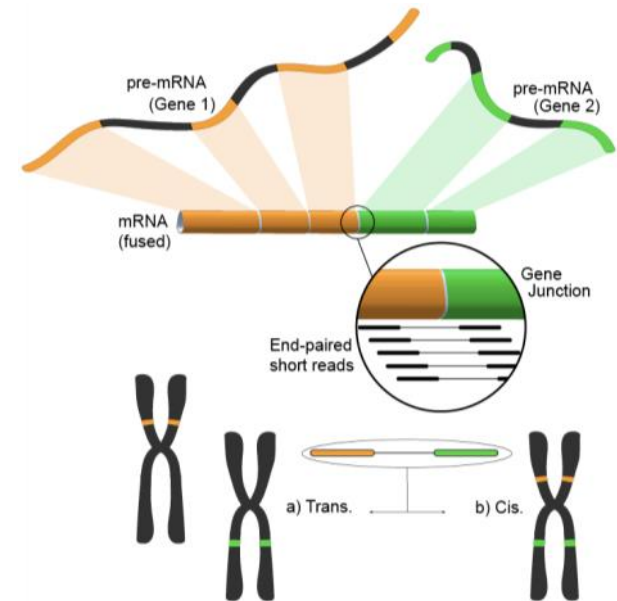
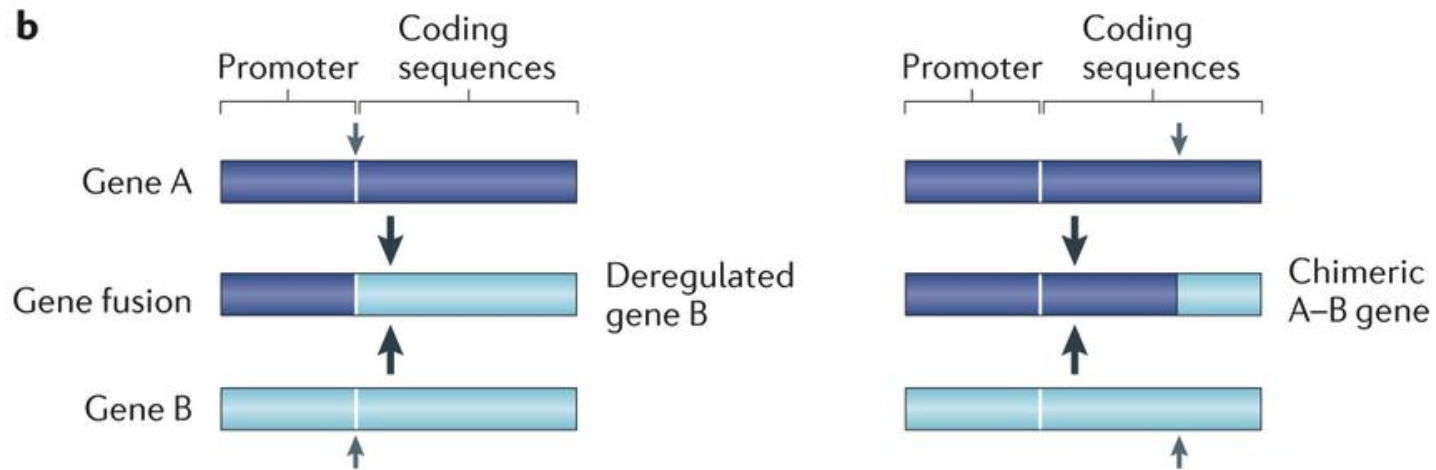


Translocatie Detectie Met Behulp Van Targeted Next Generation Sequencing In FFPE Weefsels

Tom van Wezel
Pathologie
LUMC

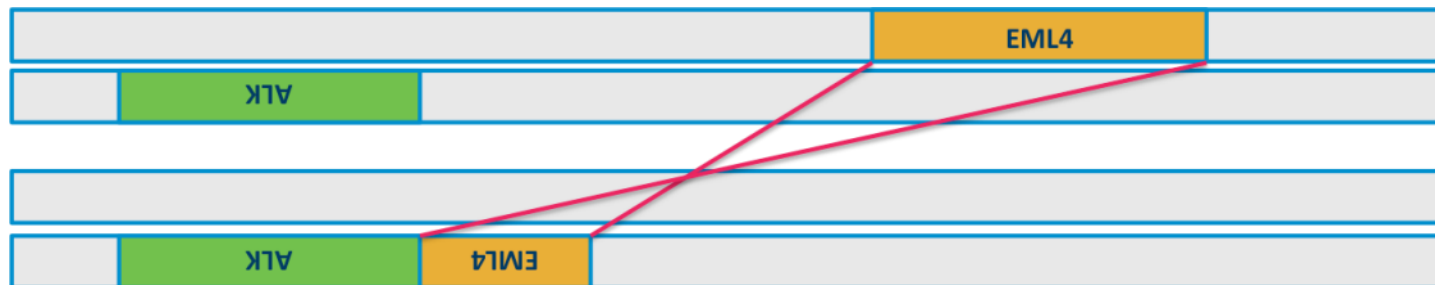


Gene fusions in cancer



Nature Reviews | Cancer

The emerging complexity of gene fusions in cancer, Mertens et.al. Nature Reviews Cancer 2015



Gene fusions in cancer

Drugable targets

Gene fusion	Drug	Disease	Year of approval
ALK fusions	Crizotinib	NSCLC	2011
	Ceritinib	NSCLC	2014 [‡]
BCR-ABL1	Imatinib	CML and ALL	2001
	Dasatinib	CML and ALL	2006
	Nilotinib	CML	2007
	Bosutinib	CML	2012
	Ponatinib	CML and ALL	2012
COL1A1-PDGFRB	Imatinib	DFSP	2006
FIP1L1-PDGFR	Imatinib	HES/CEL	2006
PDGFR fusions	Imatinib	MDS/MPN	2006

*The emerging complexity of gene fusions in cancer,
Mertens et.al. Nature Reviews Cancer 2015*

Improved Diagnostics

Sarcomas; numerous fusions

Thyroid, RET/ PPARG/ NTRK3 etc

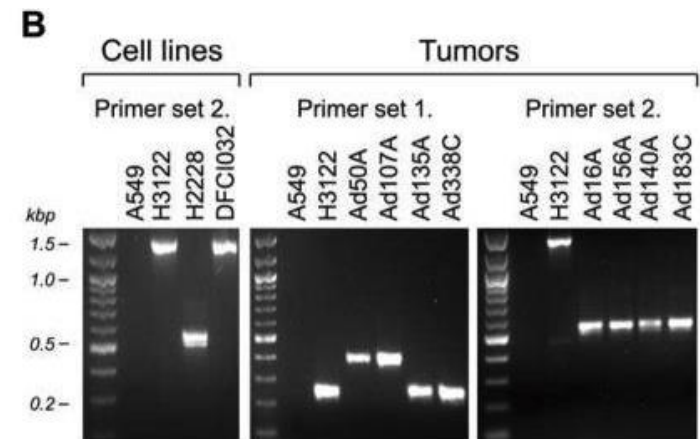
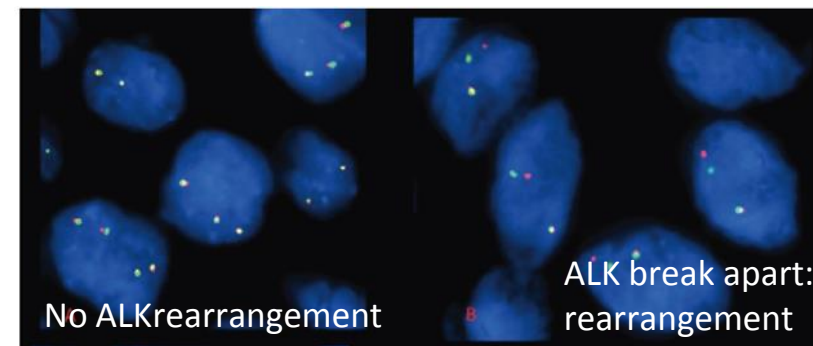
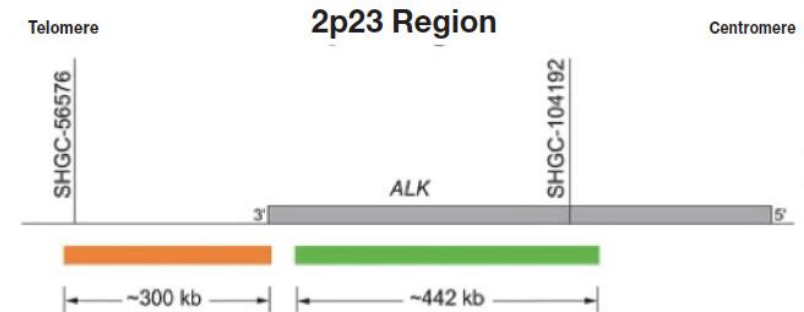
Translocation detection

Gold standard for fusion detection

FISH

RT-PCR

Fusion specific primers;
1 PCR per fusie

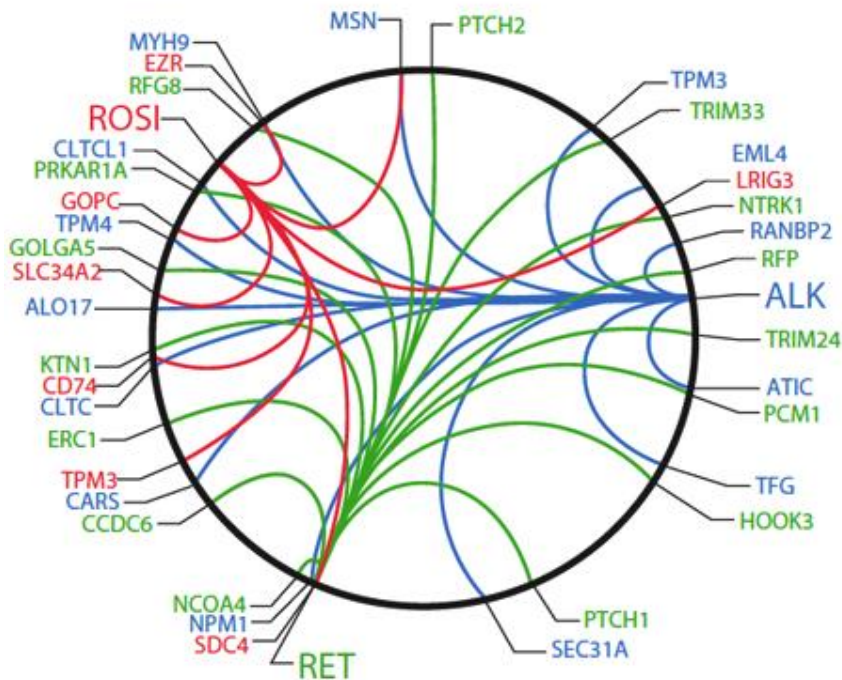


Koivunen et al, clin cancer res, 2008

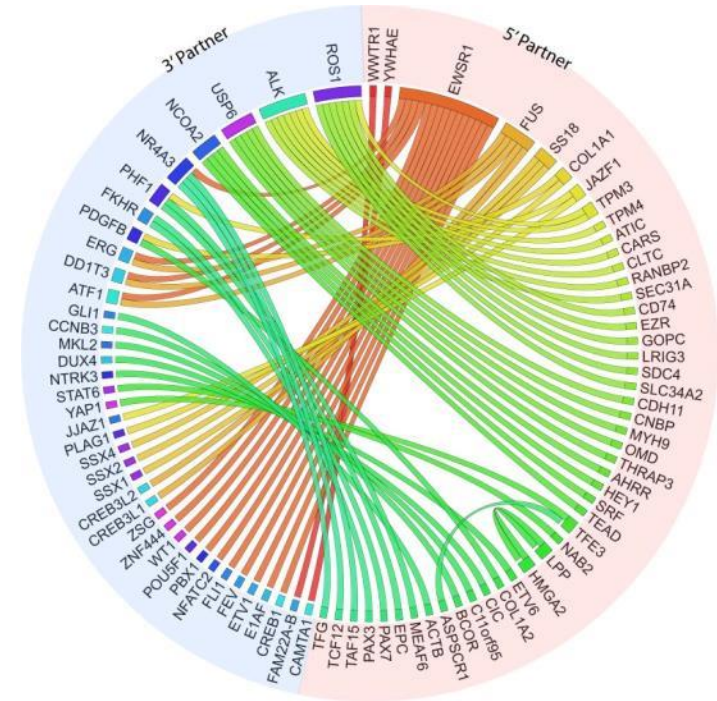
Veelvoud aan fusies

Nadeel: bewerkelijk, vele assays nodig om alle fusie te detecteren, soms materiaal daarvoor niet beschikbaar

Lung cancer fusions



Sarcoma fusions



-> Next Generation Sequencing?

Ampliseq Fusion detection

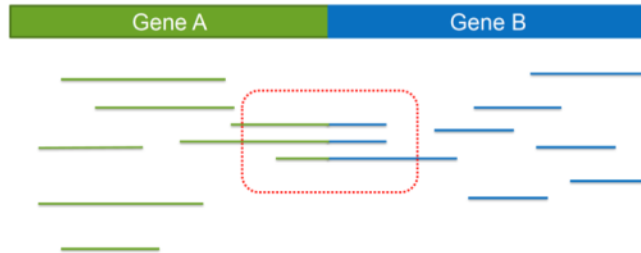
“opposed primer” approach

Multiplex amplicon based

Figure 2. Ion AmpliSeq™ RNA design strategy for detection of fusion transcripts

WT RNAseq

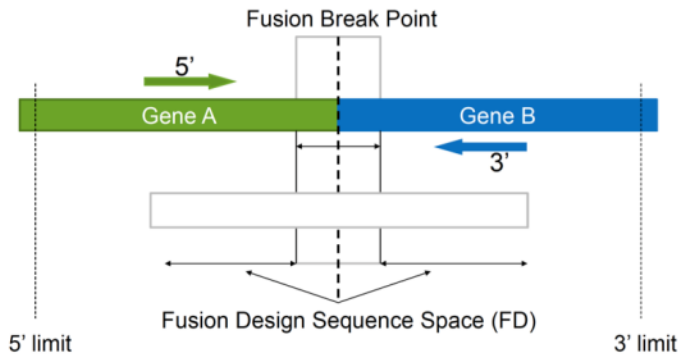
Only a fraction of the reads cross through the fusion breakpoint



Ion AmpliSeq™ RNA strategy for fusion transcripts

Focus reads around breakpoint

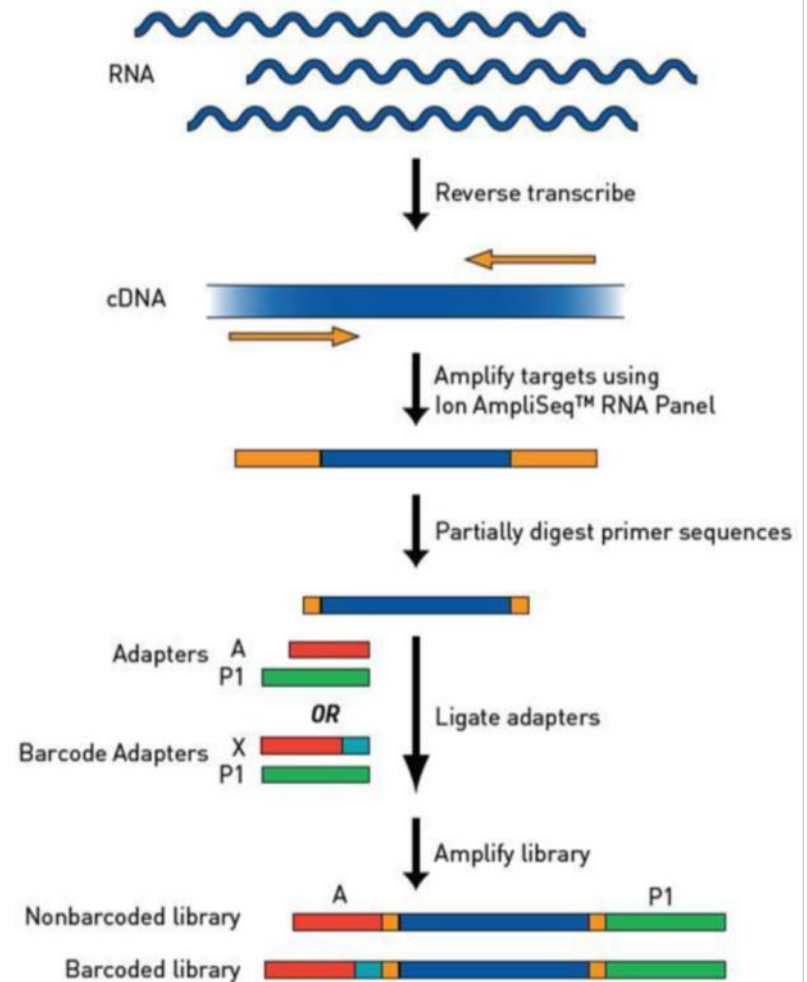
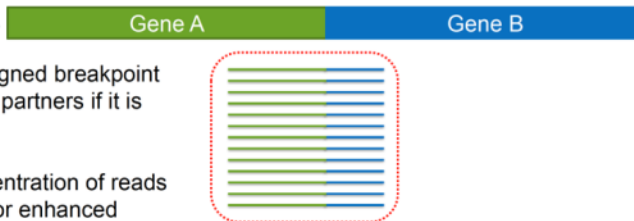
Use optimized design strategy for all fusion transcripts into single custom panel



Ion AmpliSeq™ RNA

All reads map to designed breakpoint between fusion gene partners if it is present in sample.

Allows focused concentration of reads on areas of interest for enhanced sensitivity of detection of the fusion transcript.



EML4-ALK detection by NGS

Experimental setup:

7 Diagnostic NSCLC samples **FFPE**/ 4 with ALK fusion FISH and IHC +)

Microdissected Tumor percentage 20-70%

Cell line control pool: EML4-**ALK**/ SLC34A2/**ROS1**/ CCDC6-**RET**

- Undivided nucleic acid (DNA/RNA)



Analysis Results

[Back](#)[Download](#)[Selected Variants](#)[Send to Report Role](#)[Switch To](#)[Generate Report](#)

Analysis Name: 010_c1327_1415975937984

Cancer Type: Non-Small Cell Lung Cancer

Total Mapped Fusion Panel Reads: 57220

To learn more about reviewing your results, visit the [help guide](#).

Fusions

[Search](#)

☐	☐	☐	Classification	Locus	Type	Genes (Exons)	Read Counts
☐	☐	☐	Unclassified	chr1:156104319	EXPR_CO NTROL	LMNA	27603
☐	☐	☐	Unclassified	chr1:156834532, chr1:156851323	ASSAYS_5 P_3P	NTRK1	13,0
☐	☐	☐	Unclassified	chr2:29551347, chr2:29430138	ASSAYS_5 P_3P	ALK	0,79
☐	☐	☐	Unclassified	chr2:42491871 - chr2:29446394	FUSION	EML4(6) - ALK(20)	2334
☐	☐	☐	Unclassified	chr2:42492091 - chr2:29446394	FUSION	EML4(6) - ALK(20)	991
☐	☐	☐	Unclassified	chr6:117711009, chr6:117632280	ASSAYS_5 P_3P	ROS1	0,0
☐	☐	☐	Unclassified	chr6:170871321	EXPR_CO NTROL	TBP	15690
☐	☐	☐	Unclassified	chr8:128751265	EXPR_CO NTROL	MYC	8095
☐	☐	☐	Unclassified	chr10:43606730, chr10:43622086	ASSAYS_5 P_3P	RET	0,458
☐	☐	☐	Unclassified	chr11:118960975	EXPR_CO NTROL	HMBS	1879
☐	☐	☐	Unclassified	chr12:53585786	EXPR_CO NTROL	ITGB7	60

[Click to launch IGV for this locus](#)

Filter Options

✕

Variants

- Filtered In Variants (11)
- Hidden Variants (0)
- Filtered Out Variants (69)

Samples

- Fusions Sample: 010

Chromosome

[All](#)

Filter Chains

[Default Fusions View](#)

Total Variants: 80

Default Fusions

View in Present

Variants: 11 Genes: 10

[Save Filter Chain](#)

Results

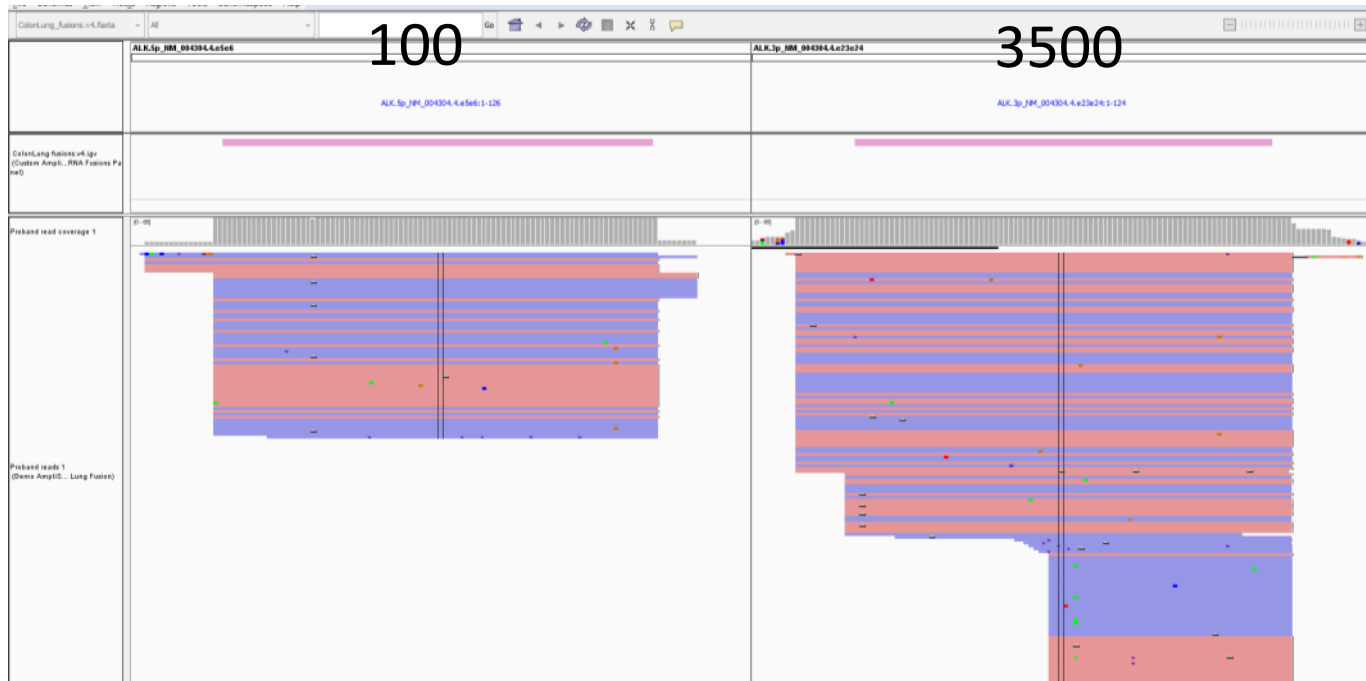
Sample	ALK break (FISH)	NGS
1	Yes	EML4 (6)-ALK (20)
2	Yes	EML4 (6)-ALK (20)
3	Yes	EML4 (6)-ALK (20)
4	Yes	EML4 (6)-ALK (20)
5	No	No
6	No	No
7	Not possible	No
Control		RET/ROS/ALK

4/4 EML ALK detected

ALK 3p/5p imbalance



EML4 ALK fusion -> increased expression of 3' ALK



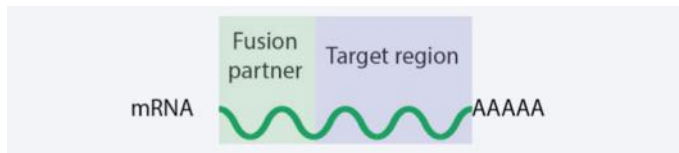
3'/5' imbalance

ALK 4/5

RET 1/1
ROS 0/1

Archer Fusionplex

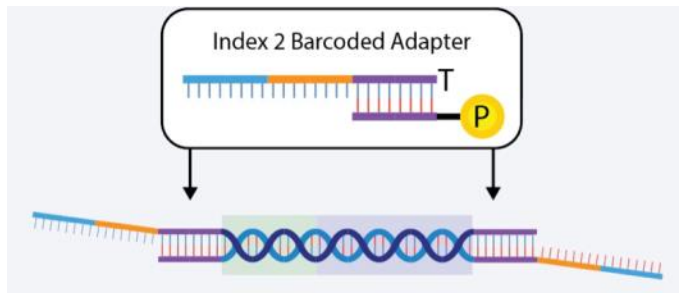
Anchored Multiplex PCR (AMP) overview



*Reverse transcription,
First and second strand cDNA synthesis*



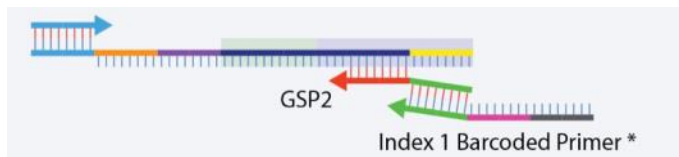
*End repair,
Phosphorylation
and dA-tailing*



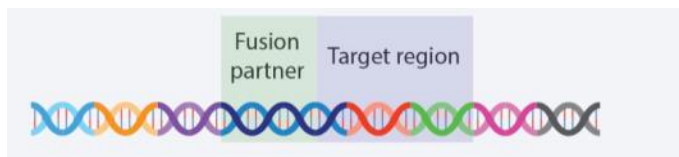
MBC Adapter ligation



Target-specific PCR 1



Target-specific PCR 2



Sequencing-ready library

RESULTS: Control (Lung Thyroid)

H2228
EML4-ALK

HCC-78
SLC34A2/ROS1

BHP 2-7
CCDC6-RET

Sample Summary RNA Expression

Download All Files

Sample Name Assay Result QC Result

ION_MBC_21.R_2016_02_03_16_33_42_user_PR1-34-MD_week05-Archer
Detailed Summary

Strong Evidence Fusions

- SLC34A2 → ROS1
- CCDC6 → RET
- EML4 → ALK
- EML4 → ALK
- SLC34A2 → ROS1
- SLC34A2 → ROS1
- SLC34A2 → ROS1

FUSION QC: PASS

Novel Isoforms

Breakpoint known
in
Quiver fusion
Database

Expression
imbalance
confirmation

Strong Evidence Weak Evidence Read Statistics Assay Targets Options

Fusion EML4 → ALK

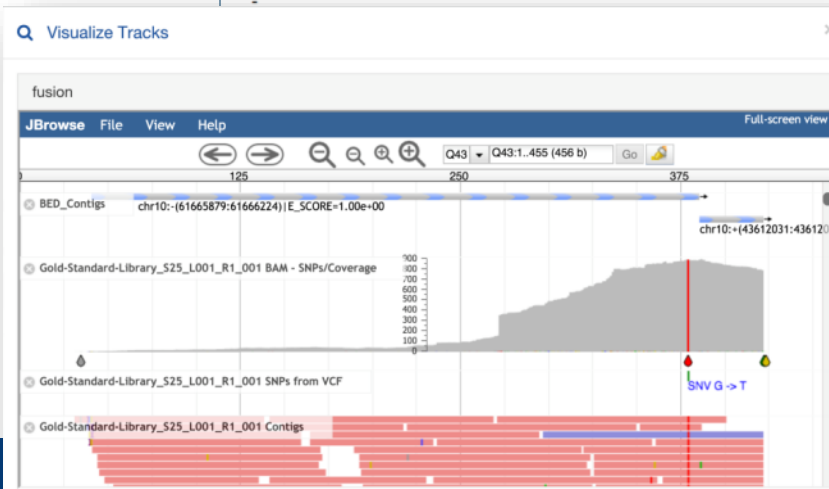
GSP2 Filters Reads (#/%) Start Sites (#/%)

ALK_chr22_29446347_28_p_GSP2 51 / 91.1 16 / 88.9

Visualize Translation Quiver Blast Misc

EML4 exon:13

ALK exon:20



Sarcoma Panel: Fusions in 26 genes

ALK breuk met onbekende fusie partner

Moleculair onderzoek:

ALK-FISH (Vysis Dual Color Break Apart Rearrangement Probe):

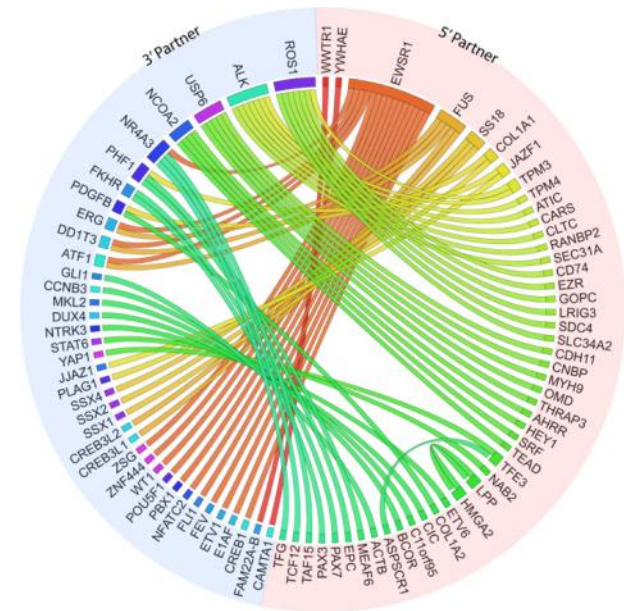
In dit materiaal wordt een **ALK breuk** gevonden binnen het onderzochte gebied.

Genfusie analyse door Next Generation sequencing:

Fusie/ibalans in aangevraagde genen

Er is EEN 3'/5' imbalans aangetoond in ALK.

Archer Sarcoma panel



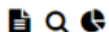
Sarcoma Panel			
ALK	CAMTA1	CCNB3	CIC
EPC	EWSR1	FKHR	FUS
GLI1	HMGA2	JAZF1	MEAF6
MKL2	NCOA2	NTRK3	PDGFB
PLAG1	ROS1	SS18	STAT6
TAF15	TCF12	TFE3	TFG
USP6	YWHAE		

VCL-ALK

Sample Name

160149T

[Detailed Summary](#)



Assay Result

Strong Evidence Fusions **1**

VCL → ALK ☒ ☒ ☒

Novel Isoforms **3**

QC Result

FUSION QC: PASS

GSP2

Filters

Reads (#/%)

Start Sites (#/%)

ALK_chr2_29446347_28_p_GSP2



32 / 100

18 / 100

[Visualize](#)

[Translation](#)

[Quiver](#)

[Blast](#)

[Misc.](#)

+ VCL exon:16

- ALK exon:20

☒ BED_GSP2

☒ BED_Annotated

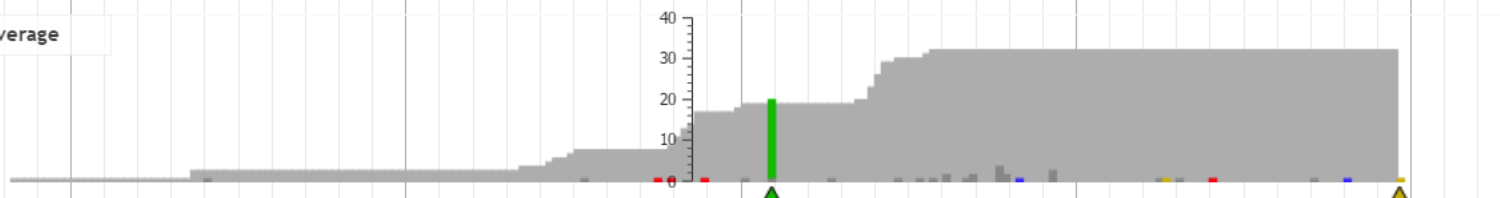
VCL(+)NM_014000|exon:16

VCL(+)NM_003373|exon:16

☒ BED_Contigs

chr10:+(75864951:75865111)|E_SCORE=QUIVER

☒ 160149T BAM - SNPs/Coverage



CARS-ALK



Prostate cancer

Prostaat biopt

ERG IHC: Sterk positief:

ERG fusie, betere respons op Androgene deprivatie therapie

Solid Tumor panel

50 genes, fusions + mutations

160350T
Detailed Summary
📄 🔍 🔄

Strong Evidence Fusions **6**

- TMPRSS2 → ERG 🔗 📄
- TMPRSS2 → ERG 🔗 📄
- TMPRSS2 → ERG ✔️ 📄
- TMPRSS2 → ERG ✔️ 📄
- TMPRSS2 → ERG ✔️ 📄
- TMPRSS2 → ERG ✔️ 📄

Novel Isoforms **14**

FUSION QC: PASS

GSP2	Filters	📄 Reads (#/%)	📄 Start Sites (#/%)					
ERG_E_r_004_17_R_0_GSP2	✔️ 📄	52 / 27.1	20 / 16.9	🔍 Visualize	📄 Translation	📄 Quiver	📄 Blast	Misc. ▼

- 🔴 **TMPRSS2 exon:1** 🔴

🔴 **ERG exon:4** 🔴

Conclusions

Fusie detectie met NGS werkt op FFPE weefsel
Totaal nucleinezuur (DNA en RNA) voldoet

Ampliseq detecteerd bekende fusies
Ion torrent platform
Relatief goedkoop
100k reads minimum (1 Cancer panel equivalent)

Archer detecteerd ook onbekende partners (van bekende genen)
platform onafhankelijk
Torrent /Illumina
1-2M reads nodig

Bioinformatica bijgeleverd bij beide
Ook lokaal mogelijk

thanks

Sandra Uljee
Karin Kleiverda
Anne-Marie Cleton Jansen
Ronald van Eijk
Melati Tamsma
Vincent Smit
Judith Bovee
Hans Morreau

