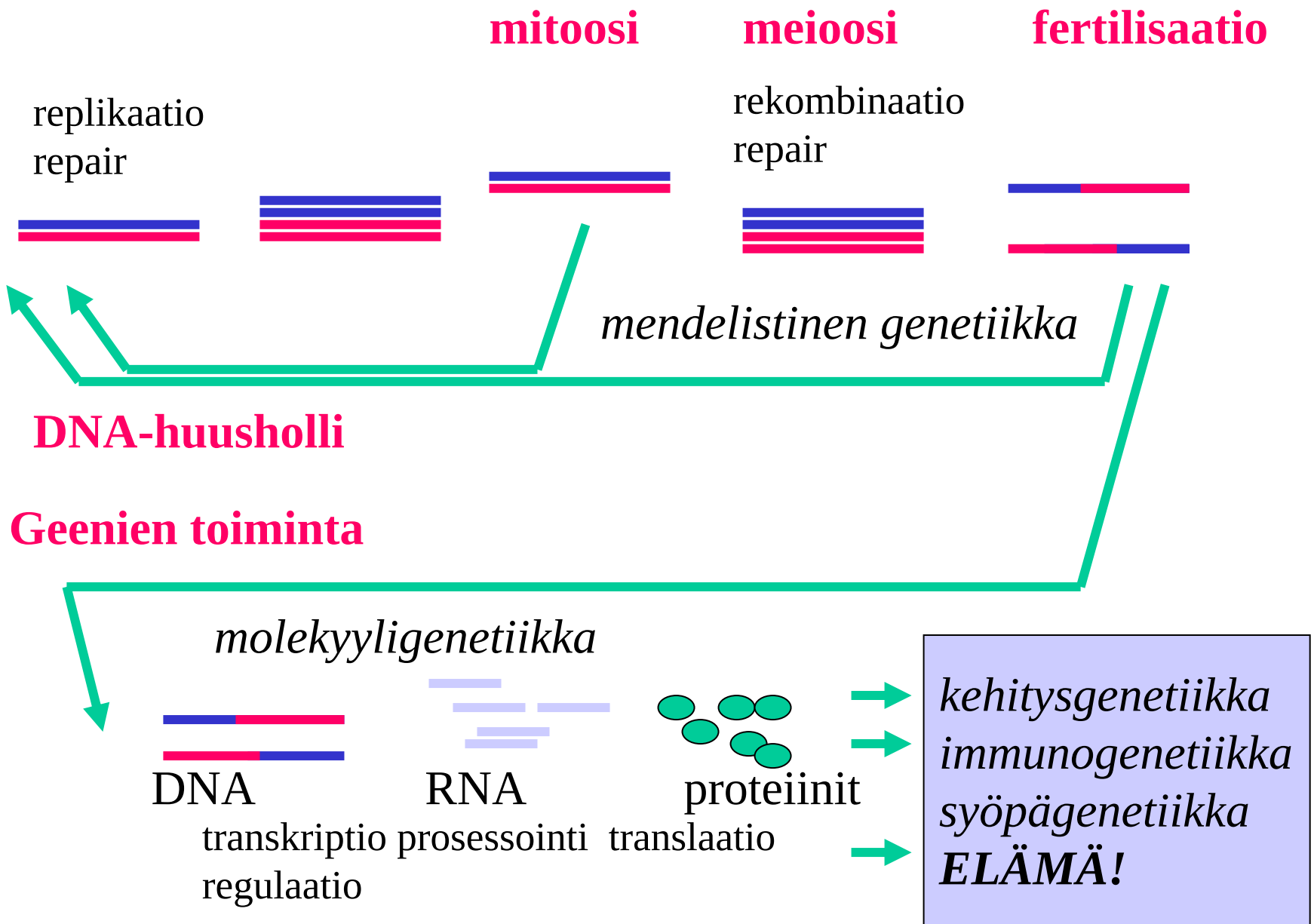




Genetiikan perusteiden miellekartta: geenien sekvensointi

Miten geeniä luetaan?

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

Sekvensointireaktio 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](#)



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Vanha kunnon Sanger- työhevonen

Tässä tosin 96
kapilaaria, kun
meillä on yksi.

[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



➤ 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

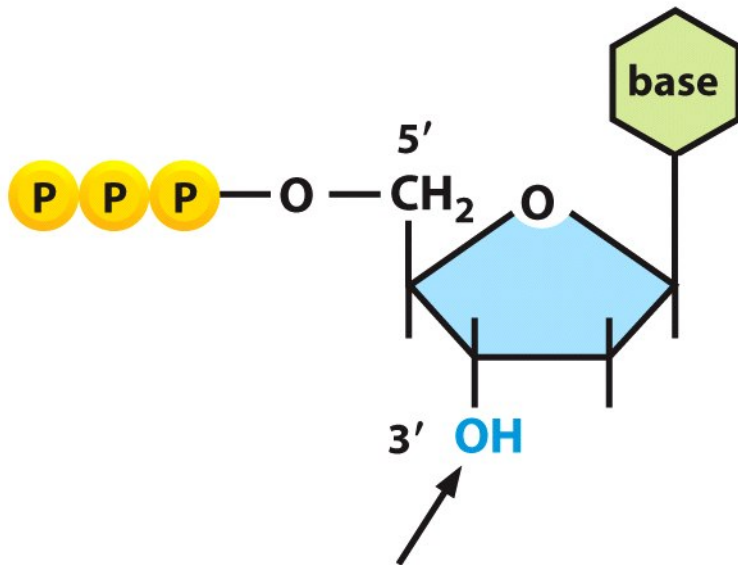
➤ Custom libraries

➤ Technology platforms

➤ Other services

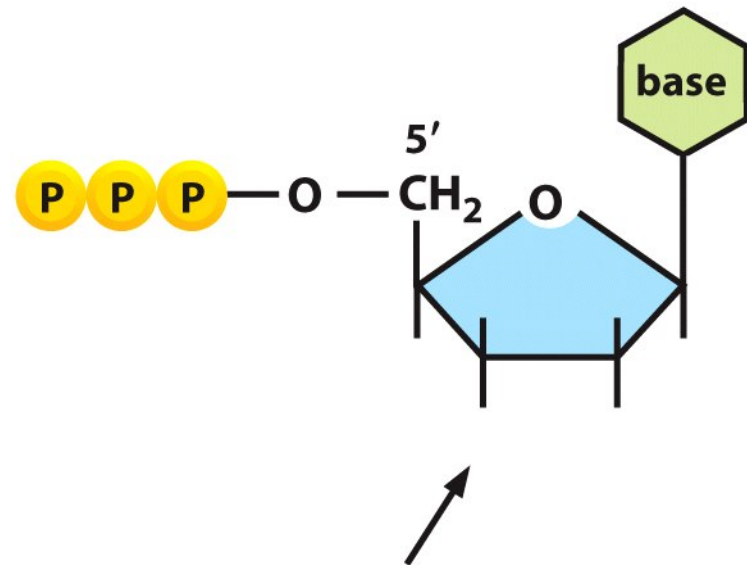
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deoxyribonucleoside triphosphate



allows strand extension at 3' end

dideoxyribonucleoside triphosphate



prevents strand extension at 3' end

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

Sangerin dideoxyribonukleotidimenetelmä

CELL 549

Nobel (kemia) Fred Sanger 1980



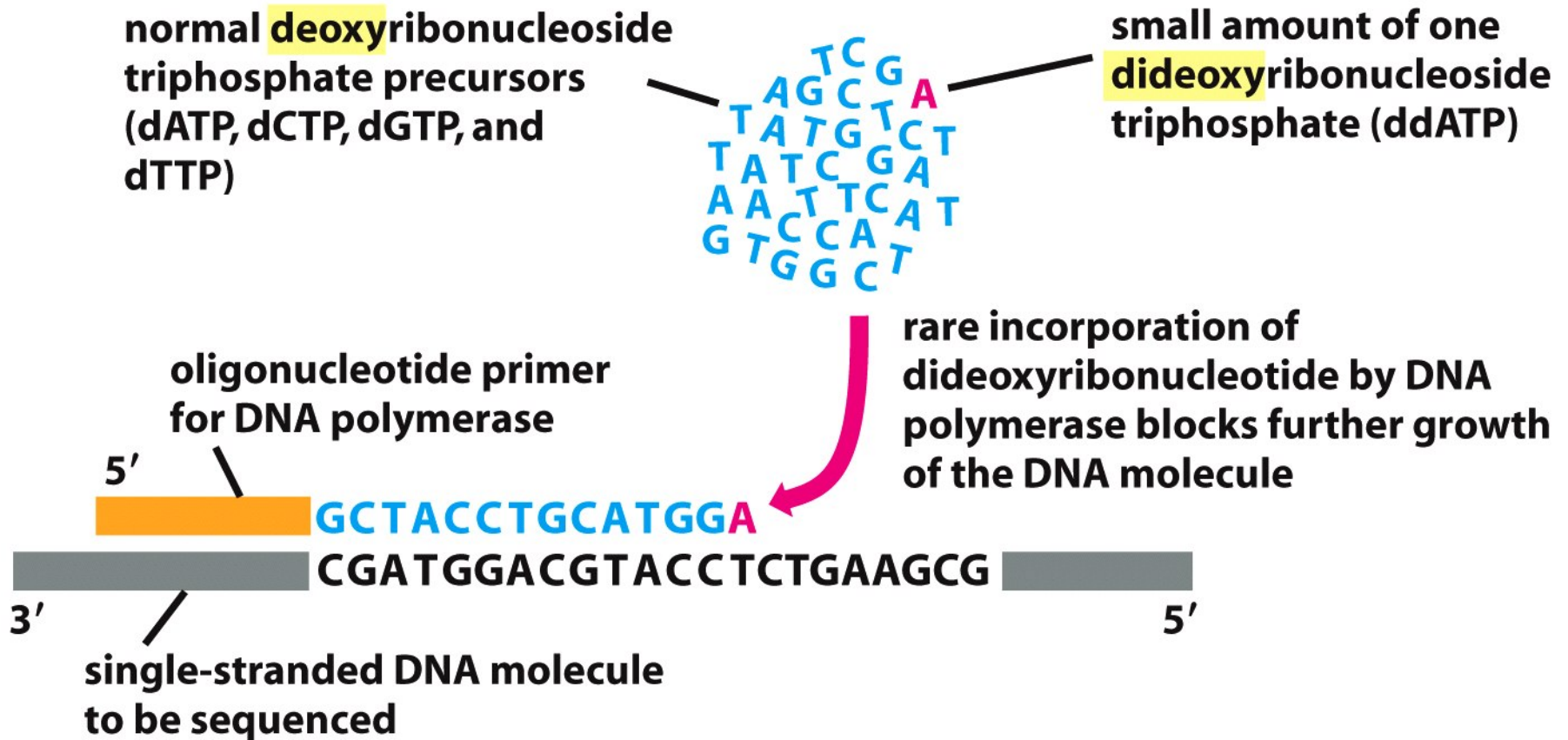
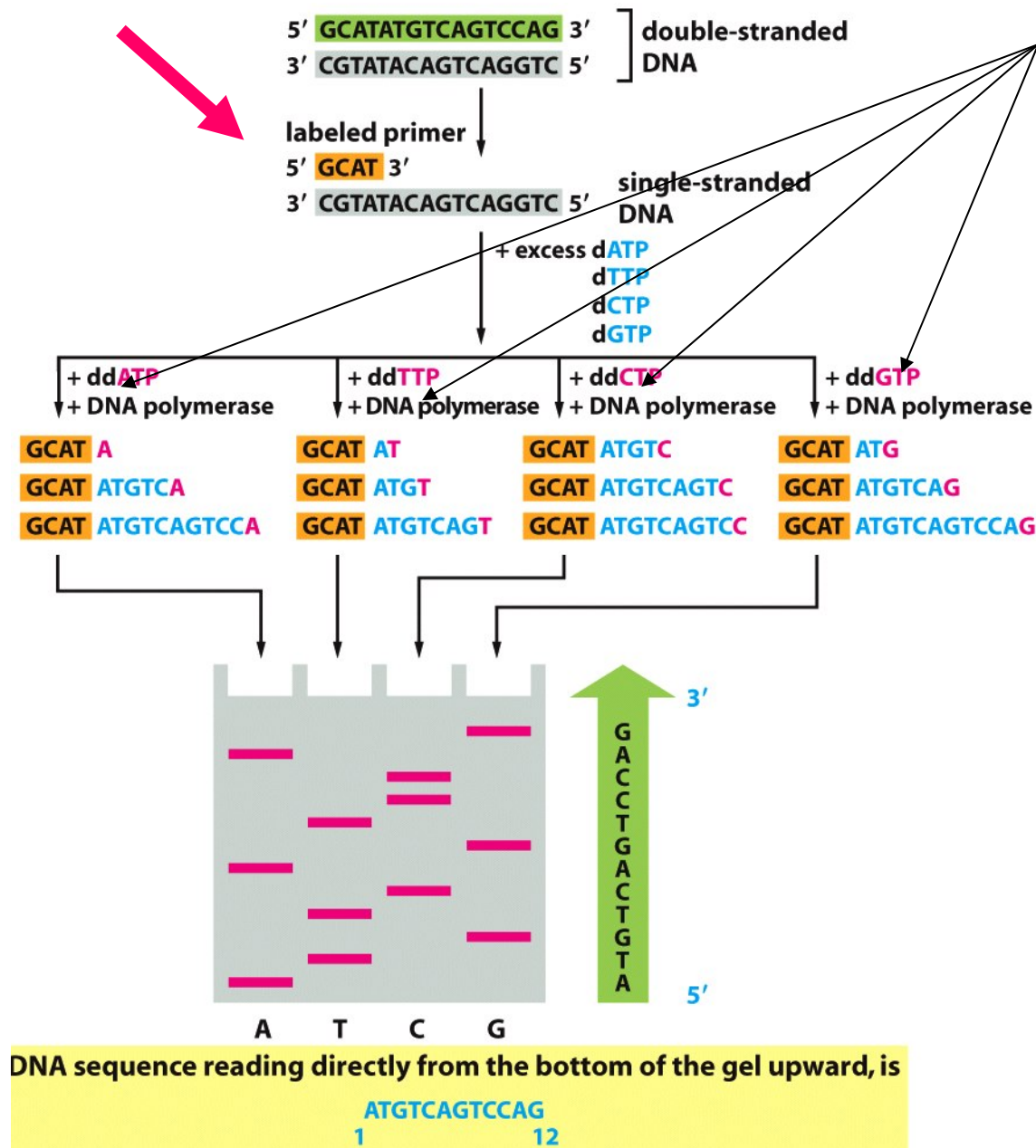


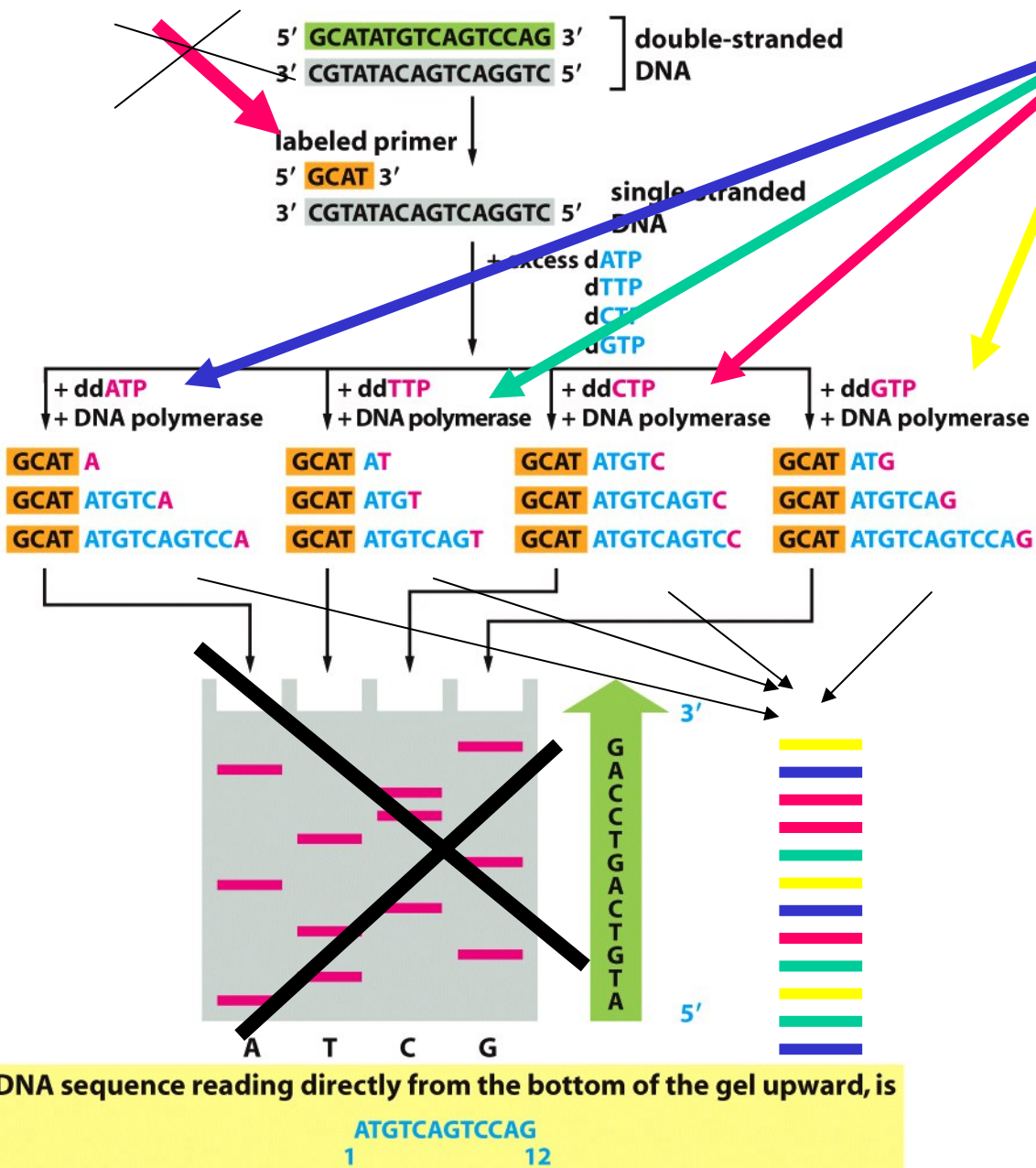
Figure 8-50b Molecular Biology of the Cell 5/e (© Garland Science 2008)



Jokaista
”kirjainta”
varten on oma
reaktioastia ja
oma kaista
geelillä

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



”Automaattisekvensseri” on elektroforeesilaitte, jossa Harmaa Laatikko ”lukee” kapillaarissa virtaavia DNA-pätkiä UV-herätteisen fluoresenssin avulla ja tekee tietokoneelle virtuaalikuva

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

Huhtikuu 2005
Viikko 14

MAANANTAI
Ulko

8

9 Marele 1 x selv.

10 J. Kiiskilä selv. 20

11 Dm S. S. 34 + 18

12 Lumi S. 8

13 Laura jaakola S. 16

14

15 kurssi psat 321

16

Marijnt 1 x psat

4

TIISTAI
Irene Irina Ira Iro

8

9 Kaikunjalte 1 x psat

10

11 David 4 x psat

12

13 Laura S. 17

14

15 Katja R. S. 24

16

17 Marijnt 1 x psat

Dm S. 2 x selv.

94-271

5

KESKIVIIKKO

Ville Vilho Vilhelm Viljami Vili Jami

8

9

10 Riika 1 x psat

11

12 Wilho 2 x selv.

13

14

15

16

17

95-270

STAI
Ahvo

DELL

Tilaukirja on täynnä, mutta prosessi on nopea



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➤ DNA sequencing

Technology Platforms

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Services

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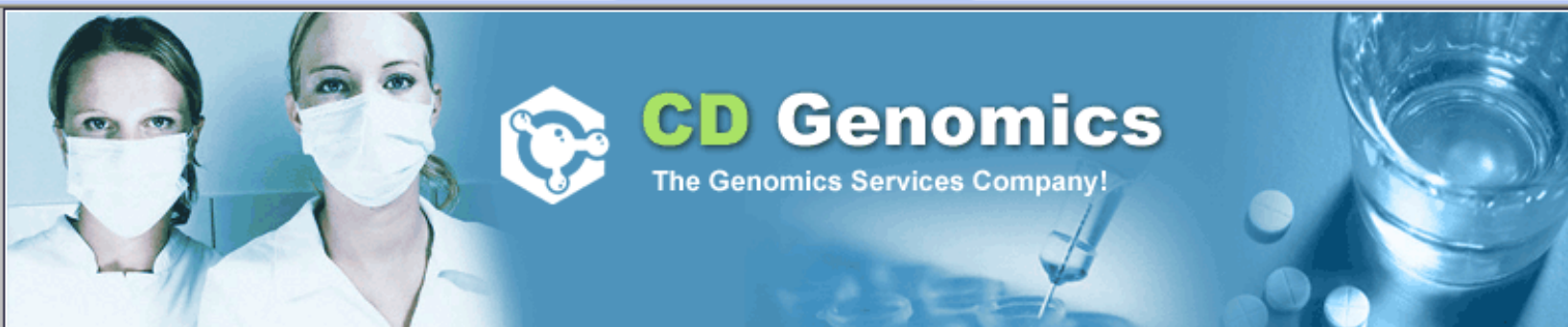
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➤ Custom libraries

➤ Technology platforms

➤ Other services

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» **DNA sequencing**

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» **Genotyping**

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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.

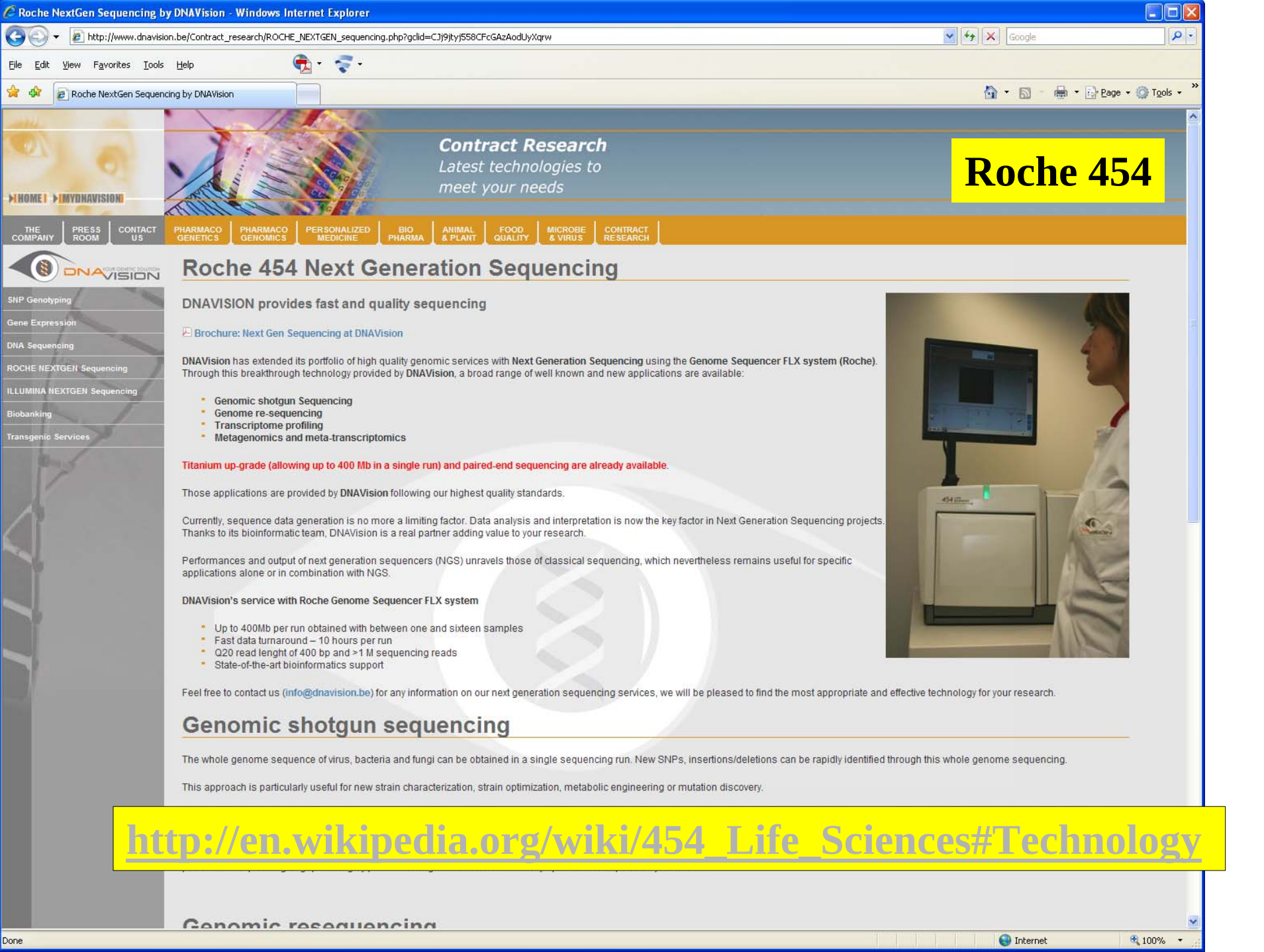
Technology Platforms

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

Services

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

[Kauppa josta saa kaikki koneet ja palvelut](#)



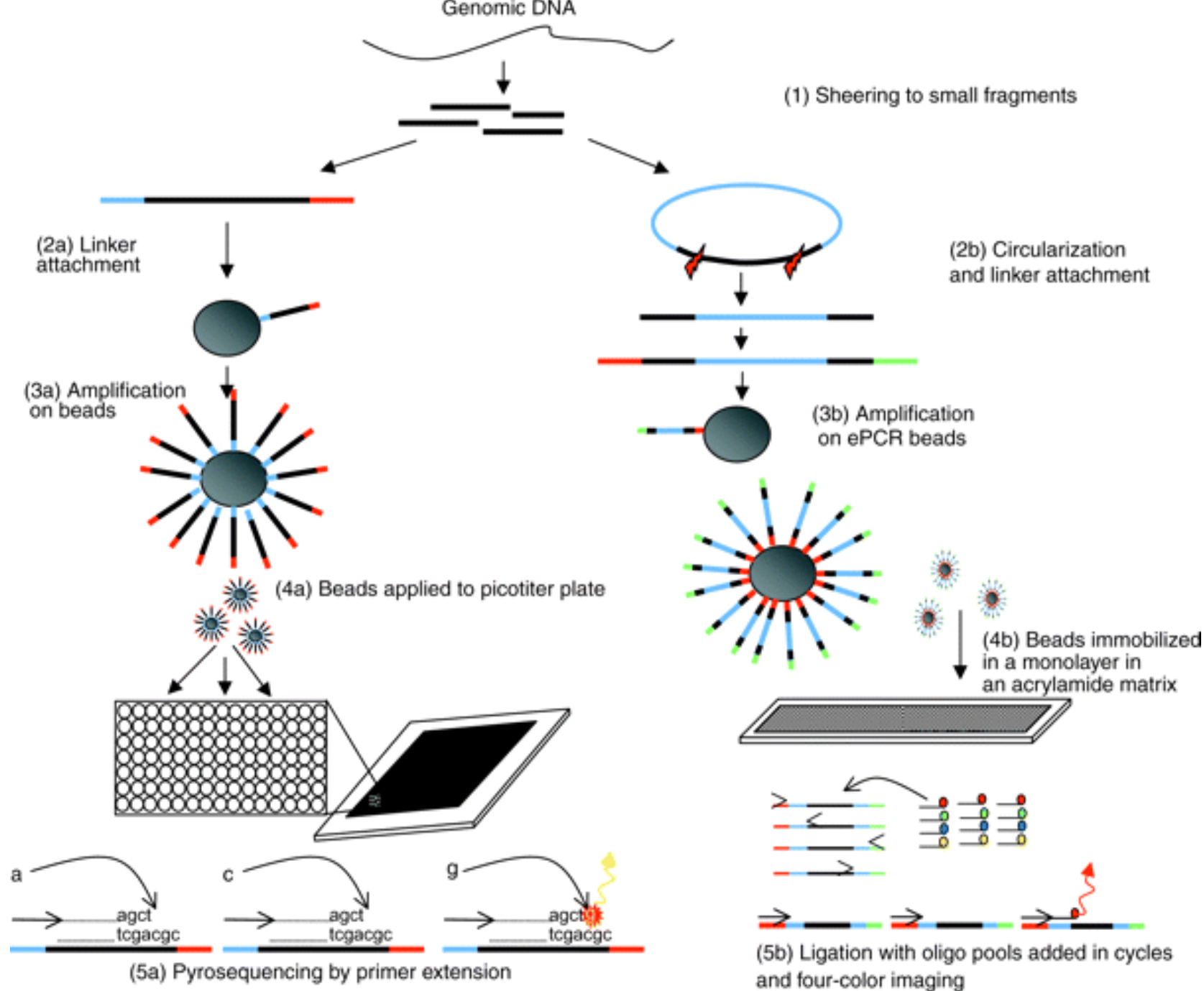


Fig. 2 Outline of the 454 and polony sequencing process. Both systems first fragment the genomic DNA (Step 1) and then use a process of *in vitro* cloning followed by amplification. The 454 process is shown on the left and Polony sequencing is shown on the right. In the 454 protocol, the linkers are 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.

DNA sequencing

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Custom libraries

Technology platforms

Other services

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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.

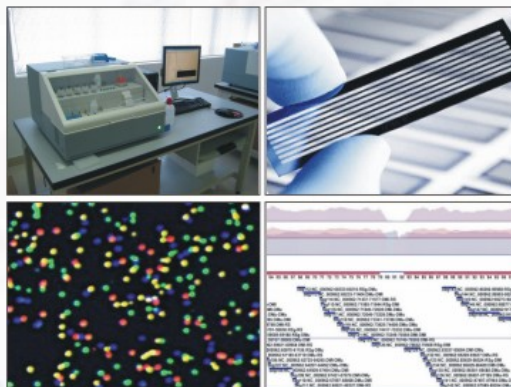


Highlights:

- *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

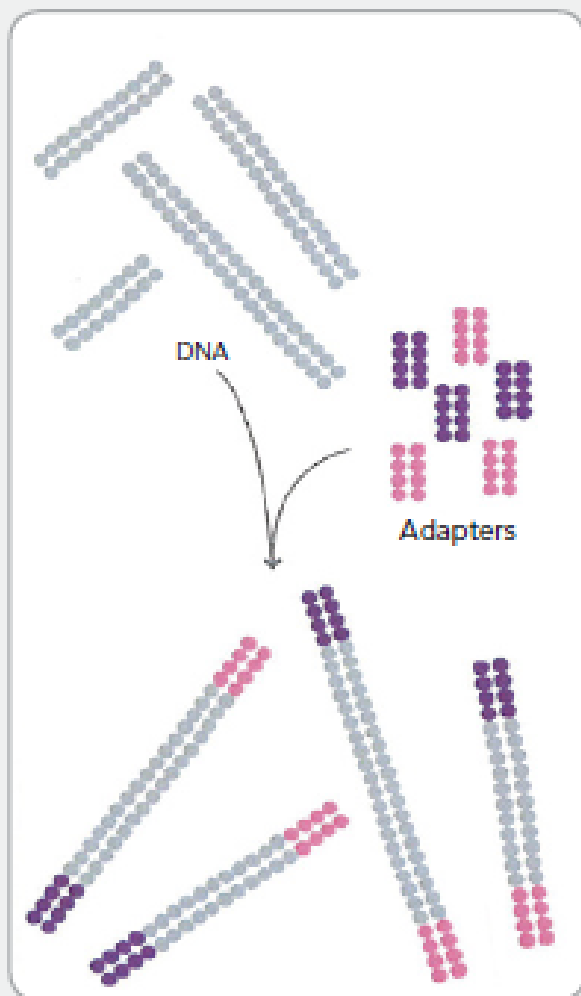


Solexa

Metodiesite

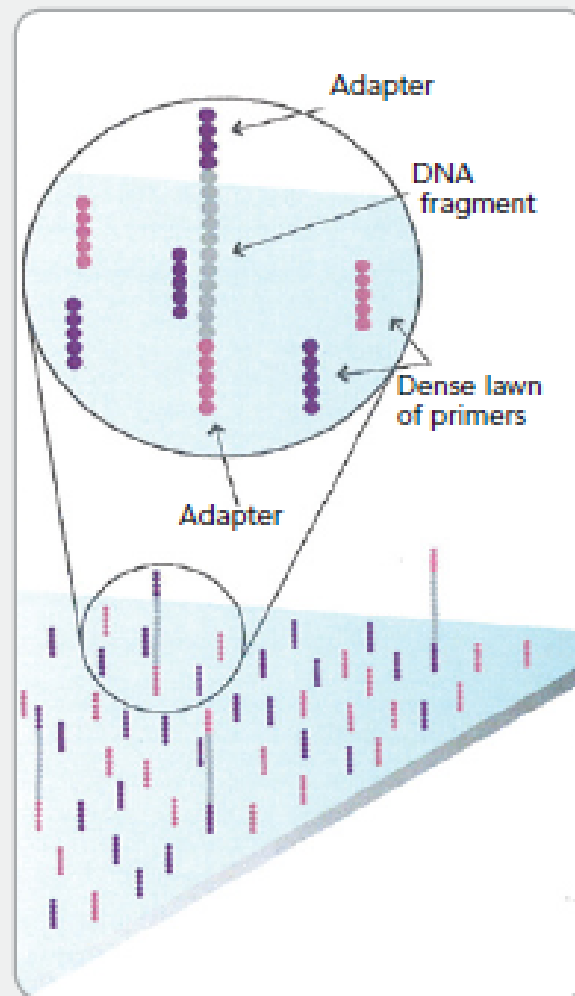
FIGURE 2: SEQUENCING TECHNOLOGY OVERVIEW

1. PREPARE GENOMIC DNA SAMPLE



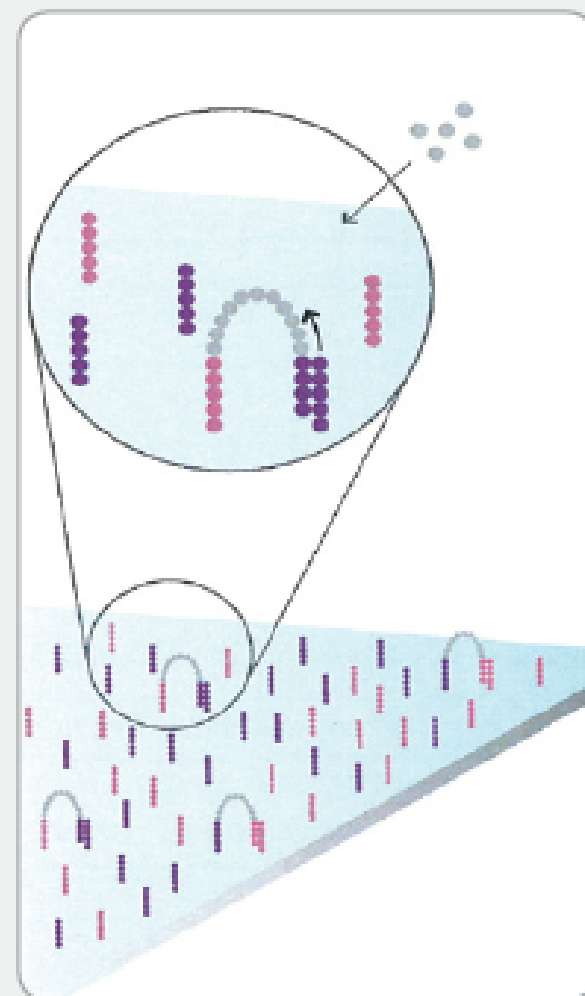
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

2. ATTACH DNA TO SURFACE



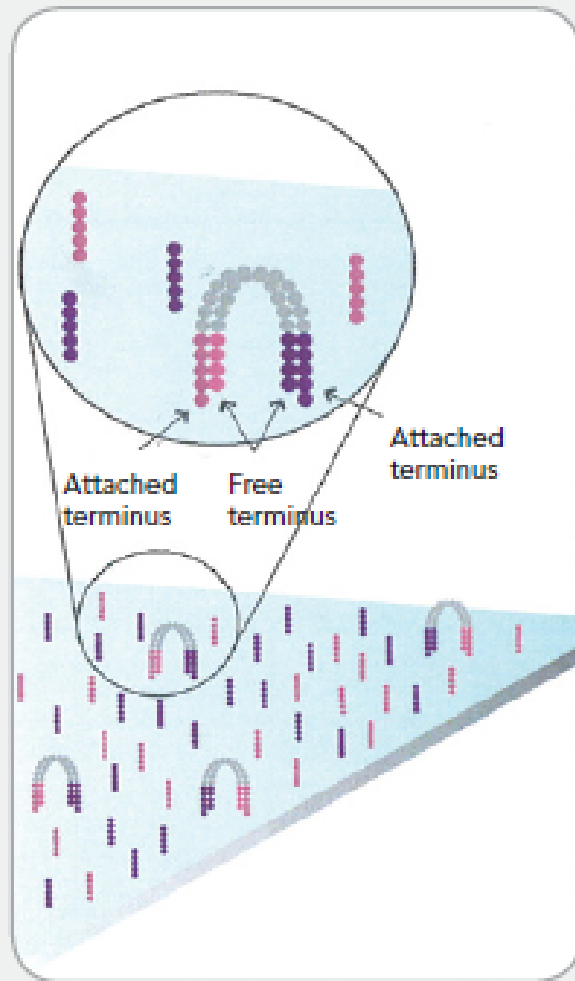
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

3. BRIDGE AMPLIFICATION



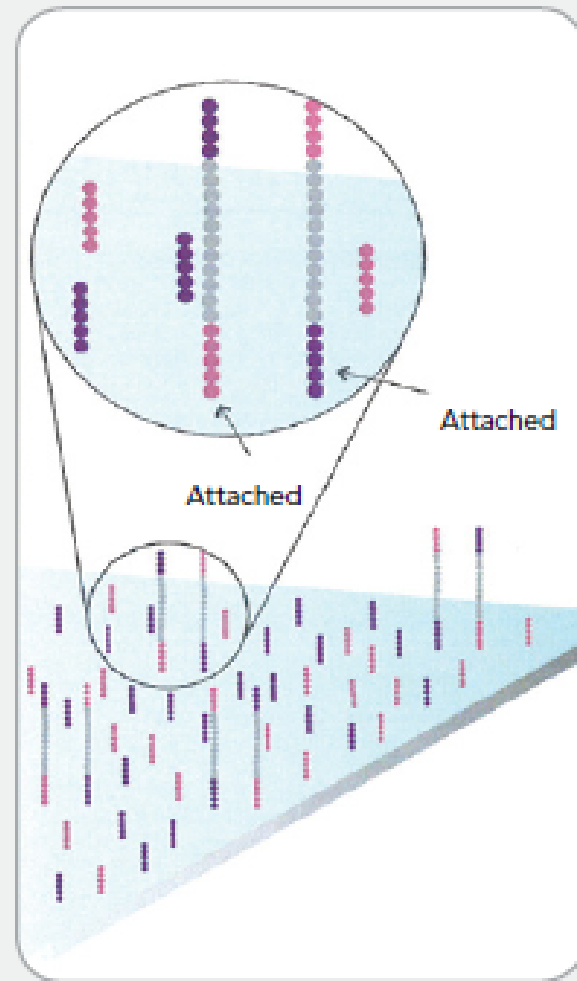
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

4. FRAGMENTS BECOME DOUBLE STRANDED



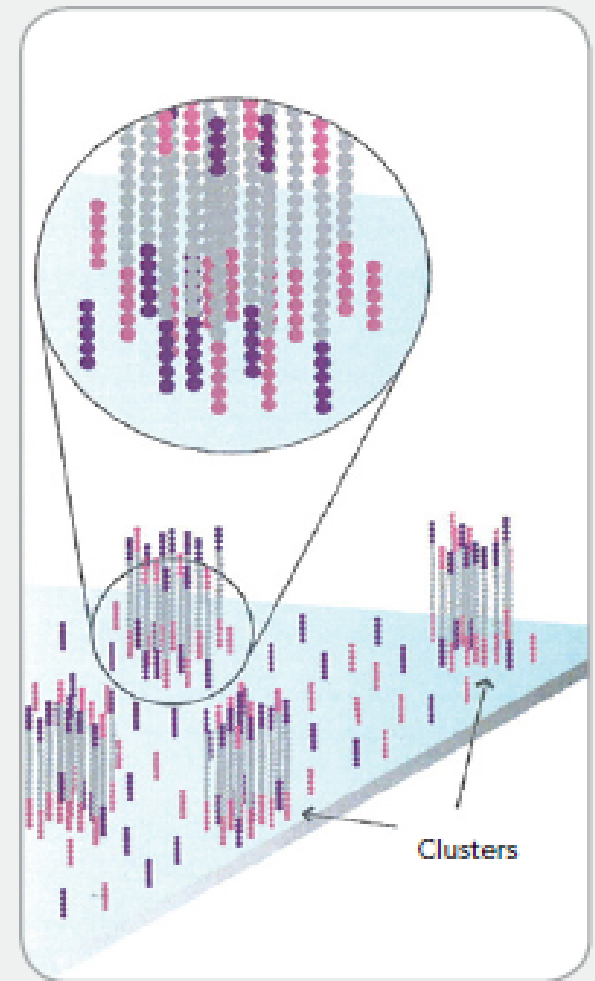
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

5. DENATURE THE DOUBLE-STRANDED MOLECULES



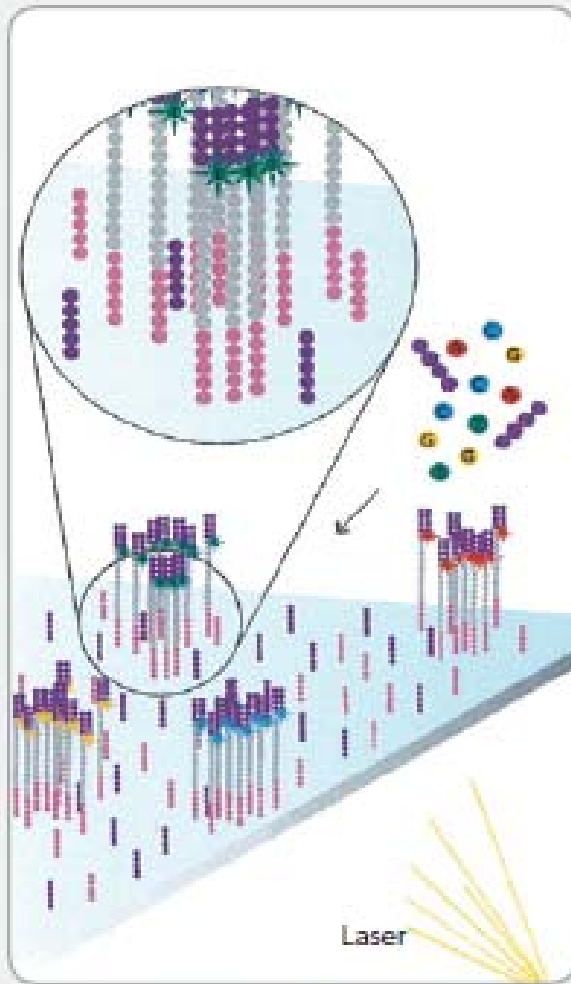
Denaturation leaves single-stranded templates anchored to the substrate.

6. COMPLETE AMPLIFICATION



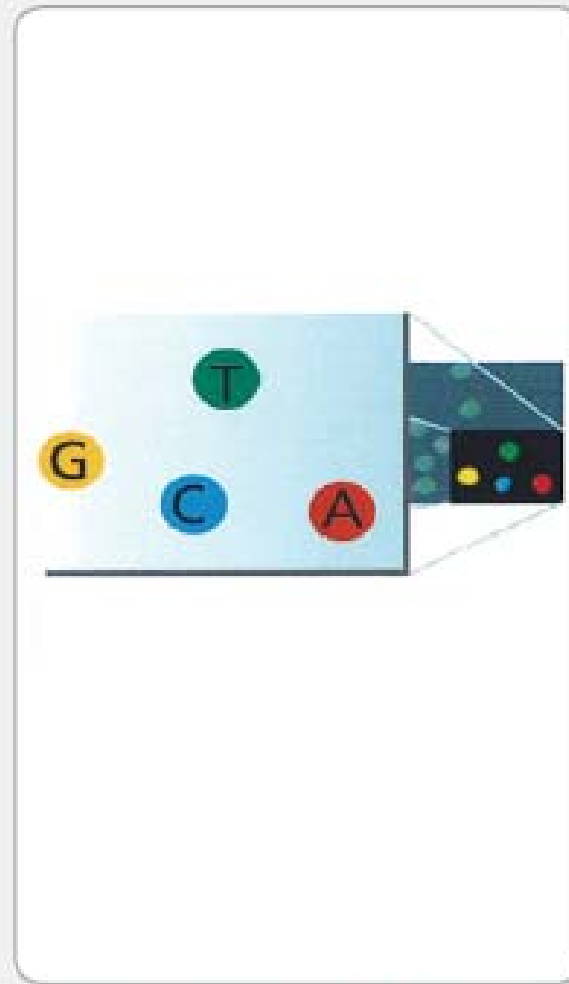
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

7. DETERMINE FIRST BASE



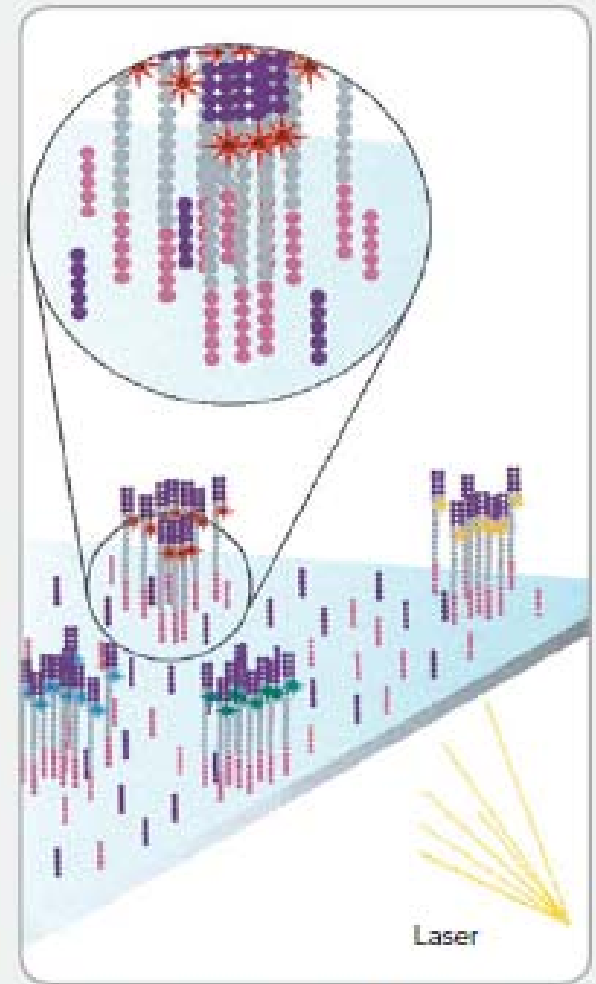
First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

8. IMAGE FIRST BASE



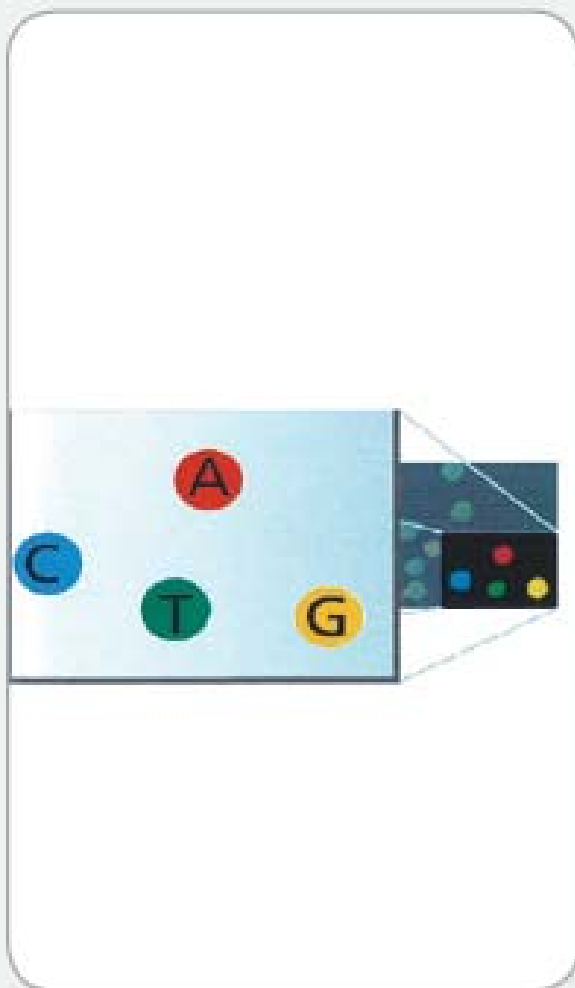
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

9. DETERMINE SECOND BASE



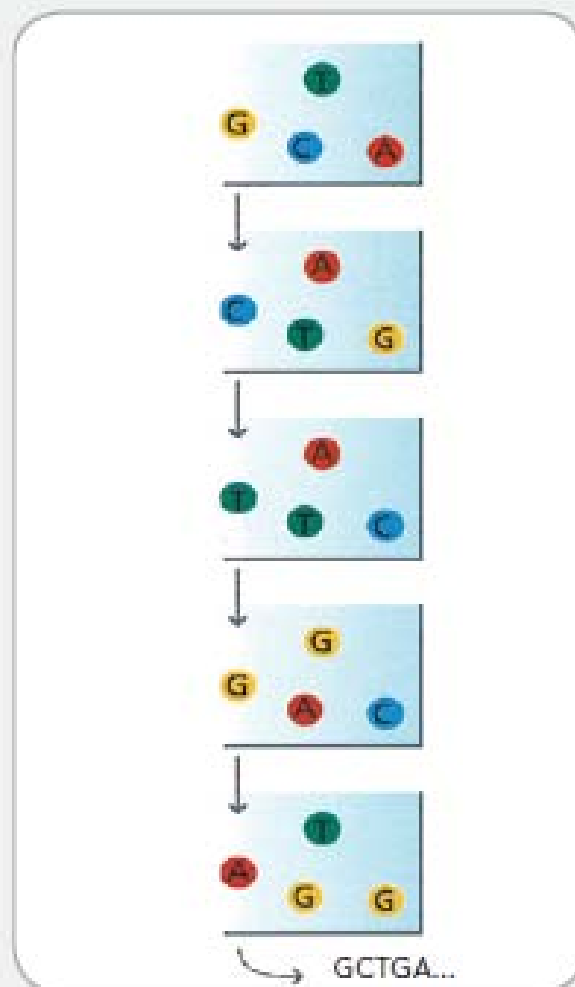
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

10. IMAGE SECOND CHEMISTRY CYCLE



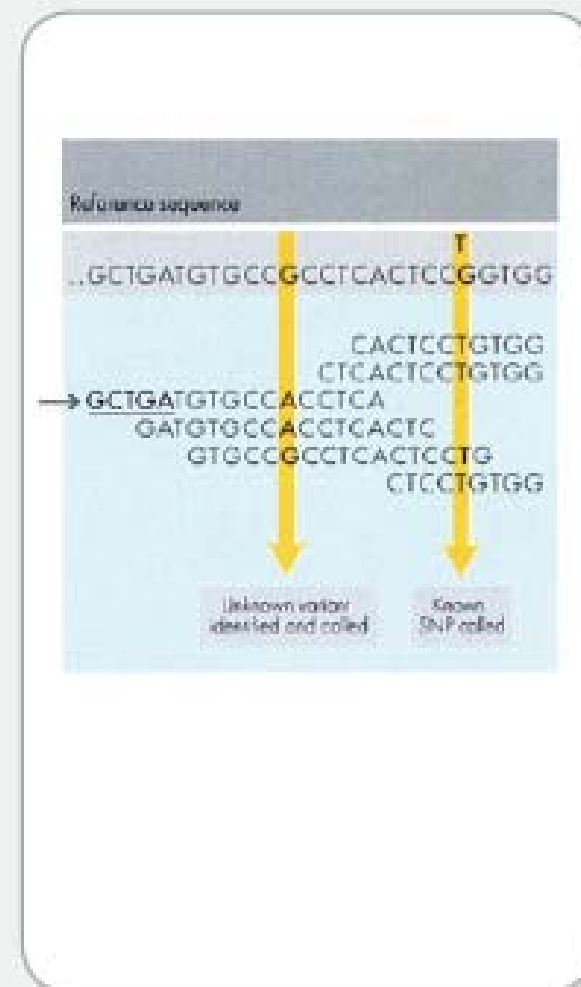
After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

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.



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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

Molekyyliomenetelmällä löytyi sellainenkin mato, joka on erikoisuutena kuvailtu Tanskassa ([Lindenström 2003](#))

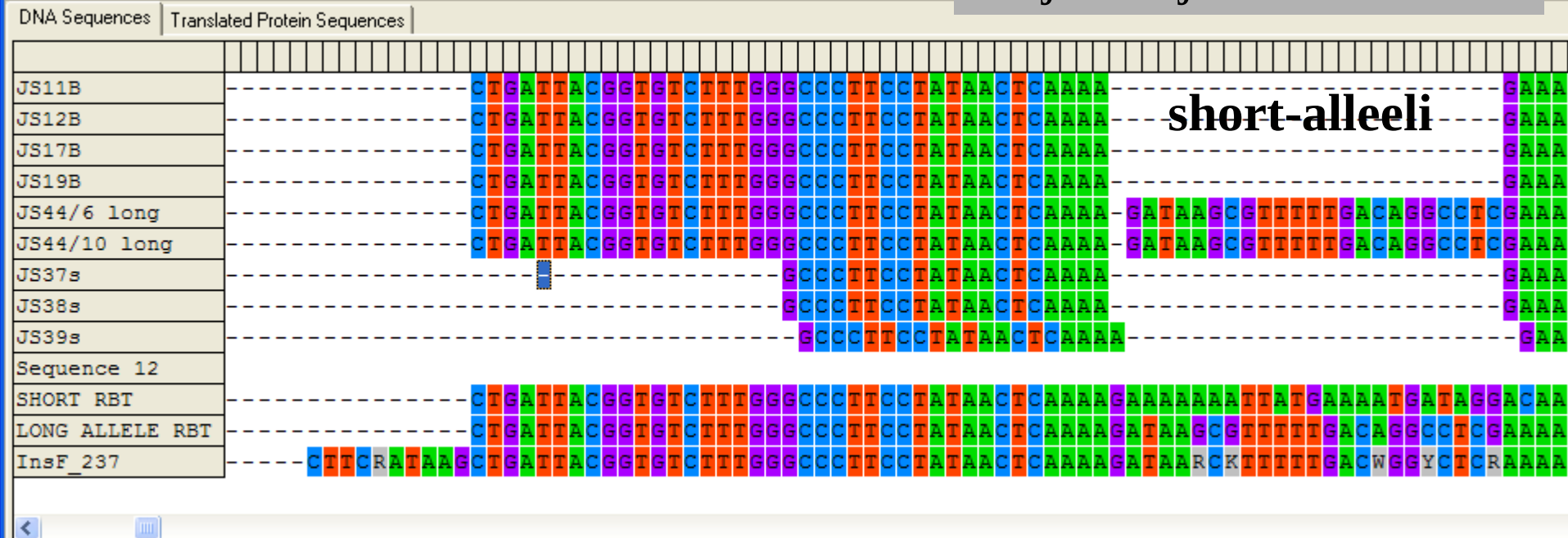
Me tutkimme siitä myös mitokondrioDNA:n, ja totesimme, että loislinjan naarasesiäiti on tosi outo (eri lajia siis)

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

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

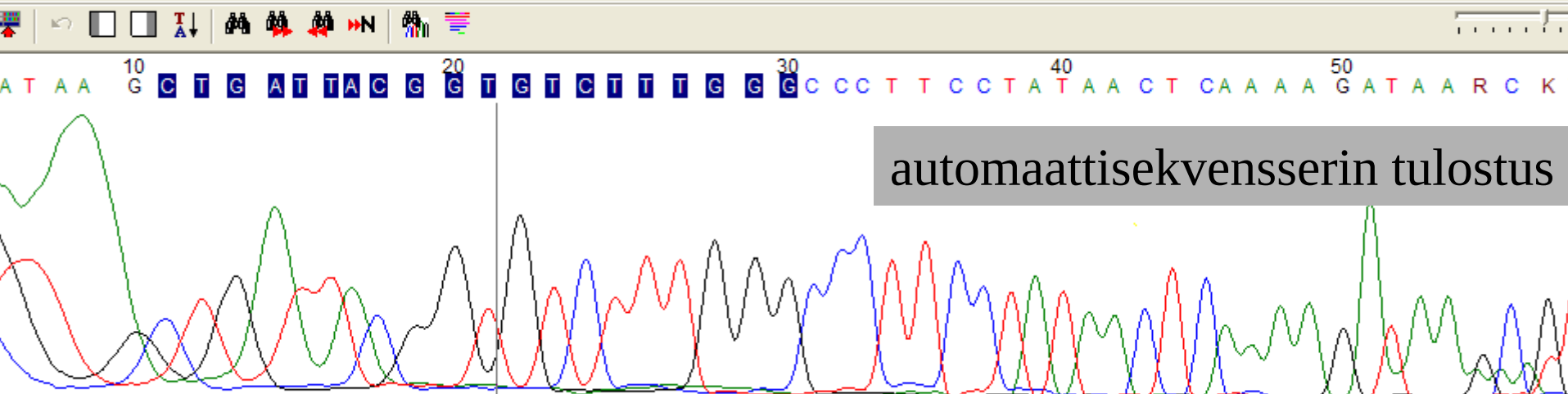
Mitokondriosekvenssien vertailu

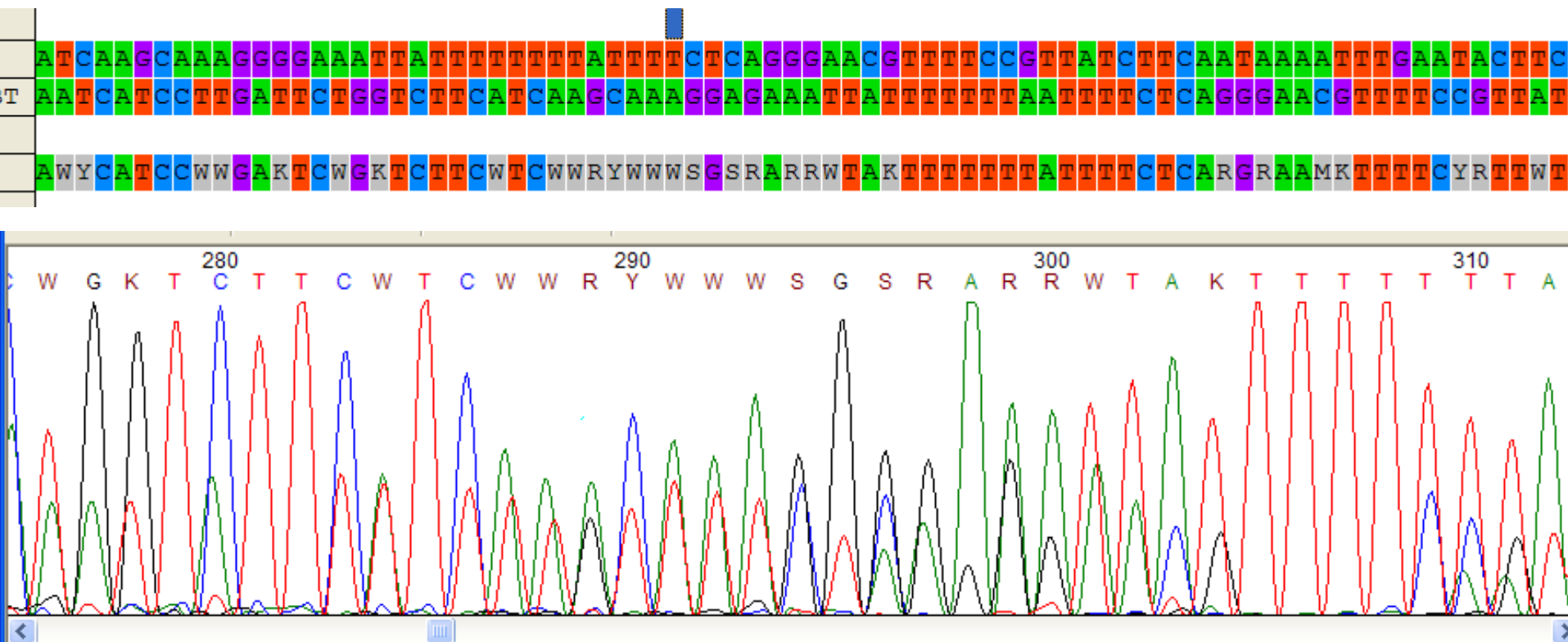




Site # 40 with w/o Gaps

Display Help





Edellisen sekvenssin vaikeampi kohta: koska yksi (kolmesta) alleelistä 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

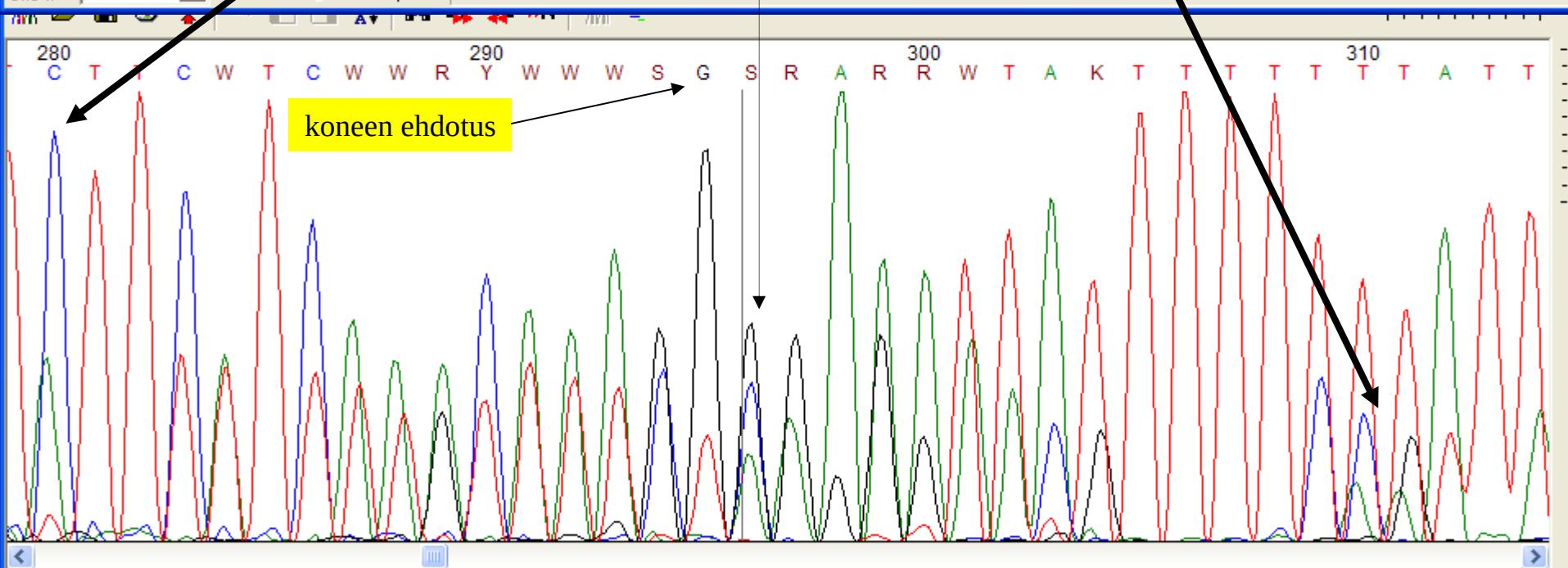
International Union of Pure and Applied Chemistry

DNA Sequences | Translated Protein Sequences

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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

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