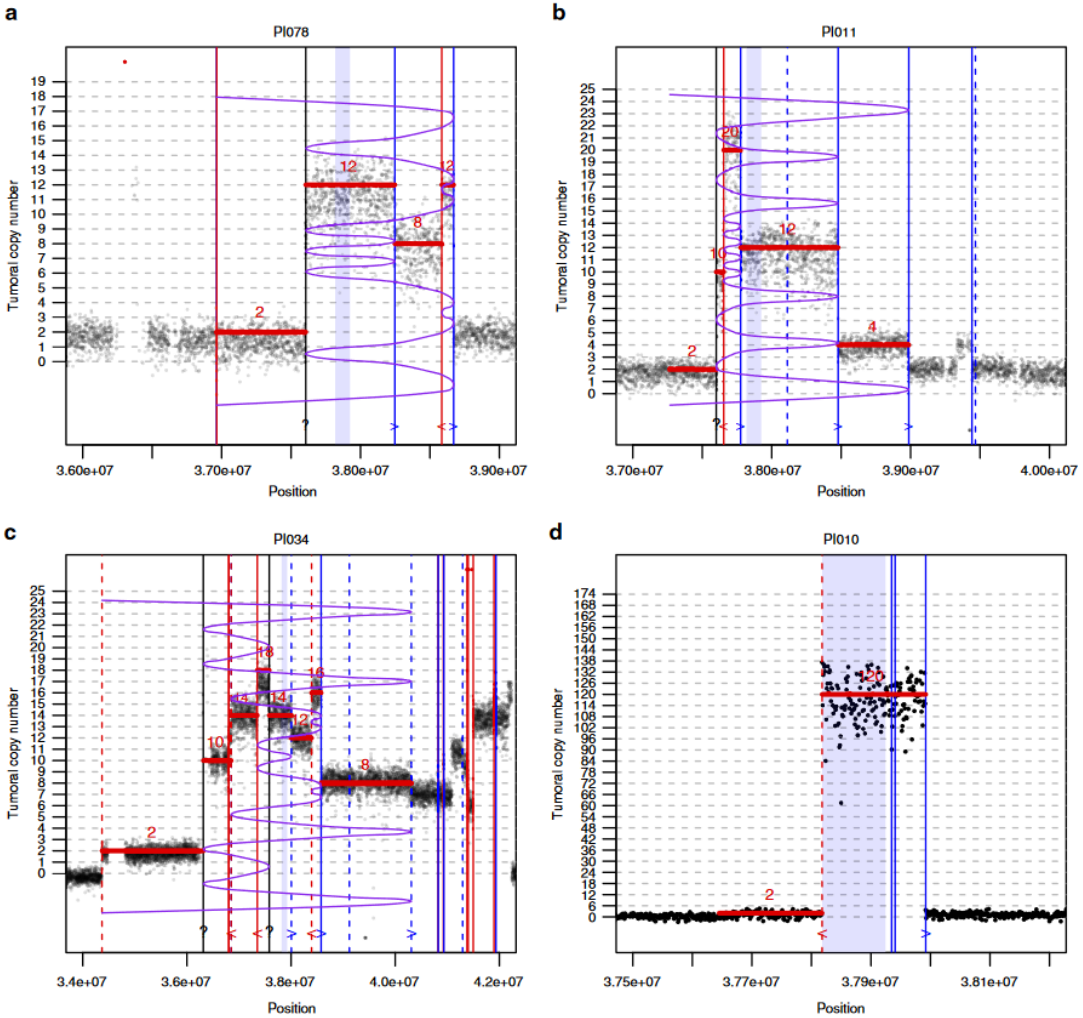


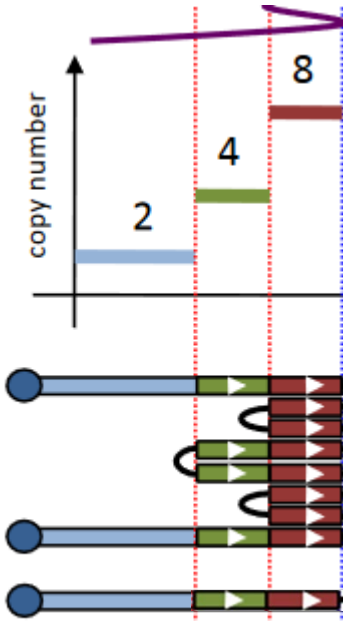
Genetically heterogeneous for
HER2 amplification
(amplified cluster)

Hanna et al . *Mod Pathol* 2014

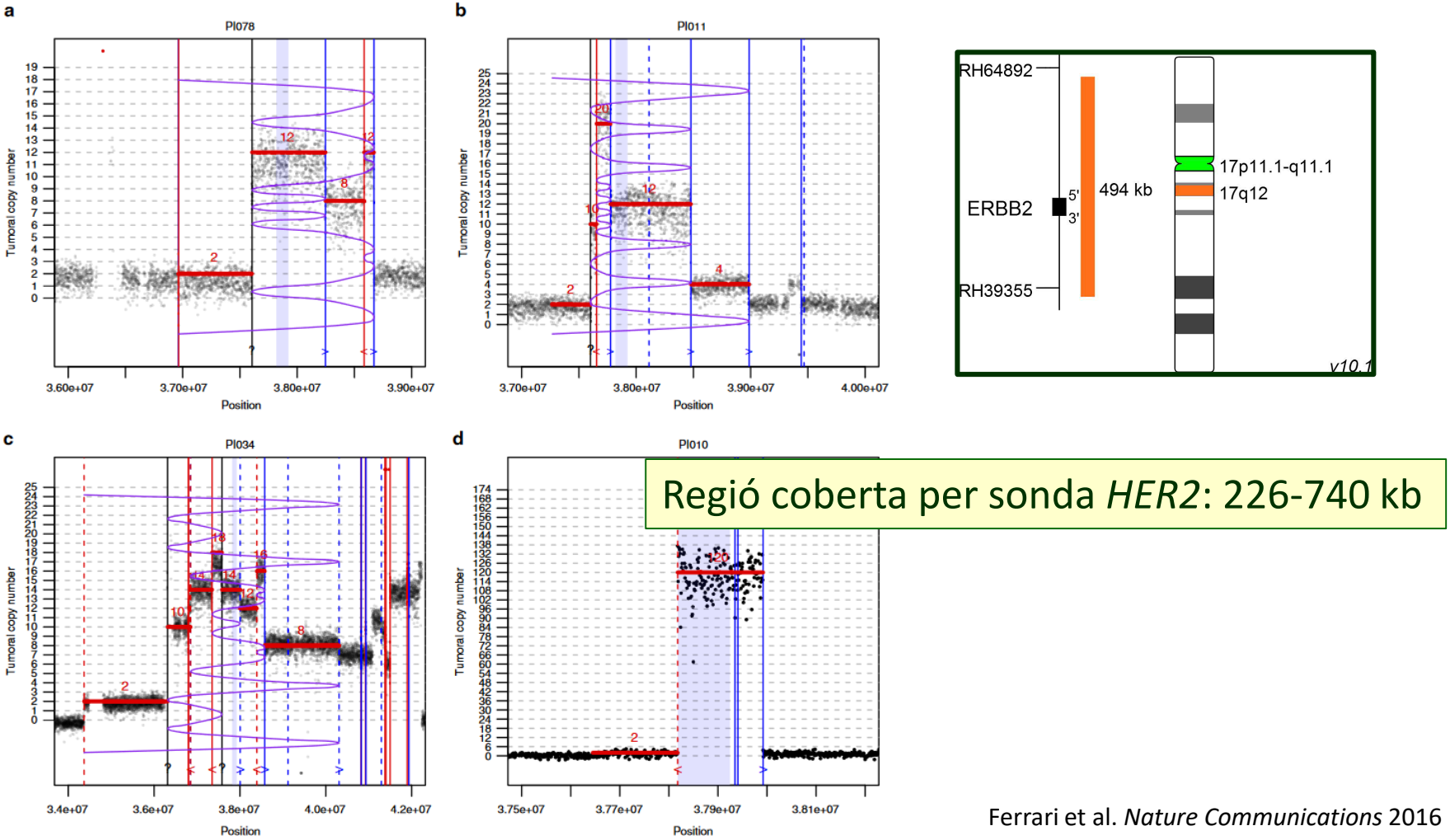


Ferrari et al. *Nature Communications* 2016





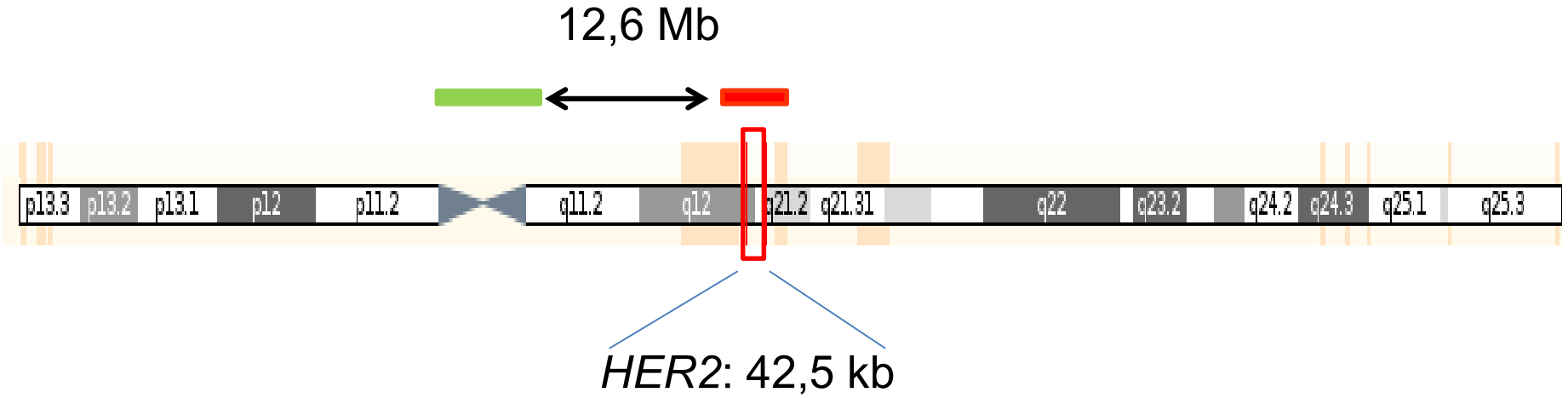
Ferrari et al. *Nature Communications* 2016



Regió coberta per sonda *HER2*: 226-740 kb

Ferrari et al. *Nature Communications* 2016

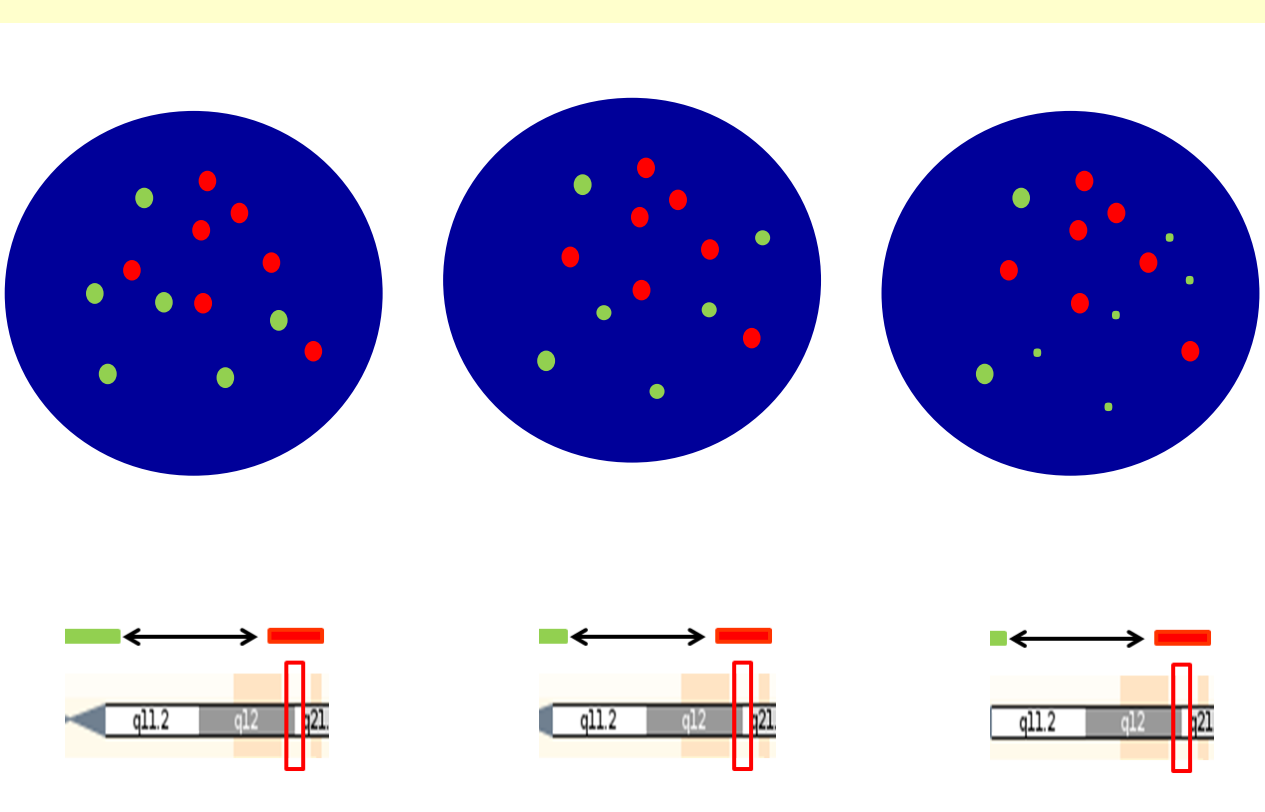
Amplicó *HER2* : 0,1 Mb-14,1 Mb (promig 1,4 Mb)

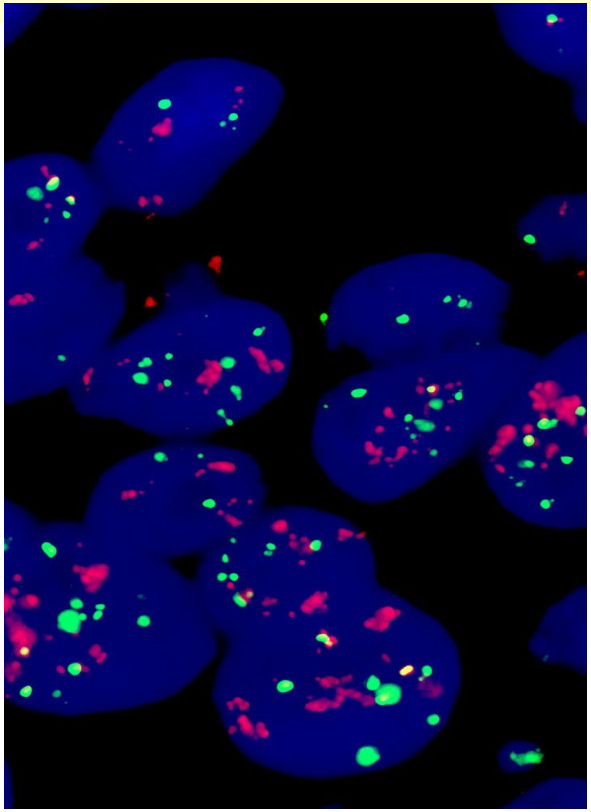


MRA (minimal region of amplification): 105 kb

Inclou altres gens (*TCAP, PMNT, PGAP3, ERBB2, MEN1, GRB7*)

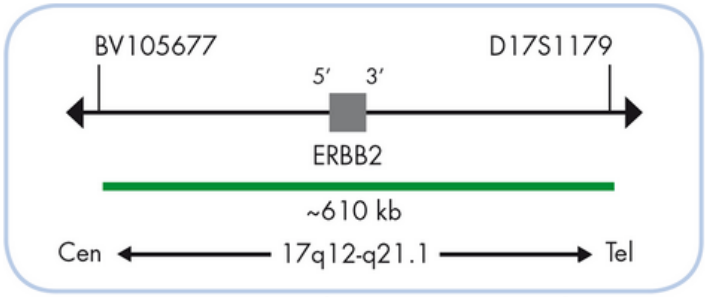
Coamplificació





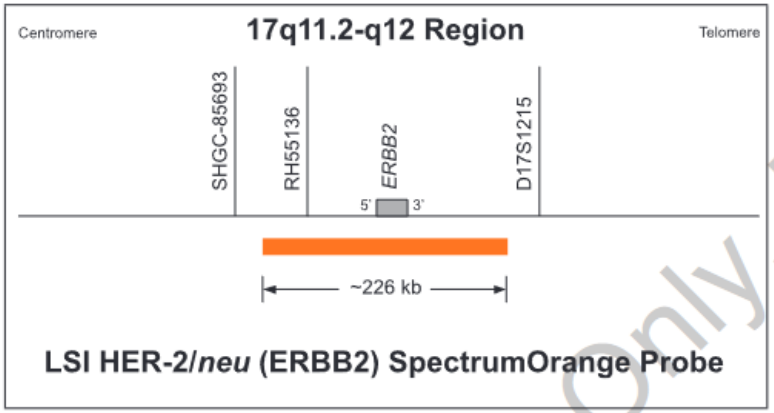
Fals negatiu si només considerem la ratio

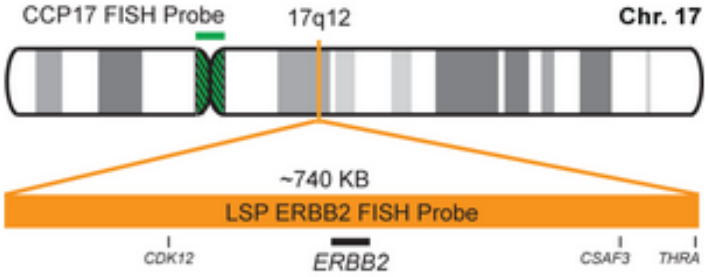
ZytoVision



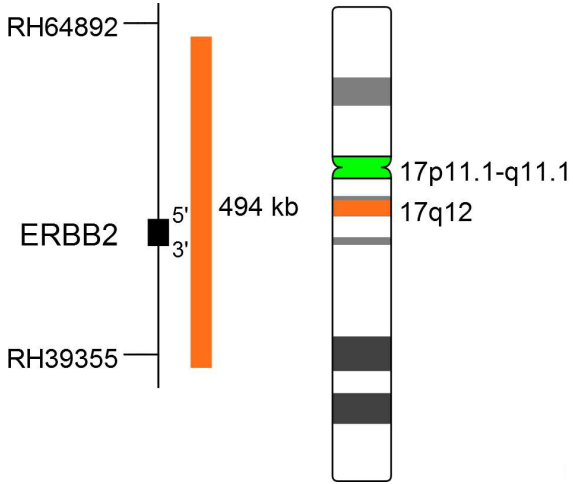
SPEC ERBB2 Probe map (not to scale).

Vysis

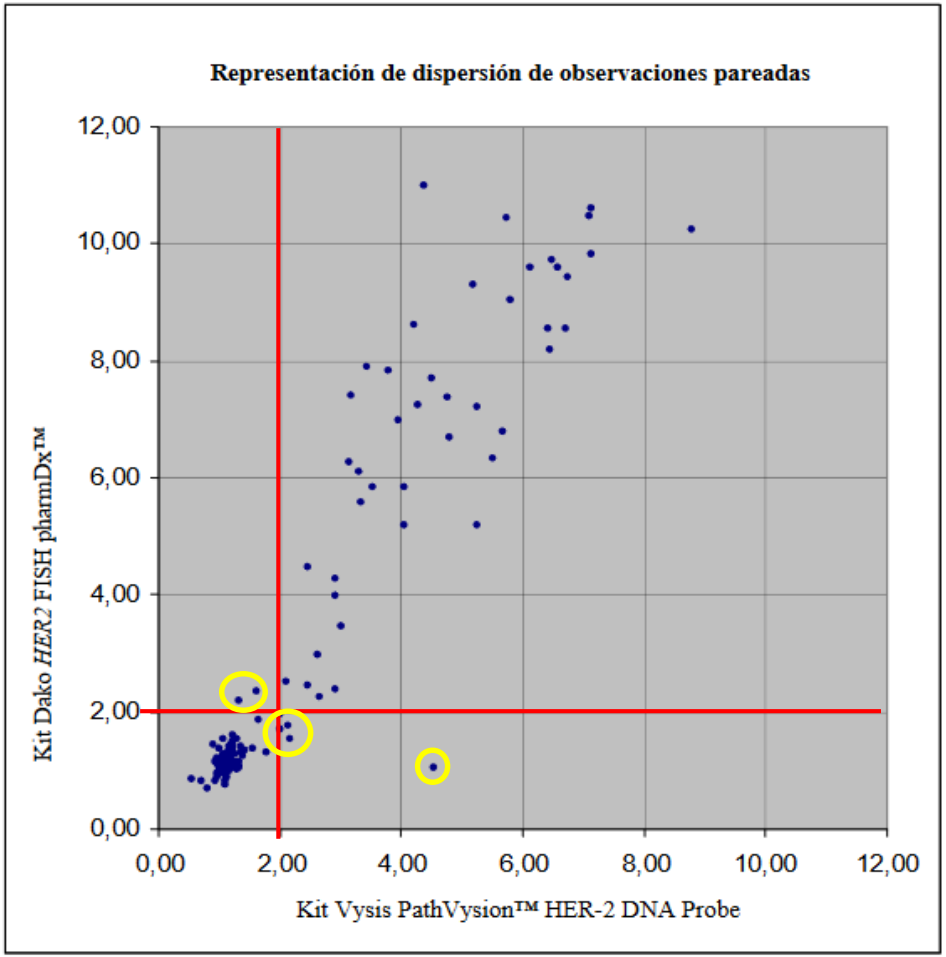




CytoTest



MetaSystems



How Do You Tell Whether a Breast Cancer is HER2 Positive? Ongoing Studies Keep Debate in High Gear

By Charlie Schmidt

"This is biology, not chemistry or physics, so there's going to be some variation and cases where you're on the **margins** with respect to making a decision. It's up to clinicians and pathologists to interpret these cases as accurately as possible, but there will always be some judgement involved"

MOLTES GRÀCIES PER LA VOSTRA ATENCIÓ!