



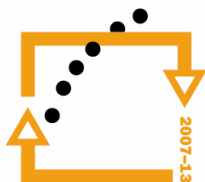
evropský
sociální
fond v ČR



EVROPSKÁ UNIE



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



**OP Vzdělávání
pro konkurenceschopnost**

INVESTICE
DO ROZVOJE
VZDĚLÁVÁNÍ

Zavádění sekvenačních metod do provozu laboratoře metalomiky a nanotechnologií

Název:

Školitel: **Kristýna Šmerková**

Datum: **20.1. 2012**

Reg.č.projektu: CZ.1.07/2.3.00/20.0148

Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik



OSNOVA

- Využití sekvenování
- Historie
- Princip Sangerovy metody
- Příprava vzorku
- Sekvenátor CEQ 8000



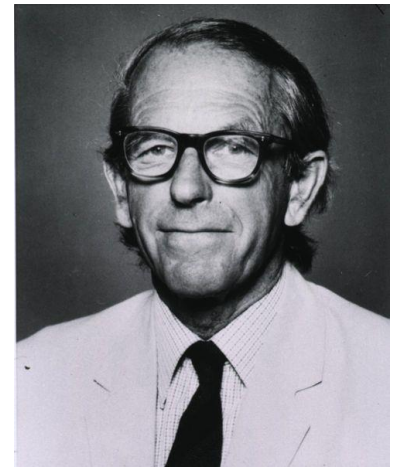
UPLATNĚNÍ ZNALOSTI SEKVENCE DNA

- Výzkum
- Forezní vědy
- Určení paternity
- Genové inženýrství, GMO
- Antropologie, fylogenetika...
- **Lékařství** – diagnostika, léčení, genová terapie



HISTORIE

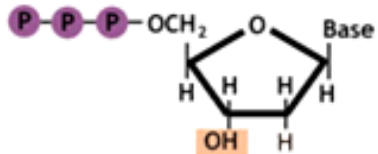
- počátek 70. let – nepřímo (sekvenováním molekul RNA nebo proteinů)
- 1. sekvenování – 1970 Ray Wu z Cornellské univerzity
 - 12 bp z okrajových oblastí fága λ během 3 let
- 1975 – Frederick **Sanger** a A. R. Coulson
- 1977 – Allan Maxam a Walter Gilbert
- 1996 – Pyrosekvenování



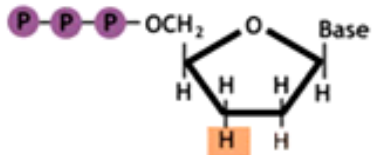
Frederick Sanger (*13. 8. 1918)



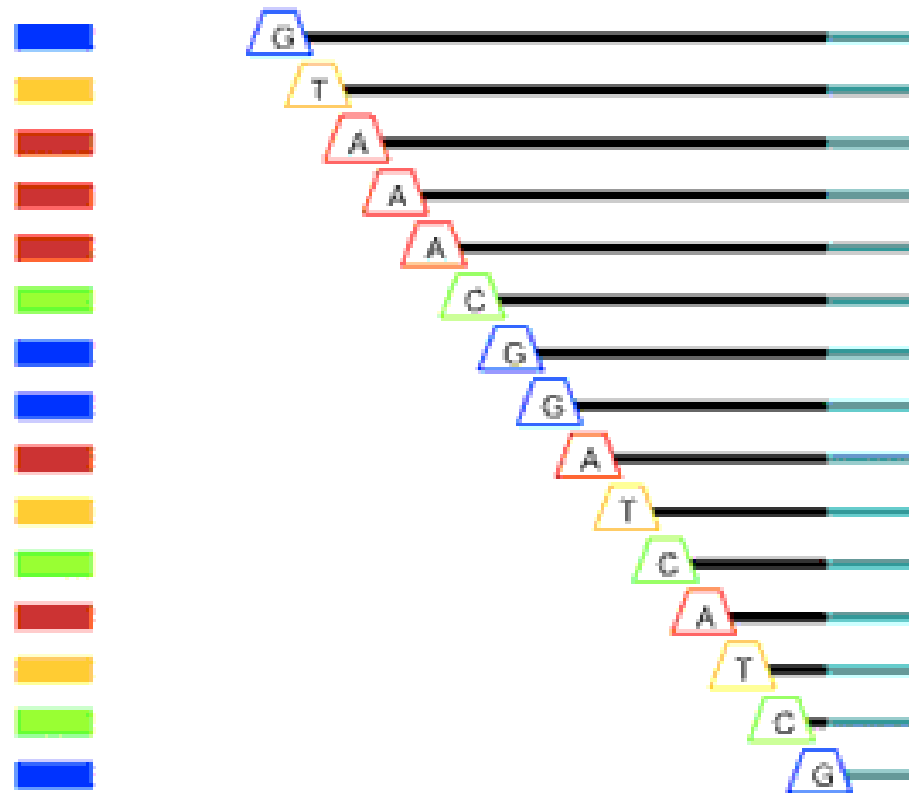
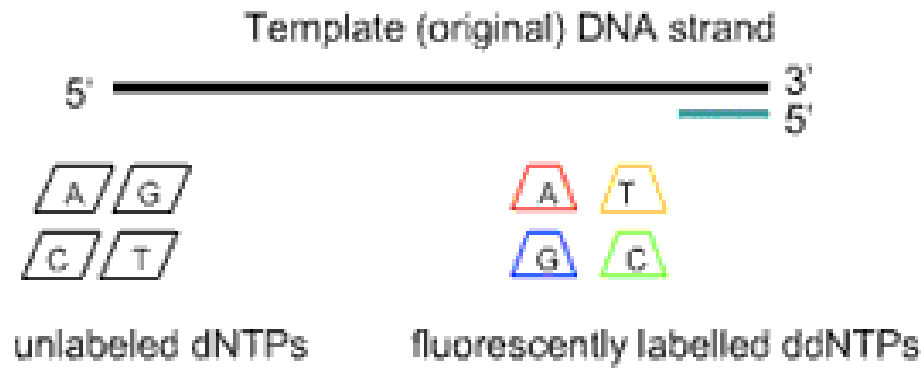
SANGEROVA METODA



deoxynucleotide (dNTP)



dideoxynucleotide (ddNTP)



G T A A A C G G A T C A T C G

PŘÍPRAVA VZORKU

- genomová DNA bakteriofágu λ
- PCR → specifický fragment

PCR (Lambda DNA)

Úvodní denaturace: 95°C/2 min

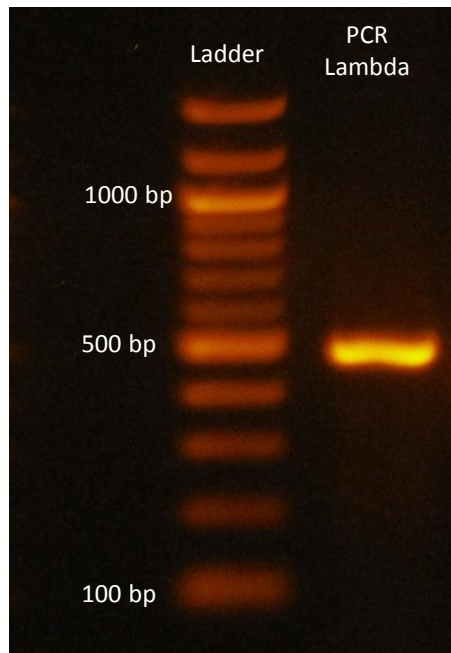
Denaturace: 95°C/15 s

Annealing: 65°C/15 s

Elongace: 72°C/45 s

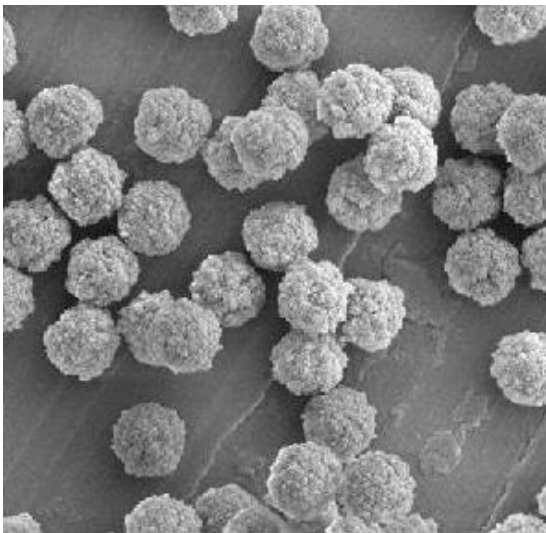
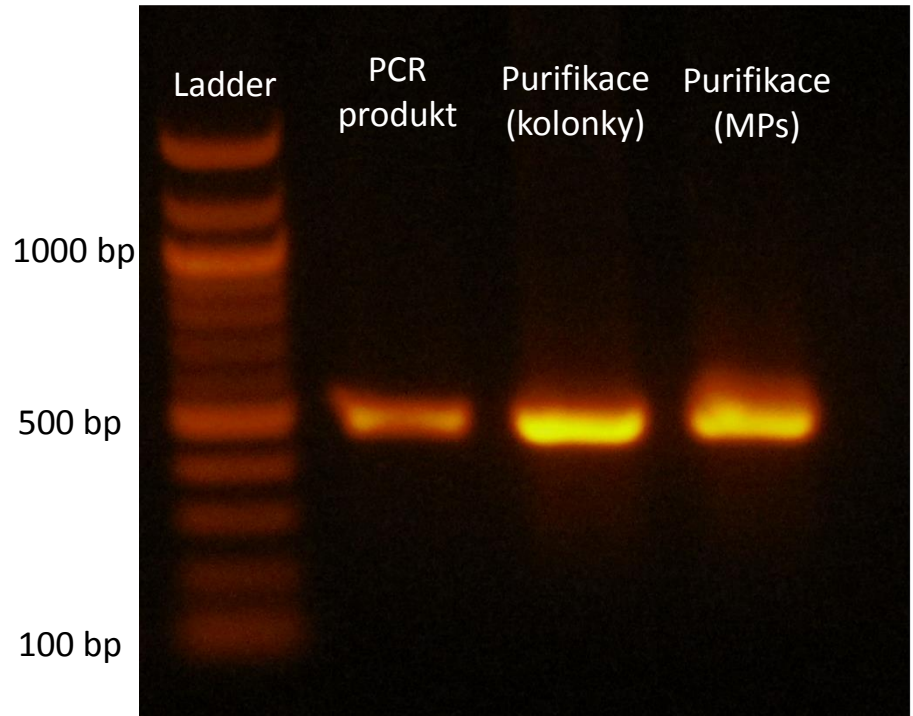
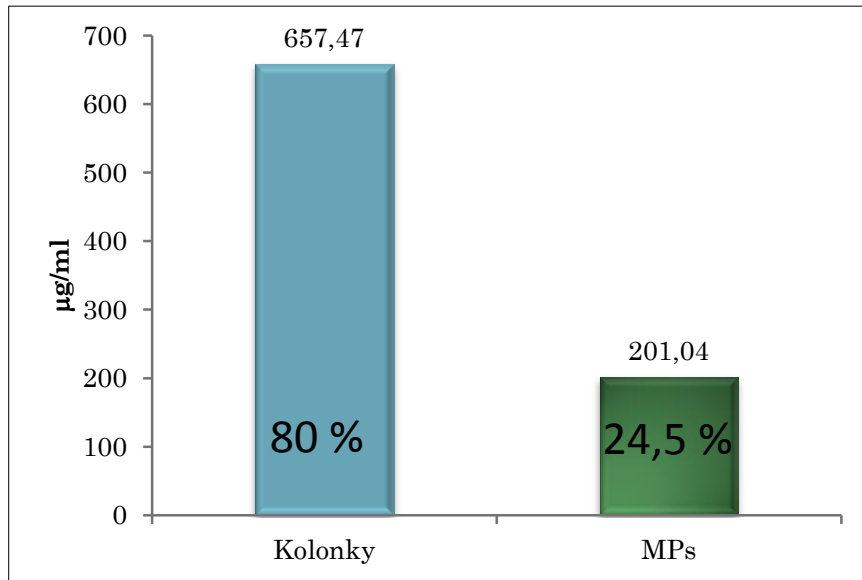
Závěrečná elongace: 72°C/5 min

} 25 cyklů



PŘÍPRAVA VZORKU II

- Izolace → odstranění zbytků reakční směsi



PŘÍPRAVA VZORKU III

- Sekvenační reakce → fluorescenčně značené fragmenty

Sekvenační PCR

Úvodní denaturace: 96°C/3 min

Denaturace: 96°C/20 s

Annealing: 50°C/20 s

Elongace: 60°C/4 min

} 30 cyklů

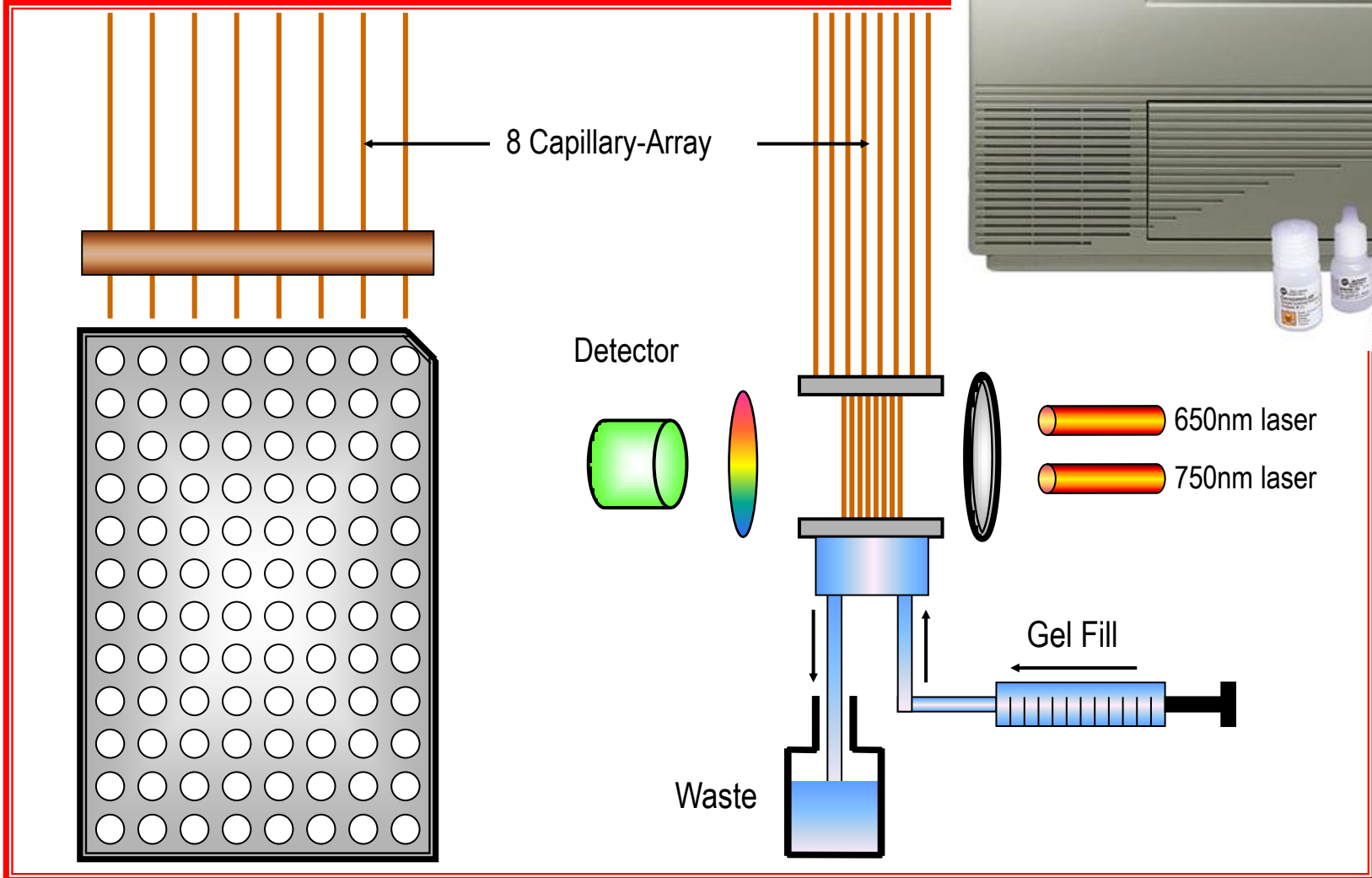


- Izolace → odstranění reakční směsi

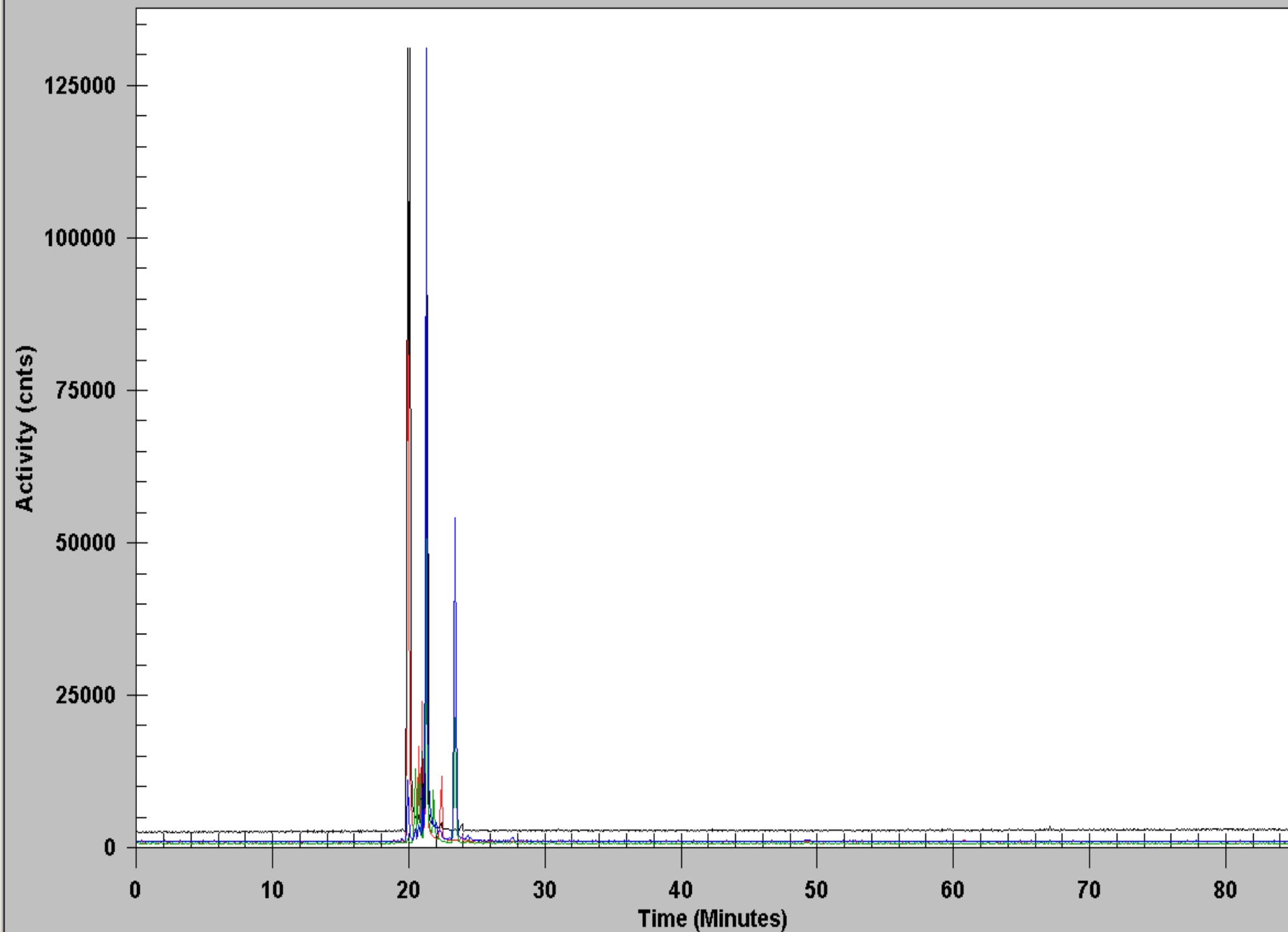


CEQ 8000

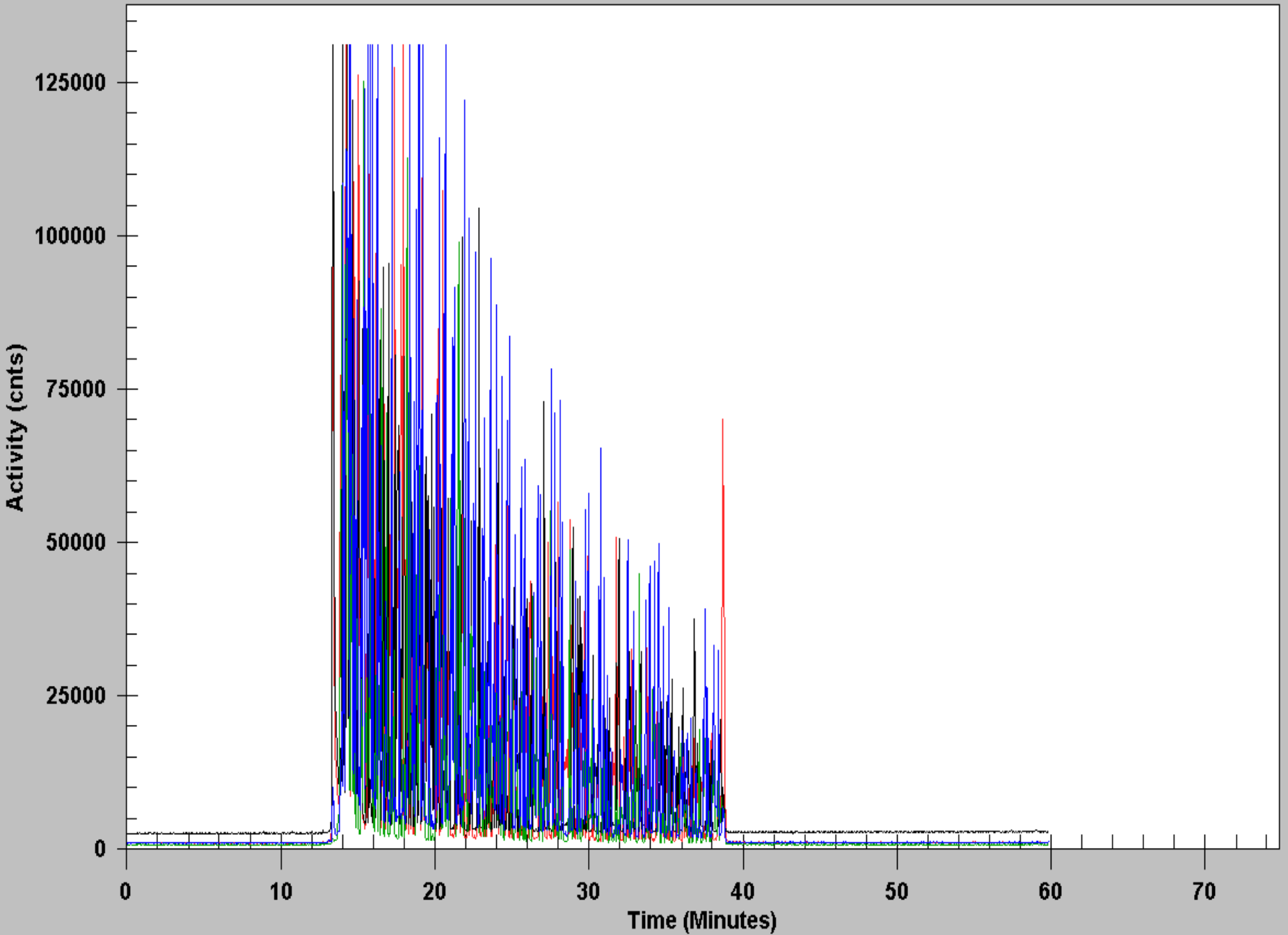
Genetic Analysis System



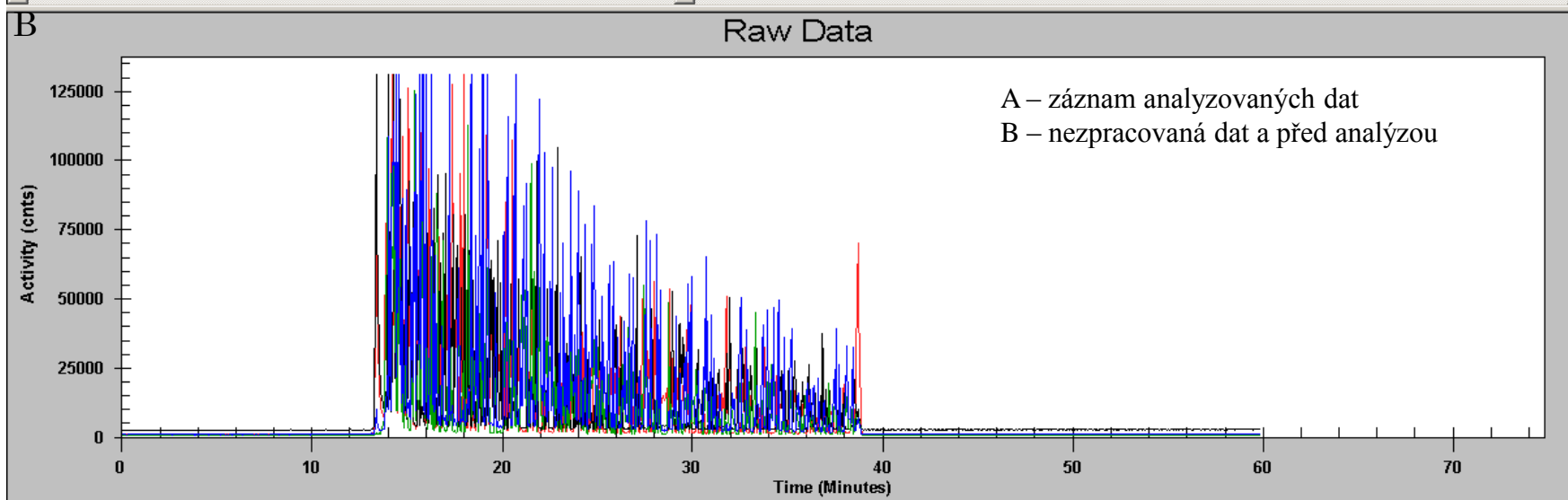
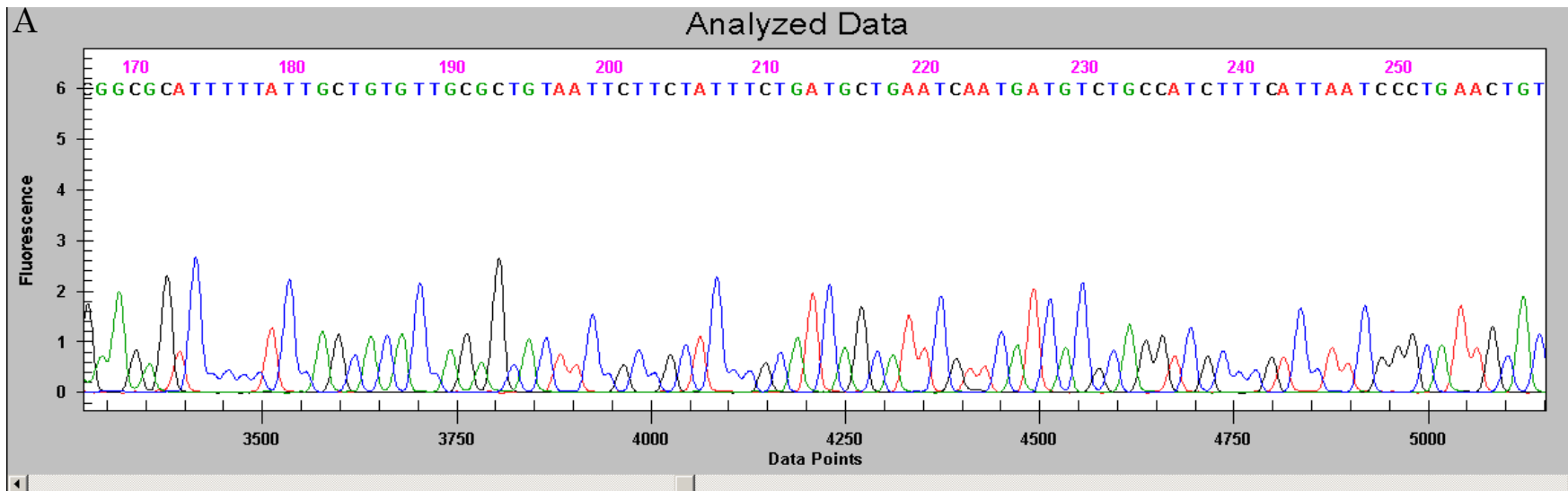
Raw Data



Raw Data



VÝSTUPNÍ DATA



Sekvence amplifikovaného úseku Lambda DNA

```
TAGTTGTCGCTCACTTCGAACCTCTCTGTTTACTGATAAGTTCAGAATTCCTCCTGGCAACTTGCACAAGTCCGACAACCTGA  
ACGAACCAGGGCGTGCTTCGTTTCATCTATCGGATCGCCACACTCACAACAATGAGTGGCAGATATAGCCTGGTGGTTCAGGCGGC  
GCATTTTTATTGCTGTGTTGCGCTGTAATTCTTCTATTTCTGATGCTGAATCAATGATGTCTGCCATCTTTCATTAATCCCTGAACT  
GTTGGTTAATACGCTTGAGGGTGAATGCGAATAATAAAAAAGGAGCCTGTAGCTCCCTGATGATTTTGCTTTTCATGTTTCATCGT  
TCCTTAAAGACGCCGTTTAAACATGCCGATTGCCAGGCTTAAATGAGTCGGTGTGAATCCCATCAGCGTTACCGTTTCGCGGTGCT  
TCTTCAGTACGCTACGGCAAATGTCATCGACGTTTTTATCCGGGA
```

○ http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome

Description	Max score	Total score	Query coverage	E value	Max ident
Escherichia coli 'BL21-Gold(DE3)pLysS AG' chromosome, complete ge	830	1527	98%	0.0	99%
Escherichia coli BW2952 chromosome, complete genome	830	830	98%	0.0	99%
Enterobacteria phage lambda, complete genome >gb J02459.1 LAM1	830	830	98%	0.0	99%
Escherichia coli 55989 chromosome, complete genome	808	808	98%	0.0	98%
Escherichia coli E24377A chromosome, complete genome >gb CP000	808	808	98%	0.0	98%
Escherichia coli ATCC 8739 chromosome, complete genome	795	795	98%	0.0	98%
Escherichia coli O157:H7 str. EC4024 scf_1109799330226 genomic sc	780	3005	98%	0.0	97%
Escherichia coli O157:H7 str. TW14588 chromosome, whole genome	780	3049	98%	0.0	97%
Escherichia coli O157:H7 str. TW14359 chromosome, complete geno	780	3005	98%	0.0	97%
Escherichia coli O157:H7 str. EC4115 chromosome, complete genom	780	3005	98%	0.0	97%
Escherichia coli O157:H7 str. EDL933 chromosome, complete genom	780	2488	98%	0.0	97%
Escherichia coli O157:H7 str. Sakai chromosome, complete genome	780	2280	98%	0.0	97%
Stx1 converting phage, complete genome	769	769	98%	0.0	97%
Stx2 converting phage II, complete genome	769	769	98%	0.0	97%
Escherichia coli O55:H7 str. CB9615 chromosome, complete genome	769	2960	98%	0.0	97%
Stx2 converting phage I, complete genome >dbj AP004402.1 Stx2	769	971	98%	0.0	97%
Enterobacteria phage VT2-Sakai, complete genome >dbj AP000363.:	769	769	98%	0.0	97%
Enterobacteria phage 933W, complete genome >gb AF125520.1 Ba	769	971	98%	0.0	97%
Escherichia coli ED1a chromosome, complete genome	763	763	98%	0.0	97%
Enterobacteria phage Min27, complete genome >gb EU311208.1 En	763	966	98%	0.0	97%
Escherichia coli IAI1 chromosome, complete genome	758	758	98%	0.0	96%
Escherichia coli 83972 genomic scaffold SCAFFOLD1, whole genome s	752	752	98%	0.0	96%
Escherichia coli O26:H11 str. 11368 chromosome, complete genome	752	1411	98%	0.0	96%
Escherichia coli CFT073 chromosome, complete genome	752	752	98%	0.0	96%
Escherichia coli O103:H2 str. 12009, complete genome	736	736	98%	0.0	95%
Stx2-converting phage 1717, complete prophage genome >gb FJ186	736	736	98%	0.0	95%
Enterobacteria phage YYZ-2008, complete prophage genome >gb FJ	736	736	98%	0.0	95%
Stx2-converting phage 86, complete genome >dbj AB255436.1 Stx:	736	736	98%	0.0	95%
Enterobacteria phage BP-4795, complete genome >emb AJ556162.1	730	730	98%	0.0	95%
Escherichia coli B354 genomic scaffold supercont1.3, whole genome s	725	725	89%	0.0	98%
Escherichia coli 536, complete genome >gb CP000247.1 Escherichia	725	725	89%	0.0	98%
Escherichia coli M605 genomic scaffold supercont1.10, whole genome	710	710	94%	0.0	96%
Escherichia fergusonii ATCC 35469 chromosome, complete genome	708	708	89%	0.0	97%
Escherichia coli IAI39 chromosome, complete genome	702	702	89%	0.0	97%
Escherichia coli S88 chromosome, complete genome	699	699	92%	0.0	96%
Escherichia coli APEC O1 chromosome, complete genome	699	699	92%	0.0	96%
Escherichia coli UTI89 chromosome, complete genome	699	699	92%	0.0	96%
Escherichia coli B str. REL606 chromosome, complete genome	697	697	89%	0.0	97%
Escherichia coli UMN026 chromosome, complete genome	697	1355	89%	0.0	97%
Escherichia coli B185 genomic scaffold supercont1.4, whole genome s	658	658	89%	0.0	95%
Escherichia coli SE11 chromosome, complete genome	654	654	85%	0.0	96%
Escherichia albertii TW07627 scf_1109867046288 genomic scaffold, s	640	640	83%	8e-180	96%

> [ref|NC_001416.1](#) **D** Enterobacteria phage lambda, complete genome
[gb|J02459.1|LAMCG](#) **E** **D** Enterobacteria phage lambda, complete genome
Length=48502

Score = 830 bits (449), Expect = 0.0
Identities = 459/463 (99%), Gaps = 4/463 (1%)
Strand=Plus/Plus

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Query 7      TCGCTCACTTCGAACCTCTCTGTTTACTGATAAGTTCAGAAATTCCTCCTGGCAACTTGC 66
            |||
Sbjct 30388   TCGCTCACTTCGAACCTCTCTGTTTACTGATAAGTTCAG-A-TTCCTCCTGGCAACTTGC 30445

Query 67     ACAAGTCCGACAACCCTGAACGACCAGGCGTGGCTTCGTTTCATCTATCGGATCGCCACAC 126
            |||
Sbjct 30446   ACAAGTCCGACAACCCTGAACG-ACCAGGCGT-CTTCGTTTCATCTATCGGATCGCCACAC 30503

Query 127    TCACAACAATGAGTGGCAGATATAGCCTGGTGGTTCAGGCGGCGCATTTTTATTGCTGTG 186
            |||
Sbjct 30504   TCACAACAATGAGTGGCAGATATAGCCTGGTGGTTCAGGCGGCGCATTTTTATTGCTGTG 30563

Query 187    TTGCGCTGTAATTCTTCTATTTCTGATGCTGAATCAATGATGTCTGCCATCTTTCATTAA 246
            |||
Sbjct 30564   TTGCGCTGTAATTCTTCTATTTCTGATGCTGAATCAATGATGTCTGCCATCTTTCATTAA 30623

Query 247    TCCCTGAACTGTTGGTTAATACGCTTGAGGGTGAATGCGAATAATAAAAAAGGAGCCTGT 306
            |||
Sbjct 30624   TCCCTGAACTGTTGGTTAATACGCTTGAGGGTGAATGCGAATAATAAAAAAGGAGCCTGT 30683

Query 307    AGCTCCCTGATGATTTTGCTTTTCATGTTTCATCGTTCCTTAAAGACGCCGTTTAAACATGC 366
            |||
Sbjct 30684   AGCTCCCTGATGATTTTGCTTTTCATGTTTCATCGTTCCTTAAAGACGCCGTTTAAACATGC 30743

Query 367    CGATTGCCAGGCTTAAATGAGTCGGTGTGAATCCCATCAGCGTTACCGTTTCGCGGTGCT 426
            |||
Sbjct 30744   CGATTGCCAGGCTTAAATGAGTCGGTGTGAATCCCATCAGCGTTACCGTTTCGCGGTGCT 30803

Query 427    TCTTCAGTACGCTACGGCAAATGTCATCGACGTTTTTATCCGG 469
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Sbjct 30804   TCTTCAGTACGCTACGGCAAATGTCATCGACGTTTTTATCCGG 30846
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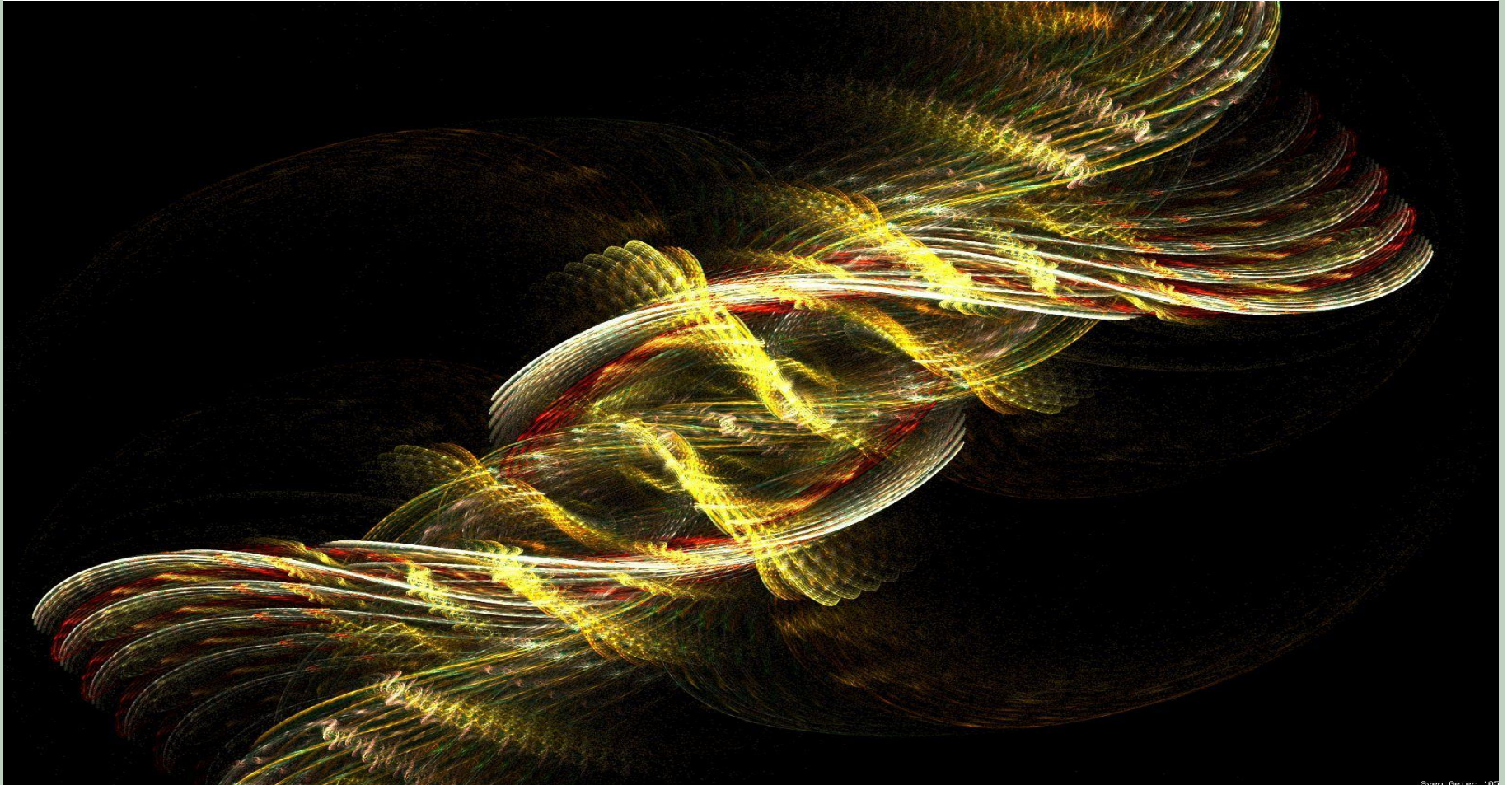


ZÁVĚR

- Zavedení sekvenační metody
- Využití MPs
- Interakce DNA + CisPt



DĚKUJI ZA POZORNOST



Ať se Vám splní všechny sny
Čínská kletba

