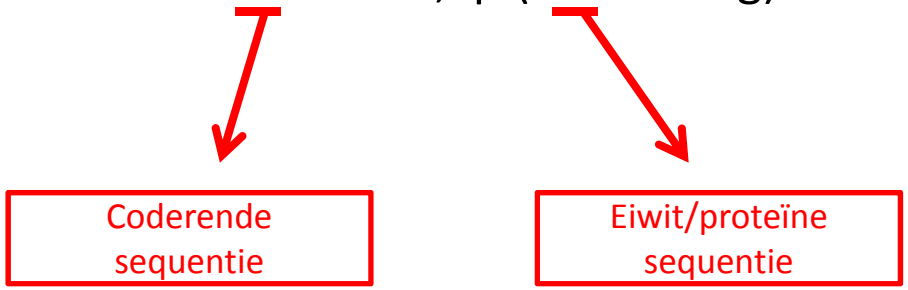
Molecular Pathology for Pathologists

Pr P. Pauwels

NGS – moleculair pathologie rapport ontcijferen

Nomenclatuur waarin gerapporteerd wordt:

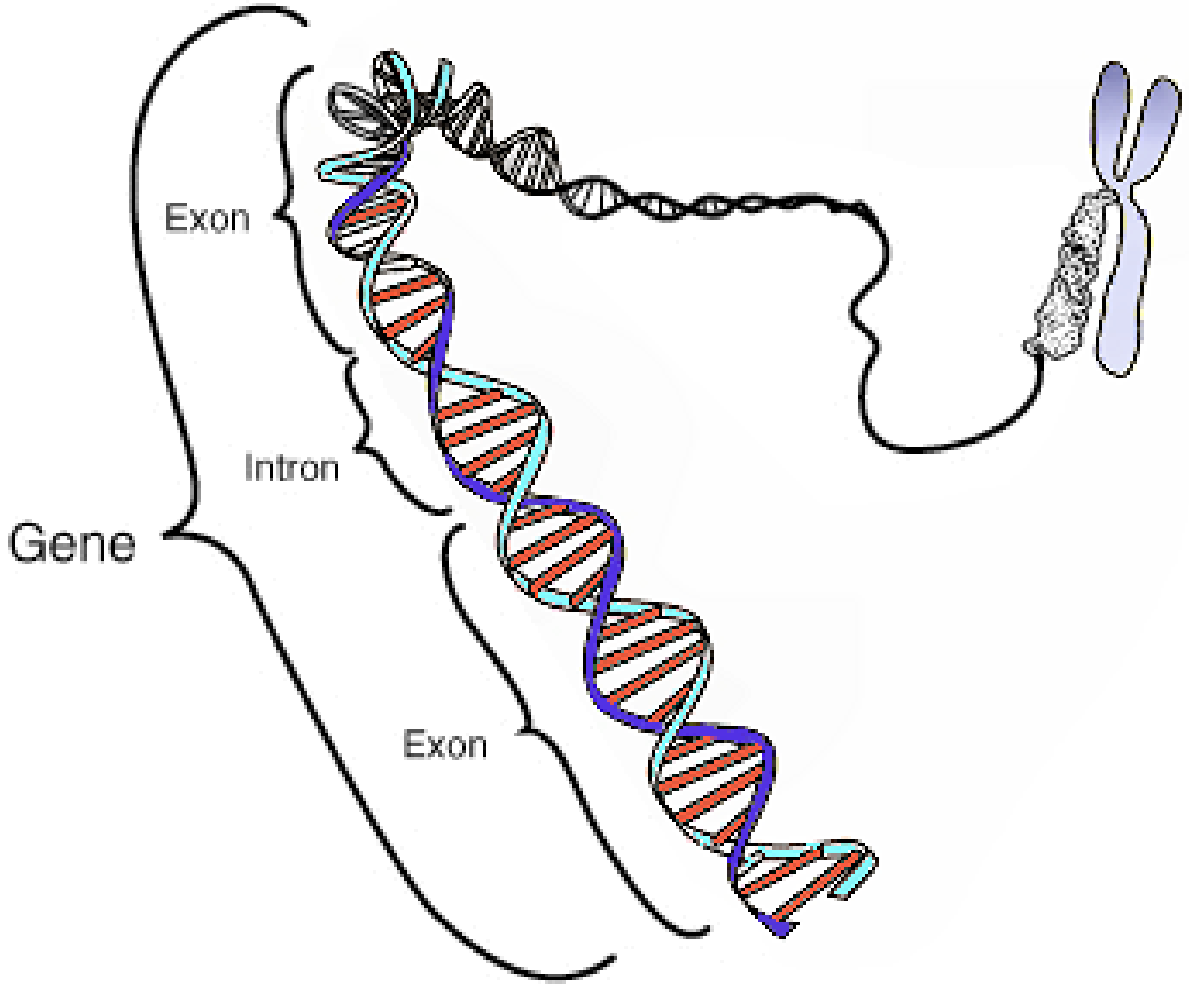
EGFR c.2573T>G, p.(Leu858Arg)

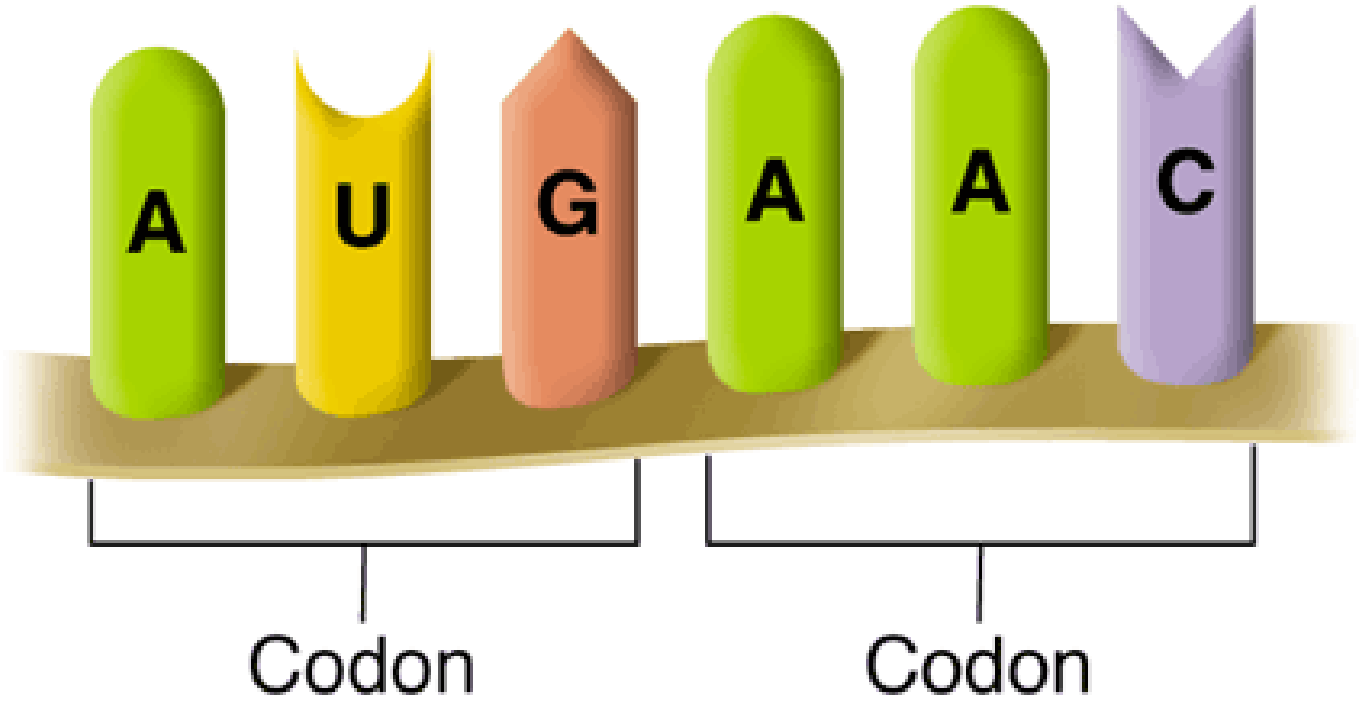


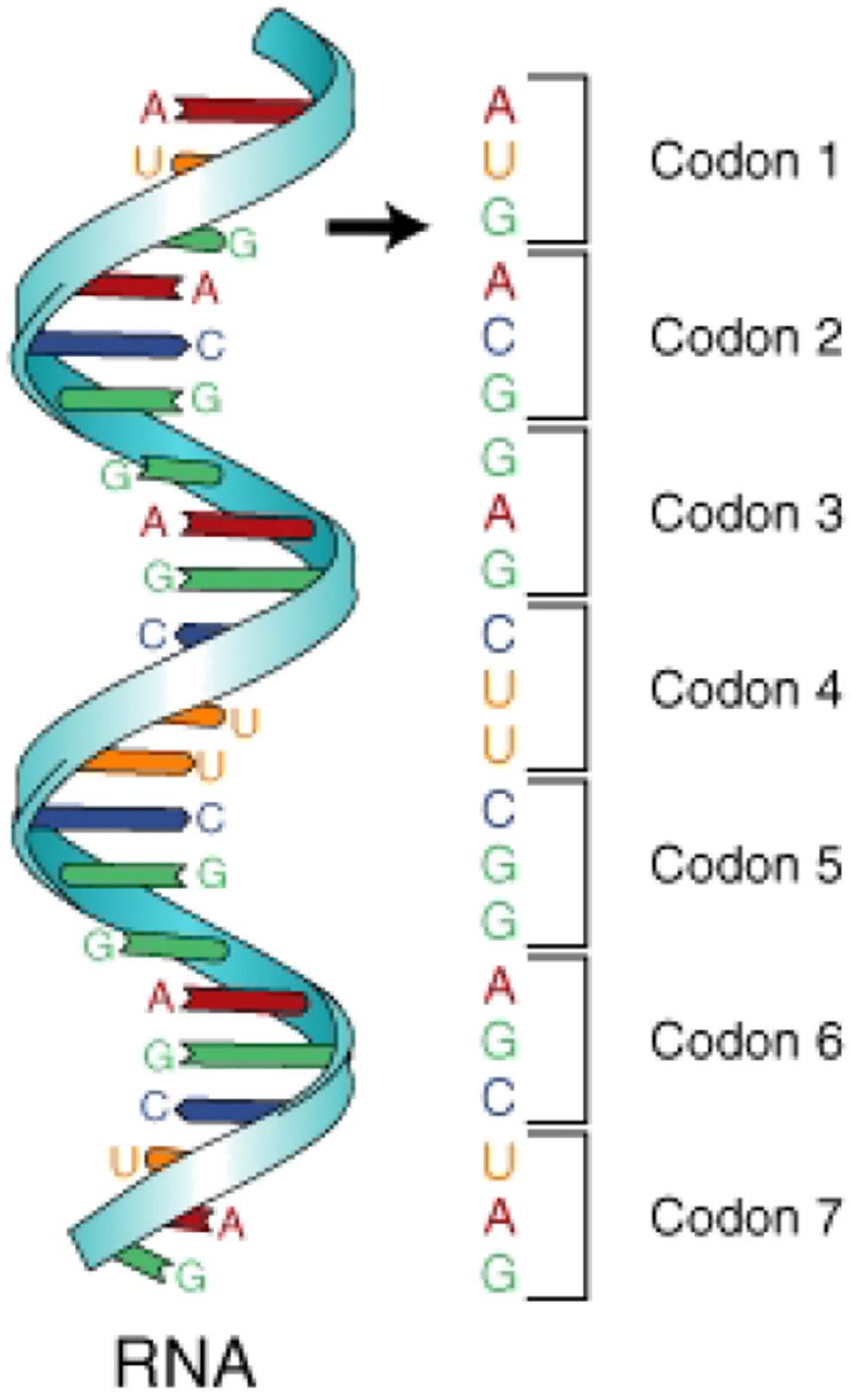
- Genomische DNA sequentie: intron + exon
- Coderende DNA sequentie: exonen only
- Eiwit sequentie : aminozuren sequentie bestaande uit een 3 lettercode (Leu ipv L)

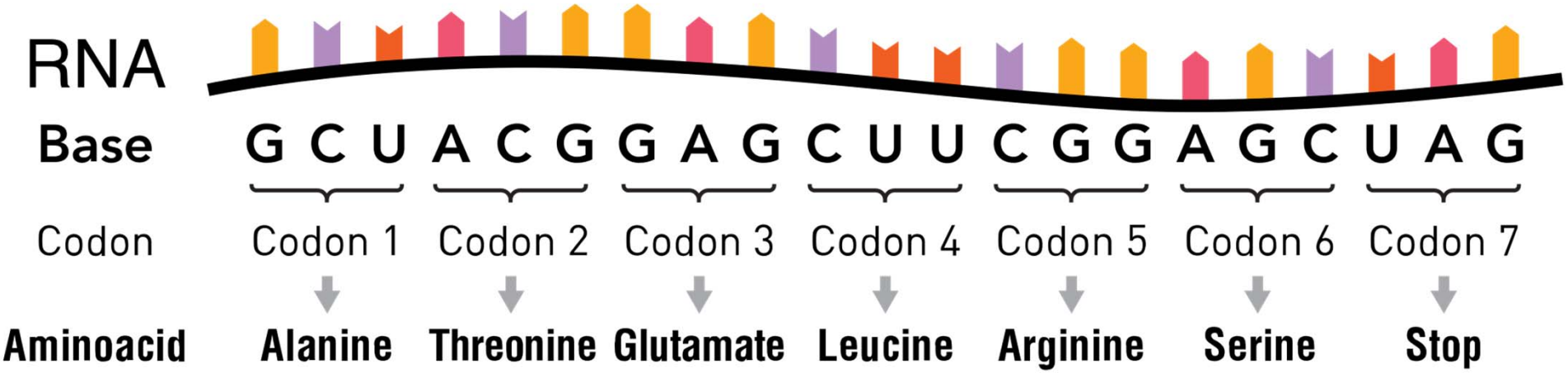
EGFR c.2235_2249del, p.(Glu746_Ala750del)

Maw, de officiële nomenclatuur refereert niet naar exonen, vandaar vele pathologie rapporten aangeven: Er werd door de test een mutatie aangetoond in exon 21 van het *EGFR* gen, nl c.2573T>G, p.(Leu858Arg).


Exon
Intron
Gene
Exon


A
U
G
A
A
C
Codon
Codon

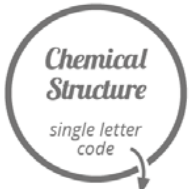

A
U
G
A
C
G
G
A
G
C
U
U
C
G
G
A
G
C
U
A
G
A
U
G
A
C
G
G
A
G
C
U
U
A
G
Codon 1
Codon 2
Codon 3
Codon 4
Codon 5
Codon 6
Codon 7
RNA


RNA
Base
G C U A C G G A G C U U C G G A G C U A G
Codon
Codon 1
Codon 2
Codon 3
Codon 4
Codon 5
Codon 6
Codon 7
Aminoacid
Alanine
Threonine
Glutamate
Leucine
Arginine
Serine
Stop

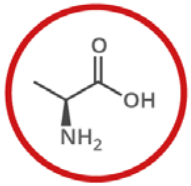
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

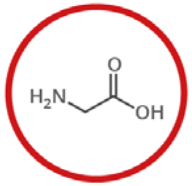
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



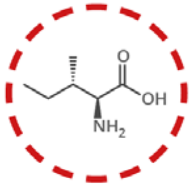
NAME A
three letter code
DNA codons



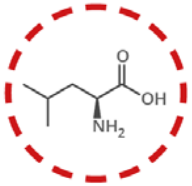
ALANINE A
Ala
GCT, GCC, GCA, GCG



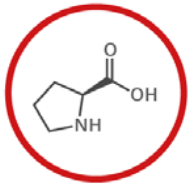
GLYCINE G
Gly
GGT, GGC, GGA, GGG



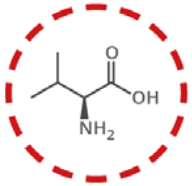
ISOLEUCINE I
Ile
ATT, ATC, ATA



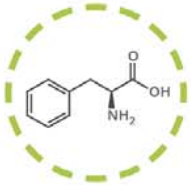
LEUCINE L
Leu
CTT, CTC, CTA, CTG, TTA, TTG



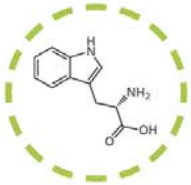
PROLINE P
Pro
CCT, CCC, CCA, CCG



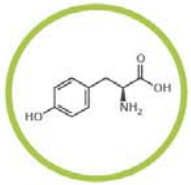
VALINE V
Val
GTT, GTC, GTA, GTG



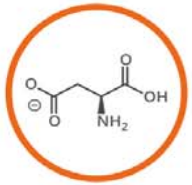
PHENYLALANINE F
Phe
TTT, TTC



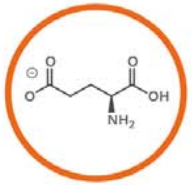
TRYPTOPHAN W
Trp
TGG



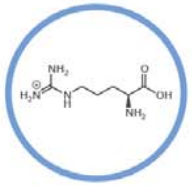
TYROSINE Y
Tyr
TAT, TAC



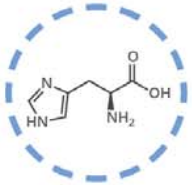
ASPARTIC ACID D
Asp
GAT, GAC



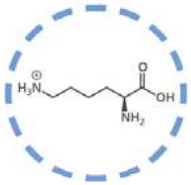
GLUTAMIC ACID E
Glu
GAA, GAG



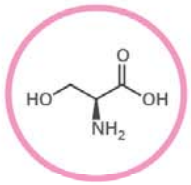
ARGININE R
Arg
CGT, CGC, CGA, CGG, AGA, AGG



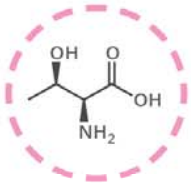
HISTIDINE H
His
CAT, CAC



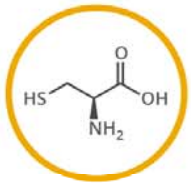
LYSINE K
Lys
AAA, AAG



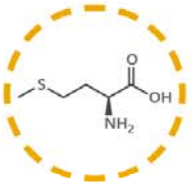
SERINE S
Ser
TCT, TCC, TCA, TCG, AGT, AGC



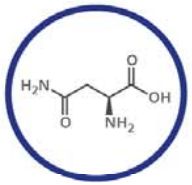
THREONINE T
Thr
ACT, ACC, ACA, ACG



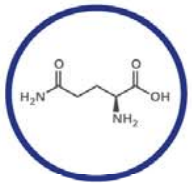
CYSTEINE C
Cys
TGT, TGC



METHIONINE M
Met
ATG



ASPARAGINE N
Asn
AAT, AAC



GLUTAMINE Q
Gln
CAA, CAG

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

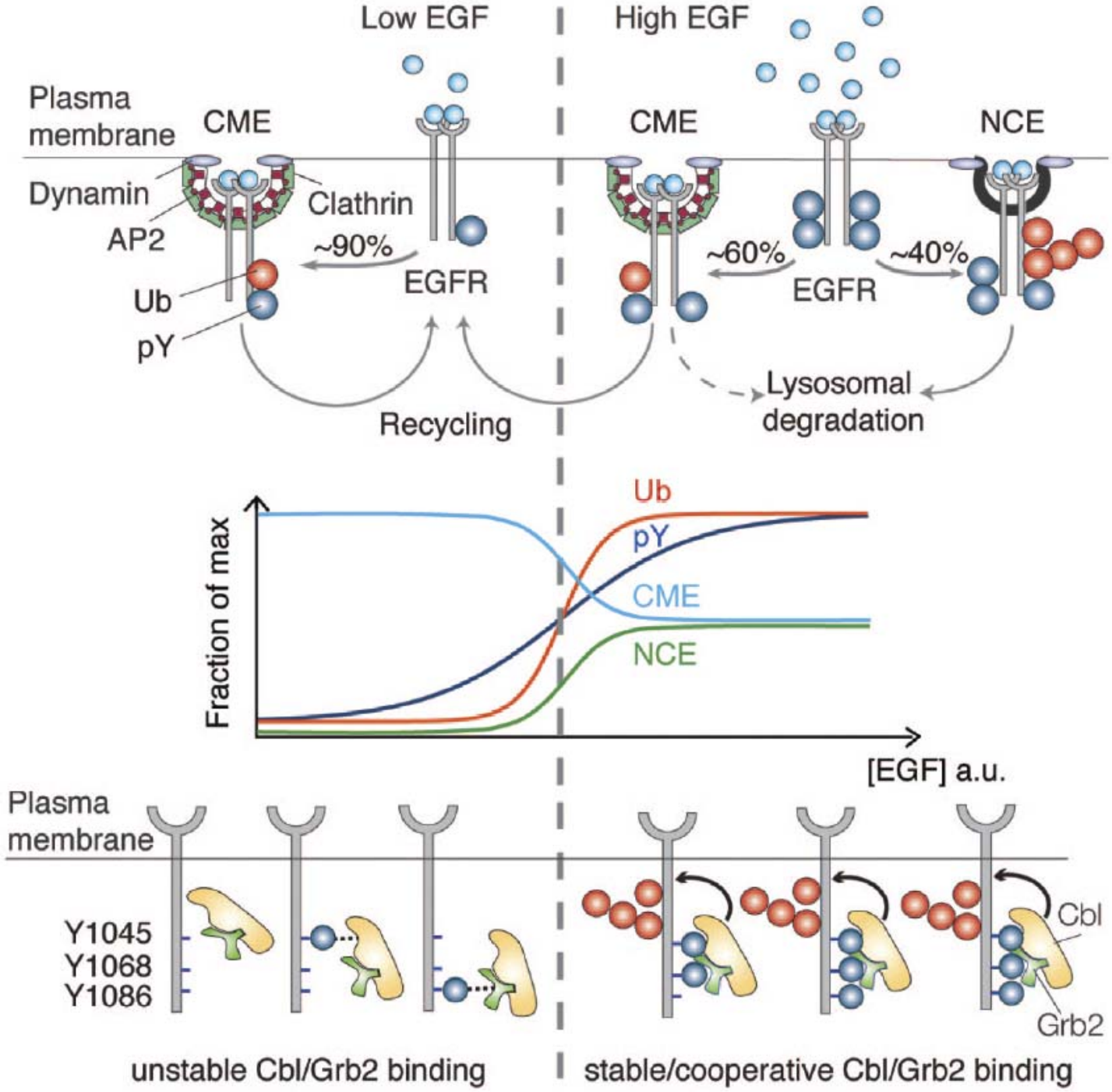
Cell Cycle 13:5, 1–2; March 1, 2014; © 2014 Landes Bioscience

Keeping EGFR signaling in check

Ubiquitin is the guardian

Simona Polo[1,2,*], Pier Paolo Di Fiore[1,2,3,*], and Sara Sigismund[1]

[1]IFOM, Fondazione Istituto FIRC di Oncologia Molecolare; Milan, Italy; [2]Dipartimento di Scienze della Salute; Università degli Studi di Milano; Milan, Italy; [3]Department of Experimental Oncology; Istituto Europeo di Oncologia; Milan, Italy


Low EGF
High EGF
Plasma membrane
CME
CME
NCE
Dynamin
AP2
Clathrin
~90%
EGFR
Ub
pY
~60%
EGFR
~40%
Lysosomal degradation
Recycling
Ub
pY
CME
NCE
Fraction of max
[EGF] a.u.
Plasma membrane
Y1045
Y1068
Y1086
Cbl
Grb2
unstable Cbl/Grb2 binding
stable/cooperative Cbl/Grb2 binding

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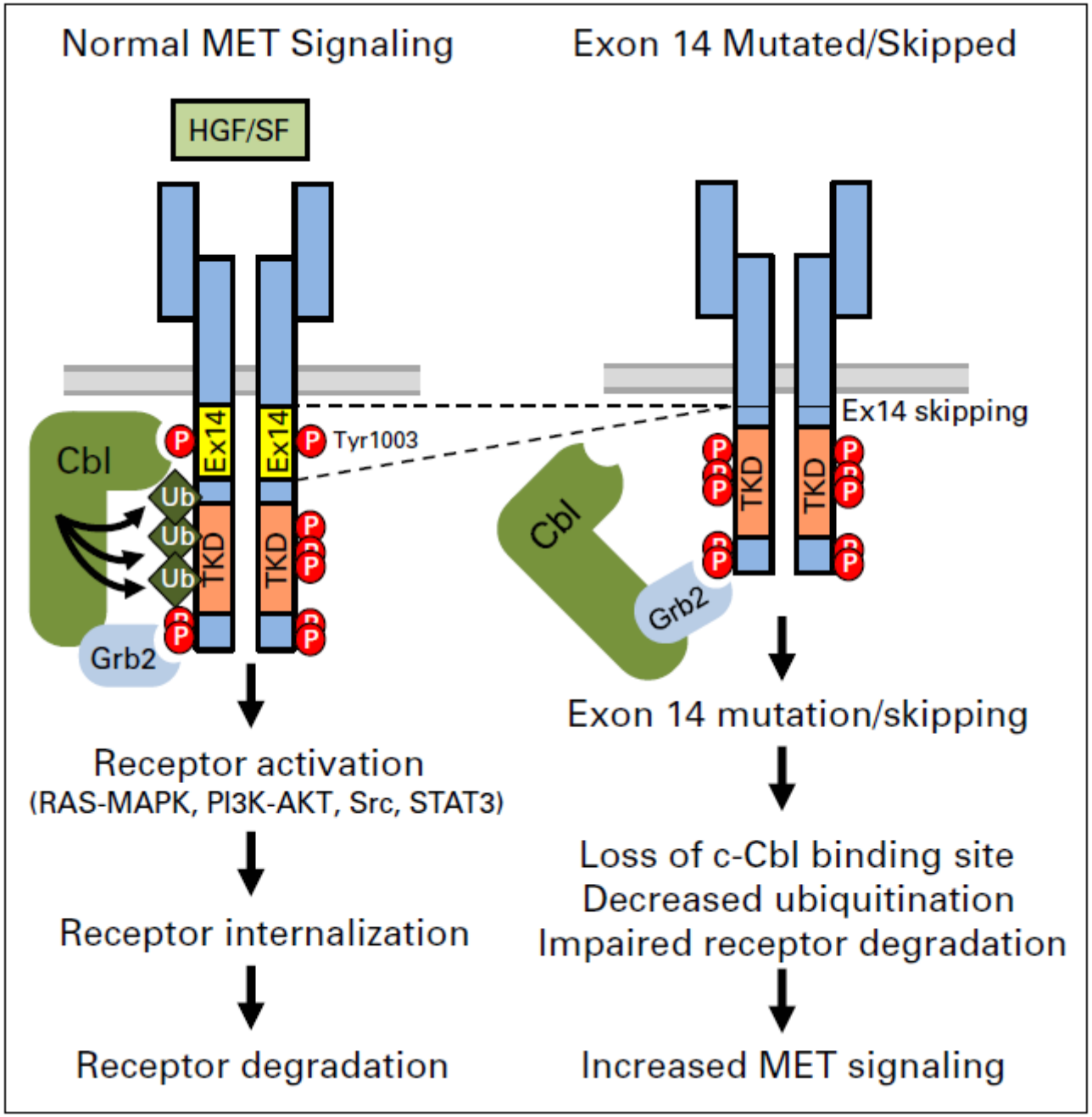
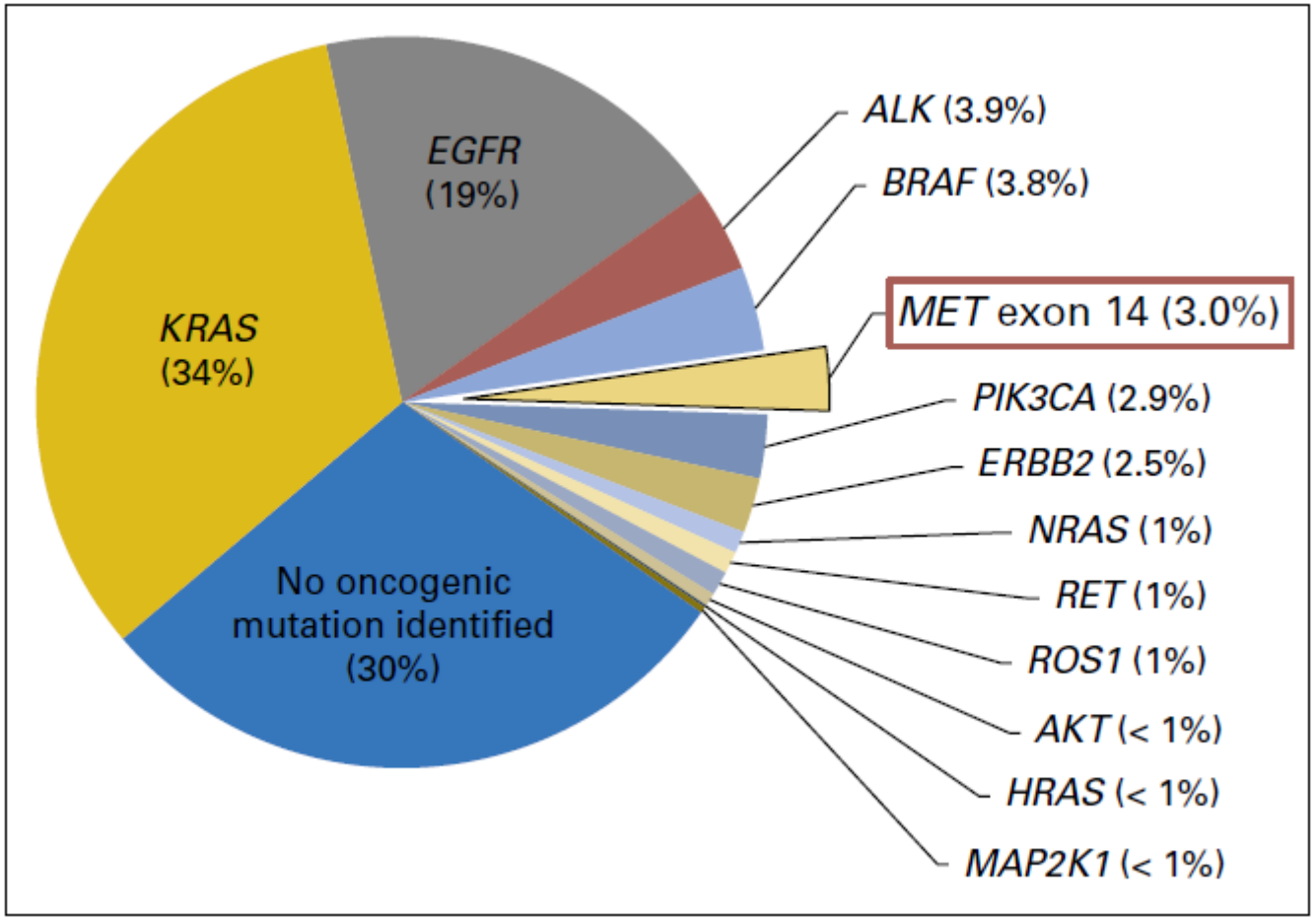
JOURNAL OF CLINICAL ONCOLOGY

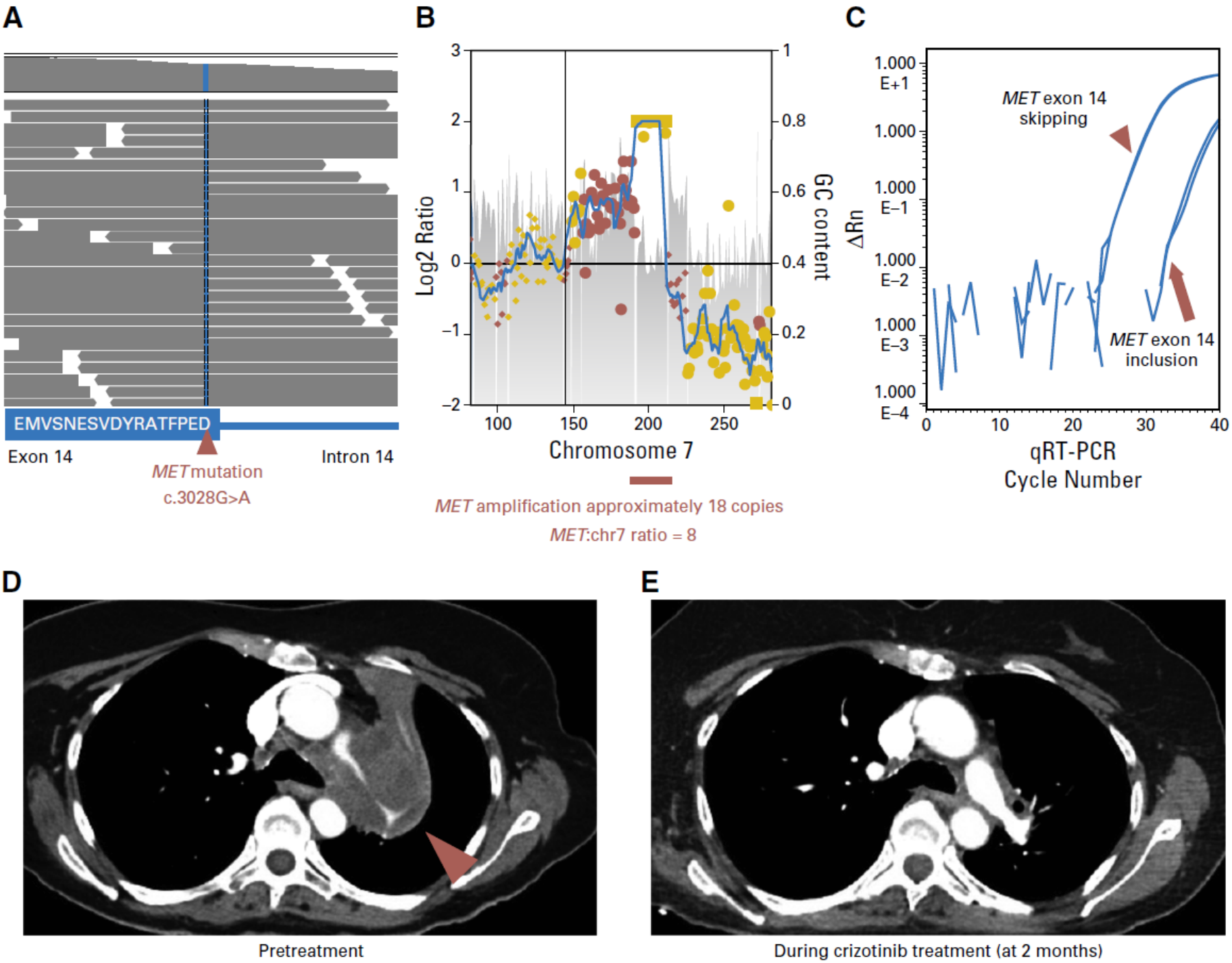
UNDERSTANDING THE PATHWAY

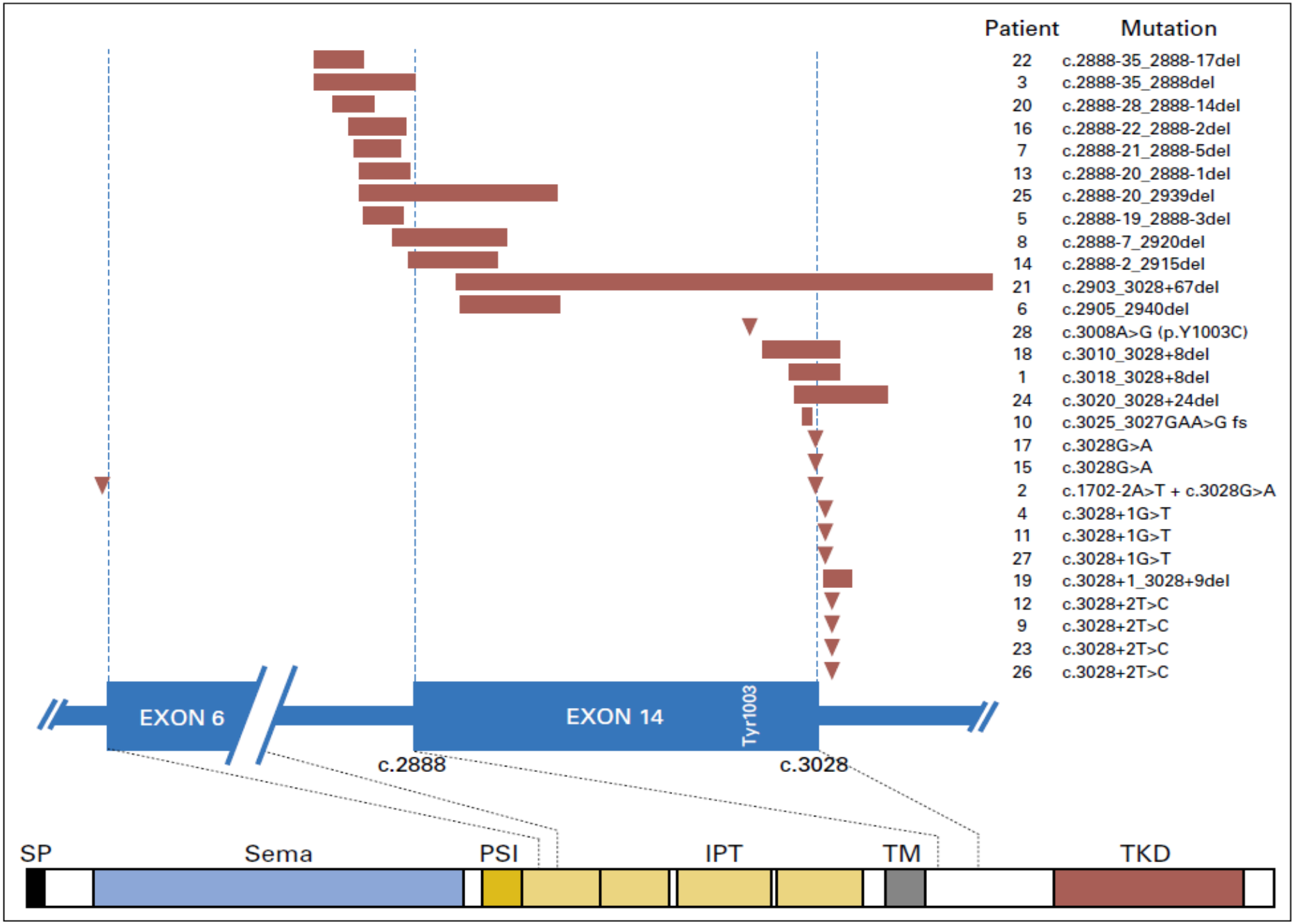
Impaired c-Met Receptor Degradation Mediated by *MET* Exon 14 Mutations in Non–Small-Cell Lung Cancer

Mark M. Awad, *Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, MA*


Normal MET Signaling
Exon 14 Mutated/Skipped
HGF/SF
Cbl
Ex14
Ex14
Tyr1003
Ub
Ub
Ub
TKD
TKD
Grb2
Ex14 skipping
TKD
TKD
Cbl
Grb2
Receptor activation
(RAS-MAPK, PI3K-AKT, Src, STAT3)
Receptor internalization
Receptor degradation
Exon 14 mutation/skipping
Loss of c-Cbl binding site
Decreased ubiquitination
Impaired receptor degradation
Increased MET signaling

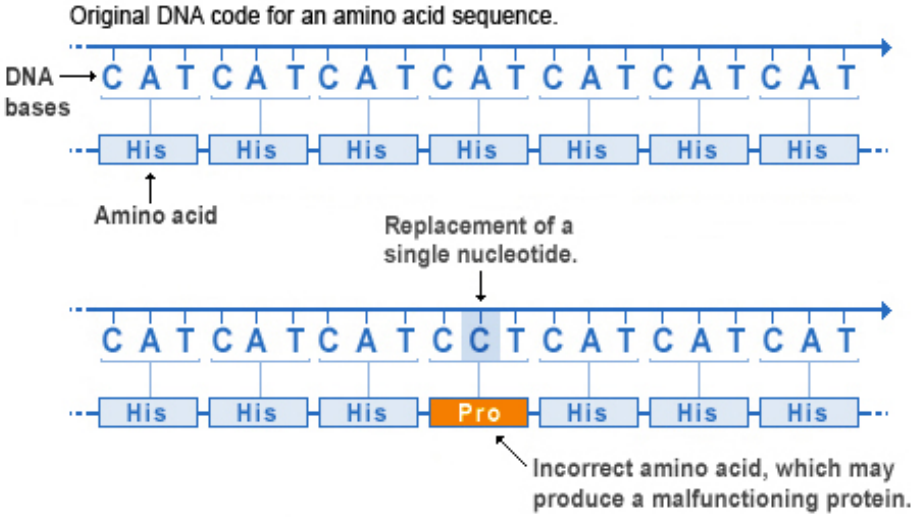

EGFR
(19%)
KRAS
(34%)
No oncogenic
mutation identified
(30%)
ALK (3.9%)
BRAF (3.8%)
MET exon 14 (3.0%)
PIK3CA (2.9%)
ERBB2 (2.5%)
NRAS (1%)
RET (1%)
ROS1 (1%)
AKT (< 1%)
HRAS (< 1%)
MAP2K1 (< 1%)


A
EMVSNESVDYRATFPED
Exon 14
Intron 14
MET mutation
c.3028G>A
B
Log2 Ratio
GC content
Chromosome 7
MET amplification approximately 18 copies
MET:chr7 ratio = 8
C
ΔRn
MET exon 14
skipping
MET exon 14
inclusion
qRT-PCR
Cycle Number
D
Pretreatment
E
During crizotinib treatment (at 2 months)


Patient
Mutation
22 c.2888-35_2888-17del
3 c.2888-35_2888del
20 c.2888-28_2888-14del
16 c.2888-22_2888-2del
7 c.2888-21_2888-5del
13 c.2888-20_2888-1del
25 c.2888-20_2939del
5 c.2888-19_2888-3del
8 c.2888-7_2920del
14 c.2888-2_2915del
21 c.2903_3028+67del
6 c.2905_2940del
28 c.3008A>G (p.Y1003C)
18 c.3010_3028+8del
1 c.3018_3028+8del
24 c.3020_3028+24del
10 c.3025_3027GAA>G fs
17 c.3028G>A
15 c.3028G>A
2 c.1702-2A>T + c.3028G>A
4 c.3028+1G>T
11 c.3028+1G>T
27 c.3028+1G>T
19 c.3028+1_3028+9del
12 c.3028+2T>C
9 c.3028+2T>C
23 c.3028+2T>C
26 c.3028+2T>C
EXON 6
EXON 14
Tyr1003
c.2888
c.3028
SP
Sema
PSI
IPT
TM
TKD

NGS – moleculair pathologie rapport ontcijferen

Soorten mutaties:



1 Silent mutatie

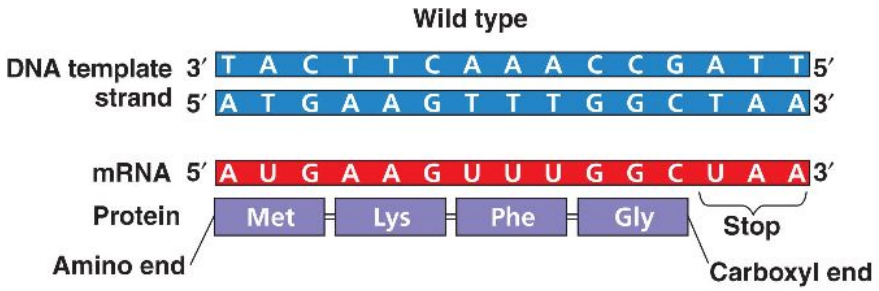
2 Nonsense mutatie

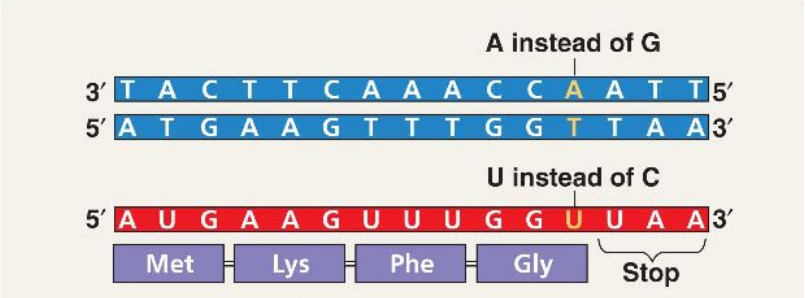
3 Missense mutatie

Mutatie van DNA veroorzaakt een verandering in de aminozuur sequentie (eiwit)

NGS – moleculair pathologie rapport ontcijferen

Soorten mutaties:





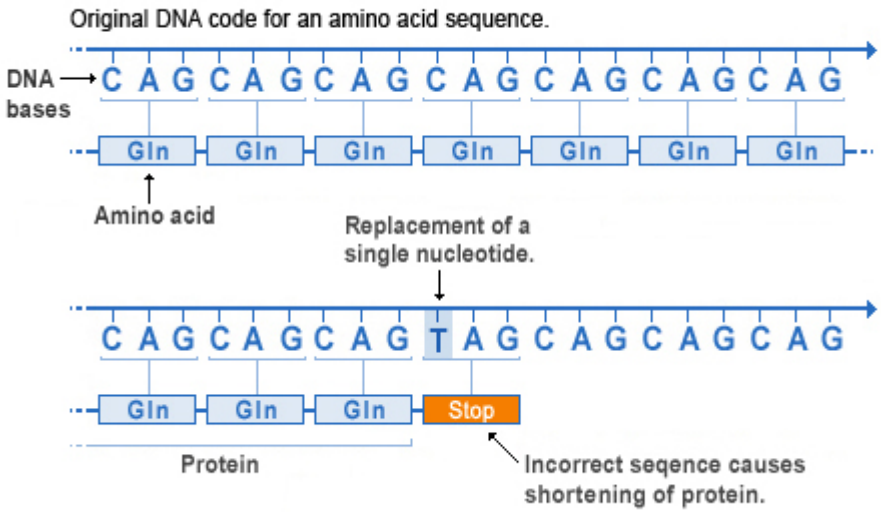
1 Silent mutatie — Mutatie van DNA veroorzaakt geen verandering in de aminozuur sequentie (eiwit)

2 Nonsense mutatie

3 Missense mutatie — Mutatie van DNA veroorzaakt een verandering in de aminozuur sequentie (eiwit)

NGS – moleculair pathologie rapport ontcijferen

Soorten mutaties:



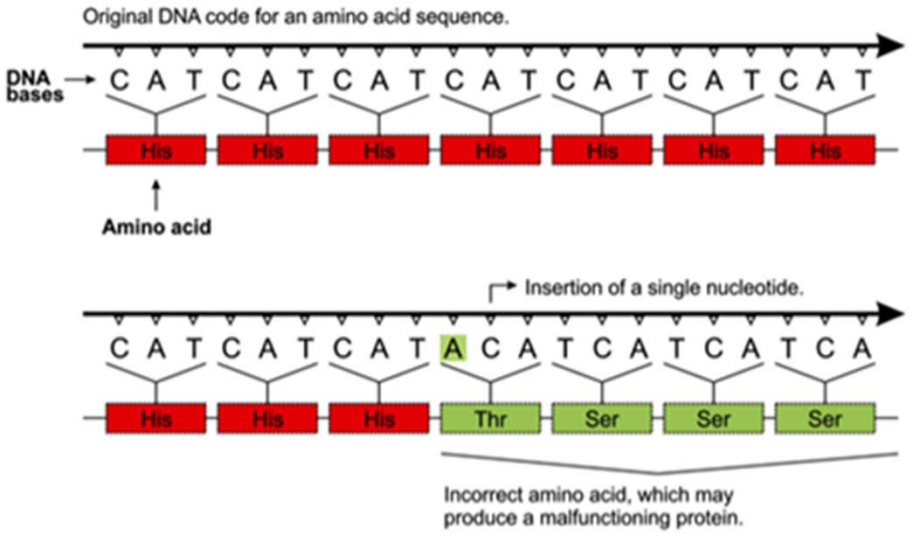
1 Silent mutatie — Mutatie van DNA veroorzaakt geen verandering in de aminozuur sequentie (eiwit)

2 Nonsense mutatie — Mutatie van DNA veroorzaakt een STOP van de aminozuur sequentie (= verkort eiwit)

3 Missense mutatie — Mutatie van DNA veroorzaakt een verandering in de aminozuur sequentie (eiwit)

NGS – moleculair pathologie rapport ontcijferen

Soorten mutaties:

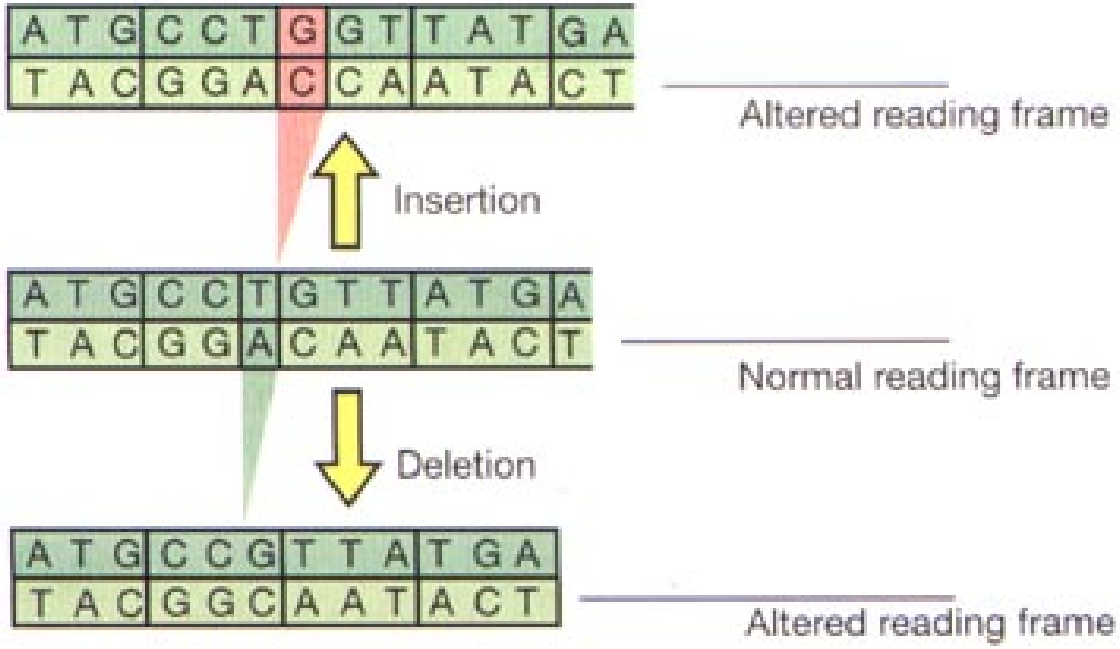


1 Silent mutatie — Mutatie van DNA veroorzaakt geen verandering in de aminozuur sequentie (eiwit)

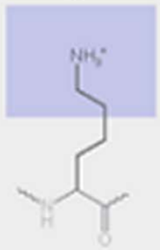
2 Nonsense mutatie — Mutatie van DNA veroorzaakt een STOP van de aminozuur sequentie (= verkort eiwit)

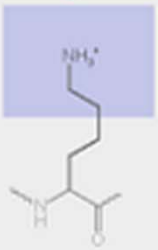
3 Missense mutatie — Mutatie van DNA veroorzaakt een verandering in de aminozuur sequentie (eiwit)

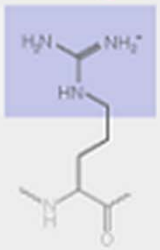
4 Frameshift mutatie — Insertie of deletie (n ≠ 3*x) van nucleotides op DNA niveau veroorzaakt een shift bij vertaling naar de aminozuur sequentie (eiwit)

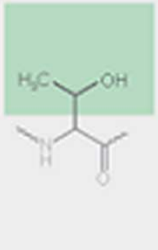

ATGCCTGGTTATGA
TACGGACCAATACT
Altered reading frame
Insertion
ATGCCTGTTATGA
TACGGACAATACT
Normal reading frame
Deletion
ATGCCGTTATGA
TACGGCAATACT
Altered reading frame

	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	**Lys**	**Lys**	**STOP**	**Arg**	**Thr**





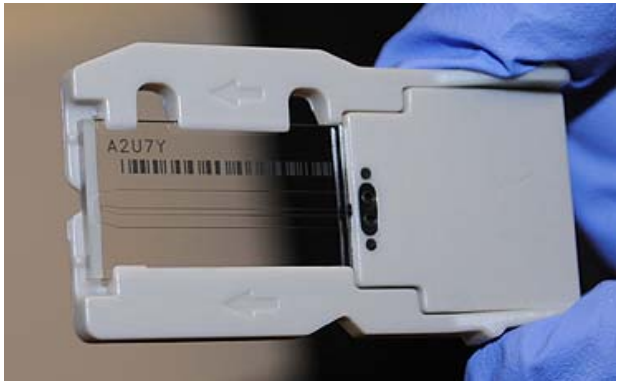


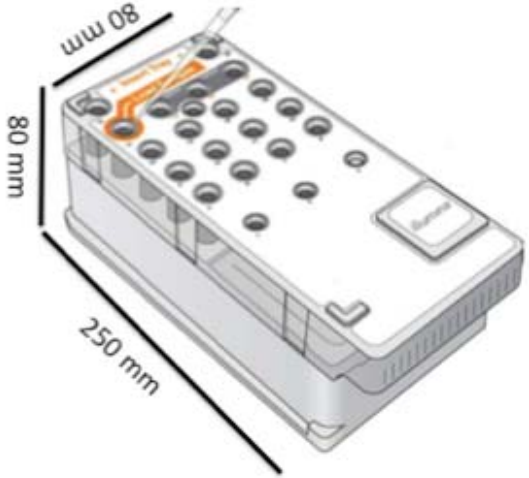


basic
polar

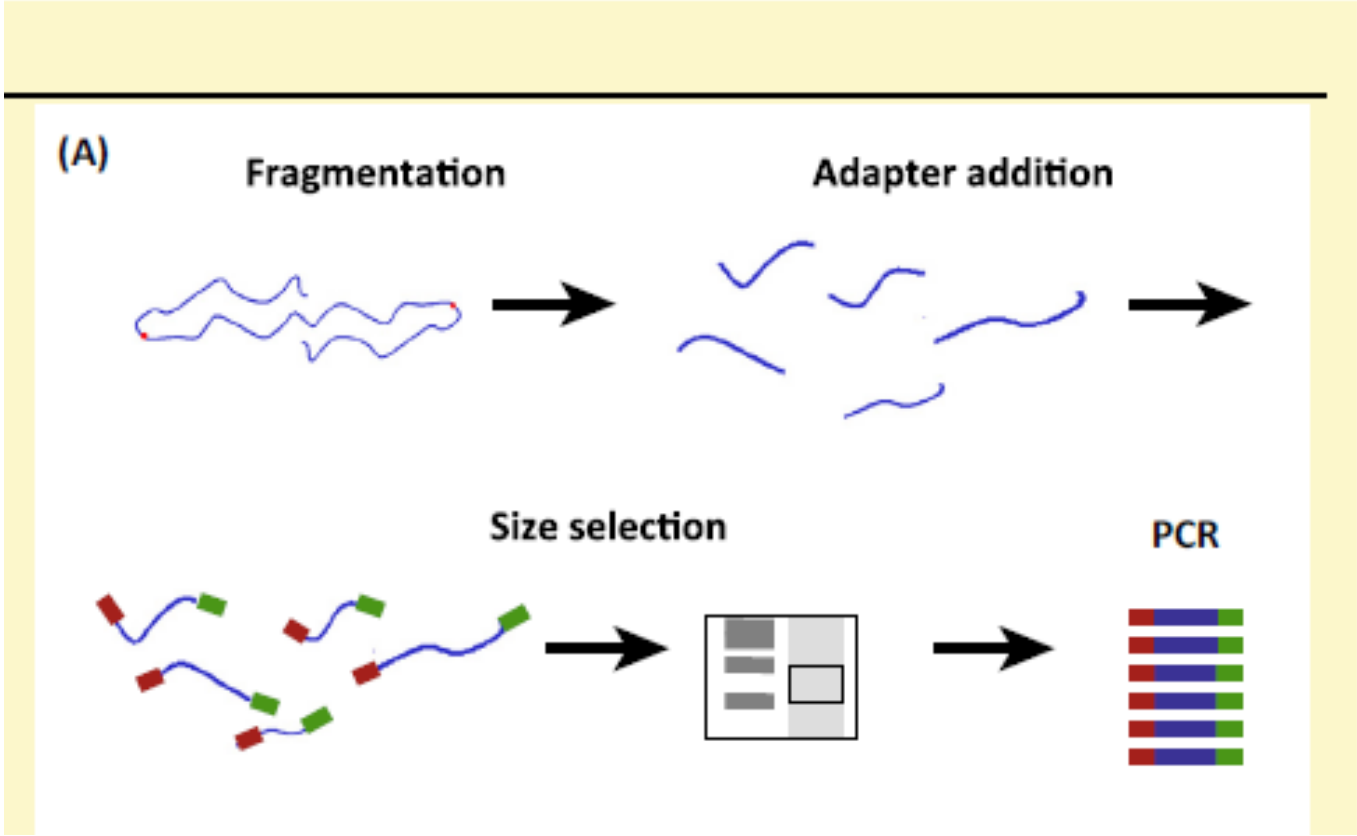
NGS – Targeted resequencing van gDNA

STAP 3: Alle stalen samen op een flow cell brengen
→ bridging amplificatie





UZA

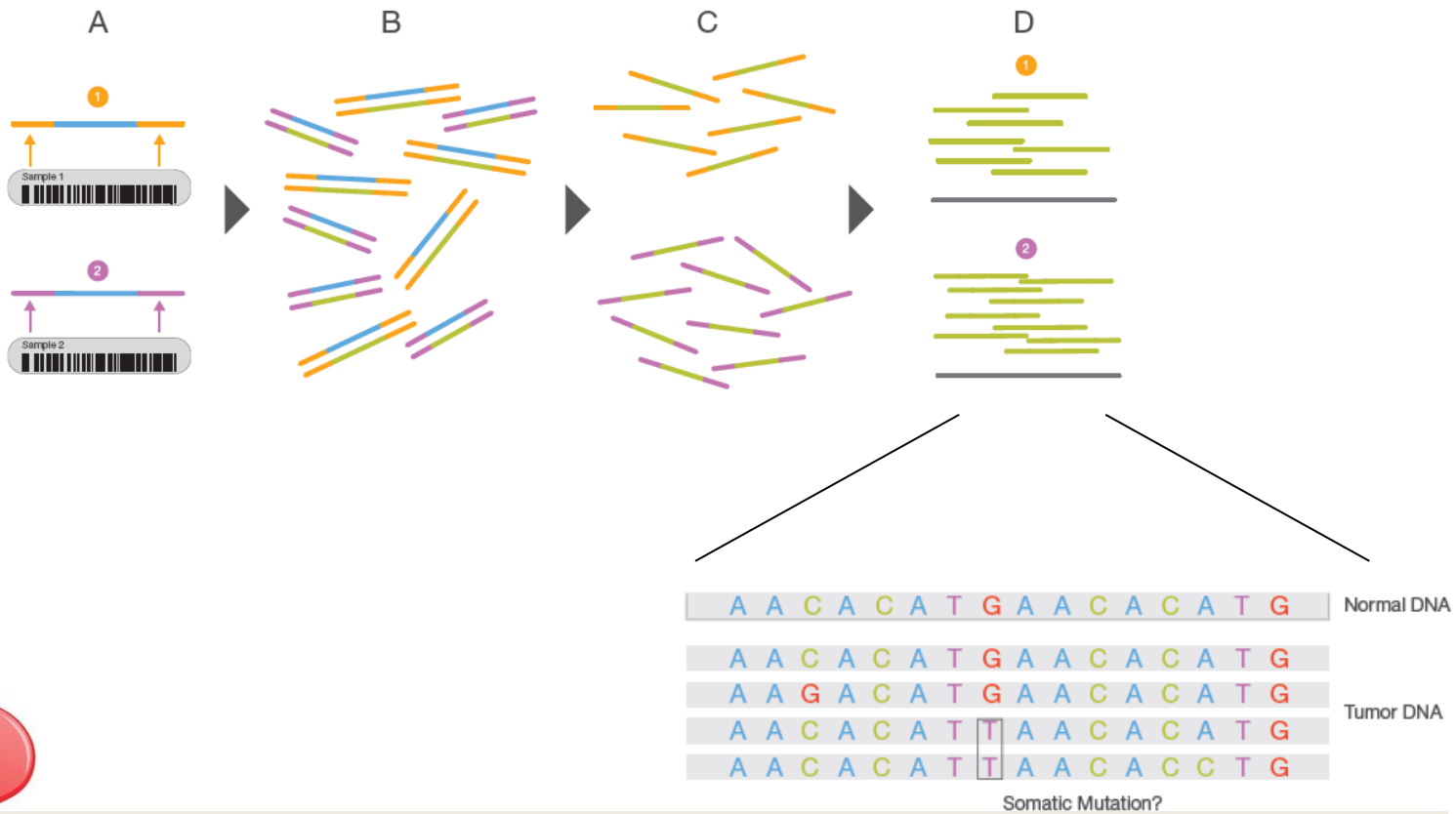

(A)
Fragmentation
Adapter addition
Size selection
PCR

NGS – Targeted resequencing van gDNA

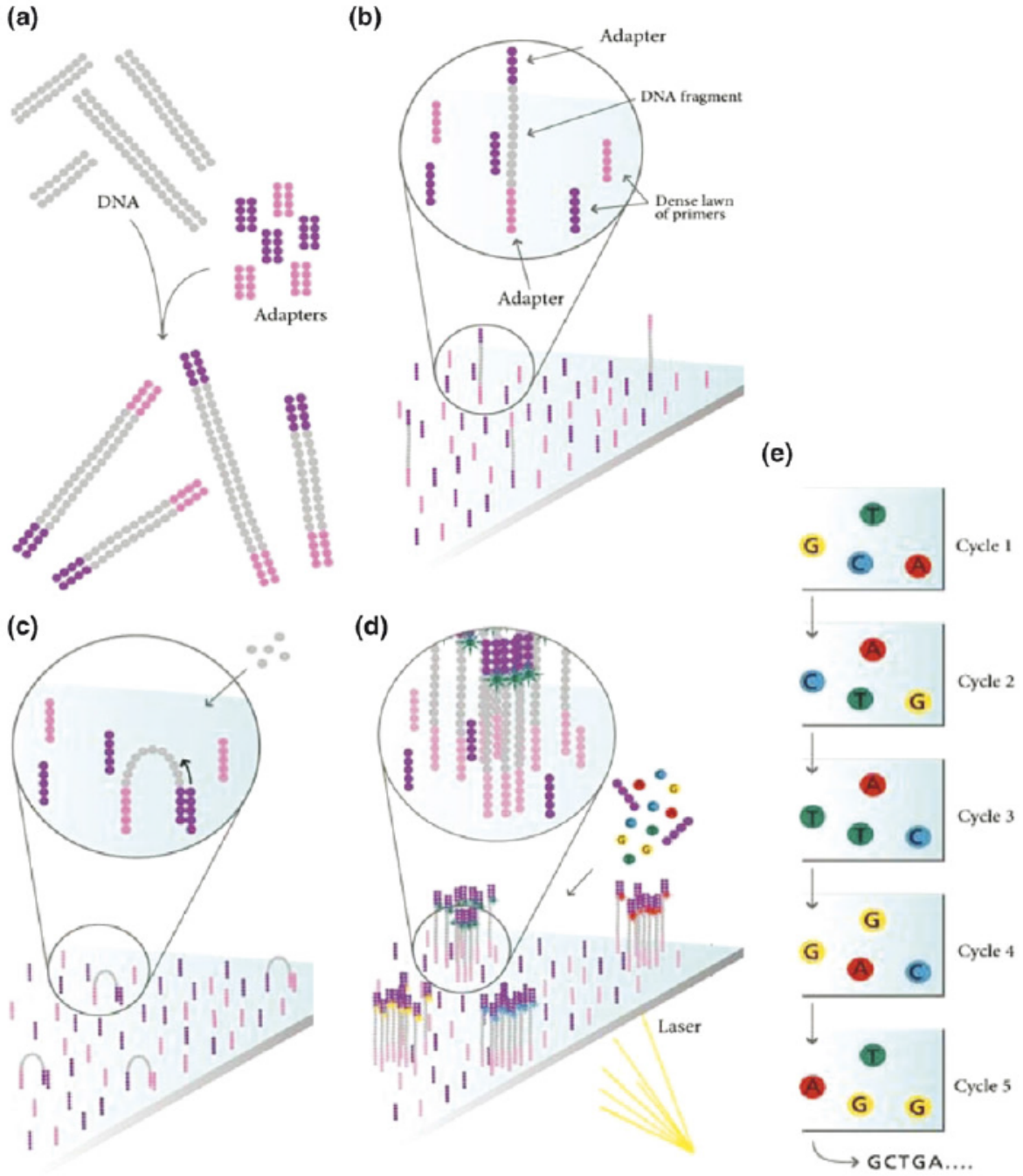
STAP 4: data analyse :

Demultiplexing: via barcode patiënten terug uit elkaar halen

Uitlijnen tov een referentie sequentie



Waarom is deze strategie niet zo geschikt om amplificatie van volledige genen te detecteren?

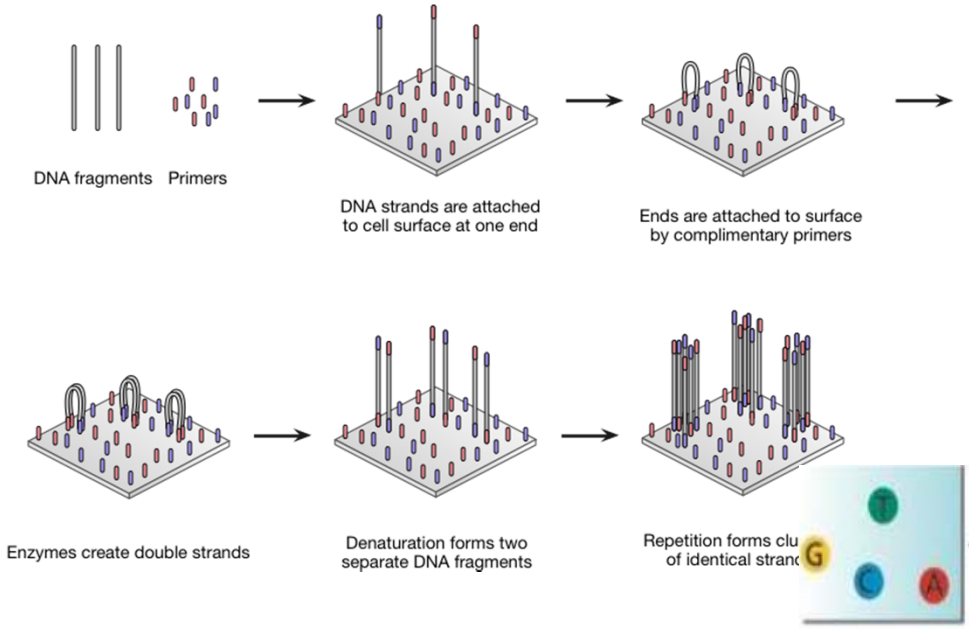


Drug Discovery Today

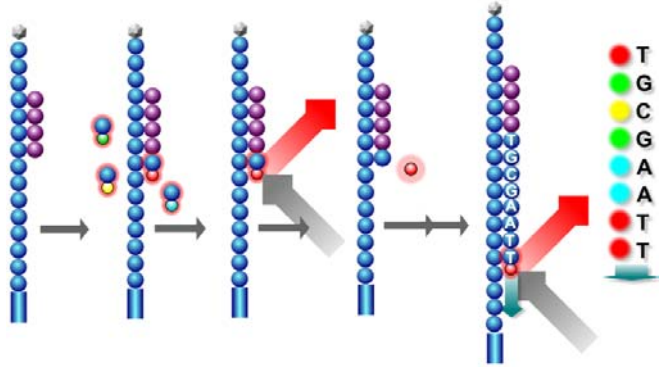
NGS – Targeted resequencing van gDNA

STAP 3: Alle stalen samen op een flow cell brengen

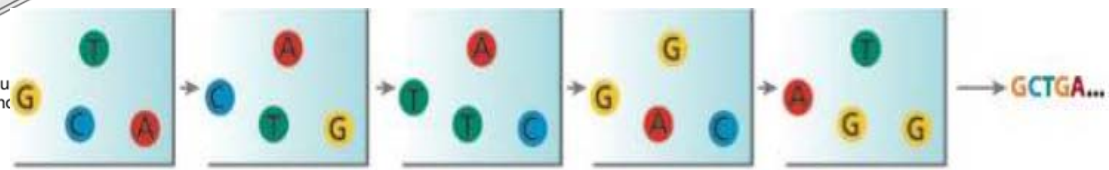
Op de flowcell worden meerdere cluster gemaakt adh bridging amplification



Per cluster: sequencing by synthesis



Een foto van iedere cluster (tijdens het sequencing by synthesis)



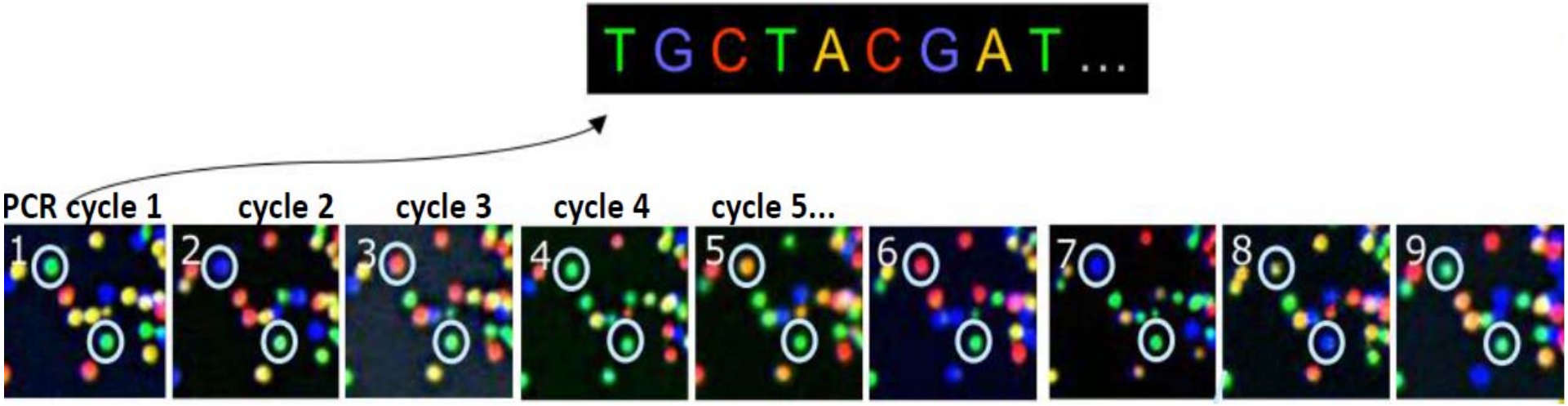
http://www.illumina.com/technology/next-generation-sequencing/sequencing-technology.ilmn

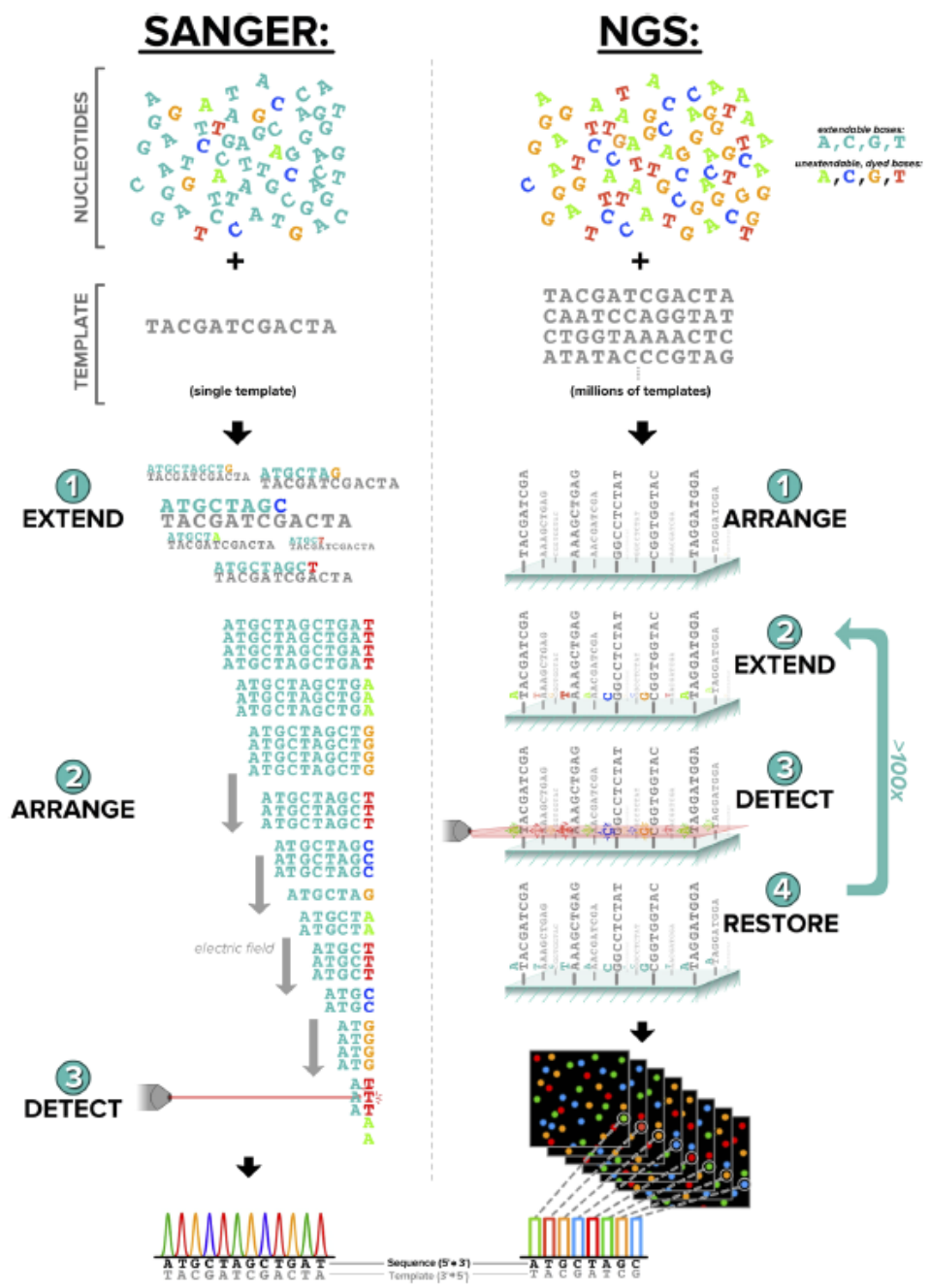
Determine the full sequence by base calling

Each PCR cycle: image tells you if A / C / G / T was incorporated in that cycle

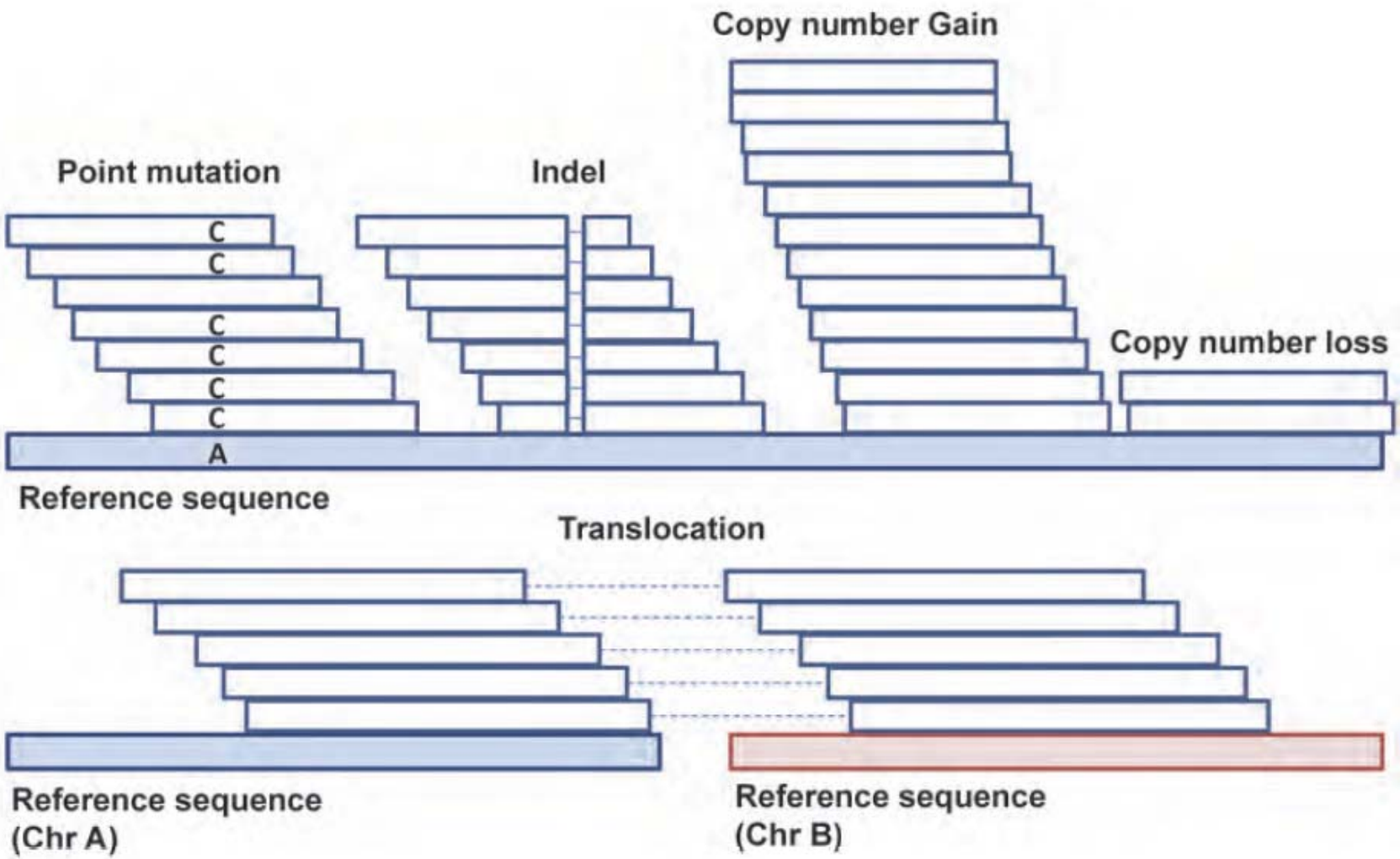
Done by Illumina software on sequencing instrument

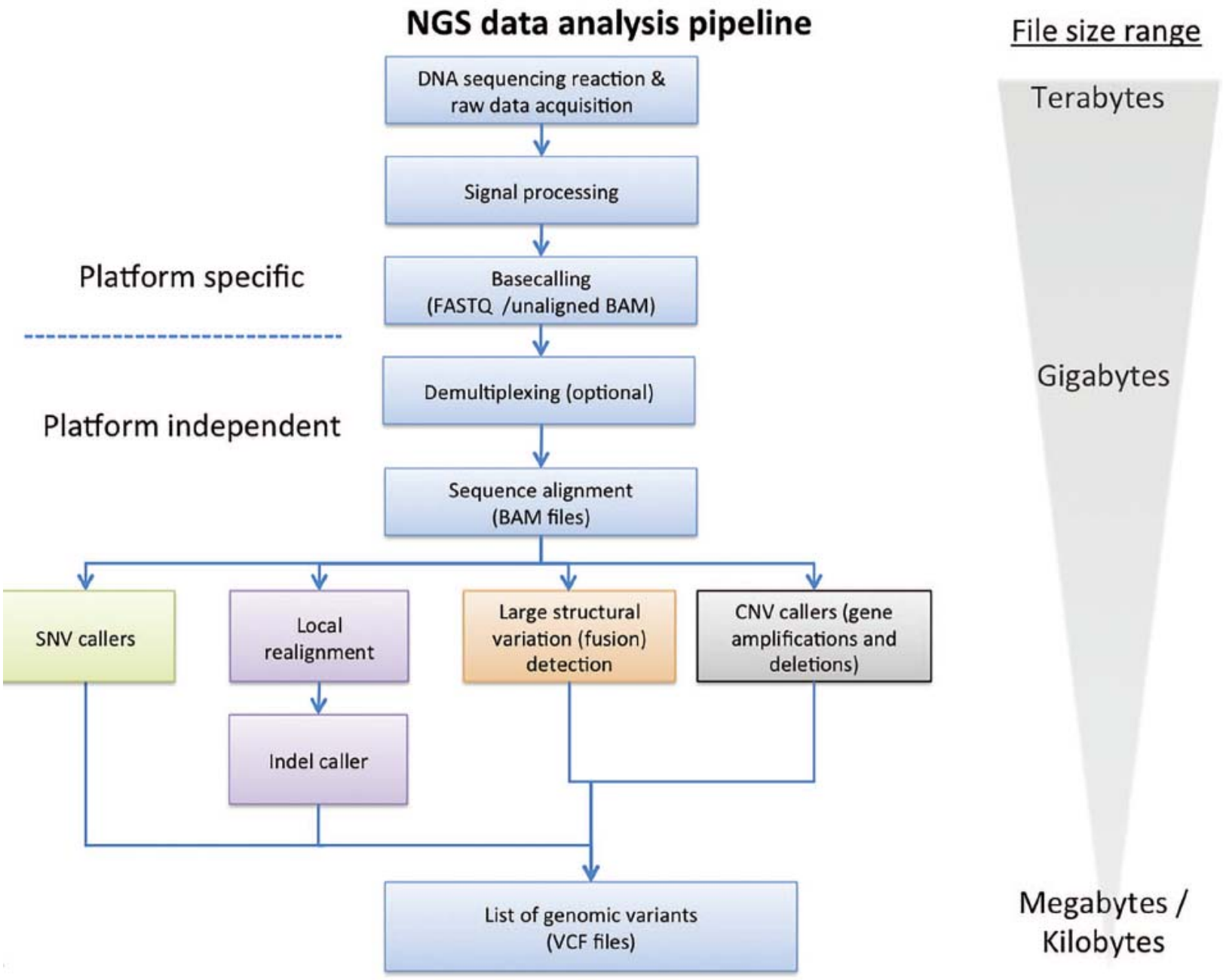
Possible for **fragments up to 150 nucleotides**



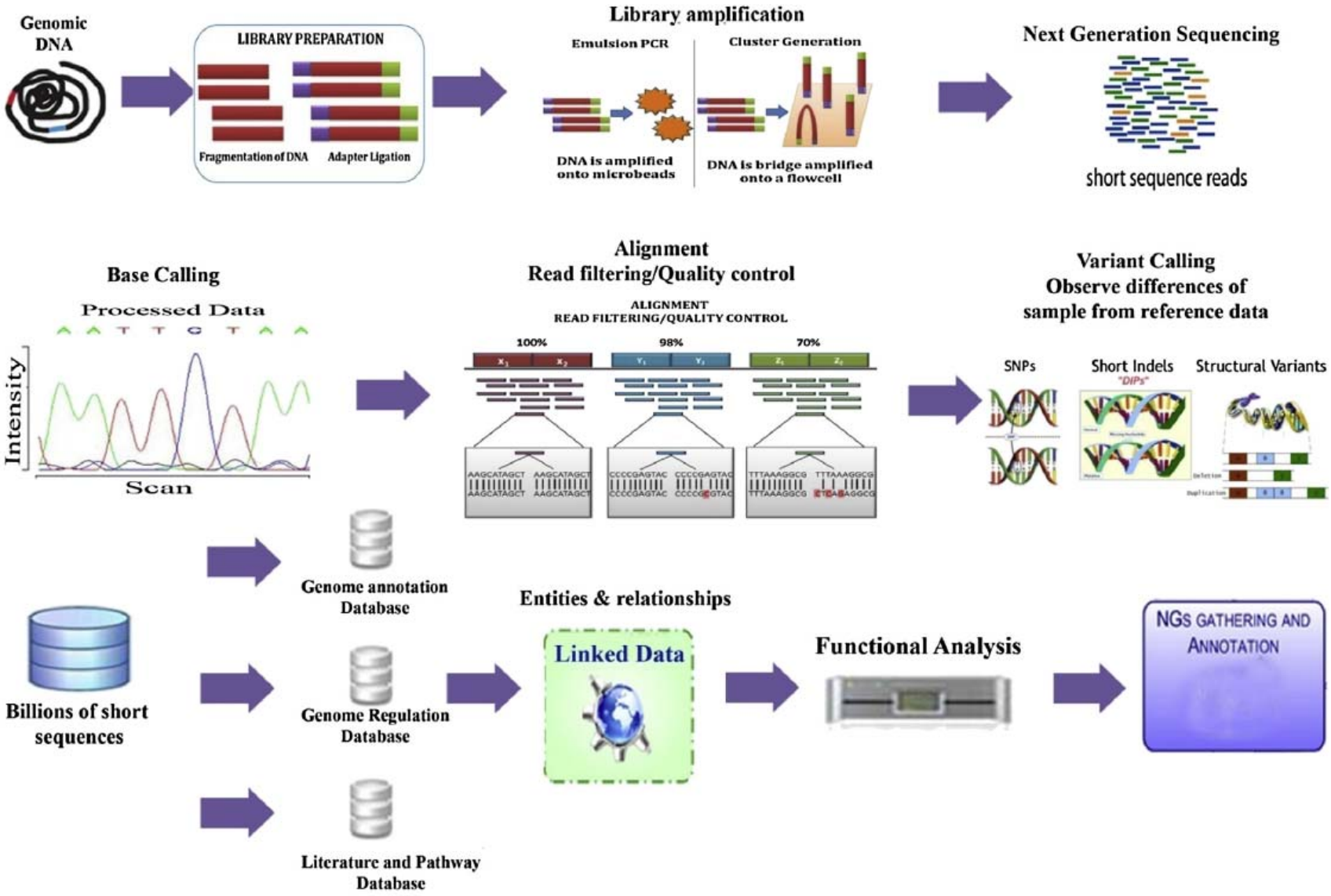

SANGER:
NGS:
NUCLEOTIDES
TEMPLATE
TACGATCGACTA
(single template)
TACGATCGACTA
CAATCCAGGTAT
CTGGTAAAACTC
ATATACCCGTAG
(millions of templates)
extendable bases:
A,C,G,T
unextendable, dyed bases:
A,C,G,T
1 EXTEND
2 ARRANGE
3 DETECT
electric field
1 ARRANGE
2 EXTEND
3 DETECT
4 RESTORE
>100x
Sequence (5'→3')
Template (3'←5')


A
GENOME REFERENCE
(3,000,000,000 bases)
...TGAAAAACTTGATTCGACACCATCAGAATTGAGACTAATCGGCTAGACATACG...
NGS READS
...TGA CTTGATTCGACACCAGCAGAATTGAGA CGGCTAGAC-TACG...
AAACTTGATTCGACACCATCAGAATTGA ATCGGCTAGACATACG...
TCGACACCATCAGAATTGAGACTAATCG GAC-TACG...
AAAAACTTGATTCGACACCAGCAGAATT AGACTAATCGGCTAGACATACG...
...TGAAAAACTTGATTCGACACCATCA ACTAATCGGCTAGAC-TACG...
AACTTGATTCGACACCAGCAGAATTGAG CTAGACATACG...
GATTCGACACCAGCAGAATTGAGACTAA GCTAGAC-TACG...
CTTGATTCGACACCATCAGAATTGAGTCG TAGAC-TACG...
AAAAACTTGATTCGACACCAGCAGAATT CATACG...
TGAAAAACTTGATTCGACACCATCAGAATTGAG AATCGGCTAGACATACG...
3x
5x
8x
DEPTH
SNP
INDEL


Copy number Gain
Point mutation
Indel
C
C
C
C
C
C
A
Copy number loss
Reference sequence
Translocation
Reference sequence
(Chr A)
Reference sequence
(Chr B)



(*Arch Pathol Lab Med.* doi: 10.5858/arpa.2015-0507-RA)


Genomic DNA
LIBRARY PREPARATION
Fragmentation of DNA
Adapter Ligation
Library amplification
Emulsion PCR
Cluster Generation
DNA is amplified onto microbeads
DNA is bridge amplified onto a flowcell
Next Generation Sequencing
short sequence reads
Base Calling
Processed Data
Intensity
Scan
Alignment
Read filtering/Quality control
ALIGNMENT
READ FILTERING/QUALITY CONTROL
100%
98%
70%
Variant Calling
Observe differences of sample from reference data
SNPs
Short Indels
"DIPs"
Structural Variants
Genome annotation Database
Billions of short sequences
Genome Regulation Database
Literature and Pathway Database
Entities & relationships
Linked Data
Functional Analysis
NGS GATHERING AND ANNOTATION

NGS – Targeted sequencing van gDNA

Betekenis van een variatie

Classificatie van somatische varianten bij mlc testing van kankerstalen – Sukhai et al, 2015

Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Open

A classification system for clinical relevance of somatic variants identified in molecular profiling of cancer

Mahadeo A. Sukhai, PhD[1,4], Kenneth J. Craddock, MD[1,4], Mariam Thomas, PhD[1,4], Aaron R. Hansen, MD[2,4], Tong Zhang, MD[1,4], Lillian Siu, MD[2,4], Philippe Bedard, MD[2,4], Tracy L. Stockley, PhD, FCCMG[1,3,4] and Suzanne Kamel-Reid, PhD, FACMG[1,3,4]

[Geen titel]

Purpose: Interpretation systems for clinical laboratory reporting of genetic variants for inherited conditions have been widely published. By contrast, there are no existing systems for interpretation and classification of somatic variants found from molecular testing of cancer. is found; (iii) recurrence of the variant; and (iv) evidence of clinical actionability. We used these factors to develop a five-category somatic variant classification for simplified reporting of variant interpretations to treating oncologists.

Wat is het verschil tussen een somatische variant en een genetische variant?

NGS – Targeted sequencing van gDNA

Betekenis van een variatie - Classificatie van somatische varianten bij mlc testing van kanker stalen – Sukhai et al, 2015

DNA varianten kunnen worden ingedeeld in 6 categorieën (klasses) gebaseerd op Sukhai *et al*, 2015:

Klasse 1: Variant die actionable is in de betreffende site/histologie

Klasse 2: Variant waarvoor (non)-actionability niet gekend is in de betreffende site/histologie, maar wel gekend is als actionable in een andere site/histologie

Klasse 3: Variant waarvoor (non)-actionability niet gekend is in de betreffende site/histologie (en is geen SNP), maar andere varianten in het gen zijn wel gekend als actionable in de betreffende site/histologie.

Klasse 4: Variant waarvoor (non)-actionability niet gekend is in de betreffende site/histologie (en is geen SNP), maar andere varianten in het gen zijn wel gekend als actionable in een andere site/histologie.

Klasse 5: variant met ongekende significantie (VUS)

Klasse 6: gekend polymorfisme (germline)

	Class 1	Class 2	Class 3	Class 4	Class 5
Variant previously reported:	Yes, pathogenic	Yes, pathogenic	No	No	No
Specific variant is actionable:	In same site/ histology	In different site/ histology	Not reported	Not reported	Not reported
Other variants in same gene are actionable:			In same site/ histology	In different site/ histology	Not reported
Variant effect from prediction tools:			3A: pathogenic 3B: unknown 3C: benign	4A: pathogenic 4B: unknown 4C: benign	

NGS – Targeted sequencing van gDNA

Betekenis van een variatie - Classificatie van **somatische varianten** bij mlc testing van kankerstalen – Sukhai et al, 2015

DNA varianten kunnen worden ingedeeld in 6 categorieën (klasses) gebaseerd op Sukhai *et al*, 2015:

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Klasse 4: Variant waarvoor (non)-actionability niet gekend is in de betreffende site/histologie (en is geen SNP), maar andere varianten in het gen zijn wel gekend als actionable in een andere site/histologie.

Klasse 5: variant met ongekende significantie (VUS)

Klasse 6: gekend polymorfisme (germline)

Belangrijke definities:

- **actionable**: variant is geassocieerd met gekende doelgerichte therapie / patiënt prognose / respons op therapie. Of de identificatie van de variant heeft implicaties op de diagnose/classificatie met een impact op de behandeling als gevolg.

-**VUS / variant with unknown significance**: de actionablility is (nog) niet gekend of werd aangetoond als zijnde niet-actionable

- **SNP / single nucleotide polymorfisme**: is een 1 veranderde nucleotidencode voor een bepaald gen die bij een dusdanig groot percentage van de populatie voorkomt dat niet meer van een mutatie kan worden gesproken. Volgens de definitie komen polymorfieën bij meer dan 1% van de populatie voor. In geval van meer zeldzame genveranderingen (voorkomen minder dan 1%) wordt van mutaties gesproken.

Genomic Analyses Reveal Potential Independent Adaptation to High Altitude in Tibetan Chickens

Ming-Shan Wang,[†,1,2,3] Yan Li,[†,1,2] Min-Sheng Peng,[†,1,2] Li Zhong,[†,4] Zong-Ji Wang,[5] Qi-Ye Li,[5] Xiao-Long Tu,[1,2,3] Yang Dong,[1,2] Chun-Ling Zhu,[1] Lu Wang,[4] Min-Min Yang,[1] Shi-Fang Wu,[1] Yong-Wang Miao,[6] Jian-Ping Liu,[7] David M. Irwin,[1,8,9] Wen Wang,[1,2] Dong-Dong Wu,[*,1,2] and Ya-Ping Zhang[*,1,2,4]

[1]State Key Laboratory of Genetic Resources and Evolution, Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China

[2]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, China

[3]University of Chinese Academy of Sciences, Beijing, China

[4]Laboratory for Conservation and Utilization of Bio-Resource, Yunnan University, Kunming, China

[5]China National Genebank-Shenzhen, BGI-Shenzhen, Shenzhen, China

[6]Faculty of Animal Science and Technology, Yunnan Agricultural University, Kunming, China

[7]Xishuangbanna Animal Husbandry and Veterinary Station of Yunnan Province, Jinghong, China

[8]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

[9]Banting and Best Diabetes Centre, University of Toronto, Toronto, ON, Canada

[†]These authors contributed equally to this work.

***Corresponding author.** E-mail: wudongdong@mail.kiz.ac.cn; zhangyp@mail.kiz.ac.cn.

Associate editor: Rasmus Nielsen

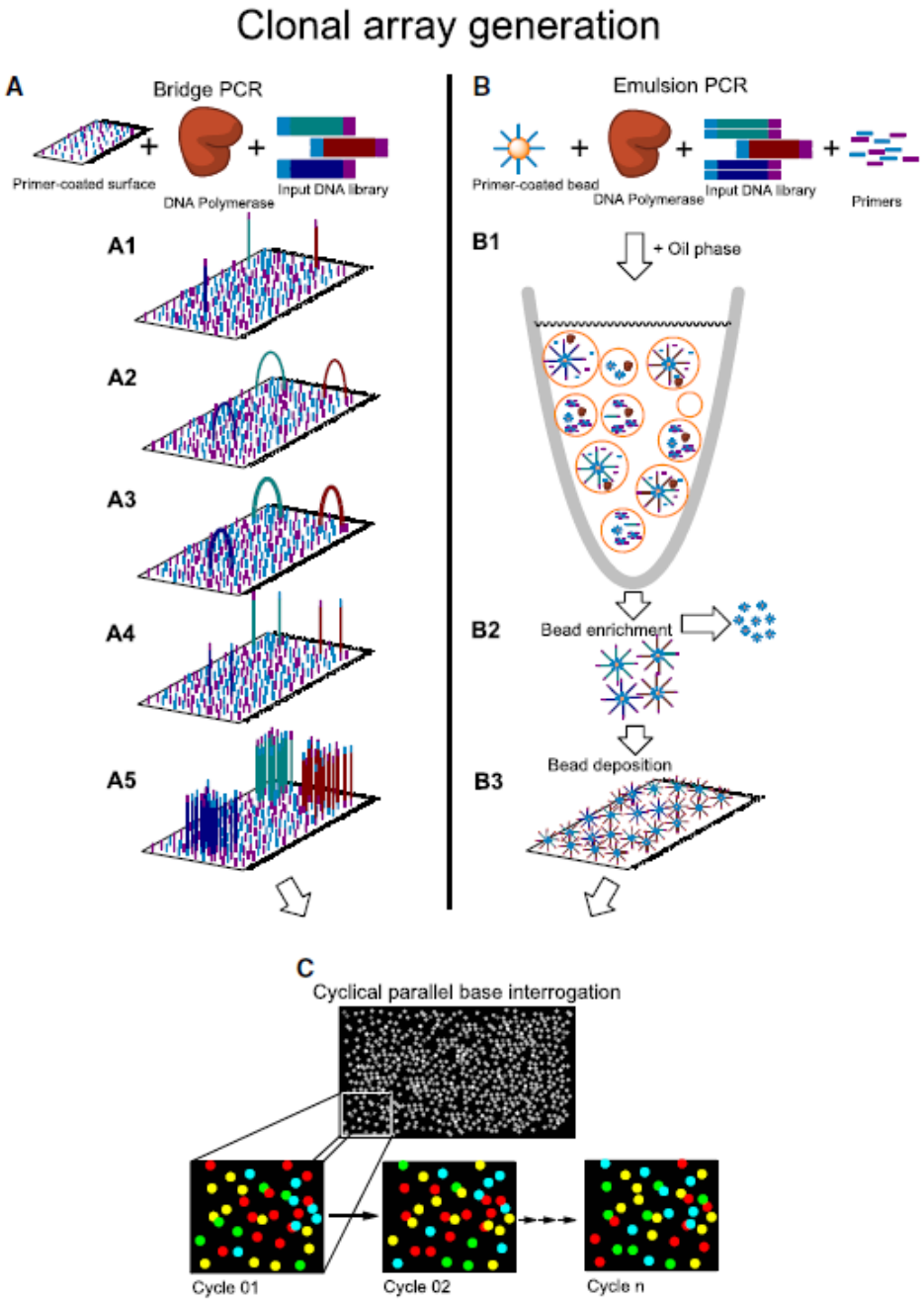


Fig. 5. Schema of the current main approaches for clonal array generation.

ARTICLE

doi:10.1038/nature12886

The complete genome sequence of a Neanderthal from the Altai Mountains

Kay Prüfer[1], Fernando Racimo[2], Nick Patterson[3], Flora Jay[2], Sriram Sankararaman[3,4], Susanna Sawyer[1], Anja Heinze[1], Gabriel Renaud[1], Peter H. Sudmant[5], Cesare de Filippo[1], Heng Li[3], Swapan Mallick[3,4], Michael Dannemann[1], Qiaomei Fu[1,6], Martin Kircher[1,5], Martin Kuhlwilm[1], Michael Lachmann[1], Matthias Meyer[1], Matthias Ongyerth[1], Michael Siebauer[1], Christoph Theunert[1], Arti Tandon[3,4], Priya Moorjani[4], Joseph Pickrell[4], James C. Mullikin[7], Samuel H. Vohr[8], Richard E. Green[8], Ines Hellmann[9]†, Philip L. F. Johnson[10], Hélène Blanche[11], Howard Cann[11], Jacob O. Kitzman[5], Jay Shendure[5], Evan E. Eichler[5,12], Ed S. Lein[13], Trygve E. Bakken[13], Liubov V. Golovanova[14], Vladimir B. Doronichev[14], Michael V. Shunkov[15], Anatoli P. Derevianko[15], Bence Viola[16], Montgomery Slatkin[2], David Reich[3,4,17], Janet Kelso[1] & Svante Pääbo[1]

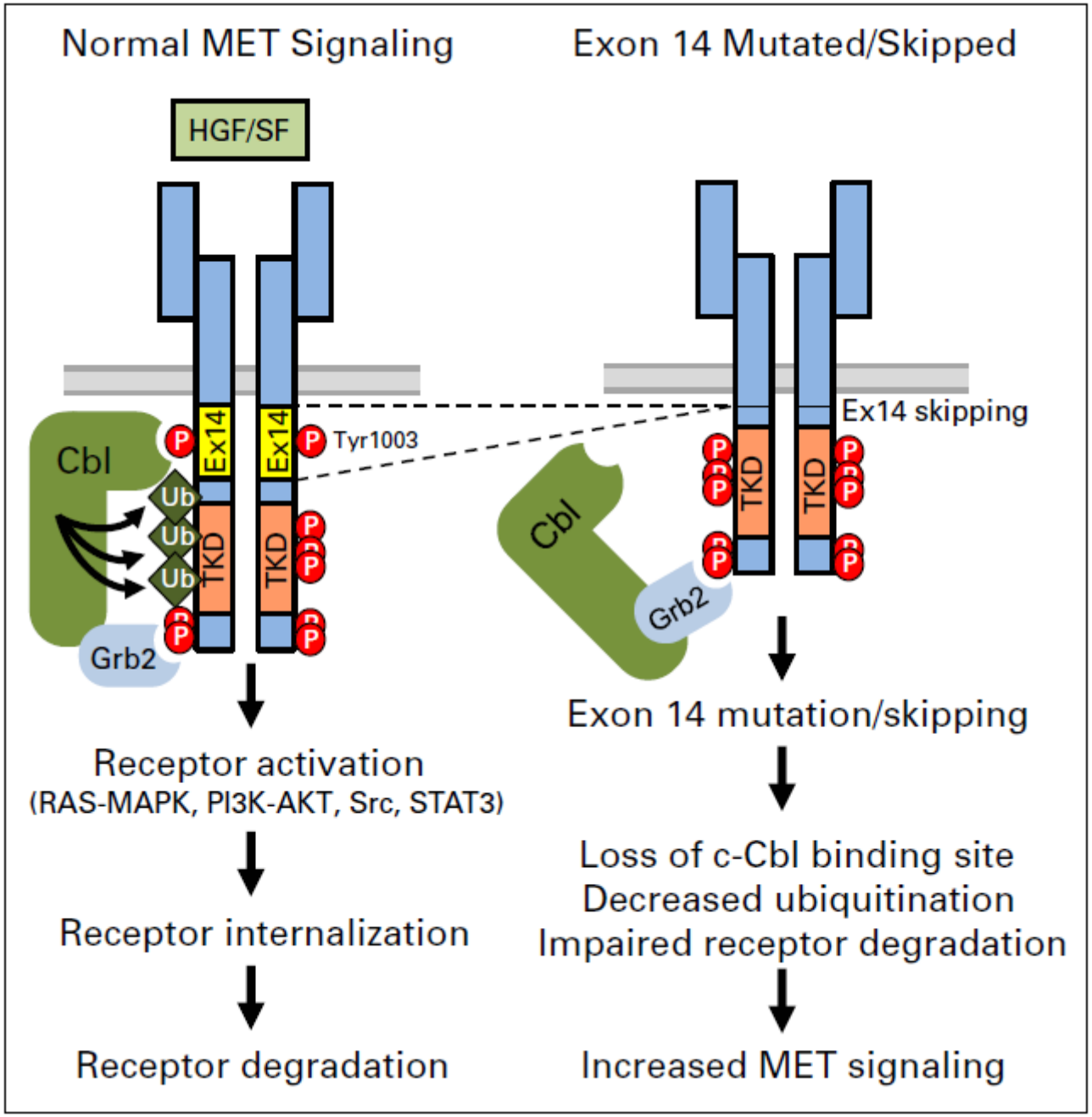
VOLUME 34 · NUMBER 8 · MARCH 10, 2016

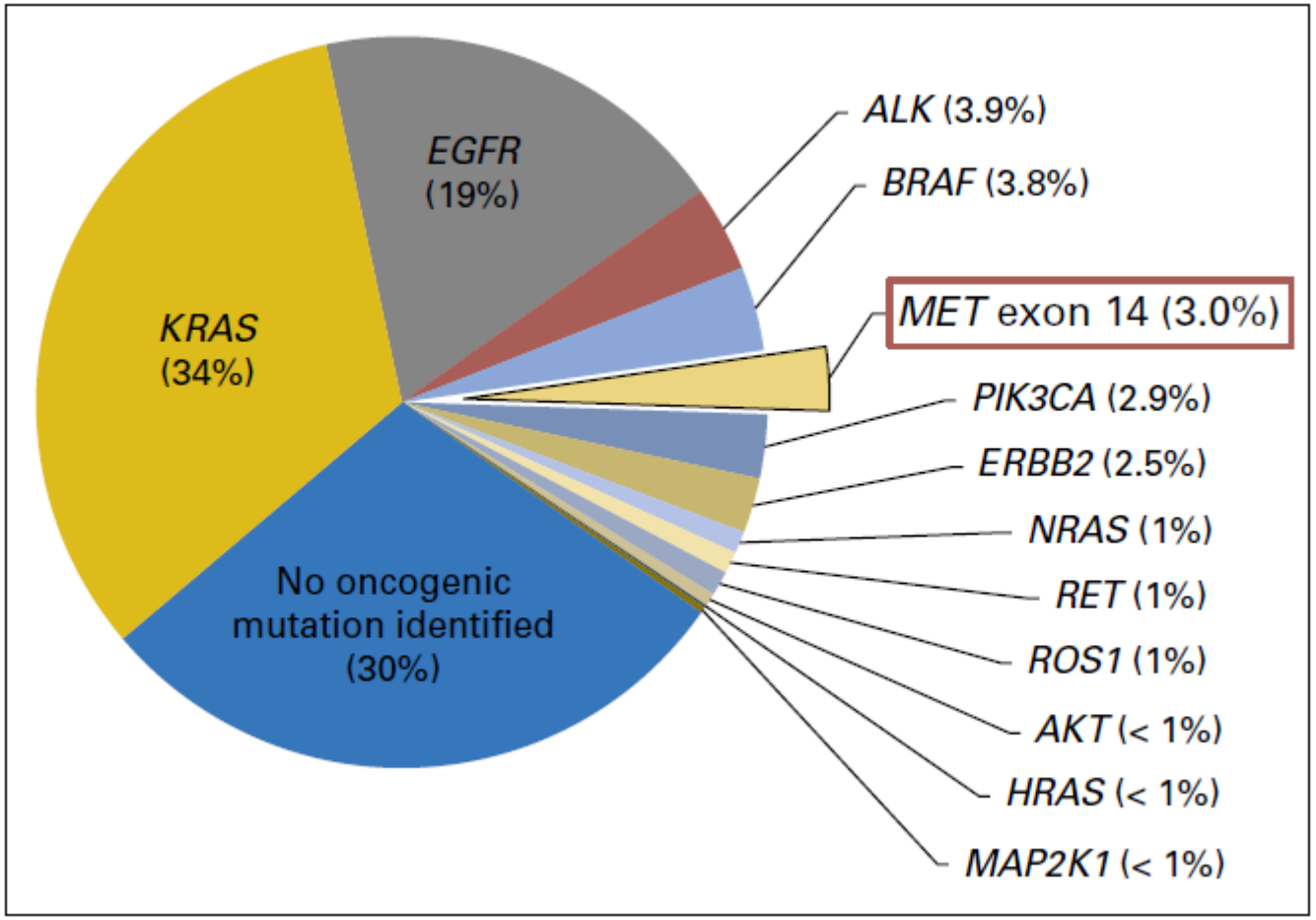
JOURNAL OF CLINICAL ONCOLOGY

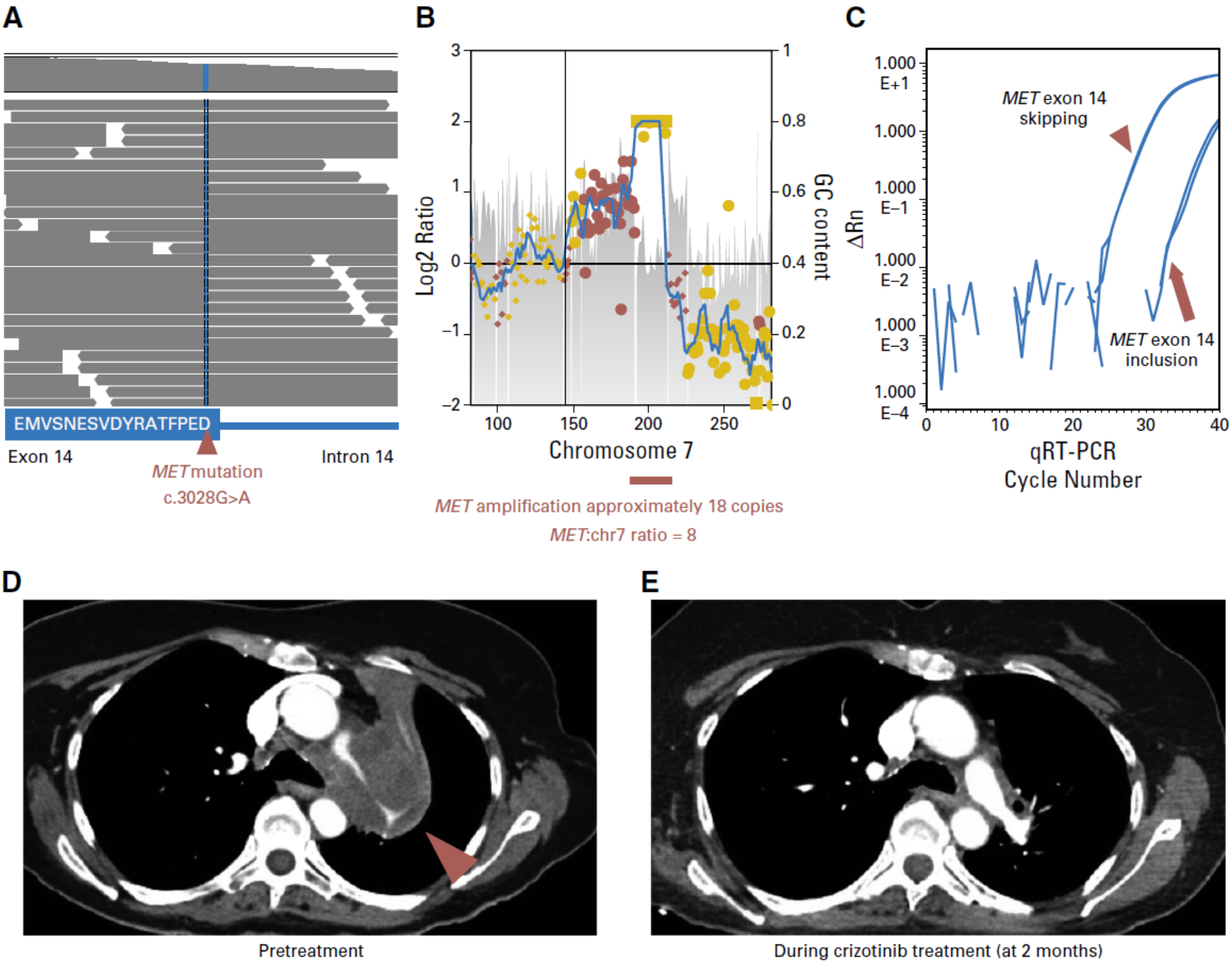
UNDERSTANDING THE PATHWAY

Impaired c-Met Receptor Degradation Mediated by *MET* Exon 14 Mutations in Non–Small-Cell Lung Cancer

Mark M. Awad, *Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, MA*


Normal MET Signaling
Exon 14 Mutated/Skipped
HGF/SF
Cbl
Ex14
Ex14
Tyr1003
Ub
Ub
Ub
TKD
TKD
Grb2
Ex14 skipping
TKD
TKD
Cbl
Grb2
Receptor activation
(RAS-MAPK, PI3K-AKT, Src, STAT3)
Receptor internalization
Receptor degradation
Exon 14 mutation/skipping
Loss of c-Cbl binding site
Decreased ubiquitination
Impaired receptor degradation
Increased MET signaling


EGFR
(19%)
KRAS
(34%)
No oncogenic
mutation identified
(30%)
ALK (3.9%)
BRAF (3.8%)
MET exon 14 (3.0%)
PIK3CA (2.9%)
ERBB2 (2.5%)
NRAS (1%)
RET (1%)
ROS1 (1%)
AKT (< 1%)
HRAS (< 1%)
MAP2K1 (< 1%)


A
EMVSNESVDYRATFPED
Exon 14
Intron 14
MET mutation
c.3028G>A
B
Log2 Ratio
GC content
Chromosome 7
MET amplification approximately 18 copies
MET:chr7 ratio = 8
C
ΔRn
MET exon 14
skipping
MET exon 14
inclusion
qRT-PCR
Cycle Number
D
Pretreatment
E
During crizotinib treatment (at 2 months)

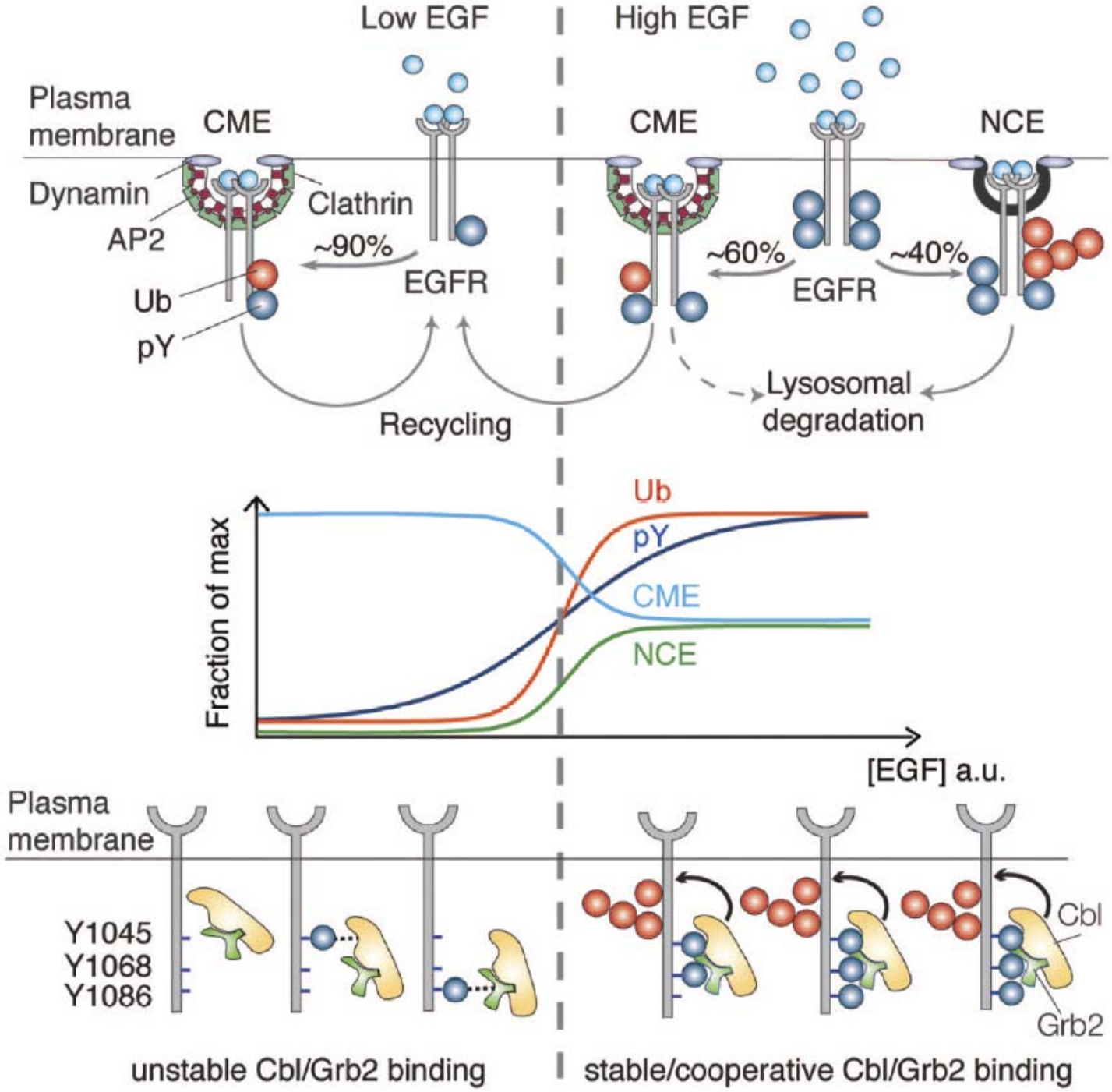
Cell Cycle 13:5, 1–2; March 1, 2014; © 2014 Landes Bioscience

Keeping EGFR signaling in check

Ubiquitin is the guardian

Simona Polo[1,2,*], Pier Paolo Di Fiore[1,2,3,*], and Sara Sigismund[1]

[1]IFOM, Fondazione Istituto FIRC di Oncologia Molecolare; Milan, Italy; [2]Dipartimento di Scienze della Salute; Università degli Studi di Milano; Milan, Italy; [3]Department of Experimental Oncology; Istituto Europeo di Oncologia; Milan, Italy


Low EGF
High EGF
Plasma membrane
CME
CME
NCE
Dynamin
AP2
Clathrin
~90%
EGFR
Ub
pY
~60%
EGFR
~40%
Lysosomal degradation
Recycling
Ub
pY
CME
NCE
Fraction of max
[EGF] a.u.
Plasma membrane
Y1045
Y1068
Y1086
Cbl
Grb2
unstable Cbl/Grb2 binding
stable/cooperative Cbl/Grb2 binding

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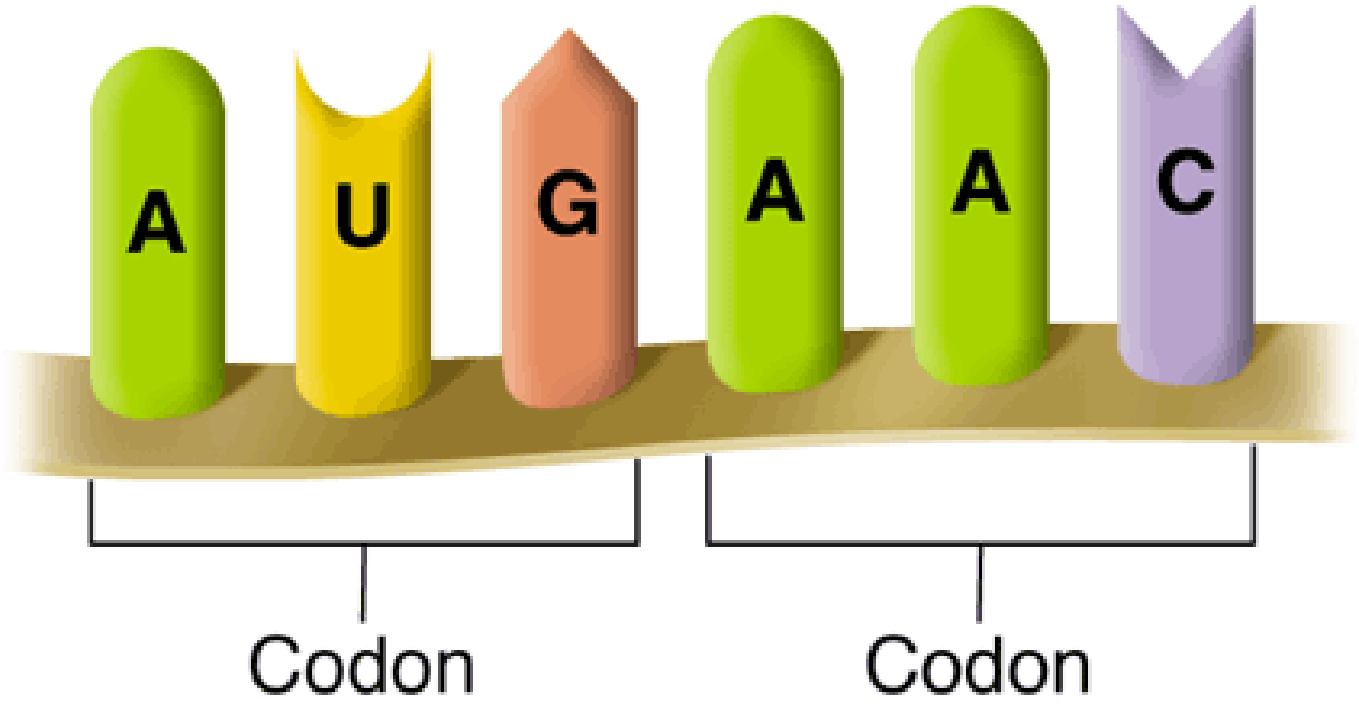
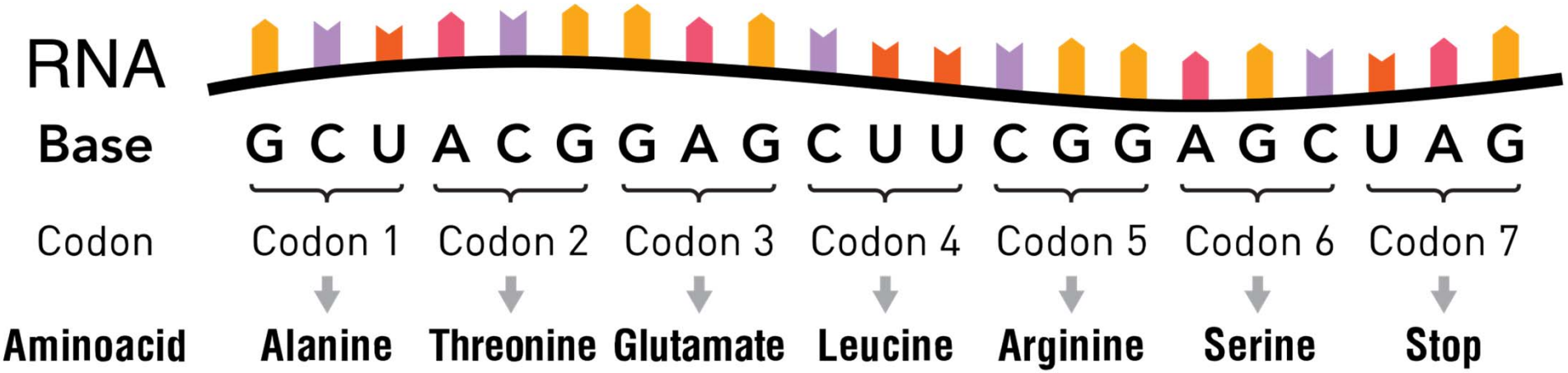
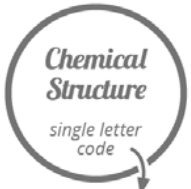

A
U
G
A
A
C
Codon
Codon


RNA
Base
G C U A C G G A G C U U C G G A G C U A G
Codon
Codon 1
Codon 2
Codon 3
Codon 4
Codon 5
Codon 6
Codon 7
Aminoacid
Alanine
Threonine
Glutamate
Leucine
Arginine
Serine
Stop

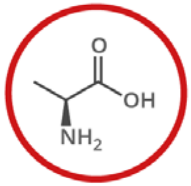
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

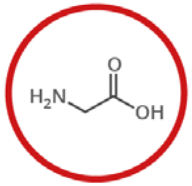
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



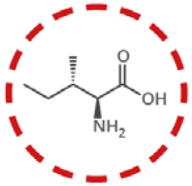
NAME A
three letter code
DNA codons



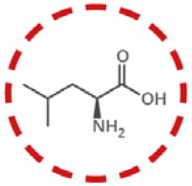
ALANINE A
Ala
GCT, GCC, GCA, GCG



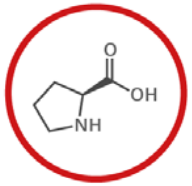
GLYCINE G
Gly
GGT, GGC, GGA, GGG



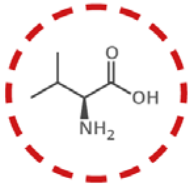
ISOLEUCINE I
Ile
ATT, ATC, ATA



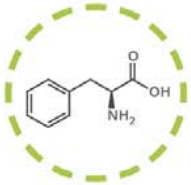
LEUCINE L
Leu
CTT, CTC, CTA, CTG, TTA, TTG



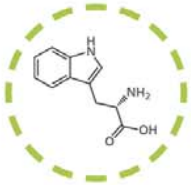
PROLINE P
Pro
CCT, CCC, CCA, CCG



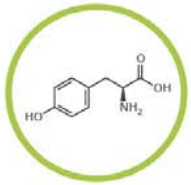
VALINE V
Val
GTT, GTC, GTA, GTG



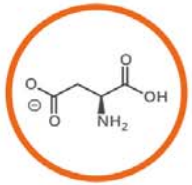
PHENYLALANINE F
Phe
TTT, TTC



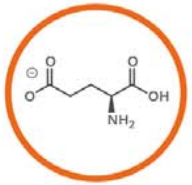
TRYPTOPHAN W
Trp
TGG



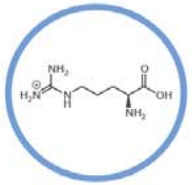
TYROSINE Y
Tyr
TAT, TAC



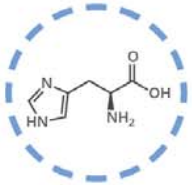
ASPARTIC ACID D
Asp
GAT, GAC



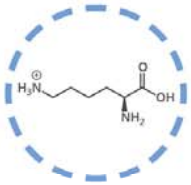
GLUTAMIC ACID E
Glu
GAA, GAG



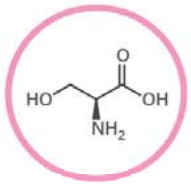
ARGININE R
Arg
CGT, CGC, CGA, CGG, AGA, AGG



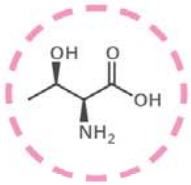
HISTIDINE H
His
CAT, CAC



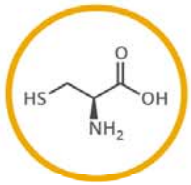
LYSINE K
Lys
AAA, AAG



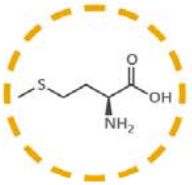
SERINE S
Ser
TCT, TCC, TCA, TCG, AGT, AGC



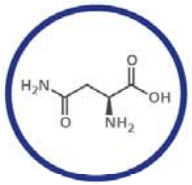
THREONINE T
Thr
ACT, ACC, ACA, ACG



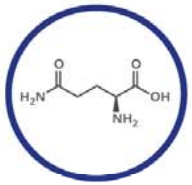
CYSTEINE C
Cys
TGT, TGC



METHIONINE M
Met
ATG



ASPARAGINE N
Asn
AAT, AAC

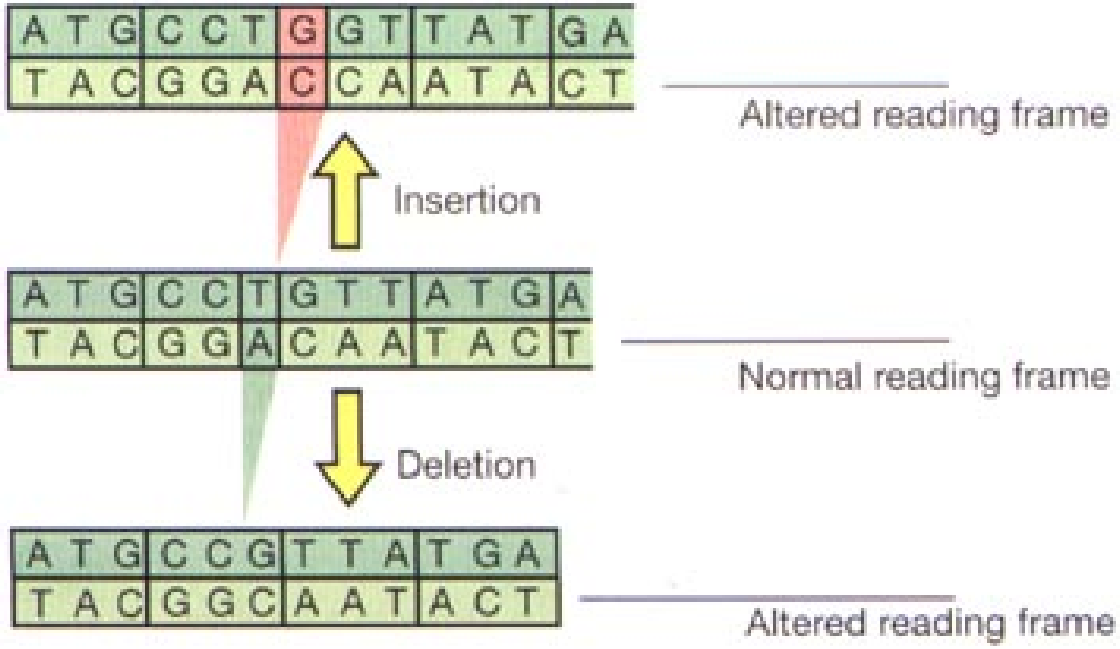


GLUTAMINE Q
Gln
CAA, CAG

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	**Lys**	**Lys**	**STOP**	**Arg**	**Thr**

basic
polar


ATGCCTGGTTATGA
TACGGACCAATACT
Altered reading frame
Insertion
ATGCCTGTTATGA
TACGGACAATACT
Normal reading frame
Deletion
ATGCCGTTATGA
TACGGCAATACT
Altered reading frame

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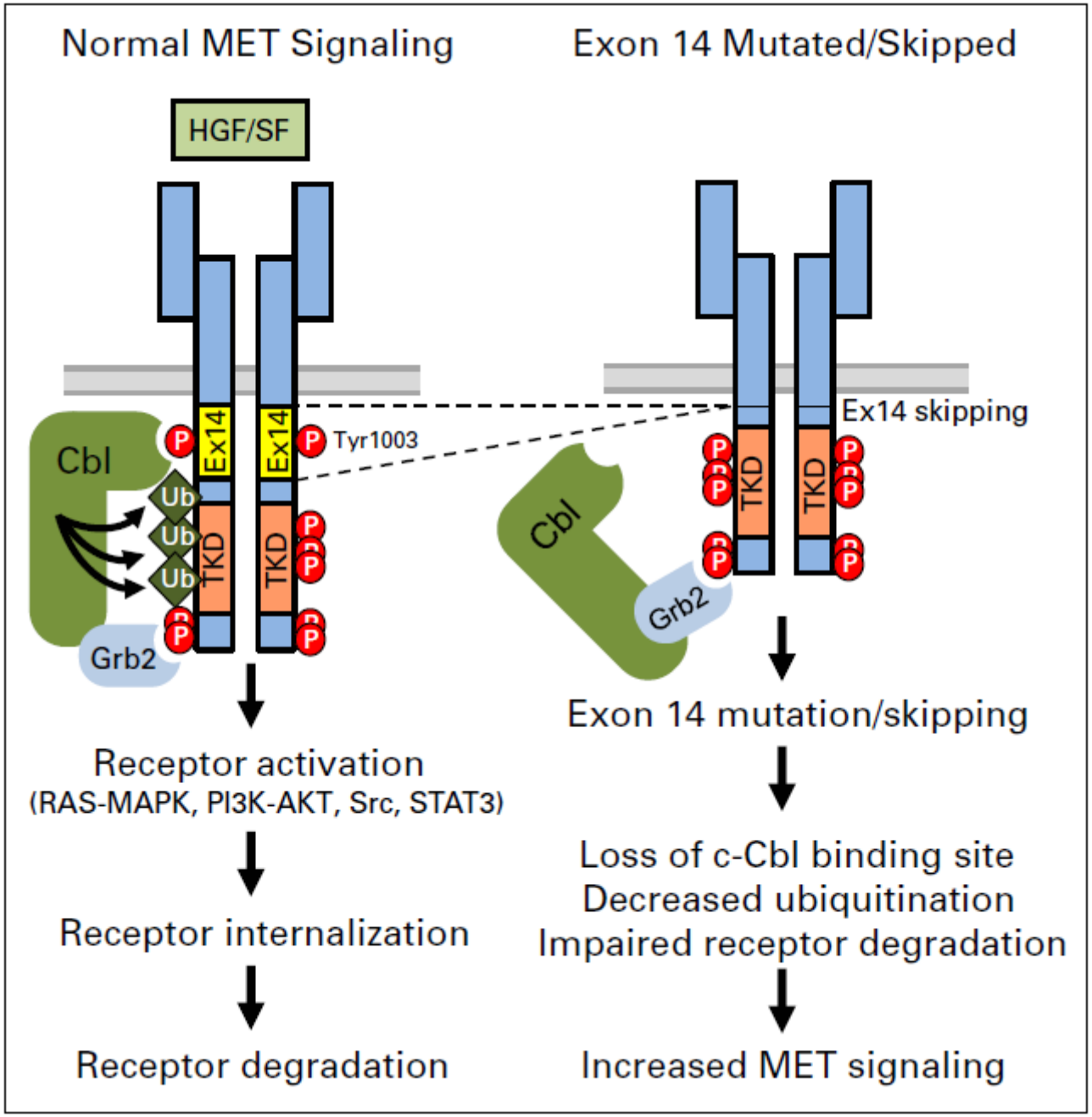
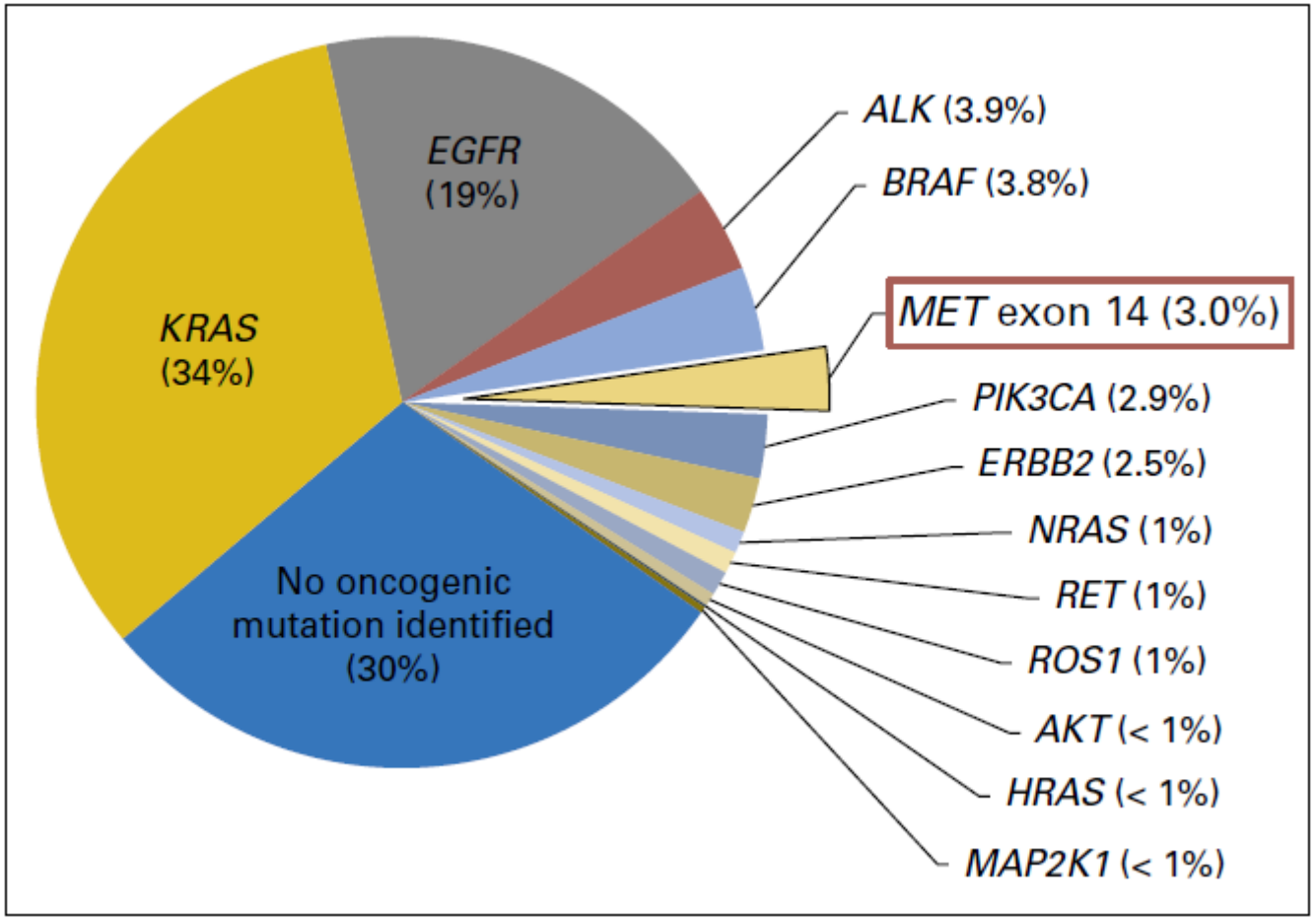
JOURNAL OF CLINICAL ONCOLOGY

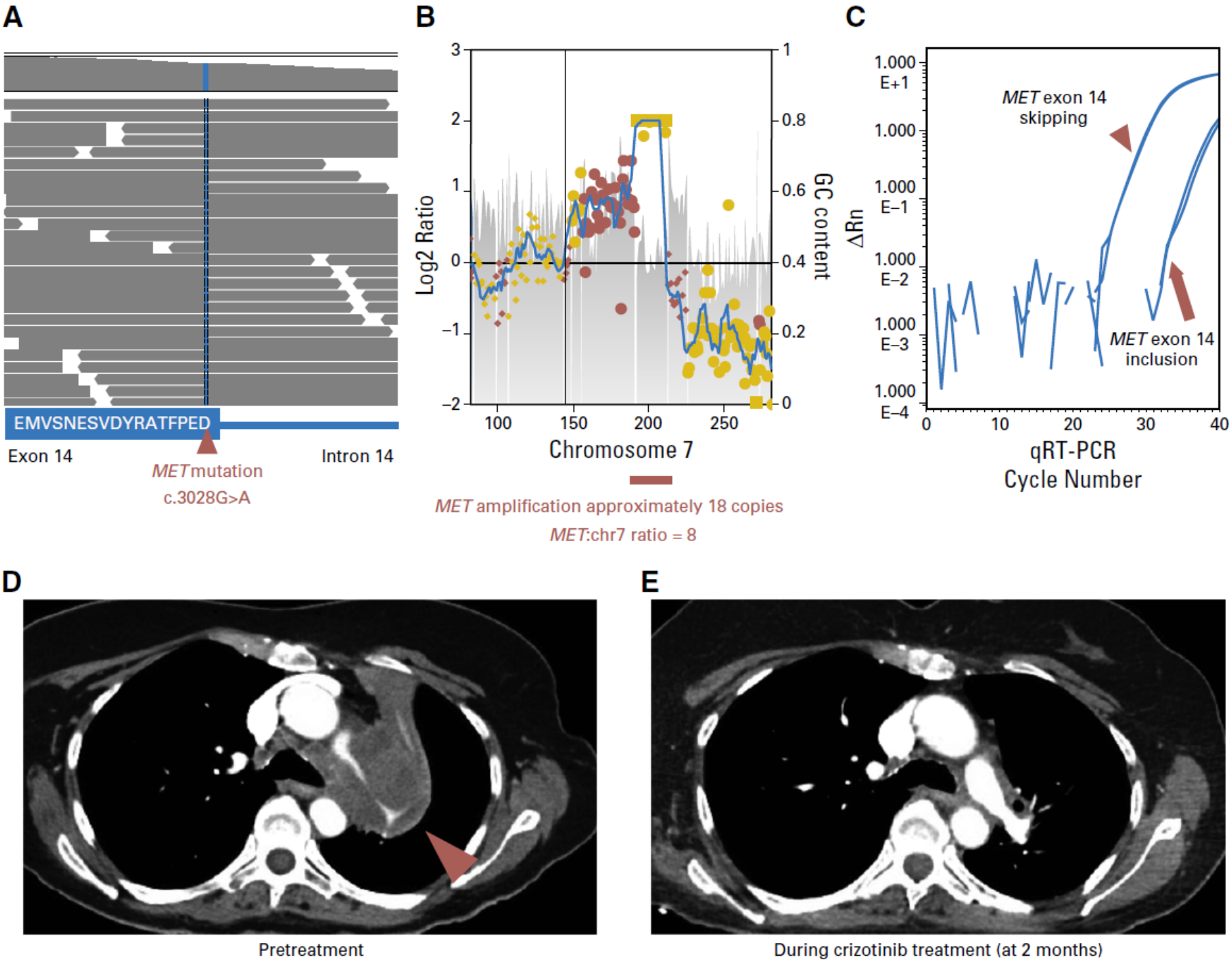
UNDERSTANDING THE PATHWAY

Impaired c-Met Receptor Degradation Mediated by *MET* Exon 14 Mutations in Non–Small-Cell Lung Cancer

Mark M. Awad, *Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, MA*

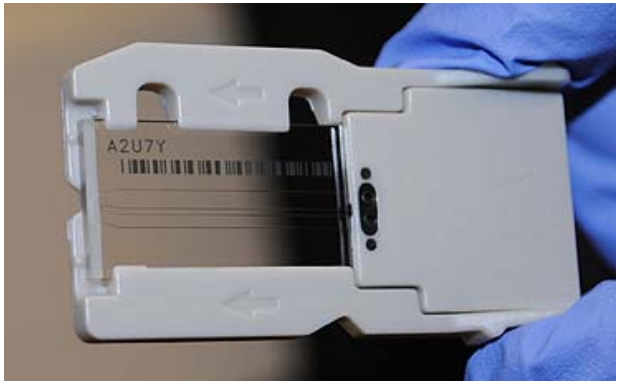

Normal MET Signaling
Exon 14 Mutated/Skipped
HGF/SF
Cbl
Ex14
Ex14
Tyr1003
Ub
Ub
Ub
TKD
TKD
Grb2
Ex14 skipping
TKD
TKD
Cbl
Grb2
Receptor activation
(RAS-MAPK, PI3K-AKT, Src, STAT3)
Receptor internalization
Receptor degradation
Exon 14 mutation/skipping
Loss of c-Cbl binding site
Decreased ubiquitination
Impaired receptor degradation
Increased MET signaling

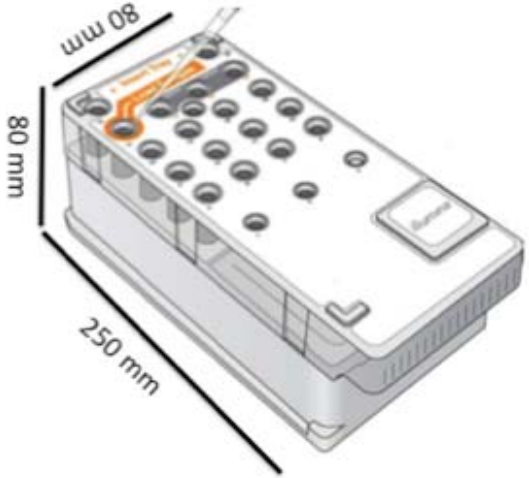

EGFR
(19%)
KRAS
(34%)
No oncogenic
mutation identified
(30%)
ALK (3.9%)
BRAF (3.8%)
MET exon 14 (3.0%)
PIK3CA (2.9%)
ERBB2 (2.5%)
NRAS (1%)
RET (1%)
ROS1 (1%)
AKT (< 1%)
HRAS (< 1%)
MAP2K1 (< 1%)


A
EMVSNESVDYRATFPED
Exon 14
Intron 14
MET mutation
c.3028G>A
B
Log2 Ratio
GC content
Chromosome 7
MET amplification approximately 18 copies
MET:chr7 ratio = 8
C
ΔRn
MET exon 14 skipping
MET exon 14 inclusion
qRT-PCR Cycle Number
D
Pretreatment
E
During crizotinib treatment (at 2 months)

NGS – Targeted resequencing van gDNA

STAP 3: Alle stalen samen op een flow cell brengen
→ bridging amplificatie





UZA

NGS – Targeted resequencing van gDNA

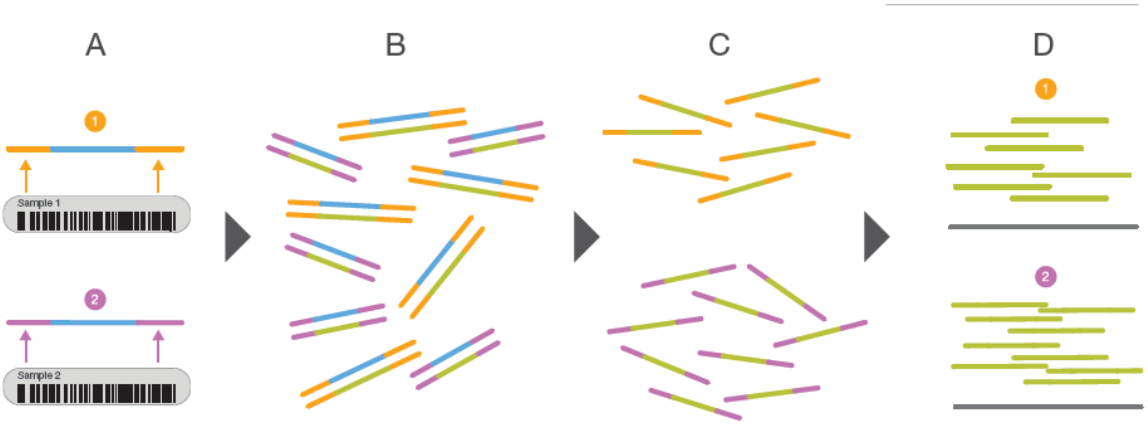
STAP 2: Verrijking van de targetsequenties uit je panel

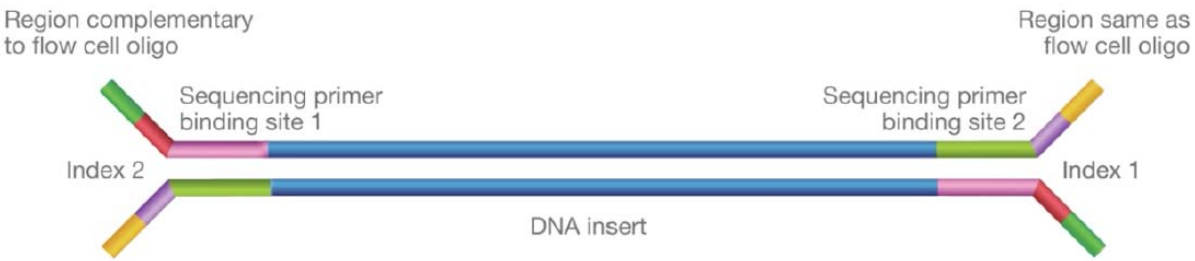
Toevoeging van adaptoren:

- binding sequences
- primer sequences

} Nodig voor 'bridging amplification'

- een identificatie barcode (specifiek voor je patiënt)

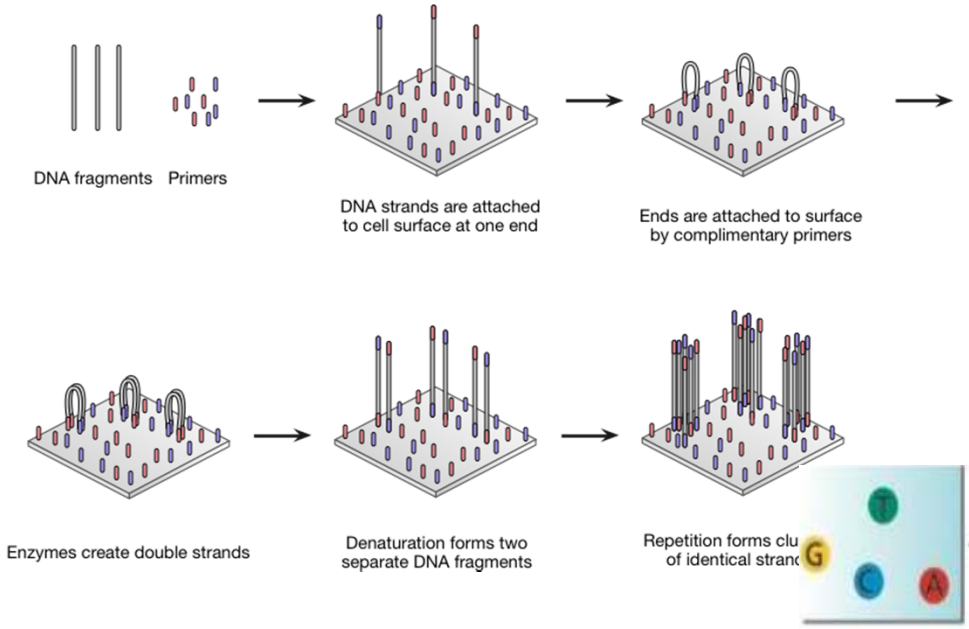




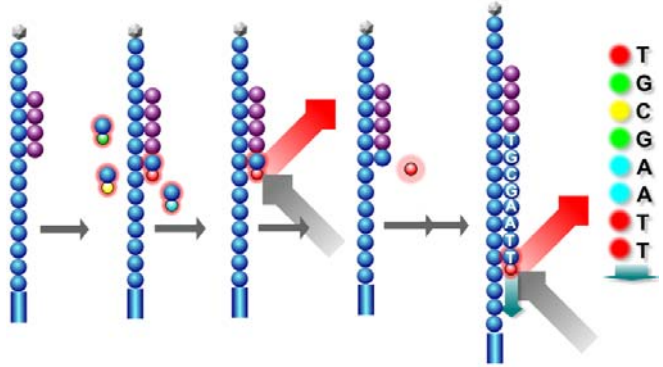
NGS – Targeted resequencing van gDNA

STAP 3: Alle stalen samen op een flow cell brengen

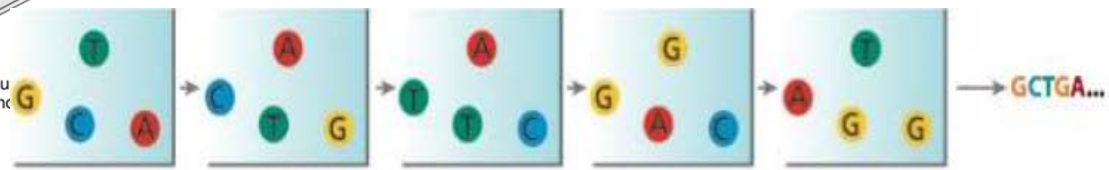
Op de flowcell worden meerdere cluster gemaakt adh bridging amplification



Per cluster: sequencing by synthesis



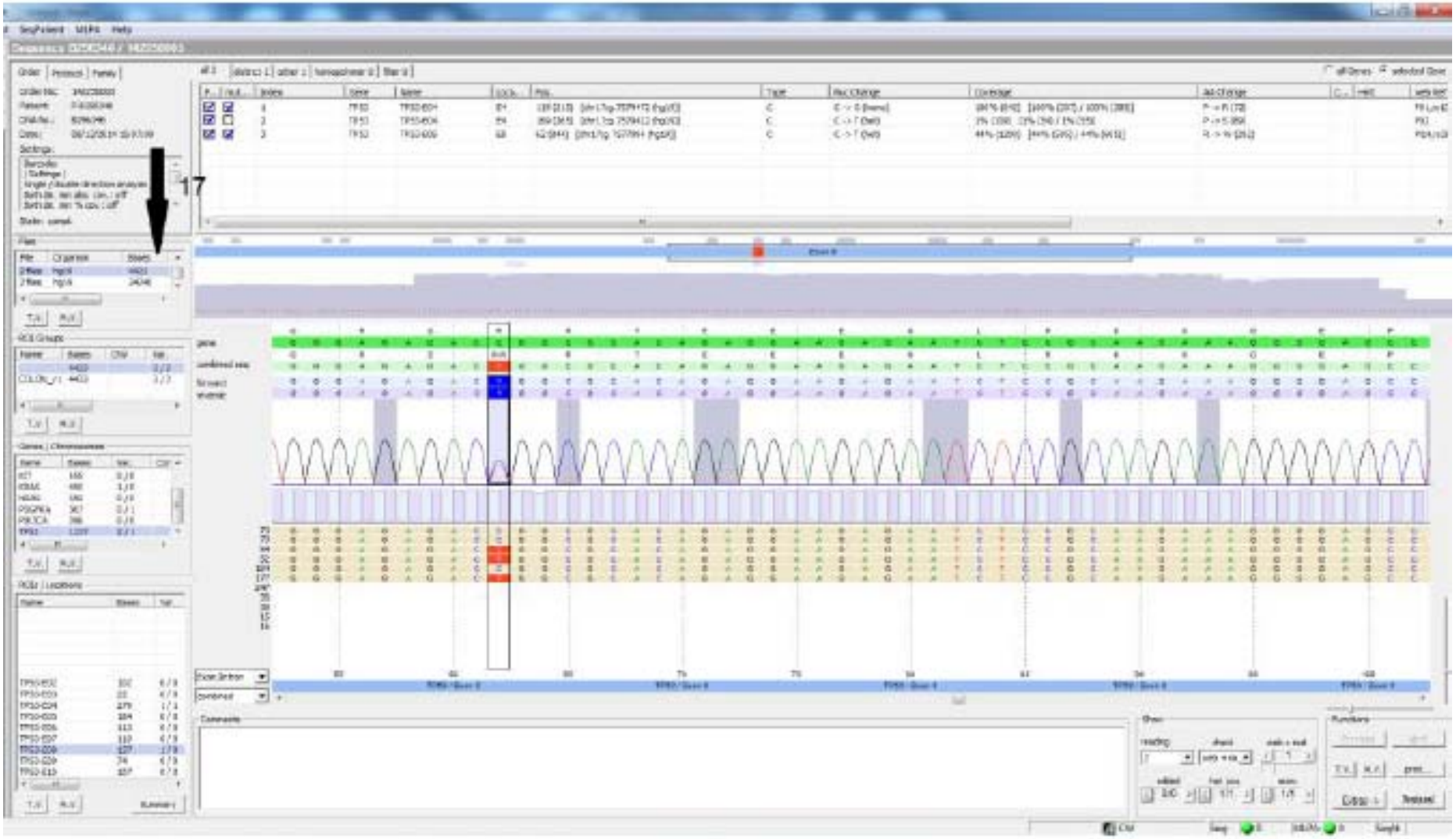
Een foto van iedere cluster (tijdens het sequencing by synthesis)



http://www.illumina.com/technology/next-generation-sequencing/sequencing-technology.ilmn

NGS – Targeted resequencing van gDNA

Betekenis van een variatie



NGS – Targeted sequencing van gDNA

Betekenis van een variatie – classificatie voor **genetische varianten** voor overdraagbare, genetische condities – Plon et al, 2008

Belangrijk bij BRCA want daar verworven/somatisch alsook genetische mutaties

Het 5-klassensysteem volgens *Plon et al.* ziet er als volgt uit:

Klasse 1 varianten zijn (frequente) polymorfismen of neutrale varianten en zijn niet pathogeen (P).

Klasse 2 varianten zijn waarschijnlijk niet-pathogeen of klinisch weinig relevant, waardoor een diagnose moleculair niet bevestigd kan worden (UVkl2).

Klasse 3 varianten zijn 'Variants of Uncertain clinical Significance' (VUS), waardoor een diagnose moleculair niet bevestigd maar evenmin uitgesloten kan worden (UVkl3 of VUS).

Klasse 4 varianten zijn waarschijnlijk pathogeen. De variant geeft (nog) geen volledige bevestiging maar ondersteunt wel de diagnose (UVkl4).

Klasse 5 varianten zijn duidelijk pathogeen waardoor een diagnose moleculair bevestigd wordt (M)

Sequence Variant Classification and Reporting: Recommendations for Improving the Interpretation of Cancer Susceptibility Genetic Test Results

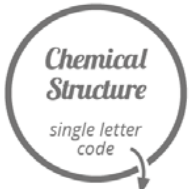
Sharon E. Plon,[1,11]* Diana M. Eccles,[2] Douglas Easton,[3] William D. Foulkes,[4] Maurizio Genuardi,[5,12] Marc S. Greenblatt,[6] Frans B.L. Hogervorst,[7] Nicoline Hoogerbrugge,[8] Amanda B. Spurdle,[9] and Sean V. Tavtigian,[10] for the IARC Unclassified Genetic Variants Working Group[†]

UZA

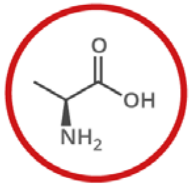
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

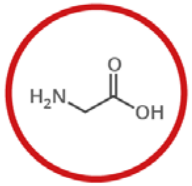
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



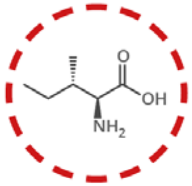
NAME A
three letter code
DNA codons



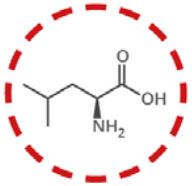
ALANINE A
Ala
GCT, GCC, GCA, GCG



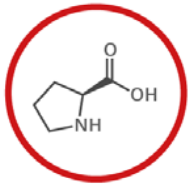
GLYCINE G
Gly
GGT, GGC, GGA, GGG



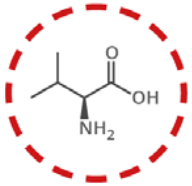
ISOLEUCINE I
Ile
ATT, ATC, ATA



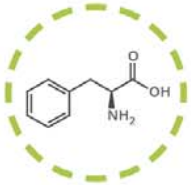
LEUCINE L
Leu
CTT, CTC, CTA, CTG, TTA, TTG



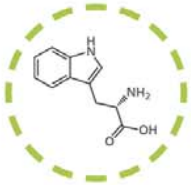
PROLINE P
Pro
CCT, CCC, CCA, CCG



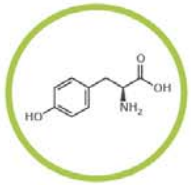
VALINE V
Val
GTT, GTC, GTA, GTG



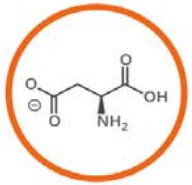
PHENYLALANINE F
Phe
TTT, TTC



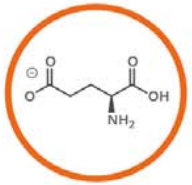
TRYPTOPHAN W
Trp
TGG



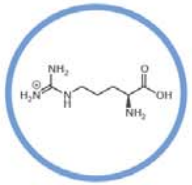
TYROSINE Y
Tyr
TAT, TAC



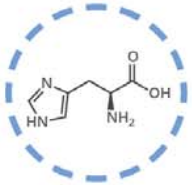
ASPARTIC ACID D
Asp
GAT, GAC



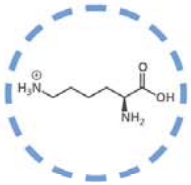
GLUTAMIC ACID E
Glu
GAA, GAG



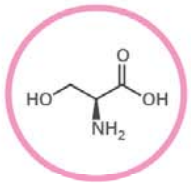
ARGININE R
Arg
CGT, CGC, CGA, CGG, AGA, AGG



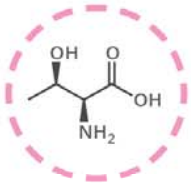
HISTIDINE H
His
CAT, CAC



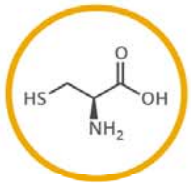
LYSINE K
Lys
AAA, AAG



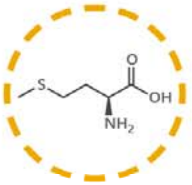
SERINE S
Ser
TCT, TCC, TCA, TCG, AGT, AGC



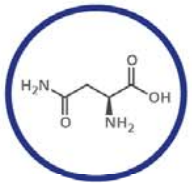
THREONINE T
Thr
ACT, ACC, ACA, ACG



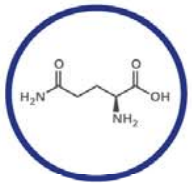
CYSTEINE C
Cys
TGT, TGC



METHIONINE M
Met
ATG



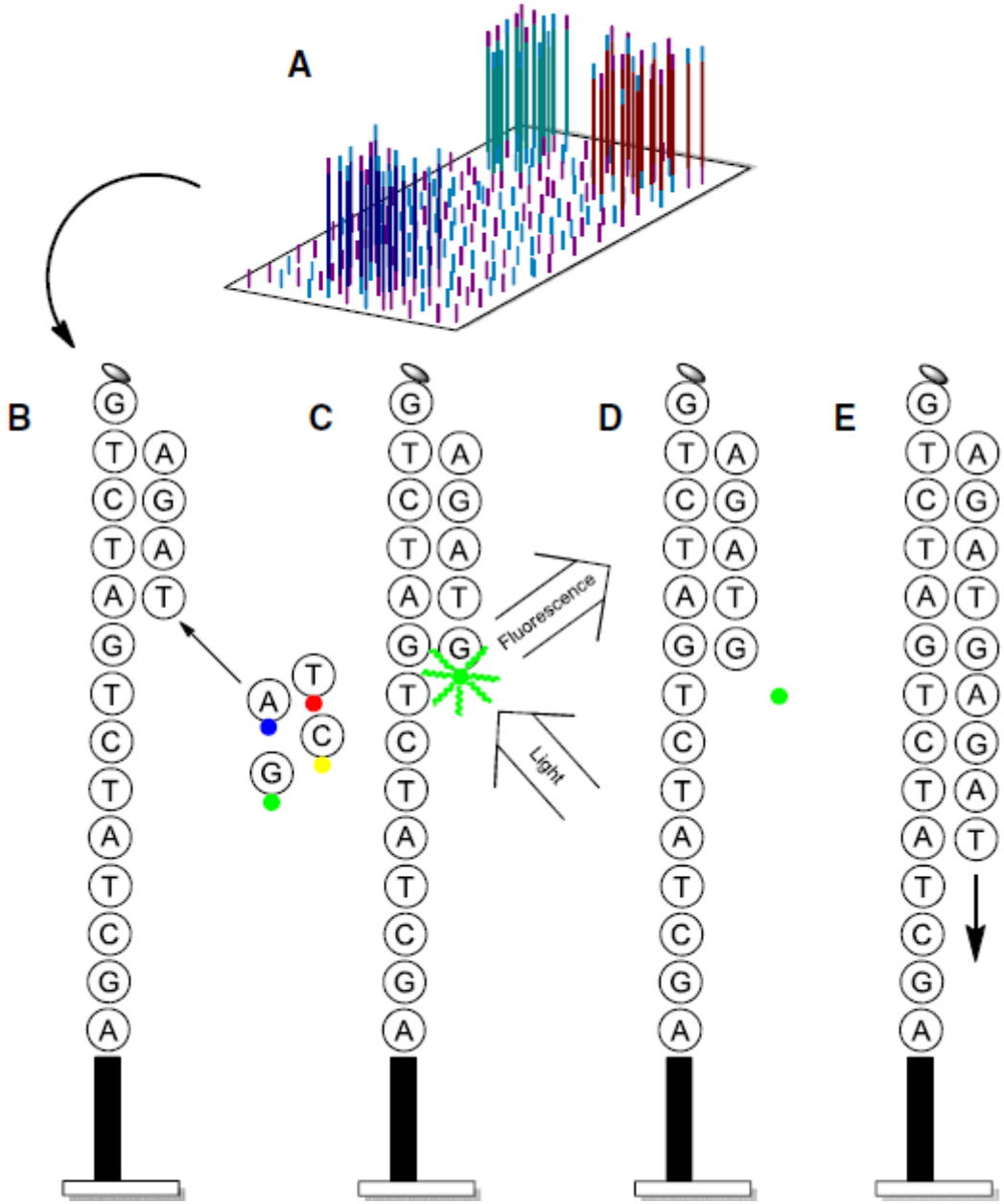
ASPARAGINE N
Asn
AAT, AAC

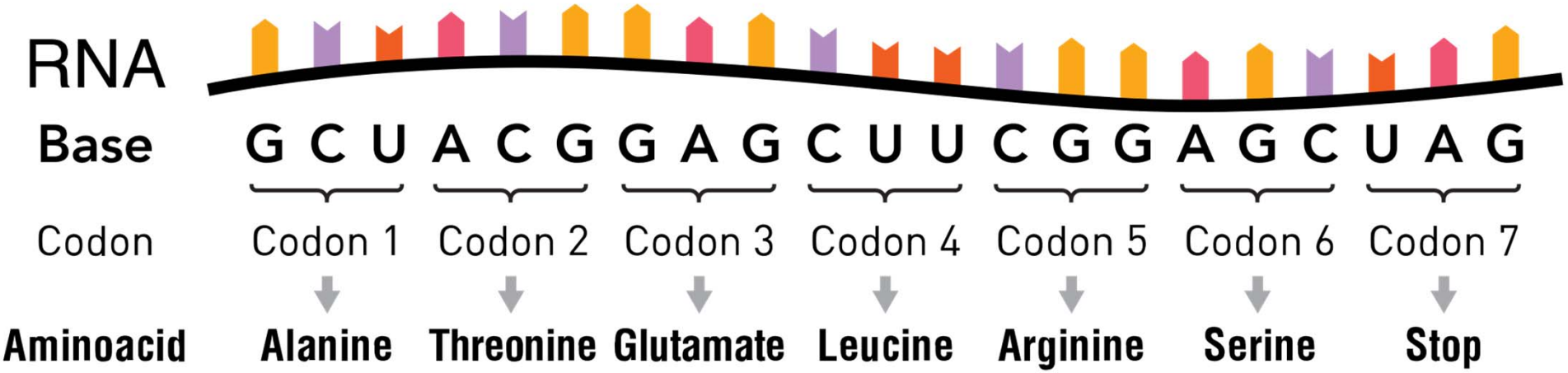


GLUTAMINE Q
Gln
CAA, CAG

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

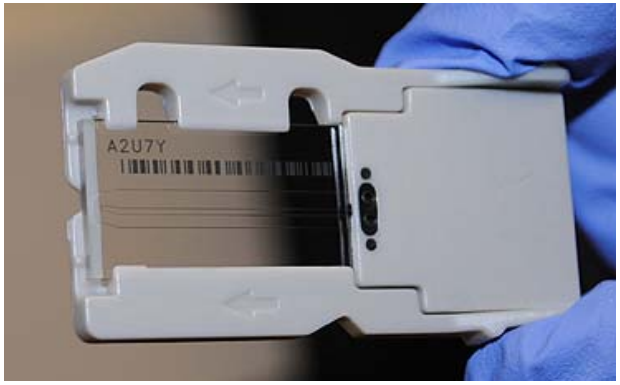
Sequencing by Synthesis

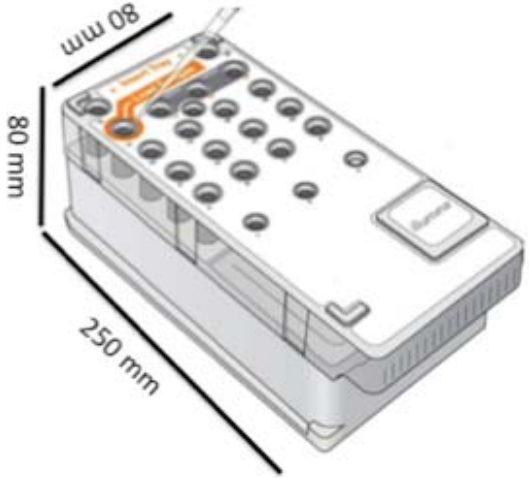



RNA
Base
G C U A C G G A G C U U C G G A G C U A G
Codon
Codon 1
Codon 2
Codon 3
Codon 4
Codon 5
Codon 6
Codon 7
Aminoacid
Alanine
Threonine
Glutamate
Leucine
Arginine
Serine
Stop

NGS – Targeted resequencing van gDNA

STAP 3: Alle stalen samen op een flow cell brengen
→ bridging amplificatie





UZA