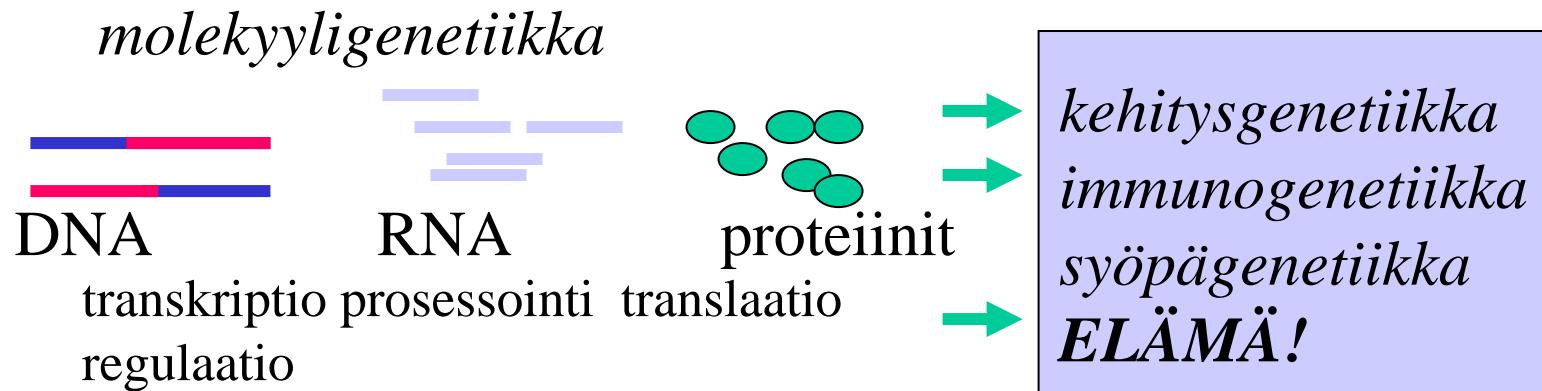


Geenien toiminta



Genetiikan perusteiden miellekartta: geenien sekvensointi

Miten geenia luetaan?

Geenien lukemisesta eli *DNA:n sekvensoinnista* on puhuttu jo niin usein, että nyt on vihdoinkin vilkaistava, mitä se tarkoittaa.

Sekvenointireaktio tuottaa erimittaisia DNA-pätkiä, joista voimme päätellä sekvenssin eli nukleotidien järjestyksen

Pitää vain saada ne erimittaiset havaituksi

Polymeraasiketjureaktio on tässäkin hommassa *aina* keskeinen menetelmä



Paul Berg: rekombinanttiDNA



Walter Gilbert: sekvensoinnista

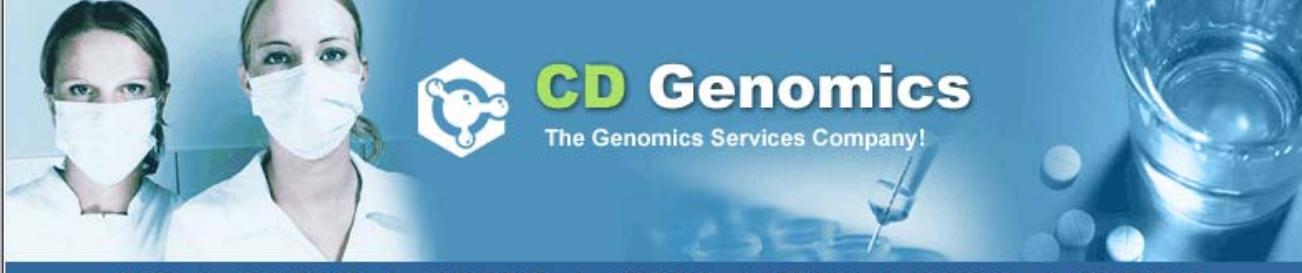


Fred Sanger: sekvensoinnista

1958

1980

[LINKKI](#)



HOME | SEQUENCING | GENOTYPING | LIBRARIES | OTHERS | ORDERING | CONTACT US

[Home](#) > [DNA sequencing](#) > [ABI 3730xl DNA Analyzer](#)

ABI 3730xl DNA Analyzer

The ABI3730xl DNA Analyzer is a fully automated, 96-capillary electrophoresis instrument. To ensure high quality base calling, assembly and SNP detection, CD Genomics uses a combination of software and manual basecalling to guarantee the sequencing quality. Our senior bioinformaticians have ever viewed more than ten thousands of trace files and accumulated abundant experience.

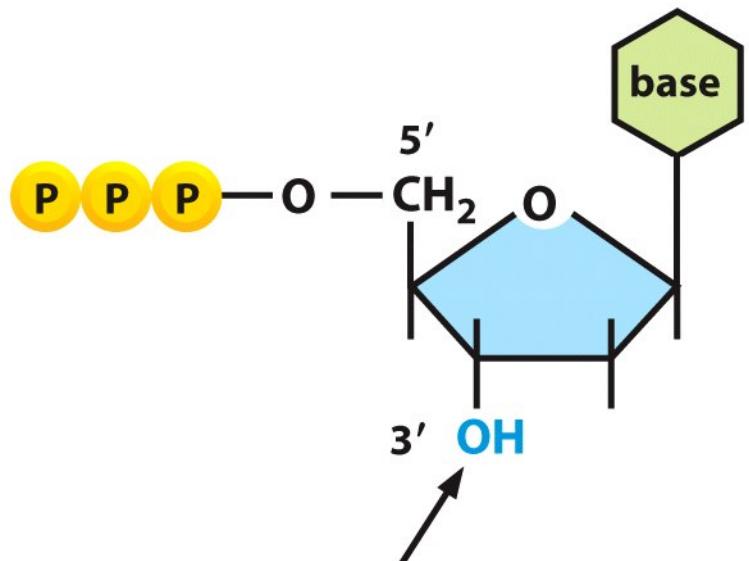
- Shotgun sequencing
- End sequencing
- SNP discovery and resequencing
- EST sequencing
- Primer walking
- STR genotyping
- Sequencing expertise



**Vanha kunnon
Sanger-
työhevonnen**

**Tässä tosin 96
kapilaaria, kun
meillä on yksi.**

deoxyribonucleoside triphosphate



dideoxyribonucleoside triphosphate

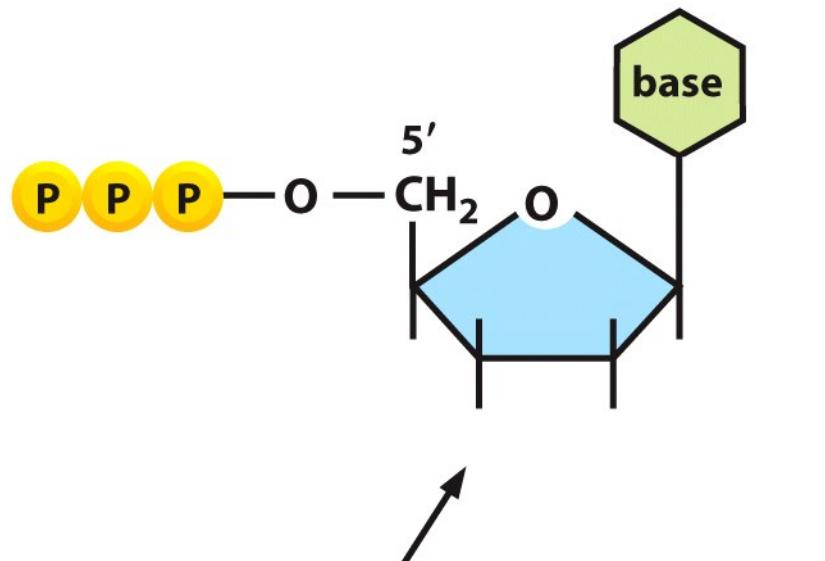


Figure 8-50a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Sangerin dideoxyribonukleotidimenetelmä

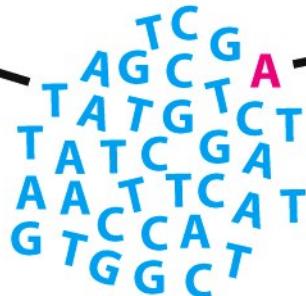
CELL 549

Nobel (kemia) Fred Sanger 1980

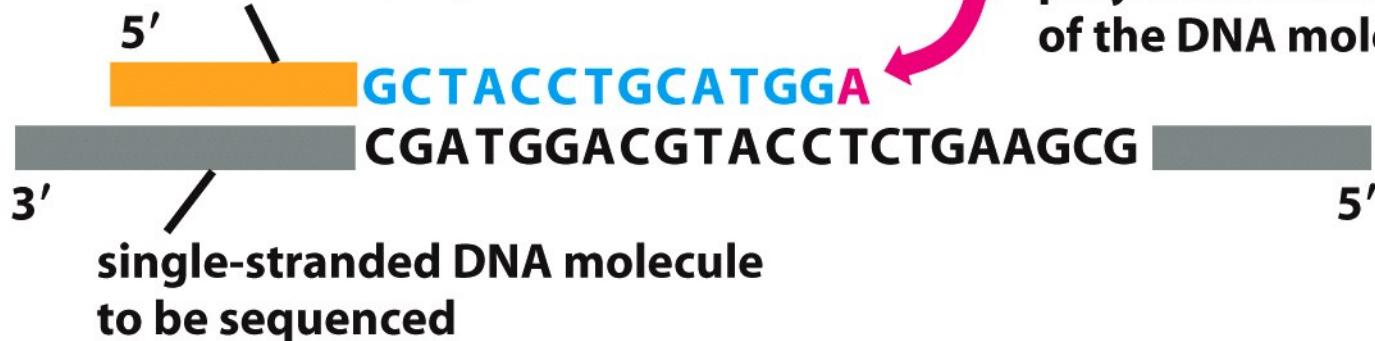


normal deoxyribonucleoside triphosphate precursors (dATP, dCTP, dGTP, and dTTP)

small amount of one dideoxyribonucleoside triphosphate (ddATP)



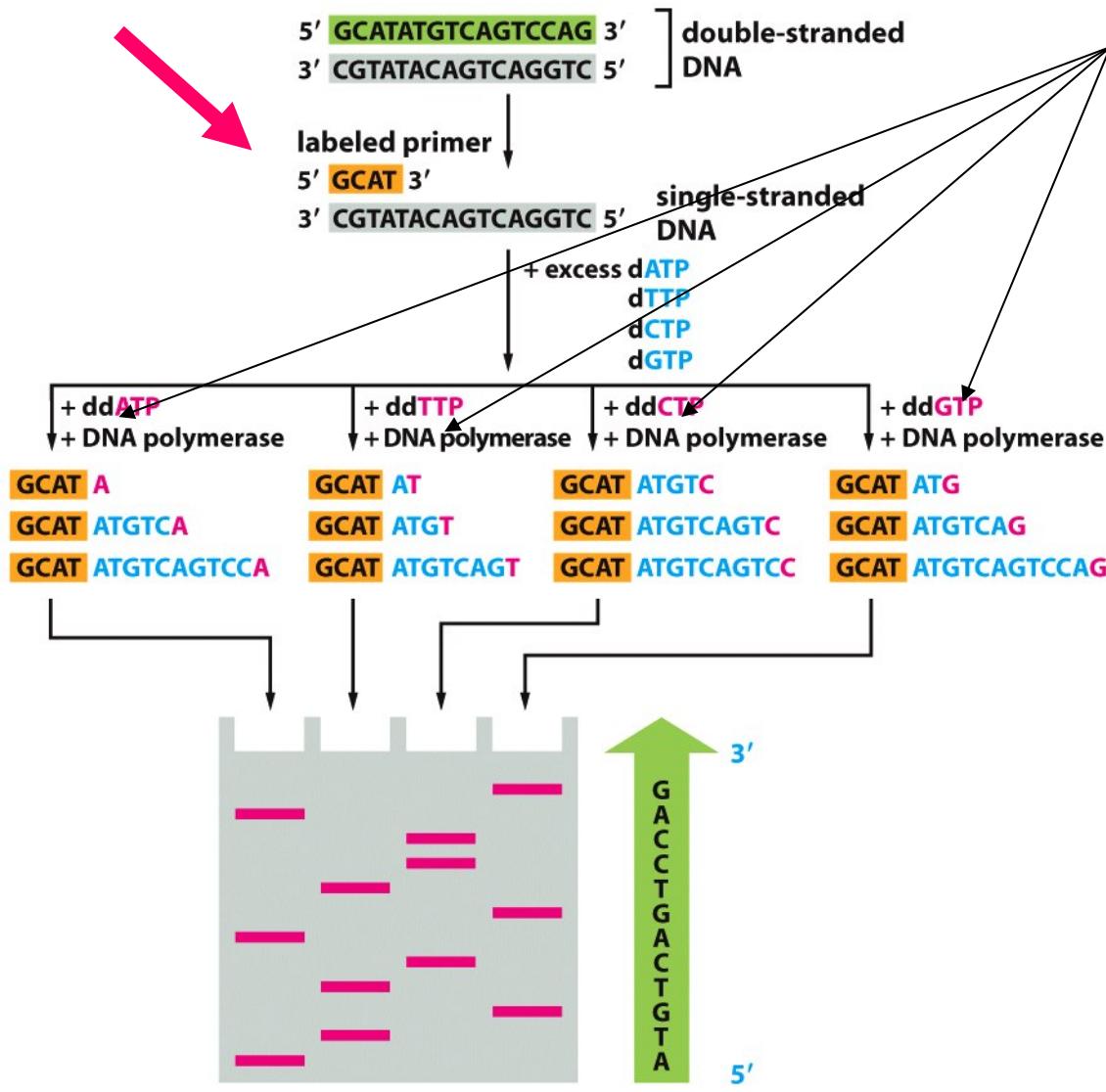
oligonucleotide primer
for DNA polymerase



rare incorporation of dideoxyribonucleotide by DNA polymerase blocks further growth of the DNA molecule

Figure 8-50b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Jokaista
"kirjainta"
varten on oma
reaktioastia ja
oma kaista
geellä



DNA sequence reading directly from the bottom of the gel upward, is

ATGTCAGTCCAG
1 12

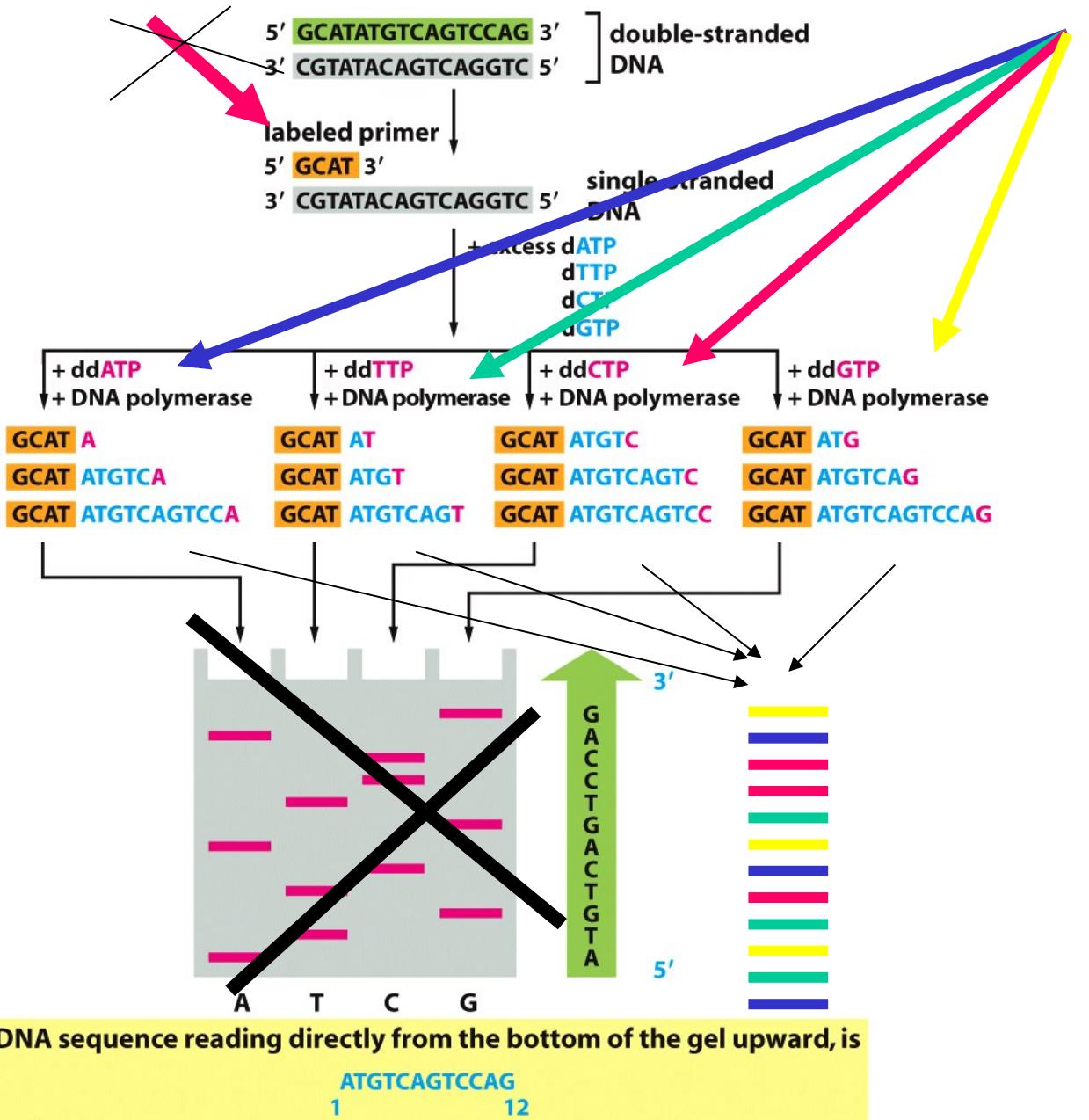
Figure 8-50c Molecular Biology of the Cell 5/e (© Garland Science 2008)

Sekvensointireaktion
tuotteet erotellaan
elektroforeesilla

Lyhyet molekyylit
kulkevat vauhdikkaasti,
pitkät hitaammin

Värillä leimattu dideoksiribonuke

Automaateissa käytettävä nykyinen muunnelma: yksi koeputki, yksi kapillaari jossa näytteet virtaavat ripeästi



CELL 549



”Automaattisekvenssori” on elektroforeesilaite, jossa Harmaa Laatikko ”lukee” kapillaarissa virtaavia DNA-pätkiä UV-herätteisen fluoresenssin avulla ja tekee tietokoneelle virtuaalikuvan

Näytteet oli annettu vastuuhenkilölle 96 reikäisessä kuoppalevyssä, ja pian ovat tulokset tilaajan omassa kansiossa

Huhtikuu 2005
Viikko 14

MAANANTAI
Ukko

- 8
9 Marele 1 x selev.
10 J. Kiiskila 2 selev. 20
11 Run S. 5.34+16
12 Lumii 5.8
13 Laura jaakola 5.16
14
15 Kurssi 1xpsat 32
16
17 Marjut 1xpsat

4

TIISTAI
Irene Irina Ira Iro

- 8
9 Kaija Viiale 1xpsat
10
11 David 4xpsat
12
13 Laura 5.17
14
15 Katja R. 5.24
16
17 Marjut 1xpsat

Run S. 2 x selev.

94-271

5

KESKIVIIKKO

Ville Vilho Vilhelm Viljami Vili Jami

STAI
Ahvo

8

9

10 Riikka 1xpsat

11

12 Wibold 2xselev.

13

14

15

16

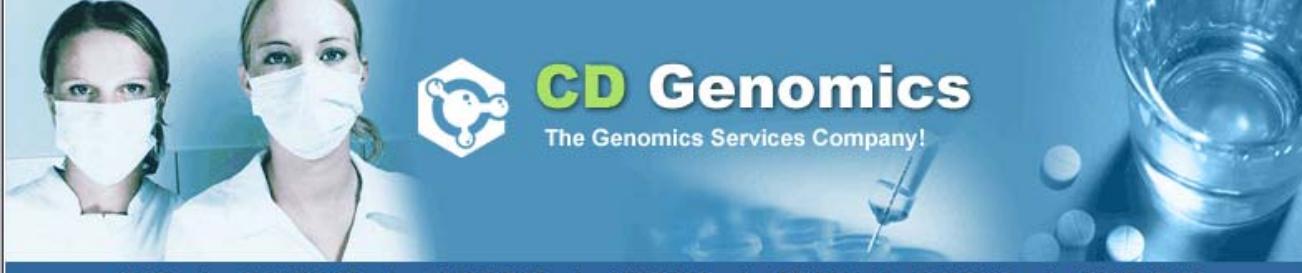
17

95-270

DELL



Tilauskirja on täynnä, mutta prosessi on nopea



HOME | SEQUENCING | GENOTYPING | LIBRARIES | OTHERS | ORDERING | CONTACT US

DNA sequencing

Technology Platforms

- ABI 3730xl platform
- Roche 454 GS-FLX platform
- ABI SOLID sequencing system
- Illumina Solexa 1G Genome Analyzer

Services

- Genomic shotgun sequencing
- BAC end sequencing
- SNP discovery and resequencing
- Large-scale EST sequencing
- Primer walking
- SAGE sequencing
- Sequencing expertise

Genotyping

Custom libraries

Technology platforms

Other services

Request a quote

[Home](#) > [DNA sequencing](#) > [ABI 3730xl DNA Analyzer](#)

ABI 3730xl DNA Analyzer

The ABI3730xl DNA Analyzer is a fully automated, 96-capillary electrophoresis instrument. To ensure high quality base calling, assembly and SNP detection, CD Genomics uses a combination of software and manual basecalling to guarantee the sequencing quality. Our senior bioinformaticians have ever viewed more than ten thousands of trace files and accumulated abundant experience.

- Shotgun sequencing
- End sequencing
- SNP discovery and resequencing
- EST sequencing
- Primer walking
- STR genotyping
- Sequencing expertise



**Vanha kunnon
Sanger-
työhevon**

**Tässä tosin 96
kapilaaria, kun
meillä on yksi.**



» **DNA sequencing**

Technology Platforms

- ABI 3730xl platform
- Roche 454 GS-FLX platform
- ABI SOLID sequencing system
- Illumina Solexa 1G Genome Analyzer

Services

- Genomic shotgun sequencing
- BAC end sequencing
- SNP discovery and resequencing
- Large-scale EST sequencing
- Primer walking
- SAGE sequencing
- Sequencing expertise

» **Genotyping**

» **Custom libraries**

» **Technology platforms**

» **Other services**

» **Request a quote**

[Home](#) > [DNA sequencing](#)

DNA Sequencing

Equipped with high-throughput sequencers such as ABI 3730xl, CD Genomics provides a complete sequencing solution. Recently we have extended our portfolio of large scale sequencing with Roche 454 GS-FLX System, ABI SOLID sequencing system and Illumina Solexa 1G Genome Analyzer using the Next-Gen sequencing technology. Highly flexible DNA sequencing packages are tailored to meet every client's needs.

Techlonogy Platforms

- + [ABI 3730xl platform](#)
- + [Roche 454 GS-FLX platform](#)
- + [ABI SOLID sequencing system](#)
- + [Illumina Solexa 1G Genome Analyzer](#)

Services

- + [Genomic shotgun sequencing](#)
- + [BAC end sequencing](#)
- + [SNP discovery and resequencing](#)
- + [Large-scale EST sequencing](#)
- + [Primer walking](#)
- + [SAGE sequencing](#)
- + [Sequencing expertise](#)

Kauppa josta saa kaikki koneet ja palvelut

Roche NextGen Sequencing by DNAVision - Windows Internet Explorer

File Edit View Favorites Tools Help

Roche NextGen Sequencing by DNAVision

Contract Research
Latest technologies to meet your needs

Roche 454

THE COMPANY PRESS ROOM CONTACT US PHARMACO GENETICS PHARMACO GENOMICS PERSONALIZED MEDICINE BIO PHARMA ANIMAL & PLANT FOOD QUALITY MICROBE & VIRUS CONTRACT RESEARCH

DNAVISION YOUR GENETIC SOLUTION

SNP Genotyping Gene Expression DNA Sequencing ROCHE NEXTGEN Sequencing ILLUMINA NEXTGEN Sequencing Biobanking Transgenic Services

Roche 454 Next Generation Sequencing

DNAVISION provides fast and quality sequencing

[Brochure: Next Gen Sequencing at DNAVision](#)

DNAVision has extended its portfolio of high quality genomic services with **Next Generation Sequencing** using the Genome Sequencer FLX system (Roche). Through this breakthrough technology provided by DNAVision, a broad range of well known and new applications are available:

- Genomic shotgun Sequencing
- Genome re-sequencing
- Transcriptome profiling
- Metagenomics and meta-transcriptomics

Titanium up-grade (allowing up to 400 Mb in a single run) and paired-end sequencing are already available.

Those applications are provided by DNAVision following our highest quality standards.

Currently, sequence data generation is no more a limiting factor. Data analysis and interpretation is now the key factor in Next Generation Sequencing projects. Thanks to its bioinformatic team, DNAVision is a real partner adding value to your research.

Performances and output of next generation sequencers (NGS) unravels those of classical sequencing, which nevertheless remains useful for specific applications alone or in combination with NGS.

DNAVision's service with Roche Genome Sequencer FLX system

- Up to 400Mb per run obtained with between one and sixteen samples
- Fast data turnaround – 10 hours per run
- Q20 read length of 400 bp and >1 M sequencing reads
- State-of-the-art bioinformatics support

Feel free to contact us (info@dnvision.be) for any information on our next generation sequencing services, we will be pleased to find the most appropriate and effective technology for your research.

Genomic shotgun sequencing

The whole genome sequence of virus, bacteria and fungi can be obtained in a single sequencing run. New SNPs, insertions/deletions can be rapidly identified through this whole genome sequencing.

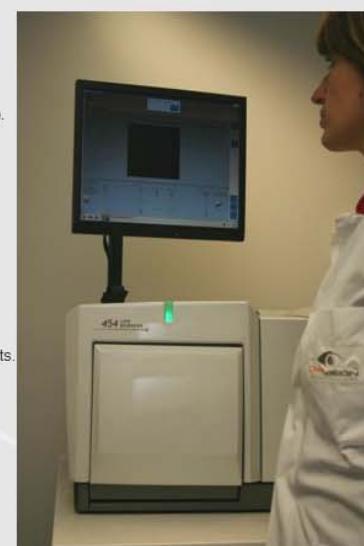
This approach is particularly useful for new strain characterization, strain optimization, metabolic engineering or mutation discovery.

http://en.wikipedia.org/wiki/454_Life_Sciences#Technology

Genomic resequencing

Done

Internet 100% Google Page Tools



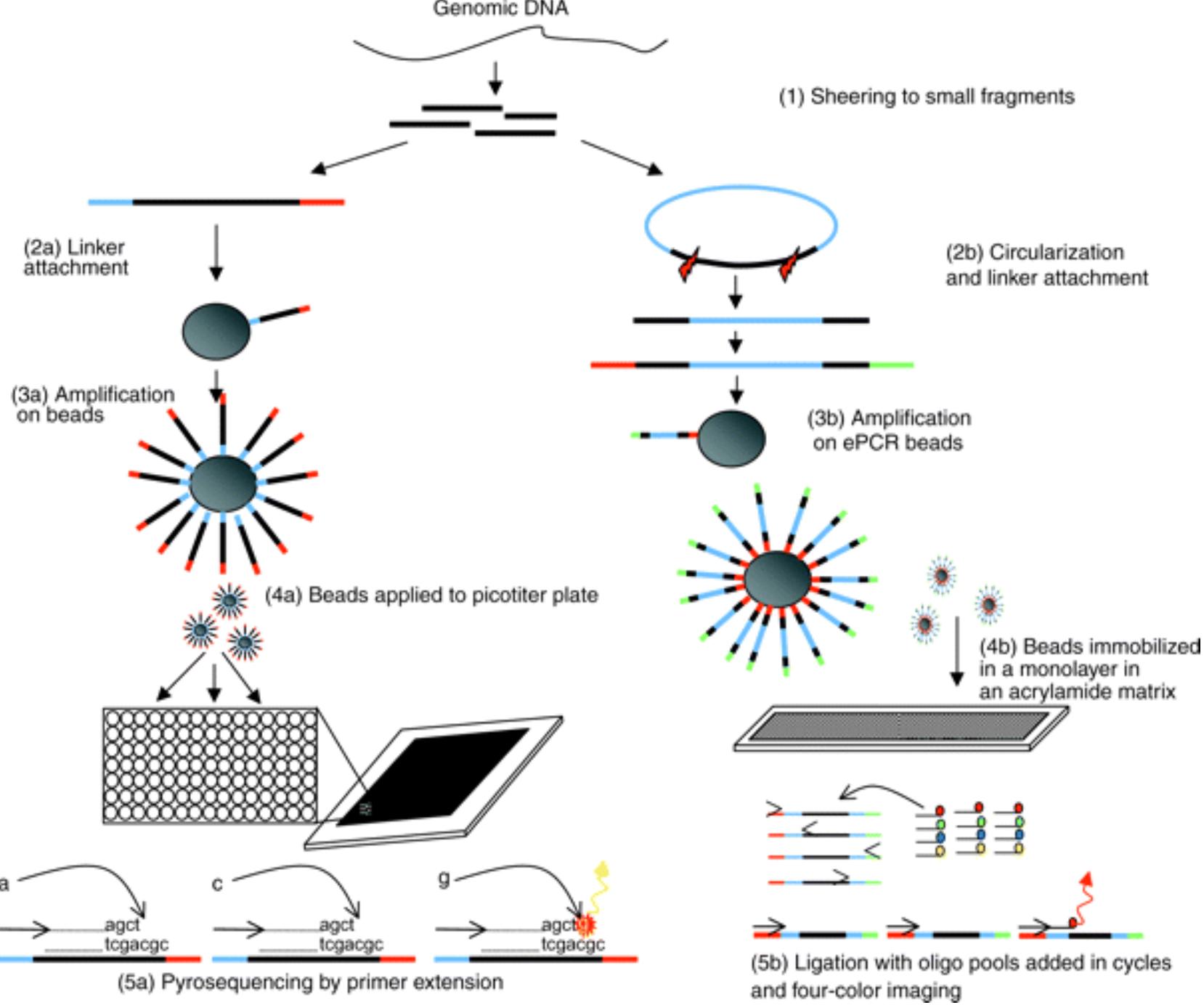


Fig. 2 Outline of the 454 and polony sequencing process. Both systems first fragment the genomic DNA (Step 1) and then use a process of *in vitro* cloning followed by amplification. The 454 process is shown on the left and Polony sequencing is shown on the right. In the 454 protocol, the linkers are ligated onto the ends of the DNA (Step 2a). Polony sequencing involves circularization followed by linearization and the addition of linkers to generate two fragments with a spacer between them and linkers at the end (Step 2B). Both processes then attach the *in vitro* clones to beads and carry out PCR in an emulsion mixture to generate beads with many clonal copies of the target fragments (Step 3a/3b). For the sequencing step, the beads must be immobilized in a single layer to allow imaging in an environment that enables the reaction reagents to be flowed across them. In the case of 454 sequencing, a picotiter plate is used, in which most cells will contain a single bead (Step 4a). The polony method immobilizes the beads in an acrylamide matrix in a dense monolayer (Step 4b). The methods are very similar up until the point of the sequencing reaction; in the case of 454 sequencing, a DNA synthesis reaction from a single sequencing primer is carried out. Bases are flowed across the picotiter plate one at a time and incorporation is detected by the release of light (Step 5a). The polony method uses ligation to anchor primers, which can be annealed in one of four positions. In each cycle, a population of degenerate nonomers, which have been fluorescently labeled, is added to the monolayer, and only complimentary oligos will anneal and ligate to the anchor primer.



HOME | SEQUENCING | GENOTYPING | LIBRARIES | OTHERS | ORDERING | CONTACT US

[Home](#) > [DNA sequencing](#) > [Illumina/Solexa Genome Analyzer II](#)

Illumina/Solexa Genome Analyzer II

Besides next generation sequencing technology by 454, CD Genomics has extended its portfolio of genomic services with Solexa technology. Generating high quality readout of one billion bases per run at less than 1% of the cost of capillary-based methods, the Illumina Genome Analyzer is designed to enable researchers to dramatically improve the efficiency of current applications.

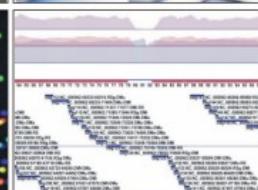
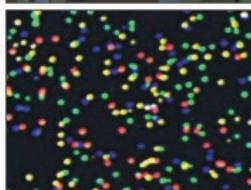


Highlights:

- *Extremely high throughput:* 2G sequences per run
- *Scalable:* Up to eight samples can be loaded onto the flow cell simultaneously
- No problems with homopolymer repeats
- High accuracy
- Cost effective
- Bioinformatics solution by professionals

Applications:

- Genome resequencing
- BAC resequencing
- Expression profiling
- Small RNA identification
- ChIP sequencing
- Paired ends sequencing

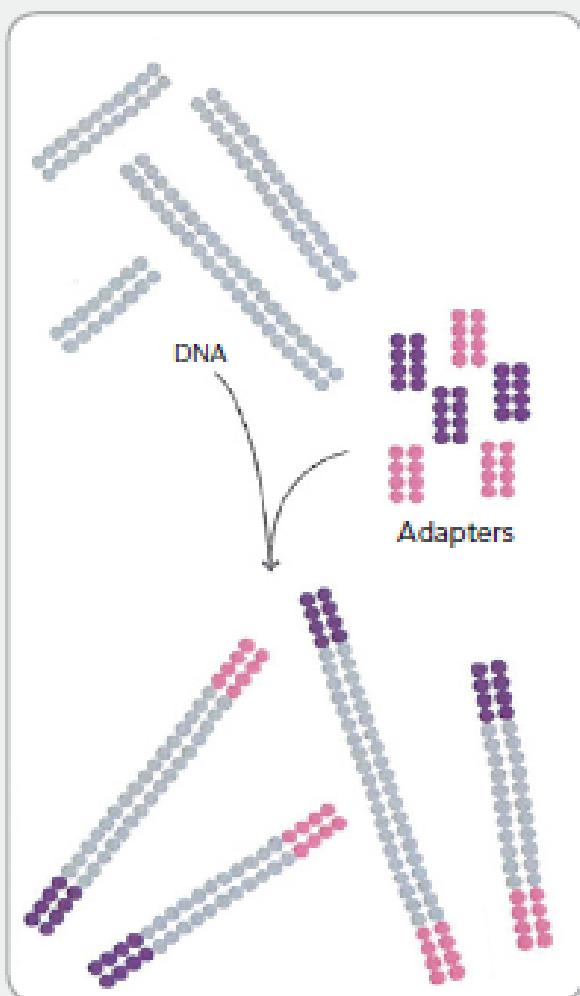


Solexa

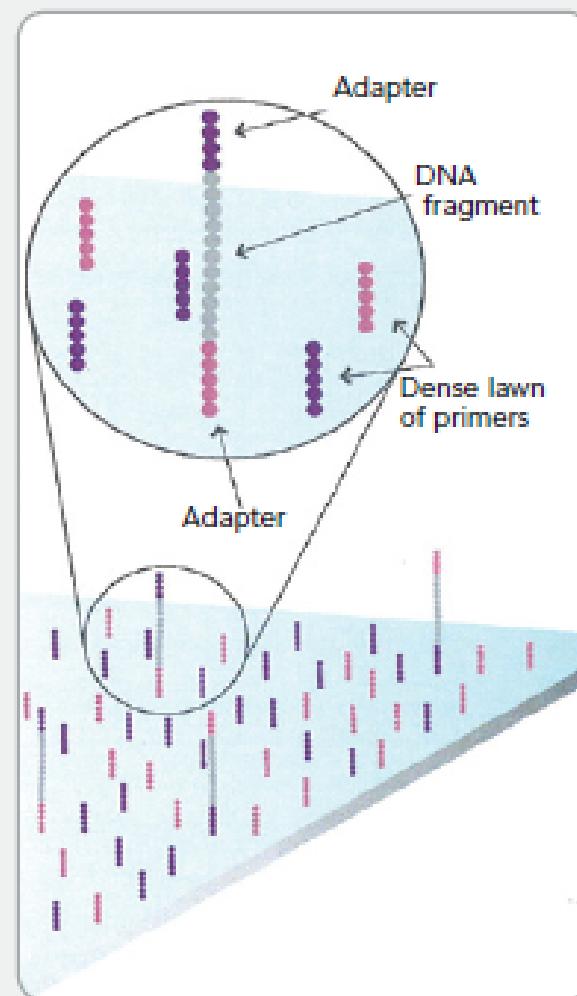
Metodiesite

FIGURE 2: SEQUENCING TECHNOLOGY OVERVIEW

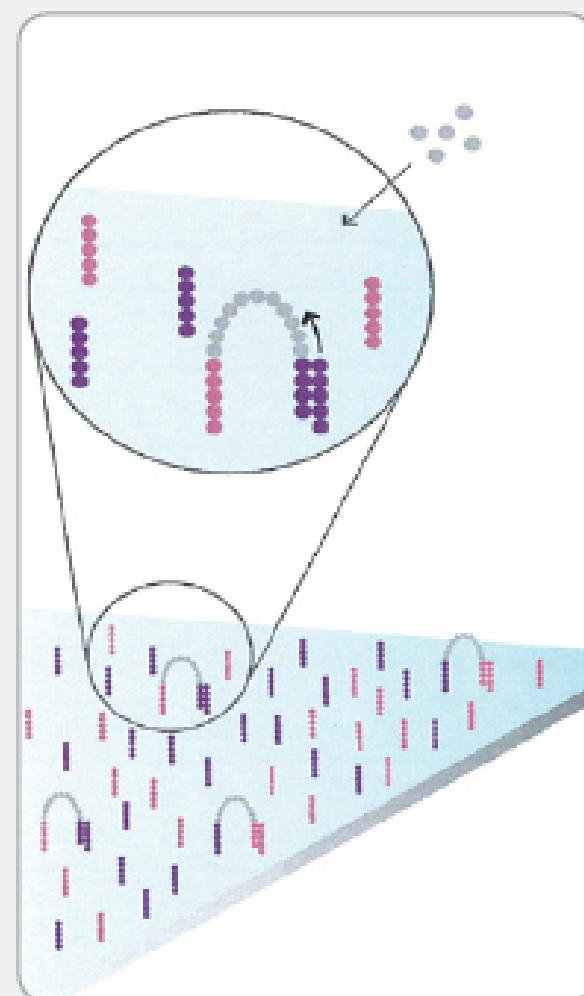
1. PREPARE GENOMIC DNA SAMPLE



2. ATTACH DNA TO SURFACE



3. BRIDGE AMPLIFICATION

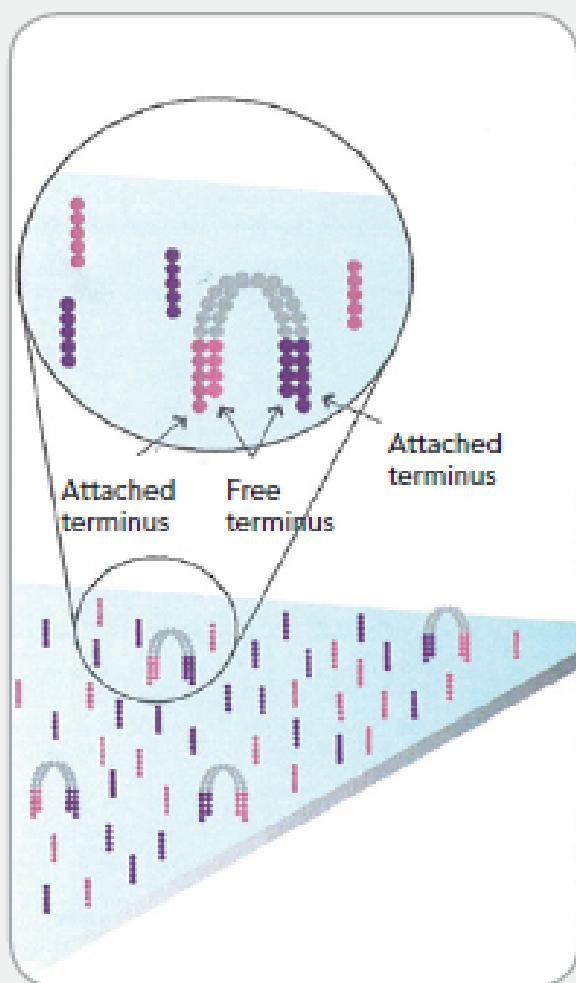


Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

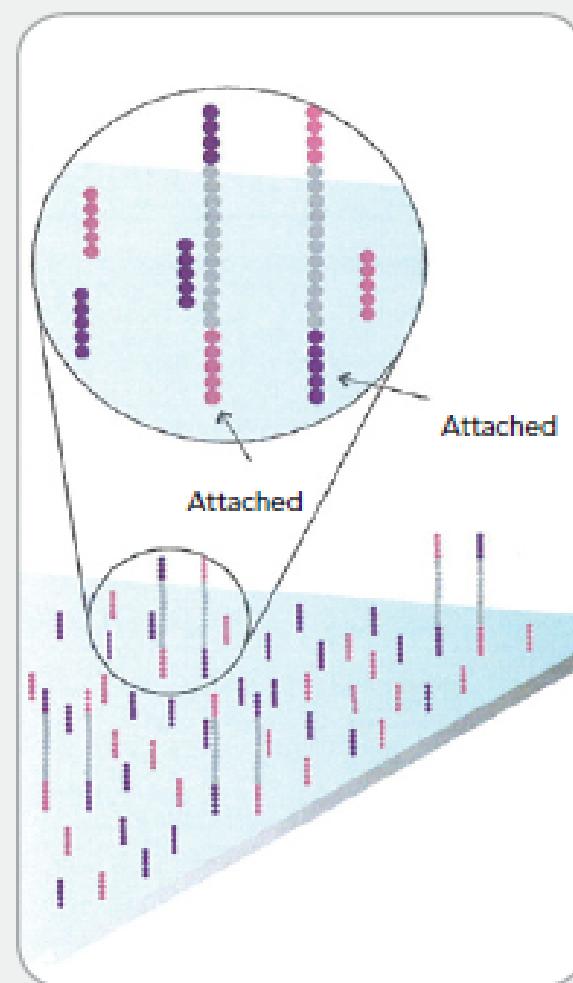
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

4. FRAGMENTS BECOME DOUBLE STRANDED



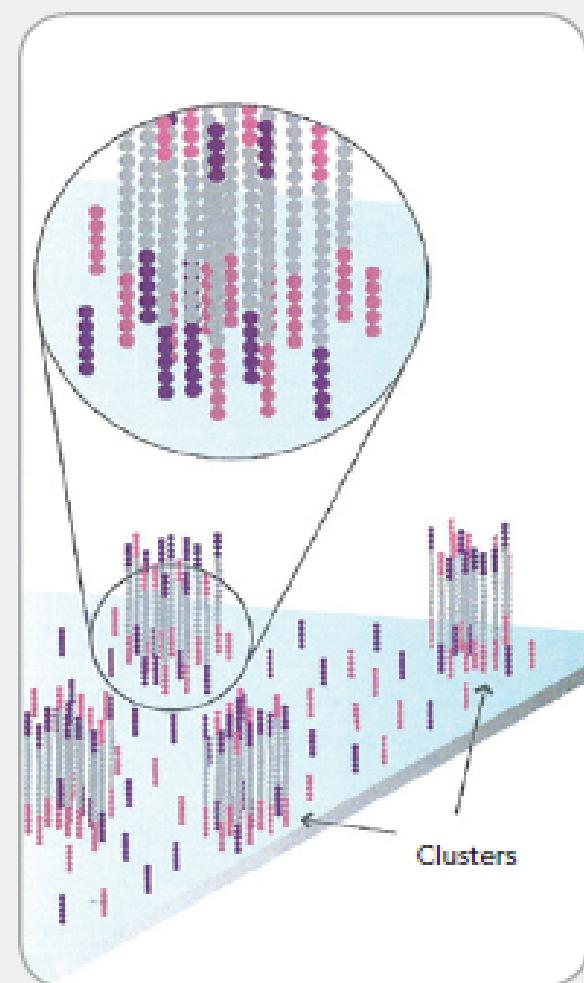
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

5. DENATURE THE DOUBLE-STRANDED MOLECULES



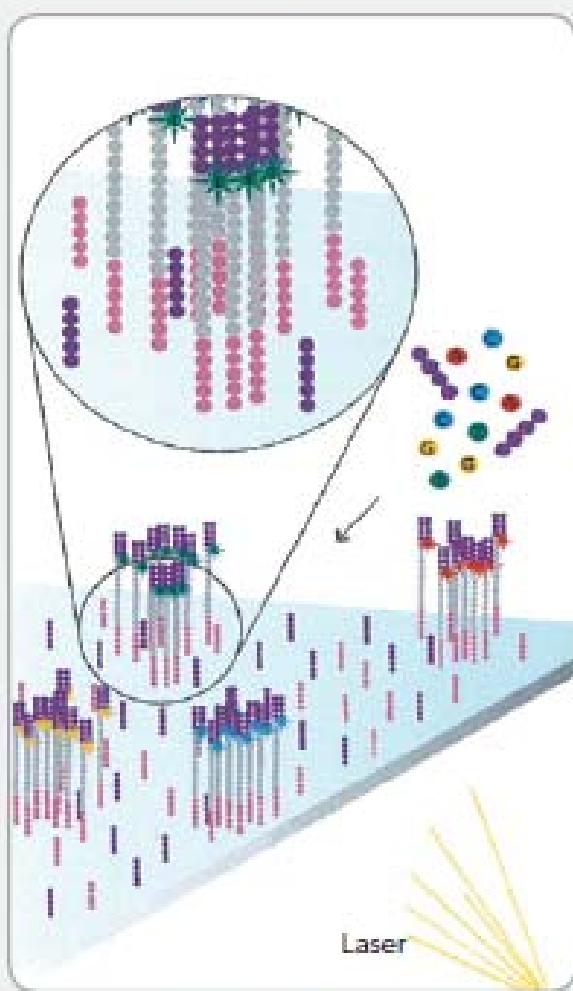
Denaturation leaves single-stranded templates anchored to the substrate.

6. COMPLETE AMPLIFICATION



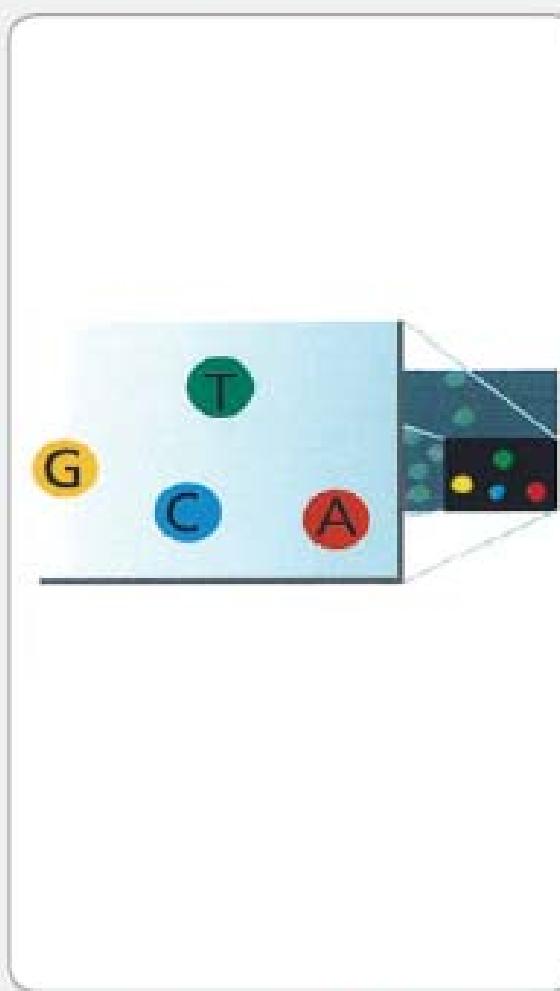
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

7. DETERMINE FIRST BASE



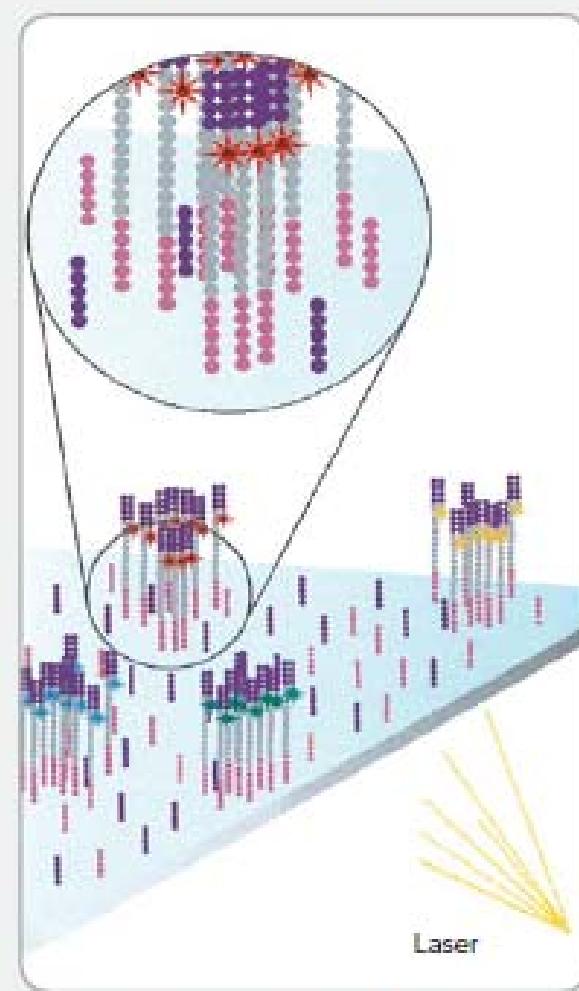
First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

8. IMAGE FIRST BASE



After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

9. DETERMINE SECOND BASE



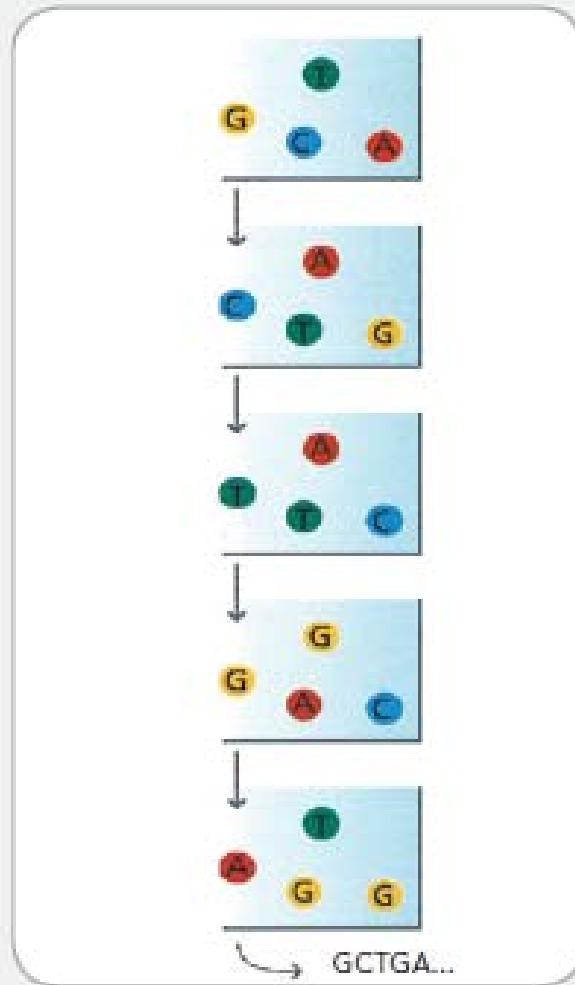
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

10. IMAGE SECOND CHEMISTRY CYCLE



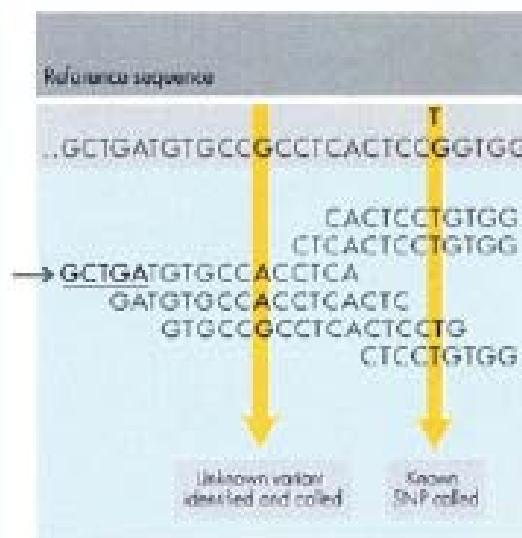
After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES



Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at time.

12. ALIGN DATA



Align data, compare to a reference, and identify sequence differences.



DNA sequencing

Technology Platforms

- ABI 3730xl platform
- Roche 454 GS-FLX platform
- ABI SOLiD sequencing system
- Illumina Solexa 1G Genome Analyzer

Services

- Genomic shotgun sequencing
- BAC end sequencing
- SNP discovery and resequencing
- Large-scale EST sequencing
- Primer walking
- SAGE sequencing
- Sequencing expertise

Genotyping

Custom libraries

Technology platforms

Other services

Request a quote

[Home](#) > [DNA sequencing](#) > [Applied Biosystems SOLiD](#)

Applied Biosystems SOLiD

The Applied Biosystems (AB) SOLiD System is a highly accurate, massively parallel genomic analysis platform that supports a wide range of applications. The flexibility of two independent flow cells and multiplexing capability allow researchers to conduct multiple experiments in a single run. With unparalleled throughput and greater than 99.94% basecalling accuracy, the SOLiD System enables researchers to complete large-scale sequencing and tag experiments more cost effectively than previously possible.



Highlights:

- o Obtain more than 2 Gb of data per run
- o High quality 35 bp reads generated with high confidence di-base encoding
- o Single molecule clonal amplification of templates avoids cloning bias
- o Ability to run up to 8 samples on separate channels
- o Paired end library approaches
- o Robust chemistry for accurate base-calling

Applications:

- o De Novo Sequencing
- o Targeted Resequencing
- o Whole Genome resequencing
- o Gene Expression profiling
- o Small RNA analysis
- o Whole Transcriptome Analysis
- o Chromatin Immunoprecipitation (ChIP)
- o Methylation Analysis



SOLiD

Katso video ja nauti?

Tästä metodista ei kyllä
saa helposti selvää,
mutta kai pitäisi.
Ihmisen genomi 6000
dollarilla ei ole kallis

Pieni esimerkki

Esimerkki elävästä elämästä:

Tutkittiin puolalaisia kirjolohilaitosten loisia

Molekyylimenetelmällä löytyi sellainenkin mato, joka on erikoisuutena kuvaltu Tanskassa ([Lindenström 2003](#))

Me tutkimme siitä myös mitokondrioDNA:n, ja totesimme, että loislinjan naarasesiäiti on tosi outo (eri lajia siis)

Tutkimme myös itse keksimämme *ADNAM1* – merkkigeenin, jonka sekvenssoinnista on seuraavat kuvat. Merkkigeeni on aiemmin selvitetty Itämeren piirin lohien ja harjusten loisilla, ja tiedämme 11 eri alleelin sekvenssit

Yhdellä alleelilla on 23 bp deleetio. Tämä *short*-alleeli on kaikilla Suomen kirjolohella löydetyillä *G. salaris* -loisilla

Mitokondriosekvenssien vertailu





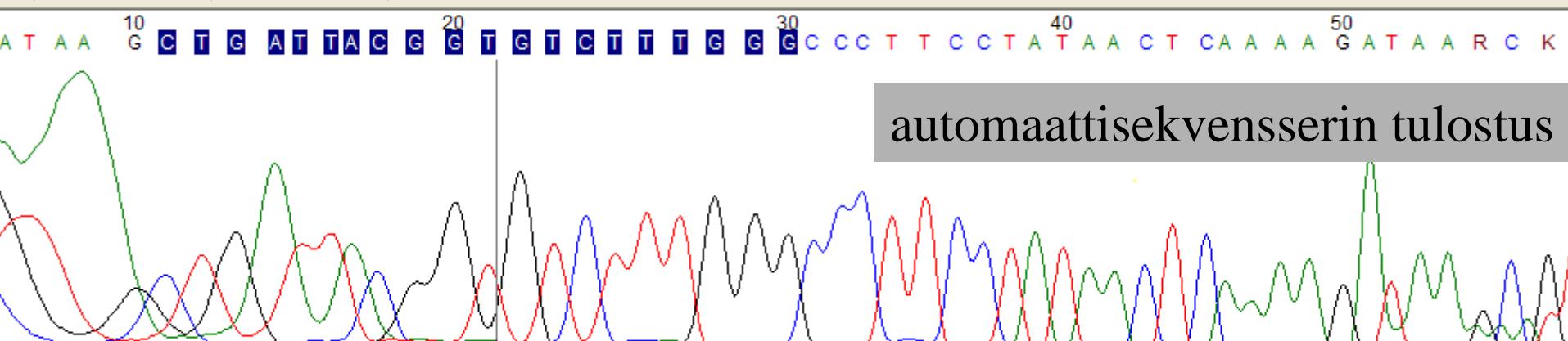
Linjausohjelma MEGA3.1

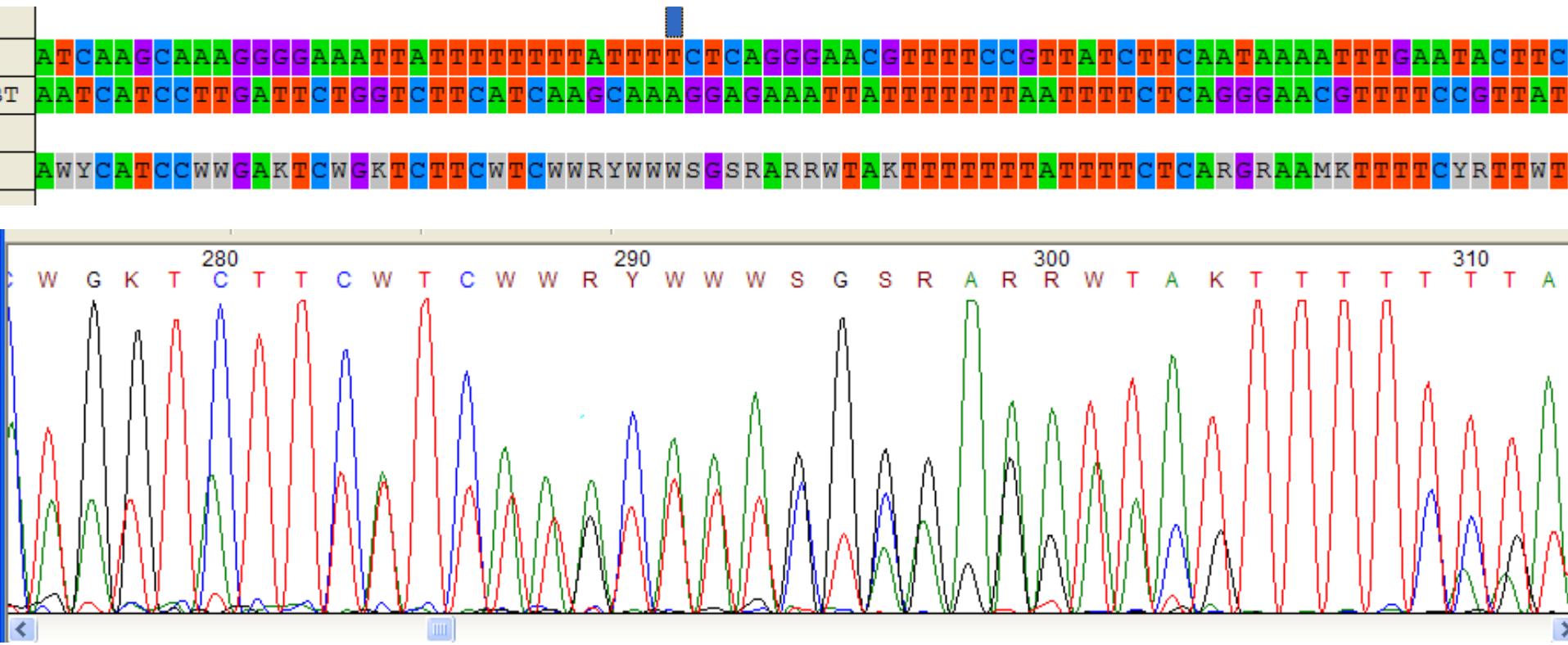
DNA Sequences | Translated Protein Sequences

JS11B	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAA	GAAAA
JS12B	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAA	GAAAA
JS17B	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAA	GAAAA
JS19B	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAA	GAAAA
JS44/6 long	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAA	GATAAAGCGTTTTGACAGGCCCTCGAAAA
JS44/10 long	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAA	GATAAAGCGTTTTGACAGGCCCTCGAAAA
JS37s	[REDACTED]	GAAAA
JS38s	[REDACTED]	GAAAA
JS39s	[REDACTED]	GAA
Sequence 12		
SHORT RBT	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAAAGAAAAATTATGAAAAATGATAAGGACAA	
LONG ALLELE RBT	CTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAAAGATAAAGCGTTTTGACAGGCCCTCGAAAA	
InsF_237	CTTCRATAAAGCTGATTAACGGTGTCTTGGGCCCTTCCTATAAACTCAAAAAGATAARCKTTTGACWGGYCTCRAAAAA	

Site # 40 with w/o Gaps

Display Help





Edellisen sekvenssin vaikeampi kohta: koska yksi (kolmesta) alleelista on 23 bp lyhyempi kuin muut, PCR tekee ja kone lukee sekvenssit päällekkäin ja tulkinta on vaikeaa (mutta ei mahdotonta, jos alleelit tunnetaan).

IUPAC –koodi löytyy osoitteesta: http://www.iupac.org/index_to.html

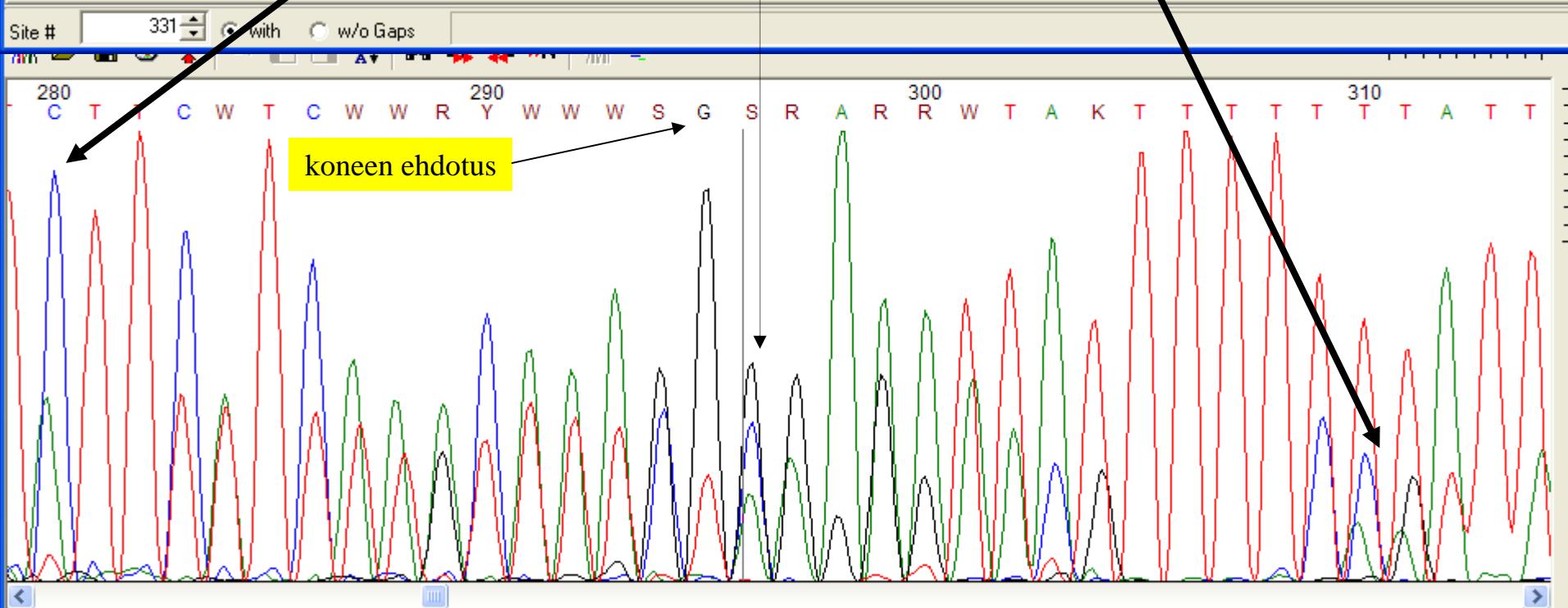
International Union of Pure and Applied Chemistry



DNA Sequences | Translated Protein Sequences |

SHORT RBT	A T T T T T T T T A T T T T C T C A G G G A A C G T T T T C C G T T A T C T T C A A T A A A A T T T T
JS44/10 long (2)	C T T C A T C A A G C A A A A G G G A G A A A T T A T T T T T T T A A T T T T C T C A G G G A A C G T T
JS38s (2)	C T T C A T C A A G C A A A A G G G G A A A T T A T T T T T T T A T T T T C T C A G G G A A C G T T
LONG ALLELE RBT	C T T C A T C A A G C A A A A G G G A G A A A T T A T T T T T T T A A T T T T C T C A G G G A A C G T T
POLSKI 237	C T T Y W T Y W W R Y W W W S K V R R R R W W M K T T T T Y Y B D W T T T C T C A R G R A A M K T T T T

Site # 331 with w/o Gaps



Mitä tuossa nähtiin?

Nähtiin, että sillä oudolla puolalaisella oli täsmälleen Suomen loisia vastaava *ADNAM1* –fenotyyppi, joka siis tuossa *ennustettiin* ja *testattiin* tunnettujen alleelien linjauksen (alignment) avulla

Puolalaisten (ja tanskalaisten) loisten historiassa on kuitenkin risteytyminen jonkun toisen lajin kanssa (vieras laji *esiäitinä*, koska mtDNA on sieltä)

Myös ribosomigeenin ITS (internal transcribed spacer) oli outo, mutta vain 3 nukleotidin verran

Tämä *ADNAM1* kertoi vielä, että mendelististä alleelien vaihdantaa on harjoitettu jossakin määrin, niin että tämä *ADNAM1* fenotyyppi on ihan suomalaisen kaltainen

International Union of Pure and Applied Chemistry - Windows Internet Explorer

http://www.iupac.org/index_to.html

NewsStand | Preferences | Search | Issues | Help |

Google IUPAC Go Bookmarks 107 blocked Check AutoLink AutoFill Send to IUPAC Settings

International Union of Pure and Applied Chemistry

I U P A C

International Union of Pure and Applied Chemistry

[Mirror Sites](#) [FAQs](#) [About IUPAC](#) [Handbook](#) [Contact](#)

The International Union of Pure and Applied Chemistry (IUPAC) serves to advance the worldwide aspects of the chemical sciences and to contribute to the application of chemistry in the service of Mankind. As a scientific, international, non-governmental and objective body, IUPAC can address many global issues involving the chemical sciences.

[News & Notices](#)

> SPOTLIGHT
updated 30 Jan 07

Here are the latest News & Notices issued by the Union and the Union's bodies.

The [latest developments of the IUPAC website](#), [News Archives](#), and the [e-news](#) are also accessible from this area.

[Organizations & People](#)

Read [about IUPAC](#) to learn who we are and what we do. View the organization chart, and locate people within various bodies of the Union. Browse the [Index of Members](#).

[Standing Committees](#)

Eight committees coordinate the work of the Union in various areas of chemistry, such as nomenclature and symbols, educational and industrial concerns.

[Divisions](#)

News & Notices

Organizations & People

Standing Committees

Divisions

Projects

Reports

Publications

Symposia

AMP

Links of Interest

Search the Site

Home Page

IUPAC –koodit löytyvät täältä