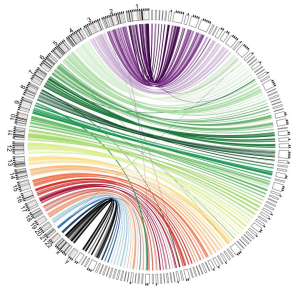
Storskalig analys av strukturella varianter

En överblick över ny teknik och stora genetiska varianter

Jesper Eisfeldt Bioinformatiker



Karolinska Institutet

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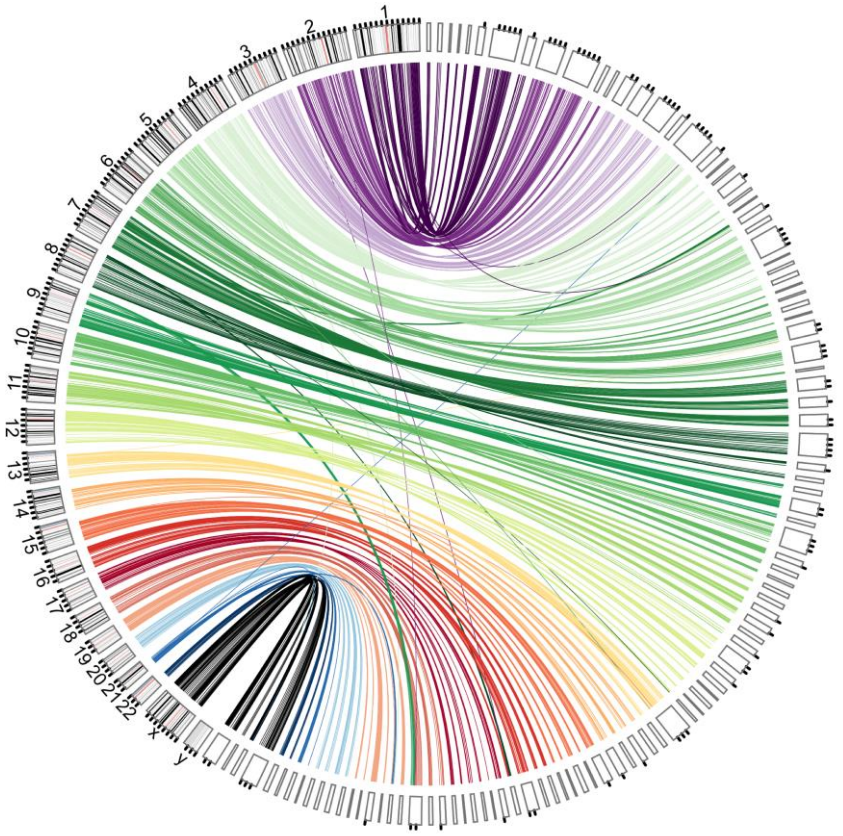
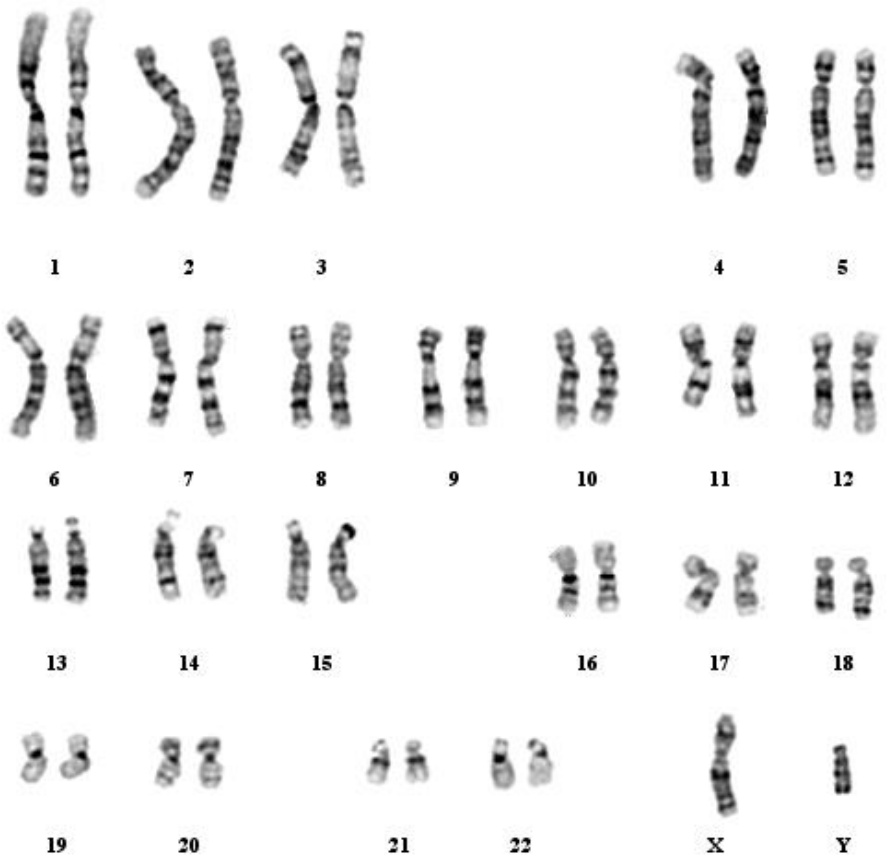
Storskalig analys av strukturella varianter

- Introduktion
  - Strukturella varianter
  - Metoder
    - Illumina
    - Nanopore
- Projekt
- Sammanfattning



Genetisk variation

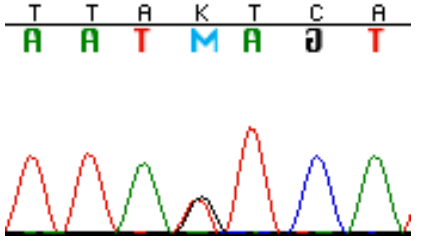


0. Mänskliga genomet

- Baser A,T,C,G
- Ca 3 (*2) miljarder baspar

1. Basparsutbyte (SNVs)

~ 5 M

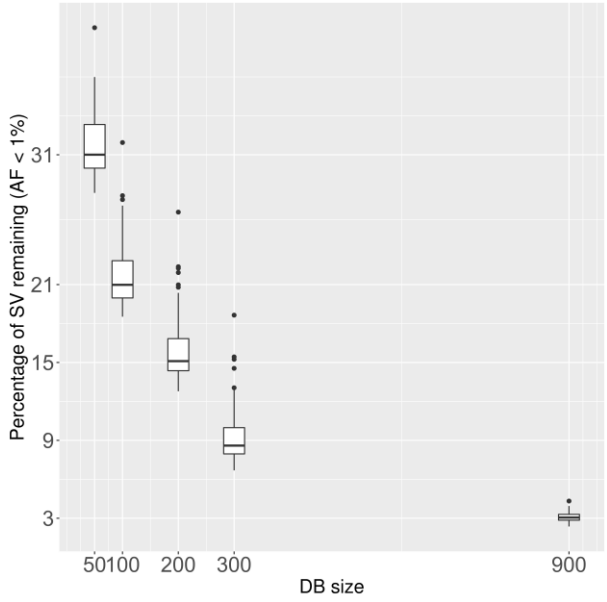


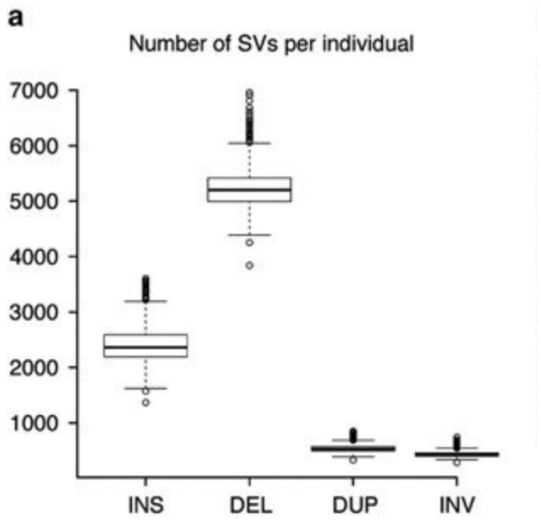
2. Strukturella varianter (> 50 baspar)

- Duplikation
- Deletion
- Translokationer
- Inversioner

Strukturella varianter

- Ca 5000 -10 000 per individ
- Vissa är sjukdomsorsakande
  - Ovanliga varianter
- Databaser
  - SweGen





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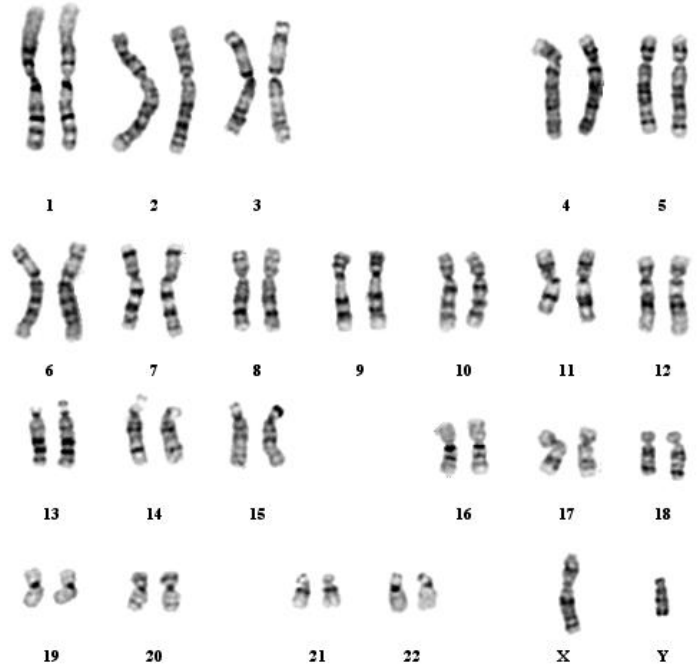
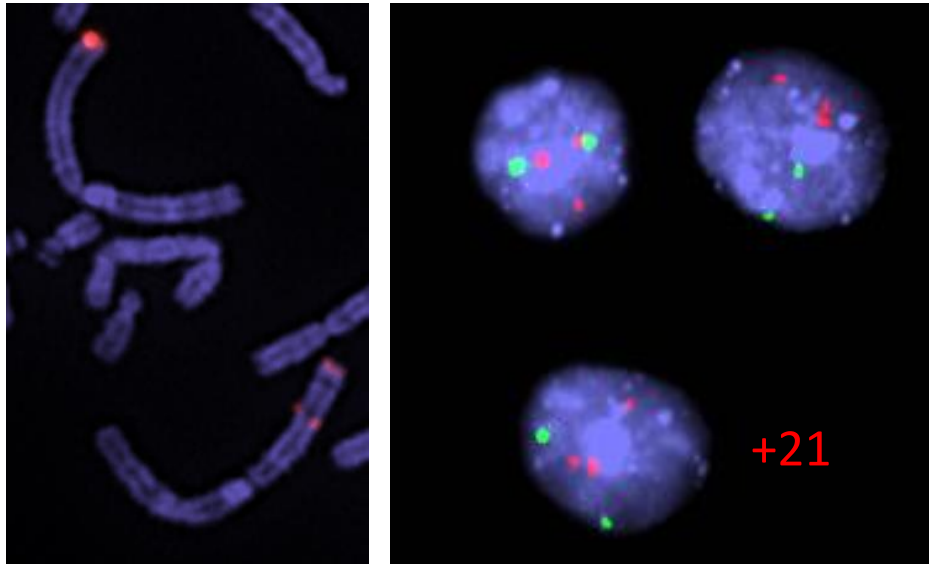
Metoder

- Vanliga metoder
  - Mikroskop (karyotyp)
    - Stora strukturella varianter
  - FISH
    - Förbestämda regioner
  - Och många andra!

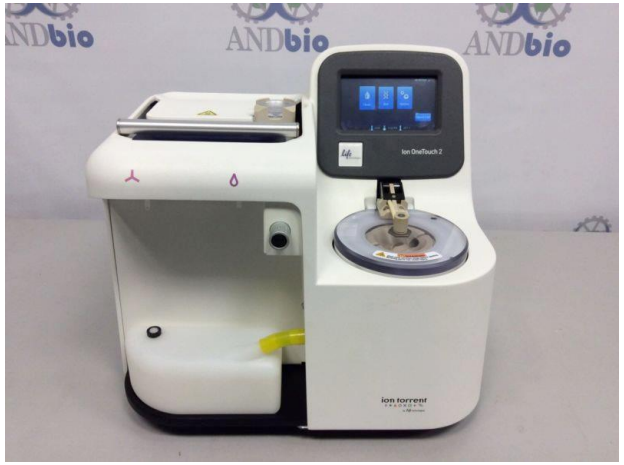
- DNA Sekvensering
  - Relativt nytt
  - Att läsa DNA
  - Hela genomet





Metoder

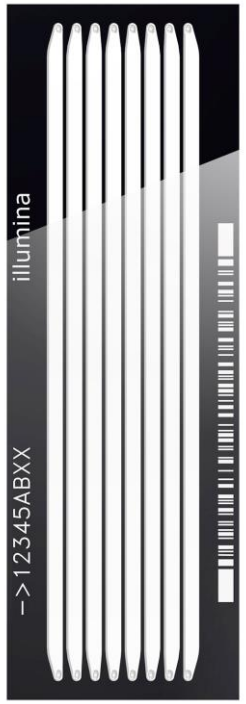
- Sekvensering
  - Att läsa DNA
  - Ofta från blodprov
- Många metoder
- För och nackdelar
  - Kostnad
  - Tid
  - Kvalitet
  - Längd på läsningarna



www.andbio.com www.pacb.com https://spectrum.ieee.org/

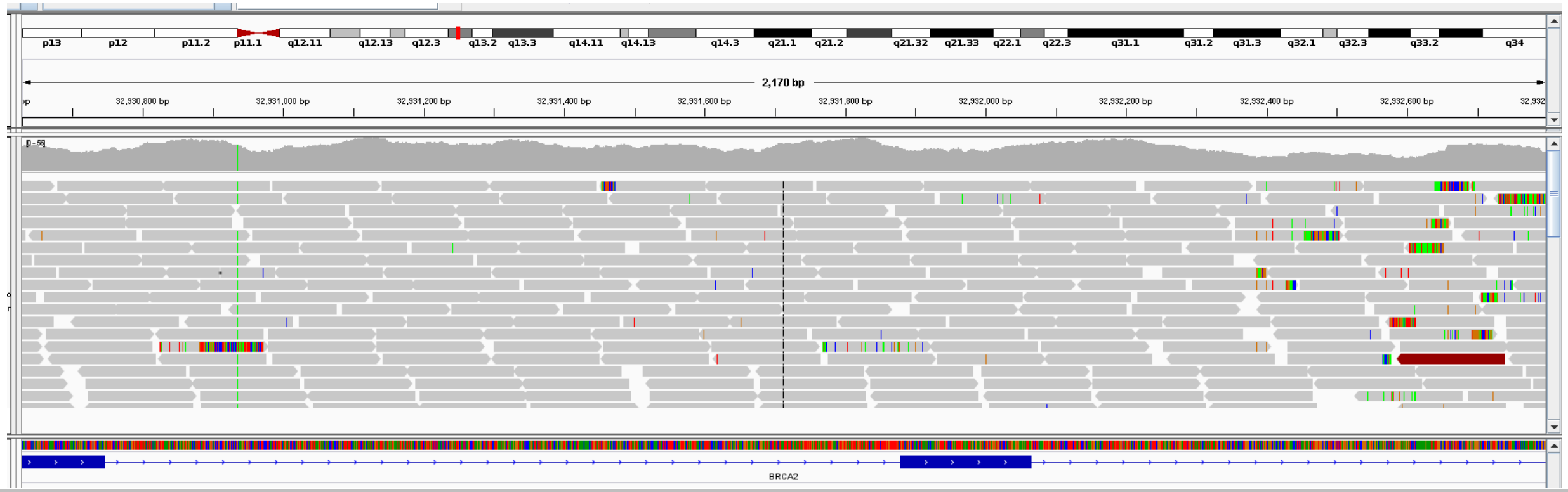
Illumina helgenomsekvensering

- Ledande tekniken
  - I klinik och forskning
- Korta läsningar
  - 150 baspar
- Billigt
  - 10 000 -20 000 kronor
  - 400 miljoner parade läsningar
- Hög kvalitet
  - Mindre än 1 fel per 100 baser



Illumina helgenomsekvensering

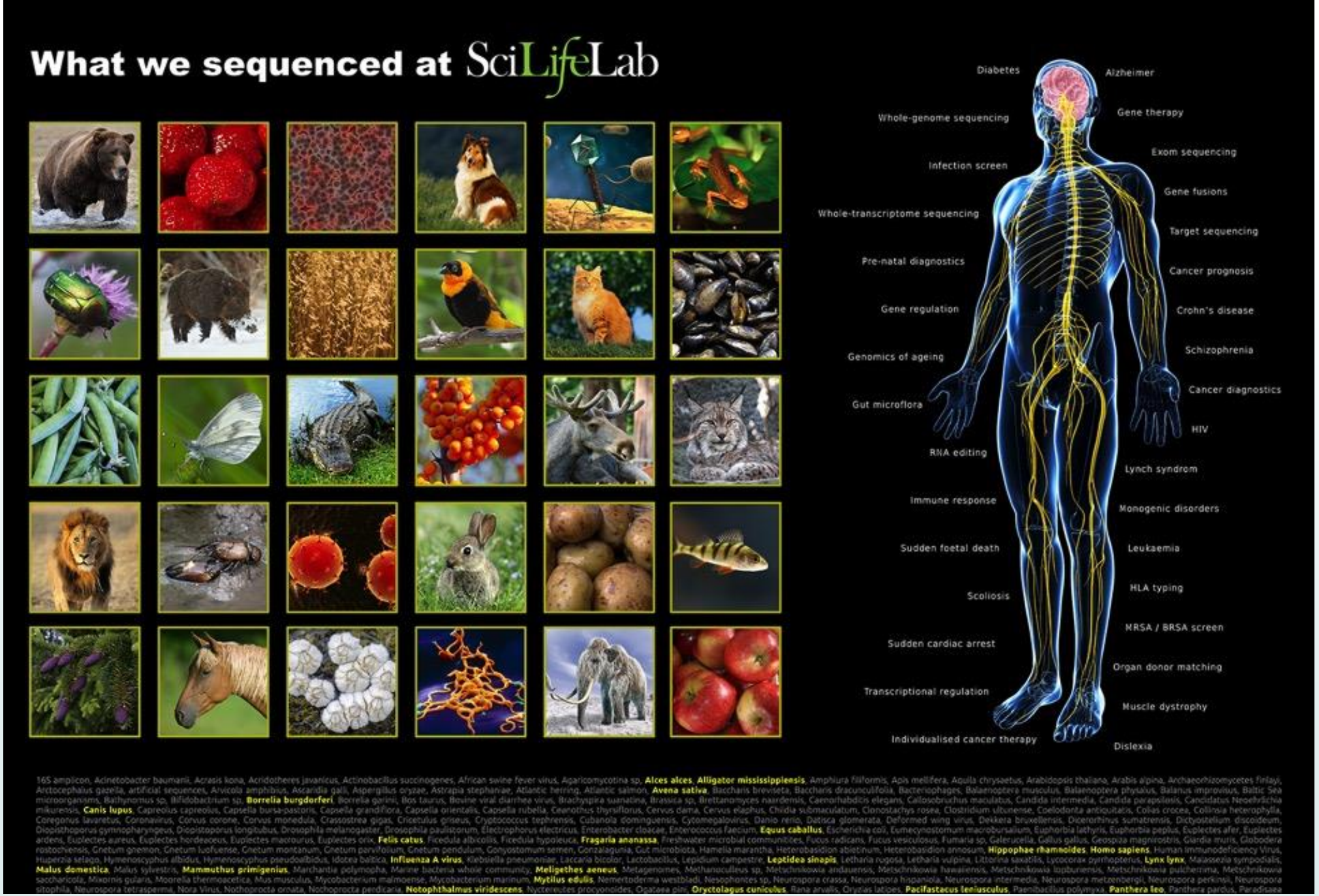
- Läs samma genom flera gånger
  - Ofta 30 gånger
  - analysera varianter
  - Hantera fel



Illumina helgenomsekvensering

- National genomics infrastructure

- Clinical genomics



https://www.scilifelab.se/platforms/ngi/

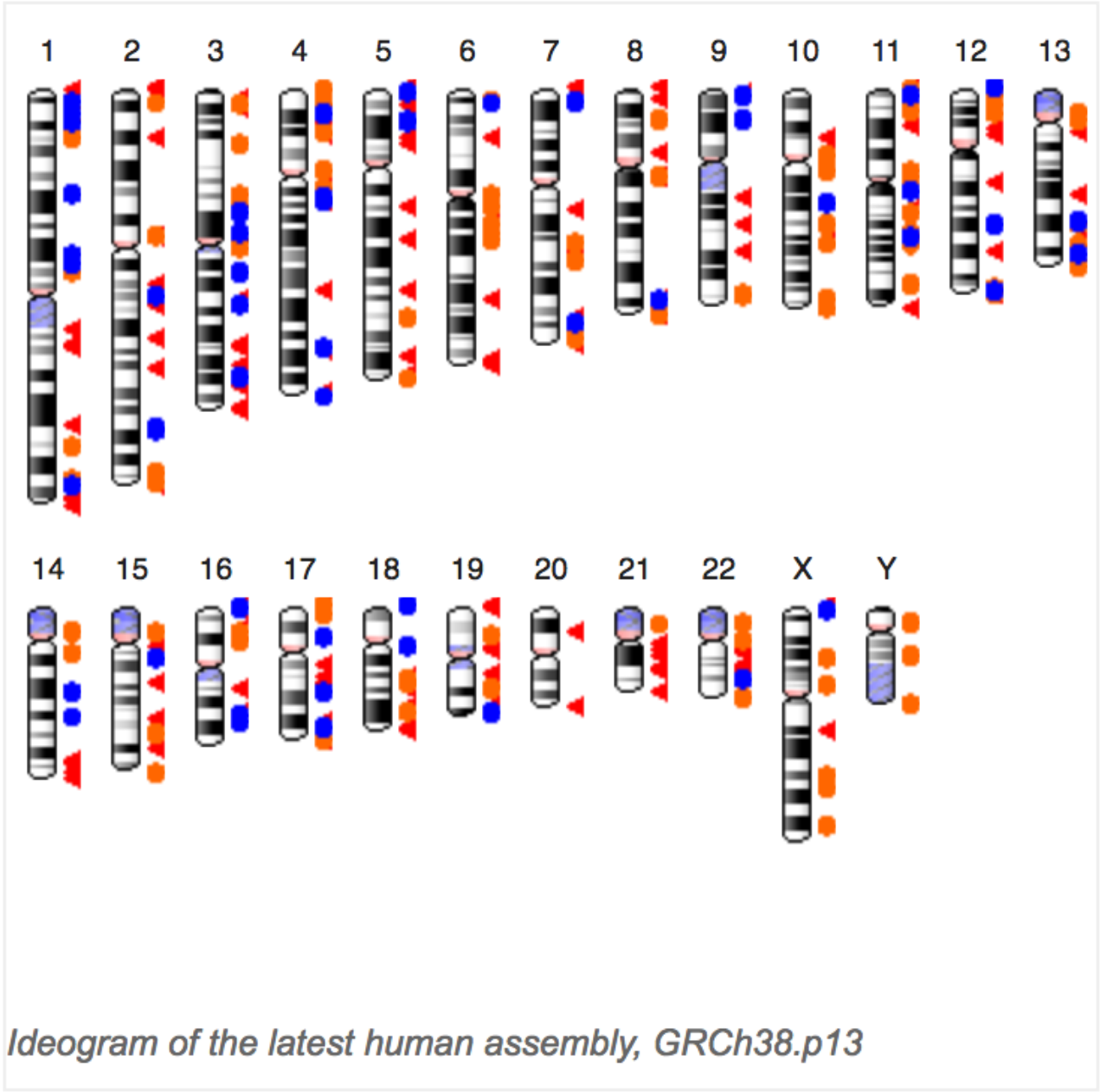
Nanopore helgenomsekvensering

- Långa läsningar
  - Längre än 10 000 baspar
- Många Storlekar
- Relativt dyrt
  - 30 000 kr
    - Mänskligt genom
  - Runt 10 miljoner läsningar
    - stora maskinen
- Sämre kvalitet
  - 10% fel av de lästa baserna

nanoporetech.com

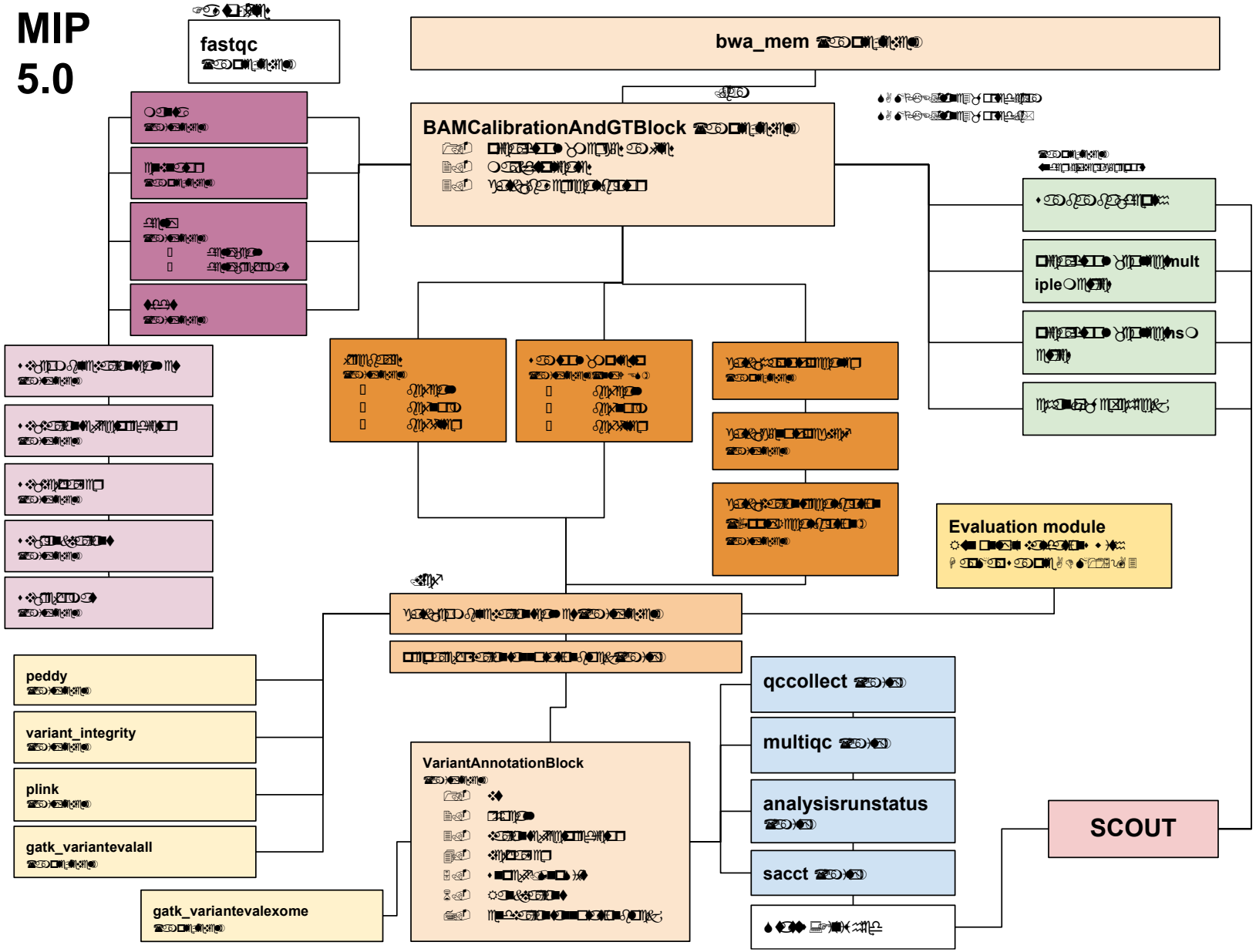
Analys

- Jämföra läsningar (sekvenser)
  - Hitta avvikelser
  - Relativt mot referensgenomet
- Referensgenom
  - En model av det mänskliga genomet
  - En stor textfil
    - 3 miljarder tecken
  - 70% från en individ
- Programvara



Ideogram of the latest human assembly, GRCh38.p13

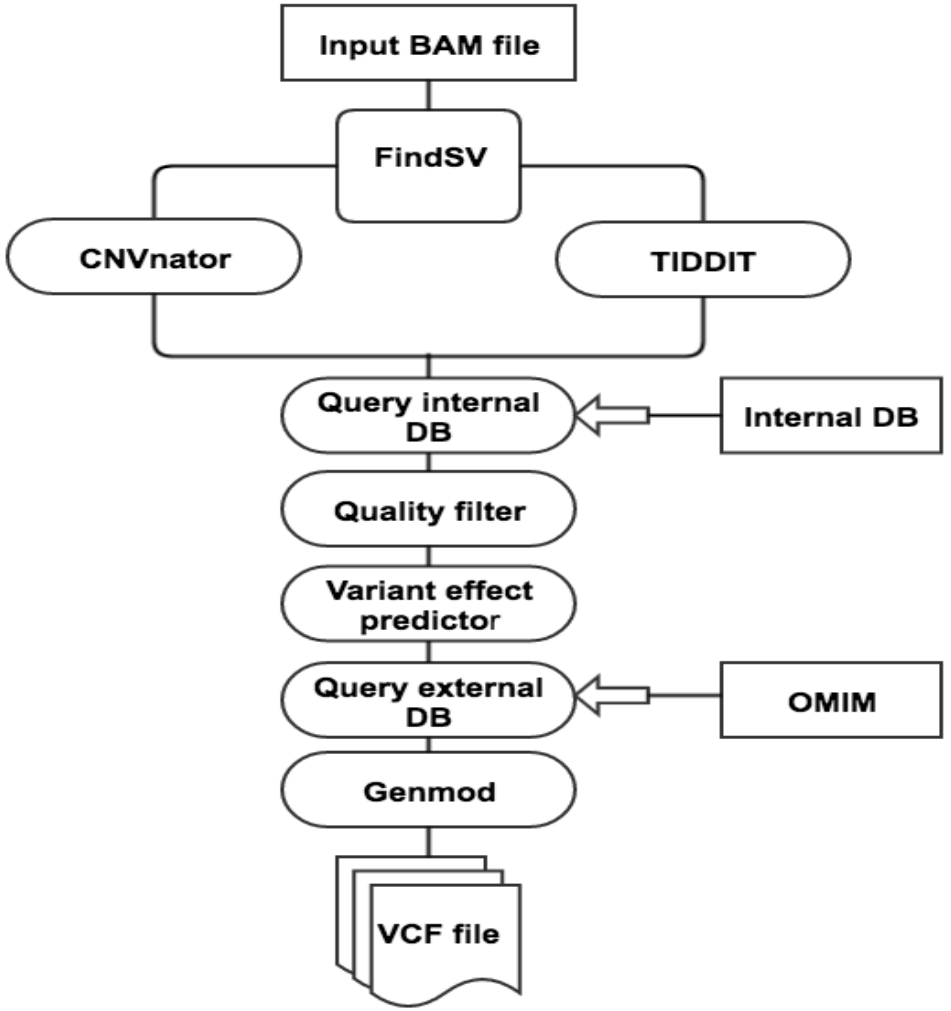
NGS pipeline: MIP



Henrik Stranneheim

Leta strukturella varianter i kliniken

- Egen pipeline
  - Hitta stora varianter
- Filtrering
  - Ta bort varianter vanliga varianter
  - Hitta vilka varianter som ligger i gener



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##svdbcmdline=/home/jesperei/anaconda2/bin/svdb --merge --overlap 0.9 --vcf P2109_123.clean.dedup.recal_FindSV.vcf
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1       86005620        SV_374_1        N       N[1:146486084[  .       PASS    SVTYPE=BND;CIPOS=-244,0;CIEND=0,213;COVM=25.5716494077;COVA=71.9725;COVB=86.03;LFA=42
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1       145382407       SV_471_1        N       N[1:148241134[  .       PASS    SVTYPE=BND;CIPOS=-142,0;CIEND=0,129;COVM=28.5746383098;COVA=113.616666667;COVB=56.15;
2       212161492       SV_3876_1       N       <DEL>   .       PASS    SVTYPE=DEL;CIPOS=-37,0;CIEND=0,8;END=212161600;SVLEN=109;COVM=20.4333333333;COVA=22.27;COVB=2
3       75276849        SV_8058_1       N       N[3:75278911[   .       PASS    SVTYPE=BND;CIPOS=-355,0;CIEND=0,238;COVM=37.16;COVA=55.9;COVB=62.91;LFA=37;LFB=40;LTE
3       75569001        CNVnator_del_156        N       <DEL>   .       PASS    END=75579000;SVTYPE=DEL;SVLEN=-10000;IMPRECISE;natorRD=0.548889;natorP1=0.00492305;na
3       103164041       SV_8101_1       N       <DEL>   .       PASS    SVTYPE=DEL;CIPOS=-64,0;CIEND=0,1;END=103164407;SVLEN=367;COVM=22.478;COVA=26.14;COVB=26.14;LF
6       57284882        SV_11122_1      N       N[6:57289357[   .       PASS    SVTYPE=BND;CIPOS=-290,0;CIEND=0,232;COVM=34.4747826087;COVA=65.1275;COVB=72.05;LFA=64
6       57423046        SV_10870_1      N       N[6:57429121[   .       PASS    SVTYPE=BND;CIPOS=-459,0;CIEND=0,204;COVM=36.1158064516;COVA=97.6933333333;COVB=84.896
8       84948937        SV_12650_1      N       <DEL>   .       PASS    SVTYPE=DEL;CIPOS=-68,0;CIEND=0,1;END=84949600;SVLEN=664;COVM=18.68125;COVA=32.42;COVB=32.03;L
9       41346001        CNVnator_del_348        N       <DEL>   .       PASS    END=41855000;SVTYPE=DEL;SVLEN=-509000;IMPRECISE;natorRD=0.42689;natorP1=3.13109e-13;n
9       136379164       SV_13046_1      N       <DUP>   .       PASS    SVTYPE=DUP;CIPOS=-72,0;CIEND=0,1;END=136379553;SVLEN=390;COVM=130.03;COVA=33.695;COVB=24.05;L
10      49865373        SV_14703_1      N       <DEL>   .       PASS    SVTYPE=DEL;CIPOS=-10,0;CIEND=0,0;END=49865532;SVLEN=160;COVM=21.43;COVA=19.75;COVB=18.19;LFA=
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14      107103001       CNVnator_dup_534        N       <DUP>   .       PASS    END=107188000;SVTYPE=DUP;SVLEN=85000;IMPRECISE;natorRD=1.52706;natorP1=0.0416581;nato
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15      61963001        CNVnator_dup_554        N       <DUP>   .       PASS    END=61989000;SVTYPE=DUP;SVLEN=26000;IMPRECISE;natorRD=1.12968;natorP1=0.00043246;nato
16      4069109 SV_28814_1      N       N[20:45929626[  .       PASS    SVTYPE=BND;CIPOS=-553,0;CIEND=0,3;COVA=32.4885714286;COVB=33.9;LFA=24;LFB=14;LTE=13;E1=0;E2=4
17      2220001 CNVnator_del_605        N       <DEL>   .       PASS    END=2485000;SVTYPE=DEL;SVLEN=-265000;IMPRECISE;natorRD=0.560547;natorP1=6.01406e-13;natorP2=2
17      2220316 SV_29905_1      N       <INV>   .       PASS    SVTYPE=INV;CIPOS=-295,0;CIEND=-226,0;END=2617815;SVLEN=397500;COVM=22.3041624748;COVA=32.385;COVB=25.
17      2618001 CNVnator_del_606        N       <DEL>   .       PASS    END=2648000;SVTYPE=DEL;SVLEN=-30000;IMPRECISE;natorRD=0.547125;natorP1=5.31242e-12;natorP2=1.
17      21321241        SV_29947_1      N       N[17:21322822[  .       PASS    SVTYPE=BND;CIPOS=-394,0;CIEND=0,220;COVM=38.3770588235;COVA=100.97;COVB=88.8133333333
17      21349689        SV_29955_1      N       ]17:25331105]N  .       PASS    SVTYPE=BND;CIPOS=0,80;CIEND=-89,0;COVM=39.686481314;COVA=61.765;COVB=84.335;LFA=9;LFB
18      50488037        SV_30743_1      N       N[18:50489494[  .       PASS    SVTYPE=BND;CIPOS=-356,0;CIEND=0,129;COVM=36.4433333333;COVA=74.894;COVB=63.46;LFA=54;
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21      11175559        SV_33613_1      N       N[22:20320513[  .       PASS    SVTYPE=BND;CIPOS=-340,0;CIEND=0,1;COVA=131.7325;COVB=53.36;LFA=12;LFB=14;LTE=7;E1=0;E
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Y       9956728 SV_35758_2      N       N[GL000199.1:5832[      .       PASS    SVTYPE=BND;CIPOS=0,265;CIEND=-7,0;COVA=158.2;COVB=509.82;LFA=34;LFB=143;LTE=6;E1=2;E2
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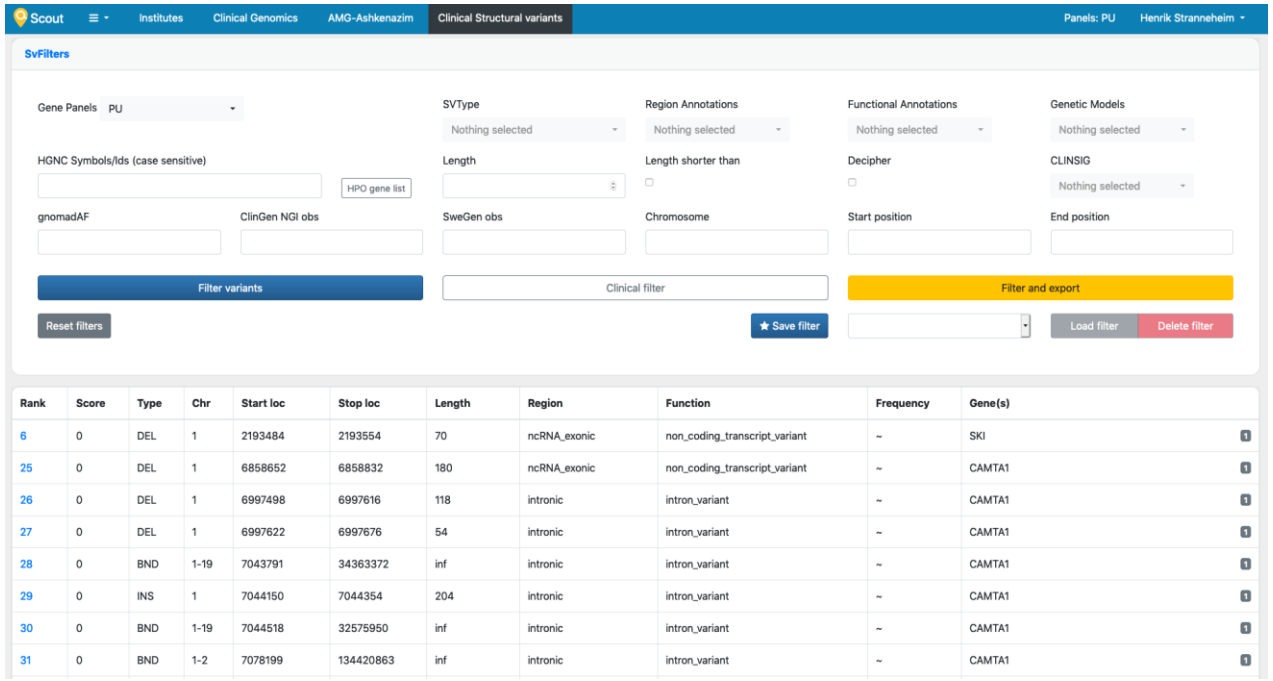
Leta strukturella varianter i kliniken



Rank	Score	Type	Chr	Start loc	Stop loc	Length	Region	Function	Frequency	Gene(s)
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25	0	DEL	1	6858652	6858832	180	ncRNA_exonic	non_coding_transcript_variant	~	CAMTA1
26	0	DEL	1	6997498	6997616	118	intronic	intron_variant	~	CAMTA1
27	0	DEL	1	6997622	6997676	54	intronic	intron_variant	~	CAMTA1
28	0	BND	1-19	7043791	34363372	inf	intronic	intron_variant	~	CAMTA1
29	0	INS	1	7044150	7044354	204	intronic	intron_variant	~	CAMTA1
30	0	BND	1-19	7044518	32575950	inf	intronic	intron_variant	~	CAMTA1
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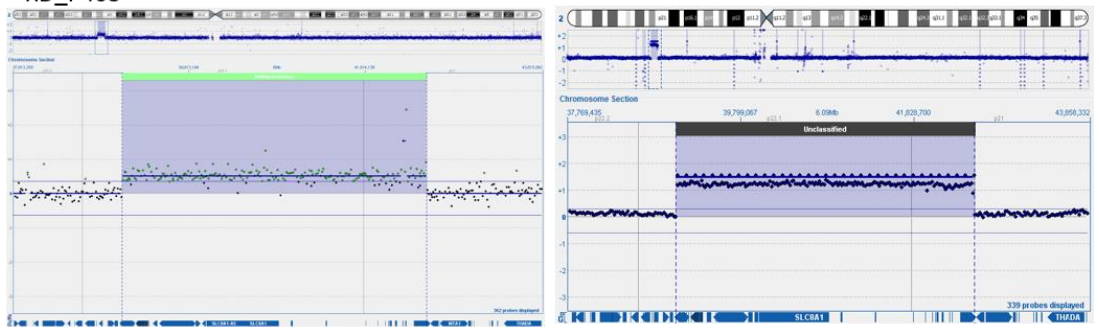
RD_P403



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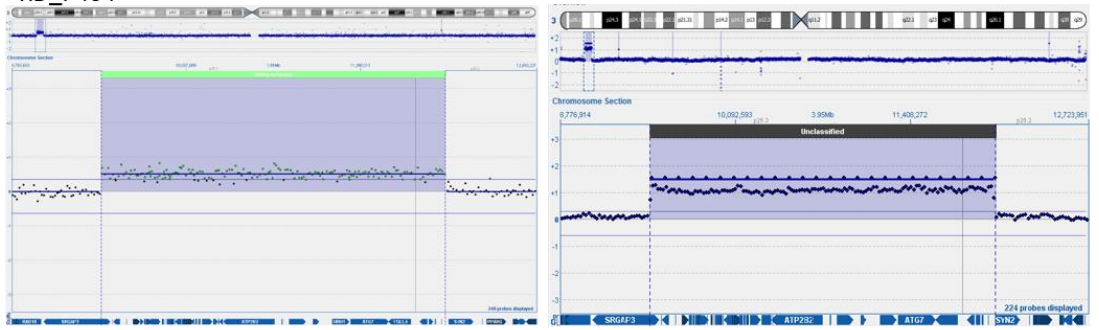

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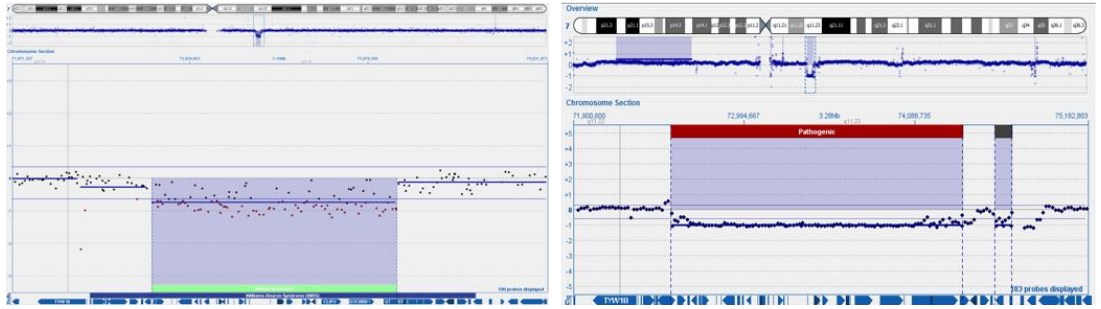
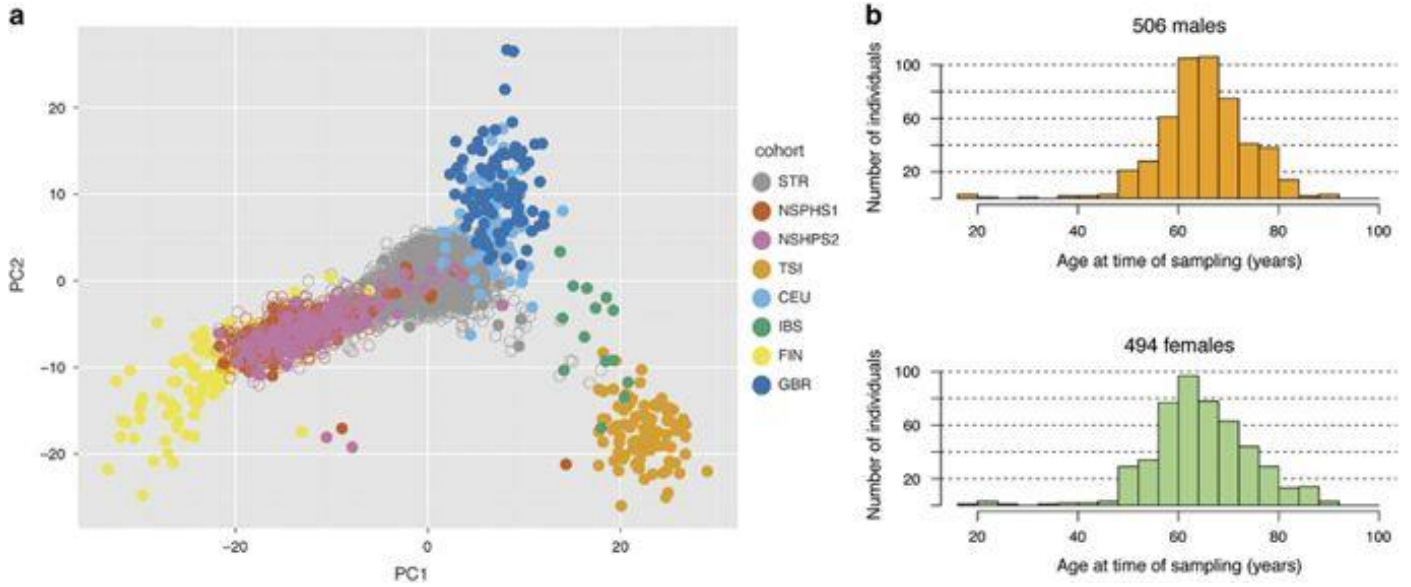


Hitta sekvenser som saknas i referens genomet

- 1000 Svenskar
  - Tvillingregistret
  - Sekvenserade 2017
- Pussla ihop genom
  - Överlappande läsningar
- Jämför mot Mänskliga referensgenomet
  - Analysera de bitar som inte matchar



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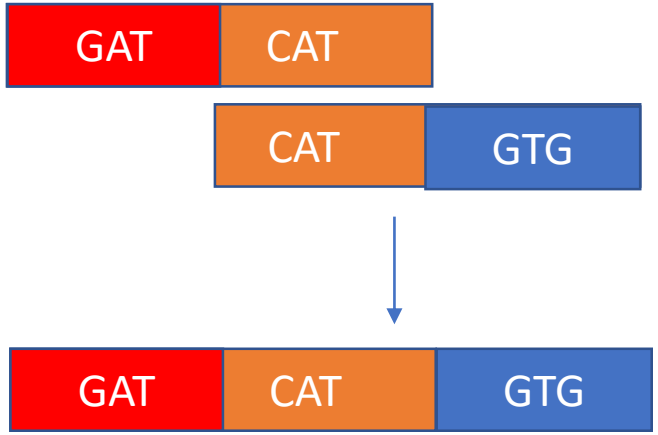
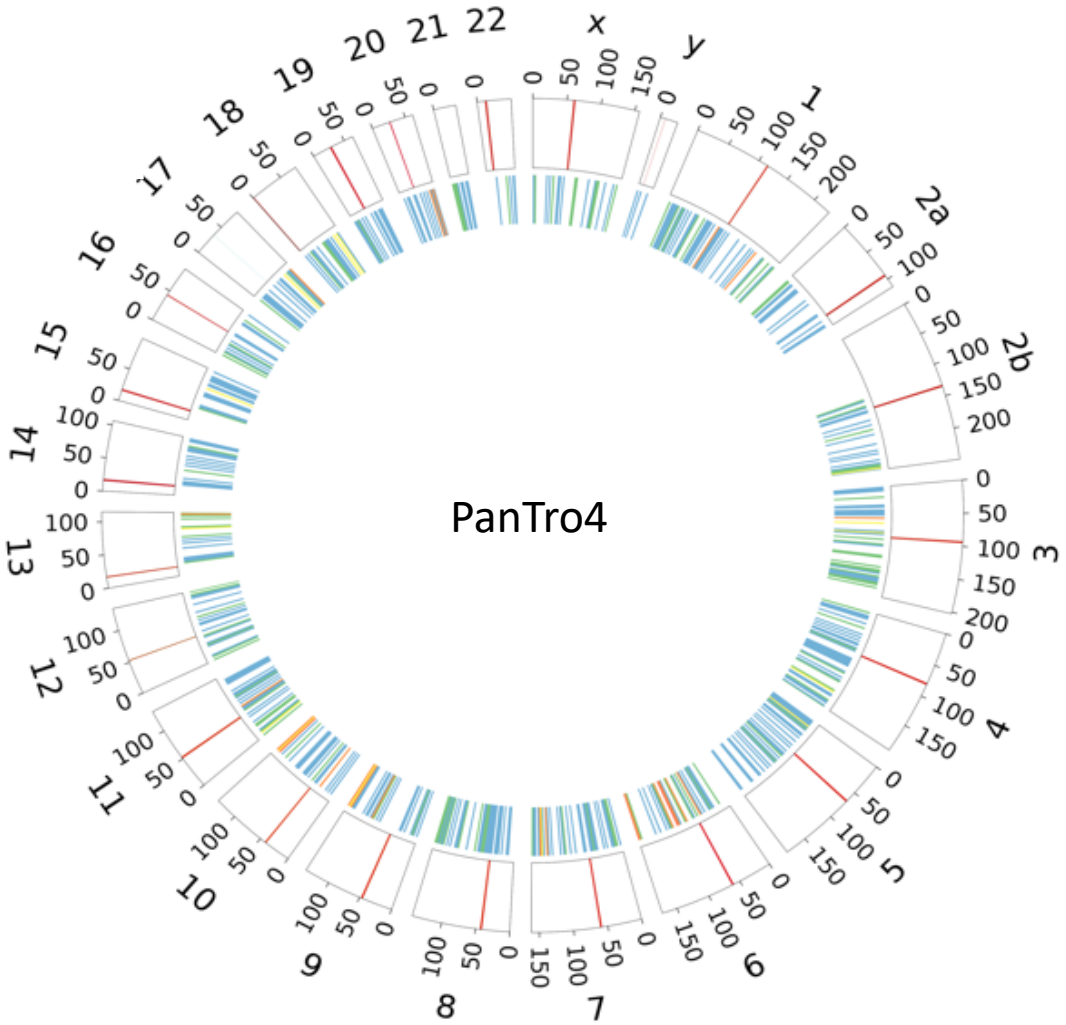


Hitta sekvenser som saknas i referens genomet

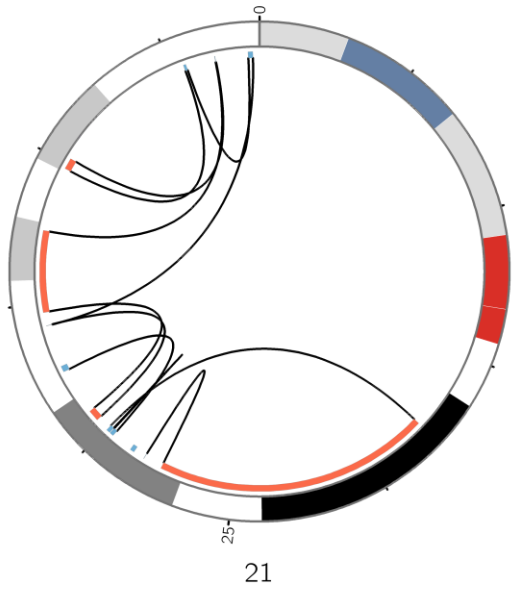
- Sekvens som saknas i referensgenomet
  - Vi hittade 46 Miljoner baser
    - Som kromosom 19
    - Ca 1 miljon baser per individ

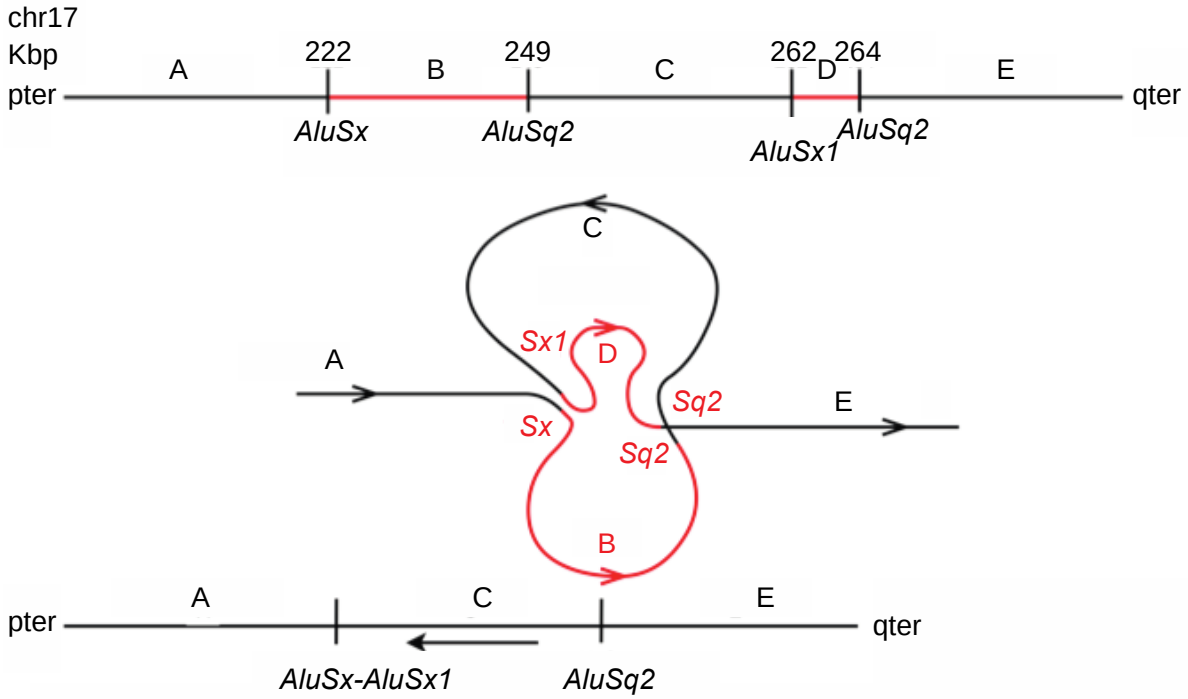
- Totalt 60 000 nya sekvenser
  - 20 000 matchar chimpansgenomet

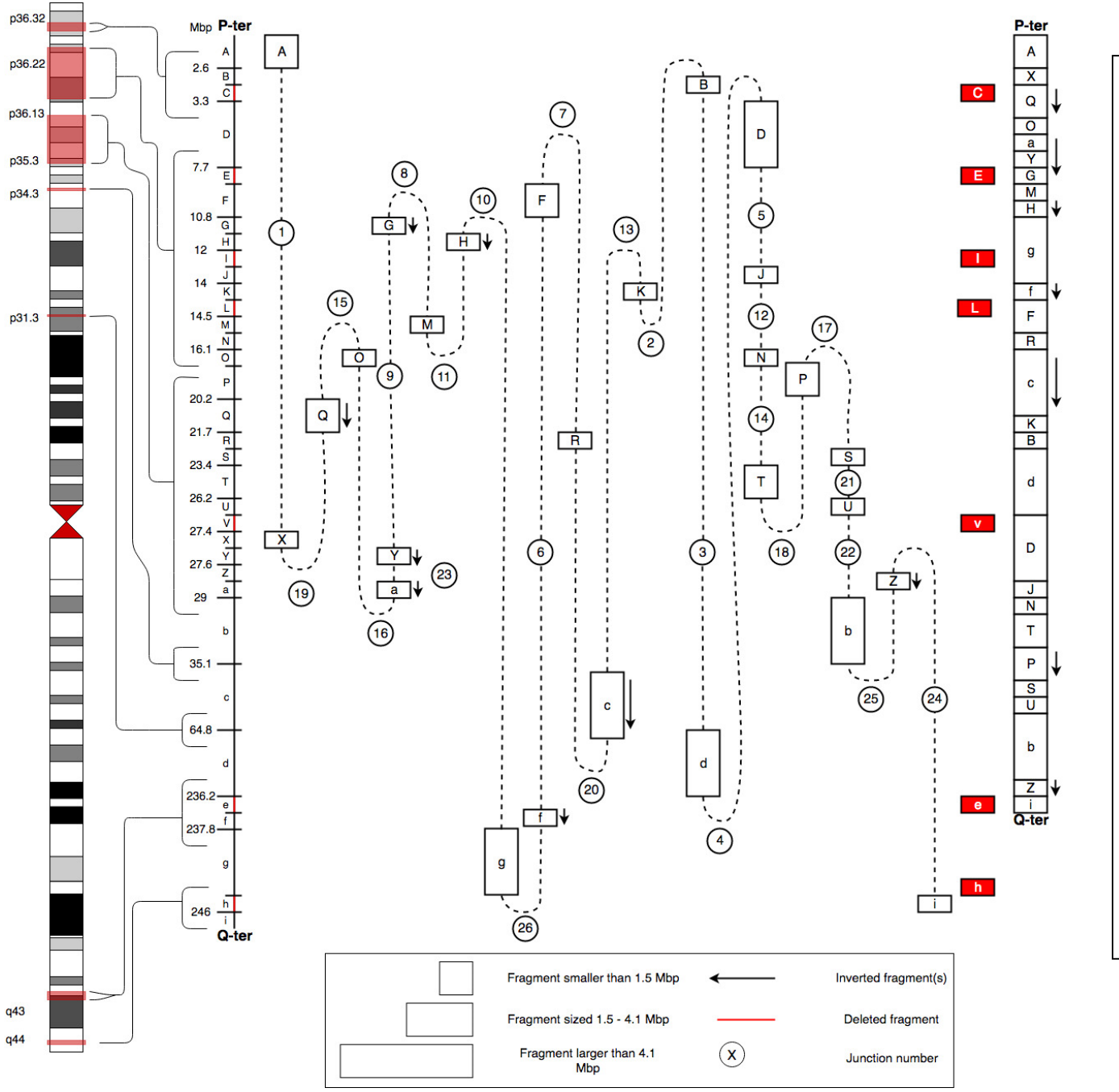


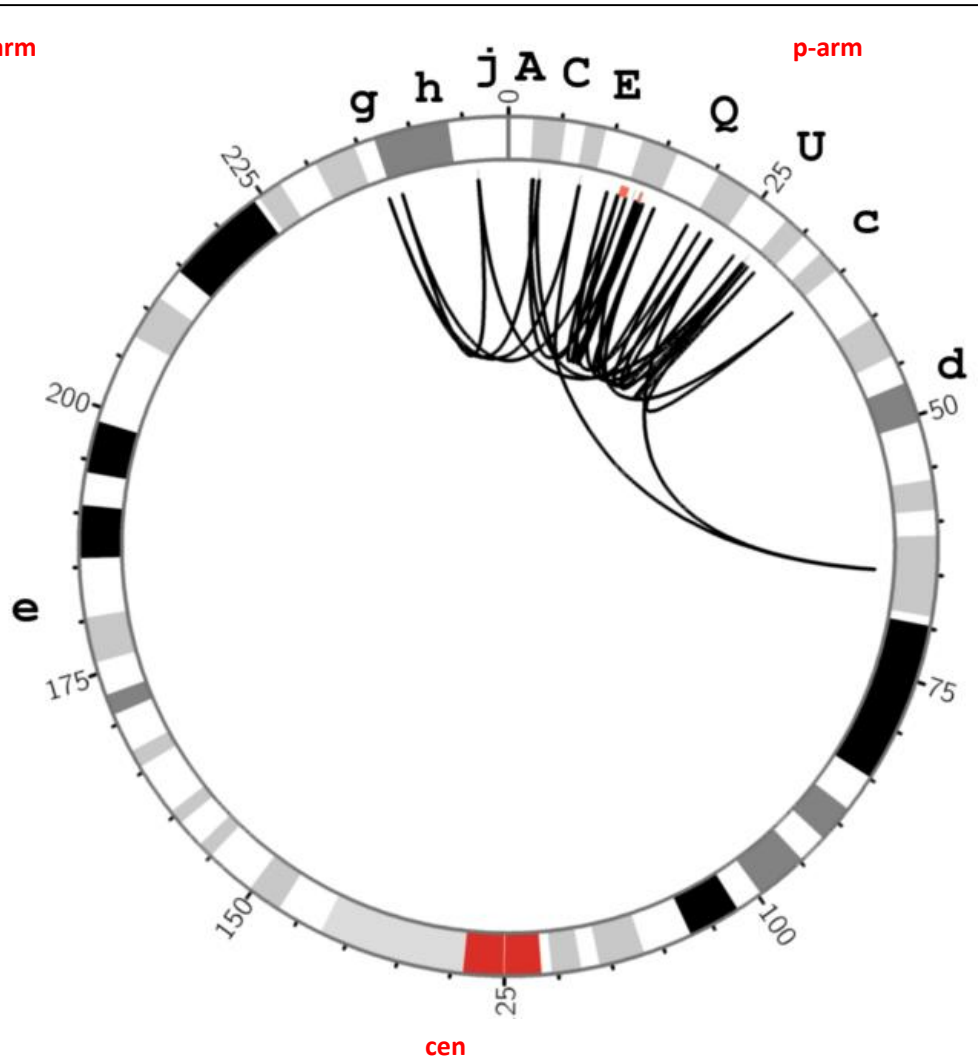
Lösa komplexa strukturella varianter

- Komplex strukturell avvikelse
  - Flera DNA fragment huller om buller
- Förstå genetiska mekanismer
- Hitta trasiga gener



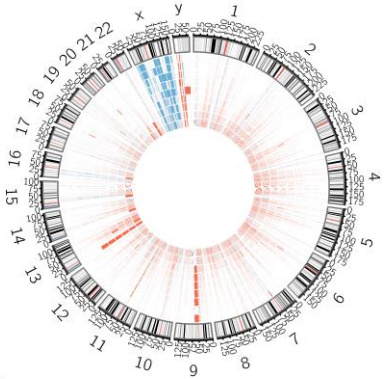


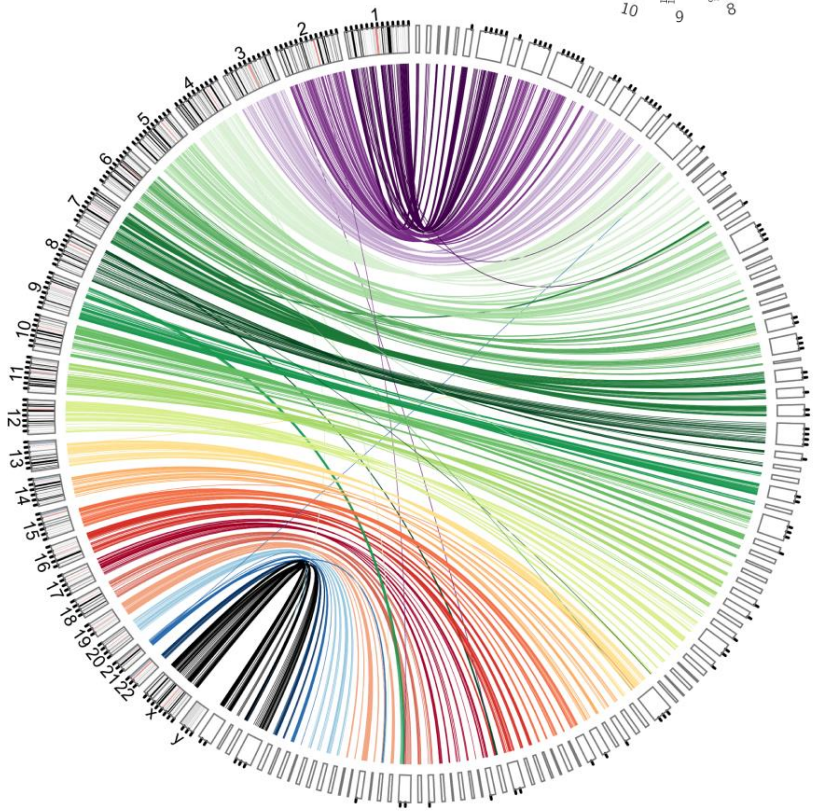

p36.32
p36.22
p36.13
p35.3
p34.3
p31.3
q43
q44
Mbp
P-ter
Q-ter
2.6
3.3
7.7
10.8
12
14
14.5
16.1
20.2
21.7
23.4
26.2
27.4
27.6
29
35.1
64.8
236.2
237.8
246
Fragment smaller than 1.5 Mbp
Fragment sized 1.5 - 4.1 Mbp
Fragment larger than 4.1 Mbp
Inverted fragment(s)
Deleted fragment
Junction number


q-arm
p-arm
cen
g h j A C E Q U c d e
0
25
50
75
100
125
150
175
200
225

Sammanfattning

- En stor variation av stora varianter
- Många sekvenseringstekniker
  - För och nackdelar
- Kliniskt relevant information
  - Sjukdomsorsakande varianter
  - Bättre verktyg





Tack!