

MOLEKÜLER TANI YAKLAŞIMLARINDA NÜKLEİK ASİT TESPİT SINIRININ DÜŞÜRÜLMESİ

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Ayten Y. KARATAŞ, *İstanbul Teknik Üniversitesi Moleküler Biyoloji ve Genetik Bölüm*

Merkez: Sarıyer Cad. 117/B ABC Plaza İstinye, Sarıyer - İSTANBUL

Şube: İ.T.Ü. ARI Teknokent ARI8 Maslak – İSTANBUL

Tel: (212) 286 2135, 36 **Faks:** (212) 286 2134

Web: www.sentromer.com

AMAÇ

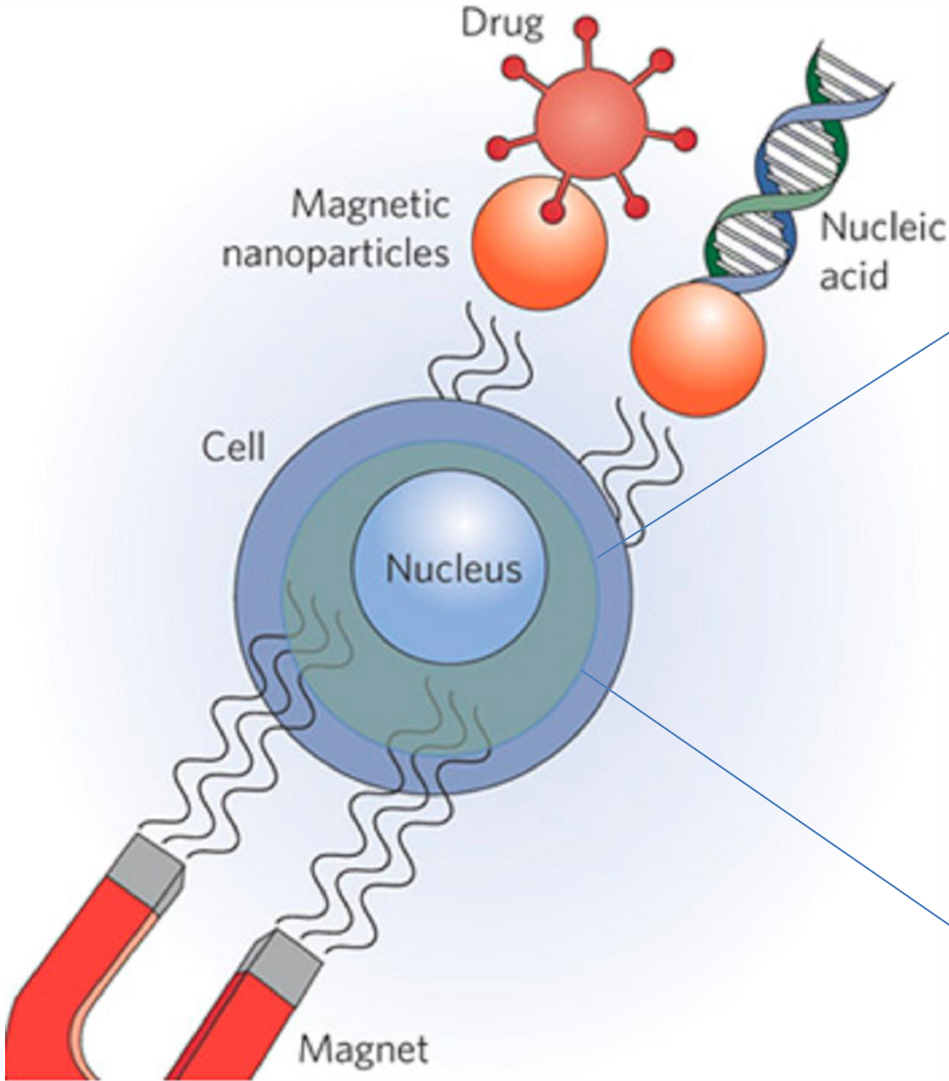
Moleküler uygulamaların başarısı nükleik asit izolasyonunun başarısına bağlıdır.

İnhibitör varlığı izolat konsantrasyonunun olduğundan düşük görünmesine ve yanlış negatif sonuca neden olur.

- İnhibitörden etkilenmeyen, seçici DNA İzolasyonu
- Hesaplı ve özel bir lab cihazı gerektirmeden tek step hedef NA saptaması (Biyosensör entegrasyonuna uygun)

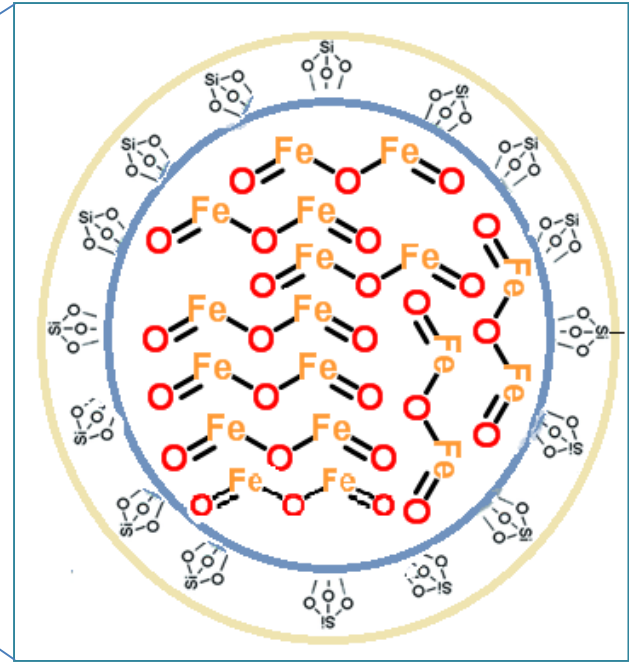


MANYETİK NANOPARTİKÜLLER (MNP)

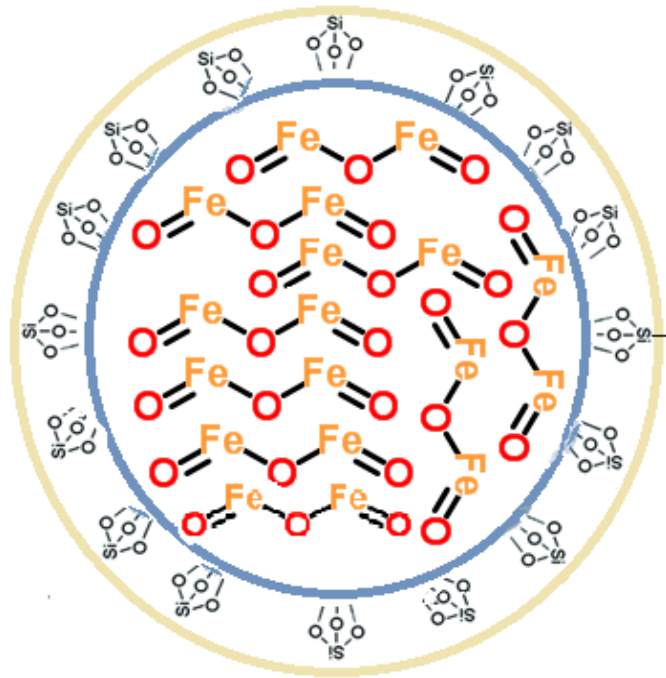


Manyetik **nanopartikül**: 5-500 nm

Manyetik **mikropartikül**: 0.5-500 μm
çapındadır

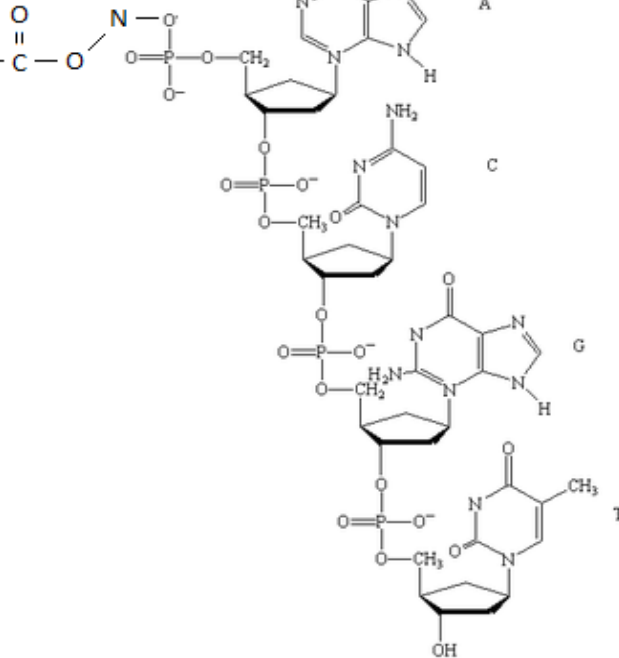


MNP2

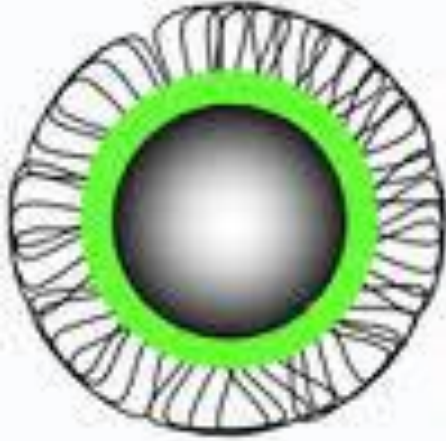


MNP-Oligo

$\text{Fe}_2\text{O}_3\text{-SiO}_2\text{-C=O-O-N-DNA}$



MALZEME VE YÖNTEM



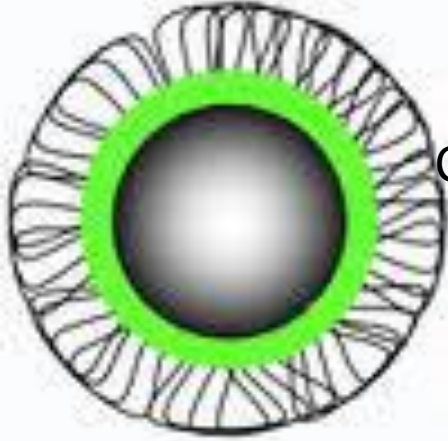
CCCCCGCTAGCTAC**GTAGCTCGTAG**

MNP-Oligo
kompleksi
(COLA)

MALZEME VE YÖNTEM

COCA: COLA-Adaptor

COLA: Carboxy Linked Amino Complex



CCCCCGCTAGCTAC **GTAGCTCGTAG**

CATCGAGCATCAAGCTTACGC...

MNP-Oligo
kompleksi
(COLA)

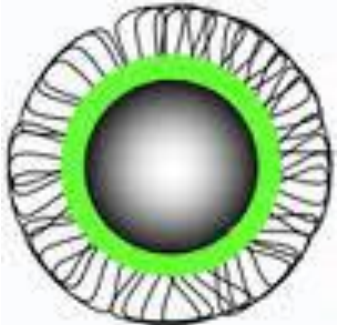
Hedef DNA'ya Özgü
Adaptör Prob (50 baz)
(COCA)



TTCGAATGCGATCTACTG

CATCGAGCATCAAGCTTACGCTAGATGAC

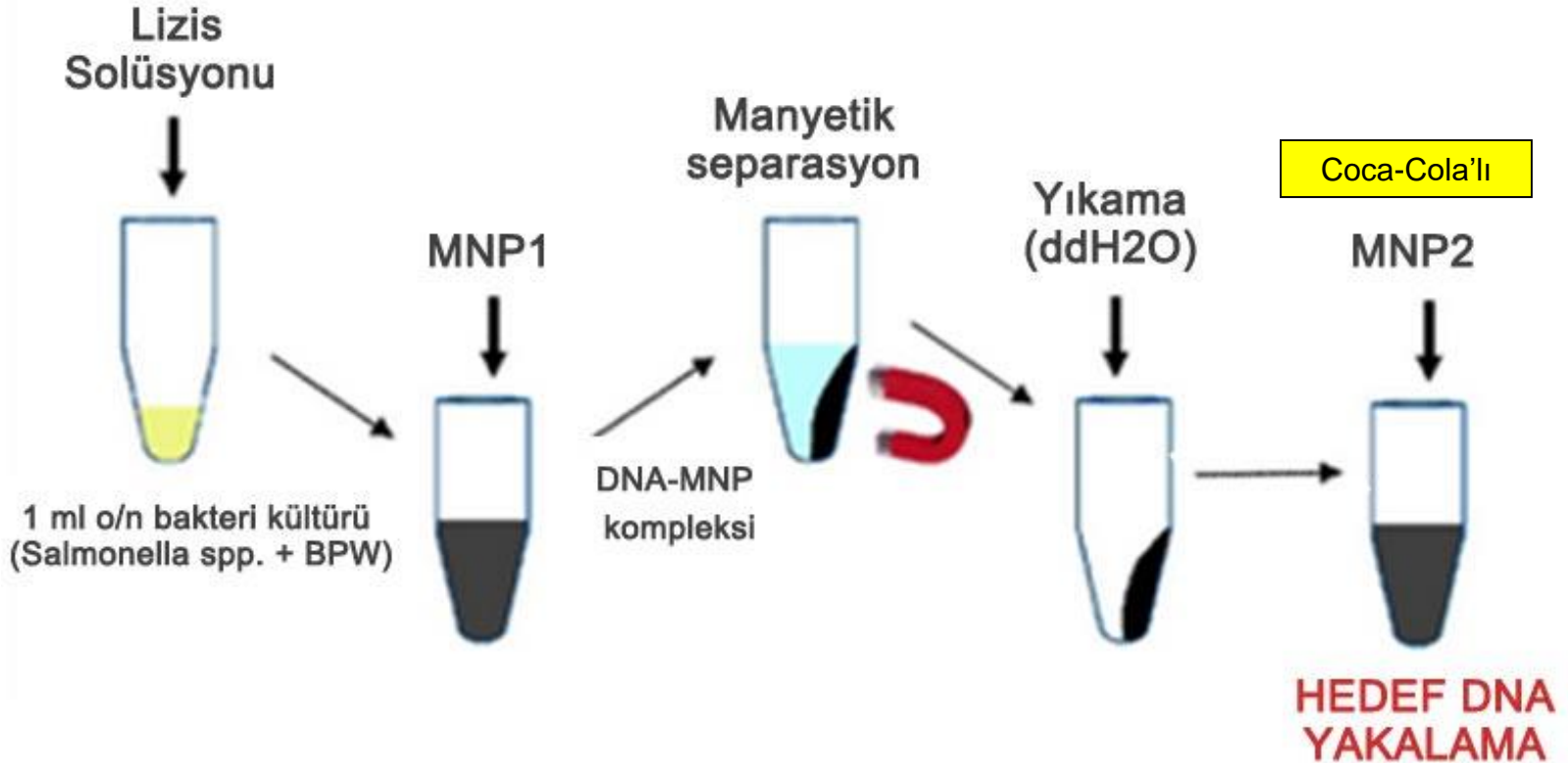
AGCTAGCTAC **GTAGCTCGTAG**



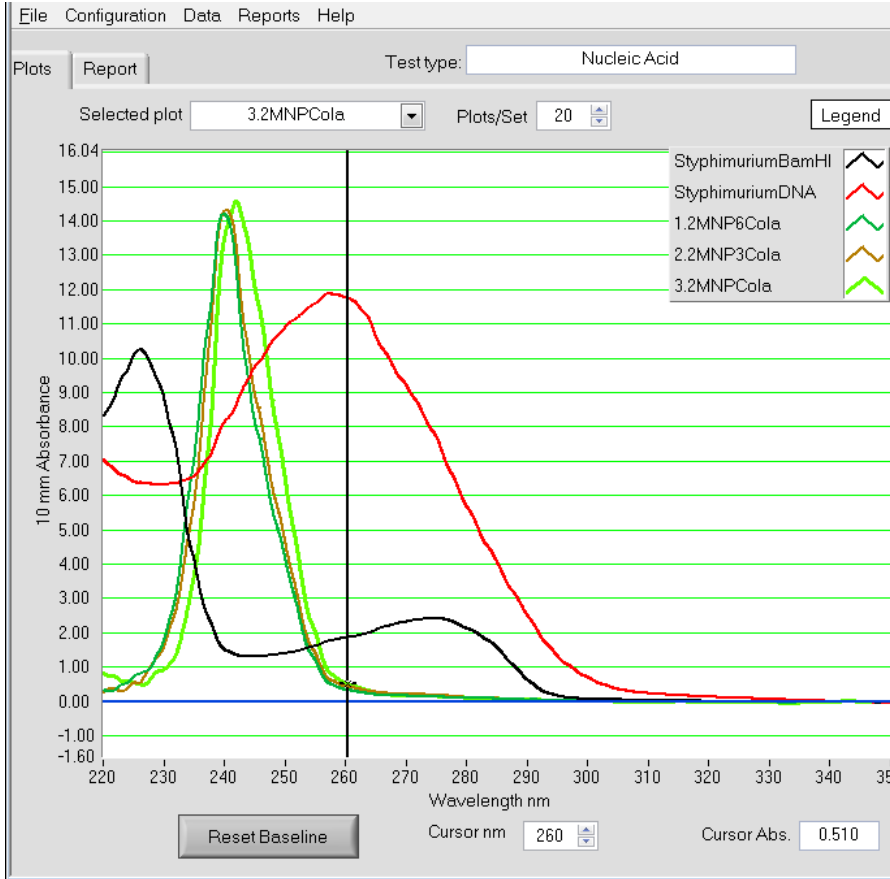
Sentromer DNA Teknolojileri

SKY-FOM009v001 14.12.2016 - 6/16

MNP İLE HEDEF YAKALAMA



MALZEME VE YÖNTEM



1.2MNP-6Cola **11 ng/ul**

2.2MNP-3Cola **14 ng/ul**

3.2MNP-Cola **17 ng/ul**

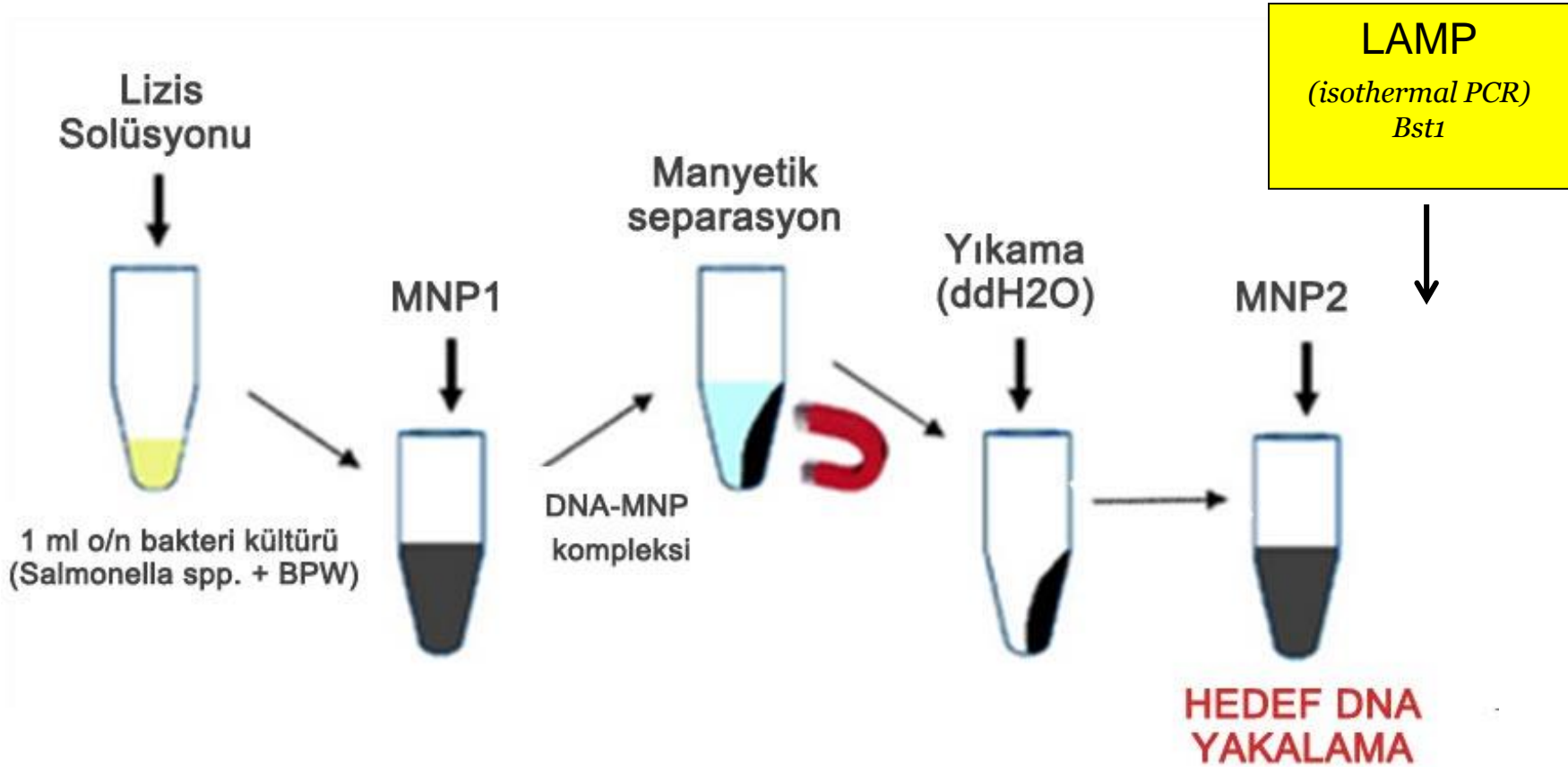
qPCR'da saptanamadı



Sentromer DNA Teknolojileri

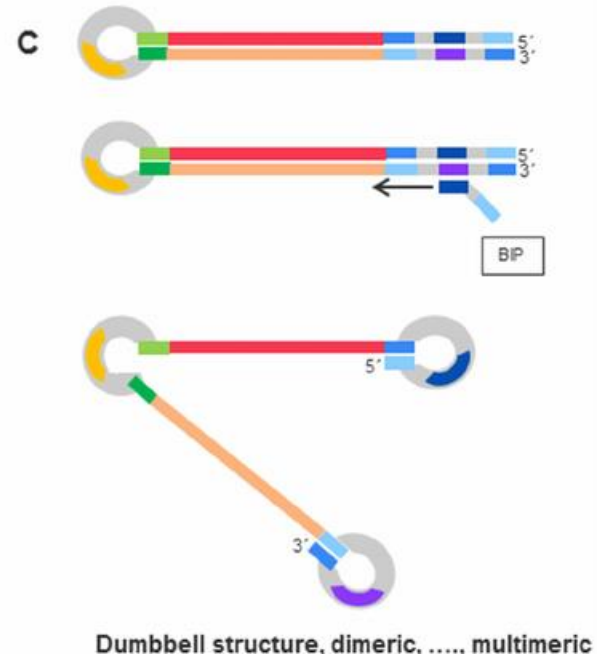
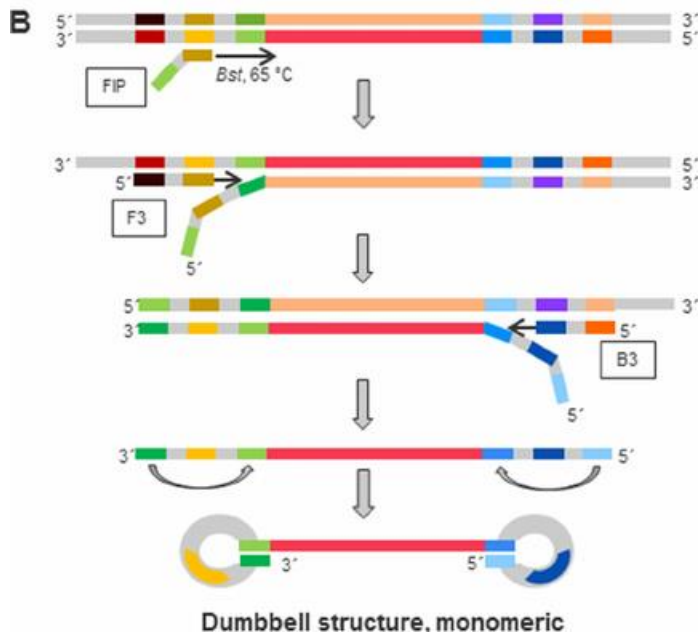
SKY-FOM009v001 14.12.2016 - 8/16

MNP İLE HEDEF YAKALAMA



MALZEME VE YÖNTEM

LAMP
(isothermal PCR)
Bst



Notomi et al. 2000



Sentromer DNA Teknolojileri

SKY-FOM009v001 14.12.2016 - 10/16

LAMP Designer - C:\PROGRA~1\LAMPDE~1.01\DemoProject\DemoProject.lpp

File Edit View Analyze Tools Online Help

3/10 GAC C TG

Sequence Information Search Status

#	Definition	Length
Z37159	Trypanosoma brucei mRNA for VSG-like pro...	1398
AY579418	Plasmodium vivax strain belem 18S ribosom...	960
U03079	Plasmodium vivax Sal 1 blood stage small s...	2064
AY327557	Plasmodium knowlesi strain Nuri A-type sm...	1622
AF145336	Plasmodium malariae small subunit ribosom...	1114
AB182489	Plasmodium ovale gene for small subunit ri...	2104

```

201 AGCCCTCAA GAAGGTTTGC AAAGTACAAA AAAACTTAGC AGACGTCGCA
251 GGAATCGCTT TGGCCAAAAT AAACAACCTG ATAAAACAAG TATCGGCAGC
301 AACCGAAGCG GAAGCAAGAA TGACCTTGGC CGCCGCAAGC ACAGACCACA
351 GCAACATCTC AGCGCTTTAT GCCGGCGCGT CAAACATAGT GACAAGATGC
401 GTACTCAACG CAGTCCACGC TTTACAAGT CTTGCGCCAA TAGCGTTAAC
451 TGCAGCGACC AACGGAGCCA AAACCAGTGG GCACATCTCA GAAGTAATCG
501 ACATTTCTGCA GARGCGTCA CAAGGTAAGA CAGAAGGAAA GTGCATAGTG
  
```


LAMP Primer Properties BLAST Information

Accession Number: Z37159 All LAMP Primers...

Status:

	Sequence	Position	Length bp	Tm °C	GC %	Rating	Hairpin ΔG kcal/mol	Self Dimer ΔG kcal/mol	Run Length bp	GC Clamp	TaOpt °C	Concentration nmol/OD
F3	GTC AAGAAGGTTTGCAAAGTAG	206	22	60.1	40.9	83.3	0	-2.4	3	1		3.8
B3	CTTACCTTGTGACGCCTG	529	18	60.1	55.6	93.1	0	0	2	3		5.6
FIP(F1c+F2)	TGTTGCTGTGGTCTGTGCTT		38									
BIP(B1c+B2)	TACTCAACGCAGTCCACGC		37									
LoopF	CATTCTTGCTCCGCTTCG	322	19	61.9	52.6	93.1						
LoopB	CTTACAAGTCTTGCGCCAATAG	422	22	61.9	45.5	88.1						
F2	ACAAGTATCGGCAGCAAC	286	18	60.1	50	93.1						
F1c	TGTTGCTGTGGTCTGTGCTT	356	20	65	50	93.1						
B2	GTTGGTCGCTGCAGTTAA	463	18	60.3	50	87.1						
B1c	TACTCAACGCAGTCCACGC	402	19	65	57.9	93.1						
Product			178	74.5	53.9	91.1						
Set						90.1						

Ready



LAMP Designer 1.14

Build 114003

PREMIER Biosoft

MALZEME VE YÖNTEM

www.sentromerdna.com/siparis/yonetici/detail.php?id=50713A242



LAMP
primerleri

OLİGONÜKLEOTİT SENTEZ RAPORU

Sentromer DNA Teknolojileri		Sipariş No: 50713A242										Sipariş Tarihi: 13.07.2015		
Müşteri Adı: Sentromer Sentromer, Lab														
Oligo No	Oligo Adı	5' Mod.	Baz Dizisi 5'-3'	3' Mod	Baz Sayısı	Saf.	Skala	MW (g/mol)	Tm(°C)	GC(%)	OD 260nm	nmol	100µM Stok (H2O/TE)µl	Not
50713A242-1	SalF3		CCA ACA GCC GAA GCA TT		17	STD	200 nmol	5148	52.8	52.9	11.37	59.8	598	
50713A242-2	SalB3		GAC TCA CCA GGA GAT TAC AAC		21	STD	200 nmol	6408	57.9	47.6	11.93	49.3	493	
50713A242-3	SalFIP_F1cF2		GCG CAA TTG ATG GCC TGT TAA CGG AAT TAA CGC ATT GCC		39	STD	200 nmol	12007	72.6	48.7	12.36	28.7	287	
50713A242-4	SalBIP_B1cB2		CCA CAT CAC GGT AGC TCA GAC ATT TAA CCC GTC GTC AGT G		40	STD	200 nmol	12201	74.6	52.5	12.18	28.5	285	
50713A242-5	SalLoopF		ACG TCC GGC AAG GTA AC		17	STD	200 nmol	5204	55.2	58.8	11.75	61.5	615	
50713A242-6	SalLoopB		AGT GAC CAT CCC ACC GA		17	STD	200 nmol	5124	55.2	58.8	13.38	73.4	734	
50713A242-7	SalF2		CGG AAT TAA CGC ATT GCC		18	STD	200 nmol	5484	53.7	50.0	11.70	59.1	591	
50713A242-8	SalF1c		GCG CAA TTG ATG GCC TGT TAA		21	STD	200 nmol	6461	57.9	47.6	14.47	62.2	622	
50713A242-9	SalB2		ATT TAA CCC GTC GTC AGT G		19	STD	200 nmol	5779	54.5	47.4	12.05	59.9	599	
50713A242-10	SalB1c		CCA CAT CAC GGT AGC TCA GAC		21	STD	200 nmol	6360	61.8	57.1	11.94	52.8	528	

Çalışmalarınızda başarılar dileriz!

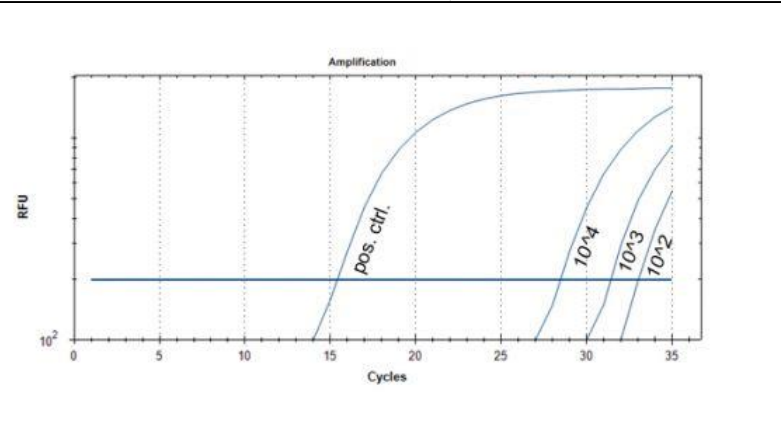
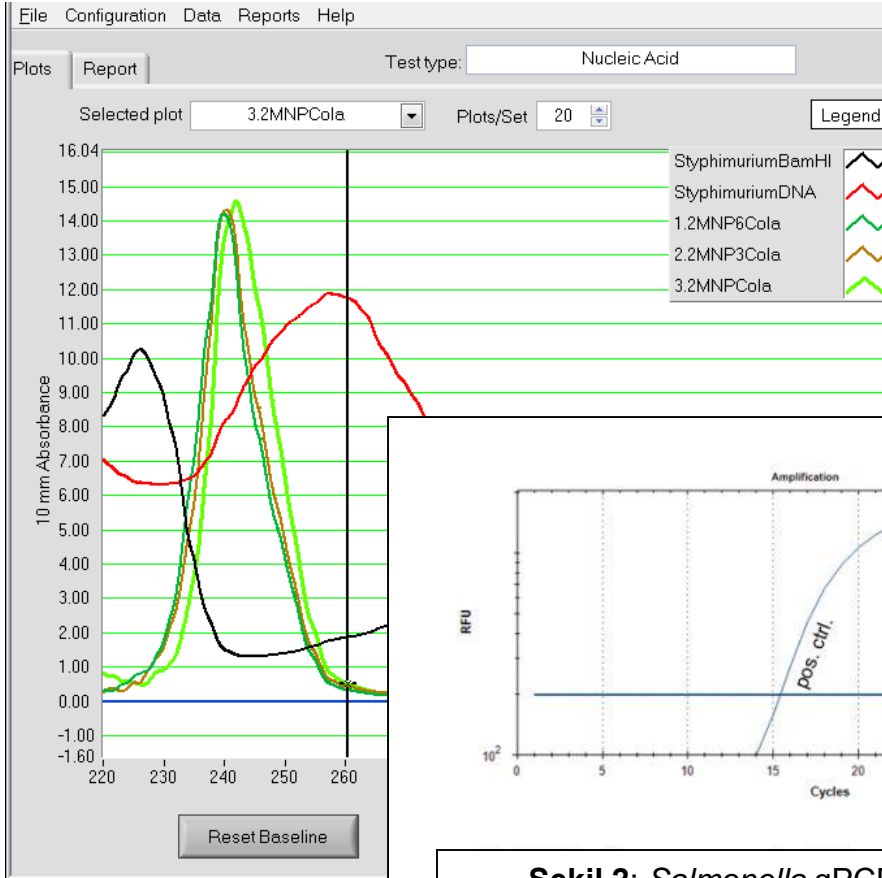
SENTROMER DNA TEKNOLOJİLERİ LTD. ŞTİ.

Sarıyer Cad. ABC Yolu ABC Plaza Kat 5 No.12 İSTİNYE 34460 İSTANBUL
T(212) 286 21 35 F(212) 286 21 34 W. www.sentromer.com E. dna@sentromer.com

MALZEME VE YÖNTEM

1.2MNP-6Cola	11 ng/ul
2.2MNP-3Cola	14 ng/ul
3.2MNP-Cola	17 ng/ul

LAMP aşaması
eklendiğinde
>100 kat artış kaydedildi

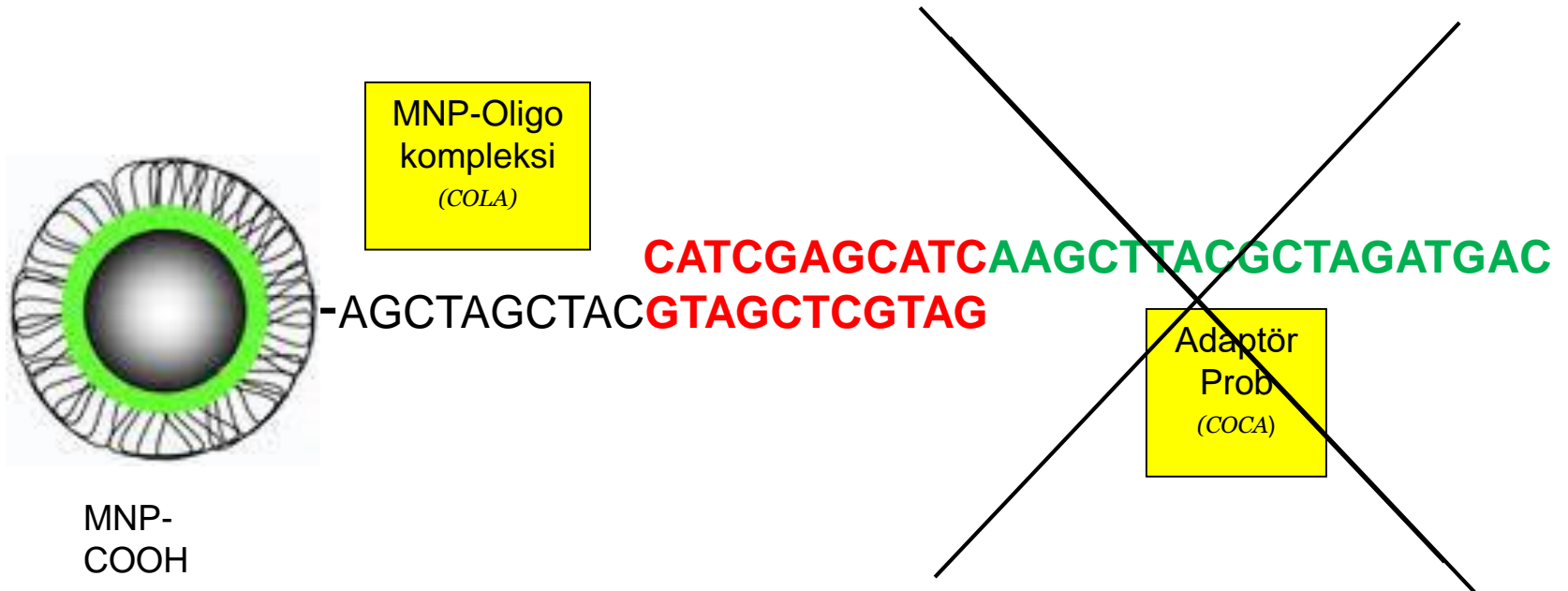


Şekil 2: Salmonella qPCR doğrulaması

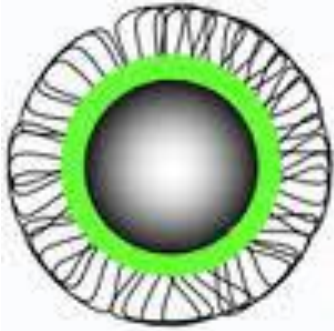


Sentromer DNA Teknolojileri

MALZEME VE YÖNTEM



MALZEME VE YÖNTEM



MNP-COOH

-AGCTAGCTACAAGCTTACGCTAGATGACGGTGAGACC...

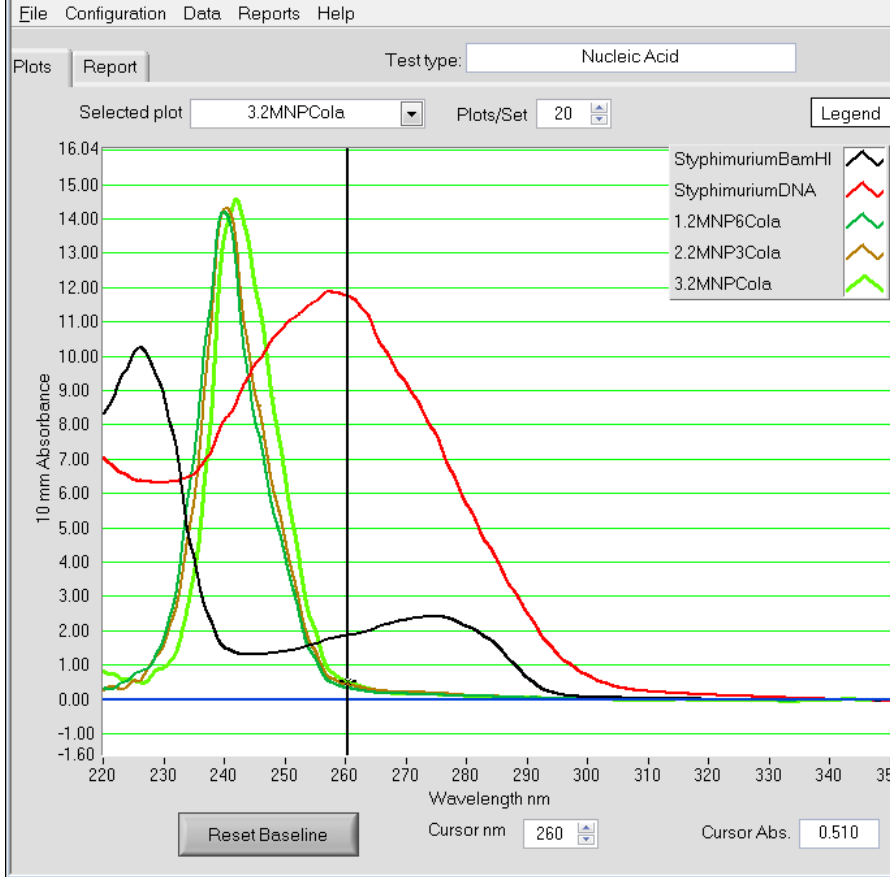
Hedefe özgü oligonükleotid bağlı MNP



Sentromer DNA Teknolojileri

SKY-FOM009v001 14.12.2016 - 15/16

MALZEME VE YÖNTEM



1.2MNP-6Cola 11 ng/ul

2.2MNP-3Cola 14 ng/ul

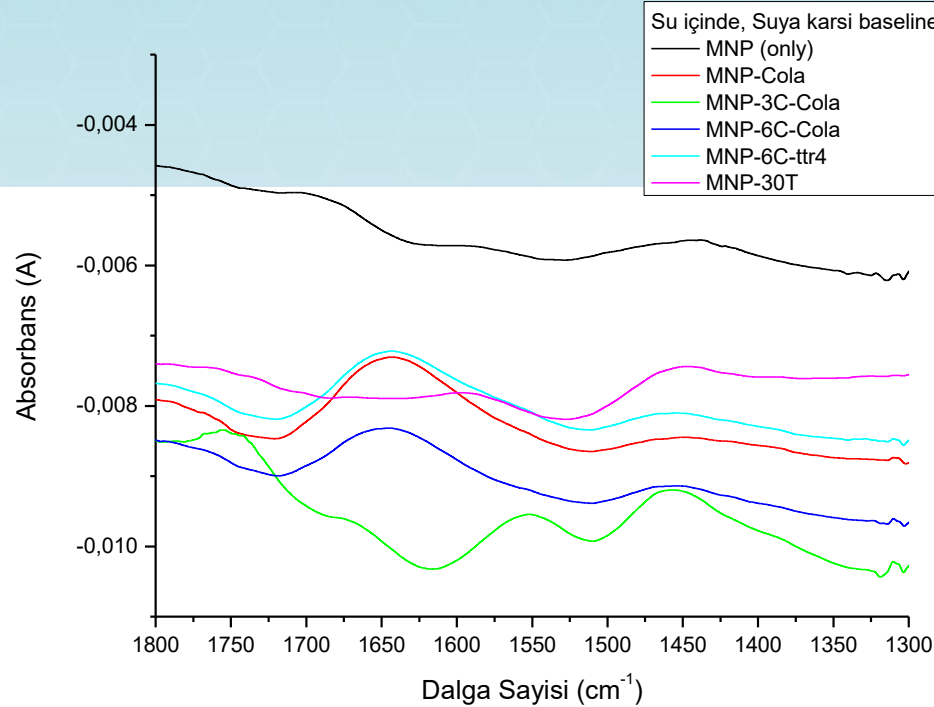
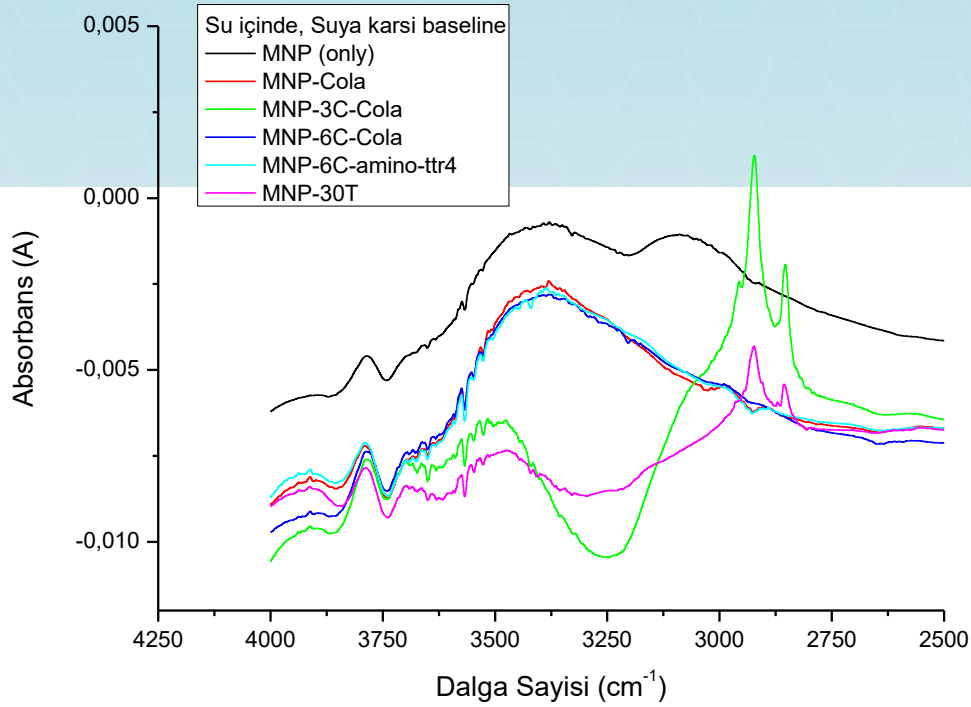
3.2MNP-Cola 17 ng/ul

Doğrudan MNP bağlı
hedef DNA ile yapılan
deneyde
10 kat artış kaydedildi



Sentromer DNA Teknolojileri

SKY-FOM009v001 14.12.2016 - 16/16



IR Absorbansları

3428 cm ⁻¹	O-H gerilme titreşimi
1680 cm ⁻¹	O-H eğilme titreşimi
1481 cm ⁻¹	O=C=O gerilme titreşimi
2963-2669 cm ⁻¹	C-H gerilme titreşimi

IR Absorbansları

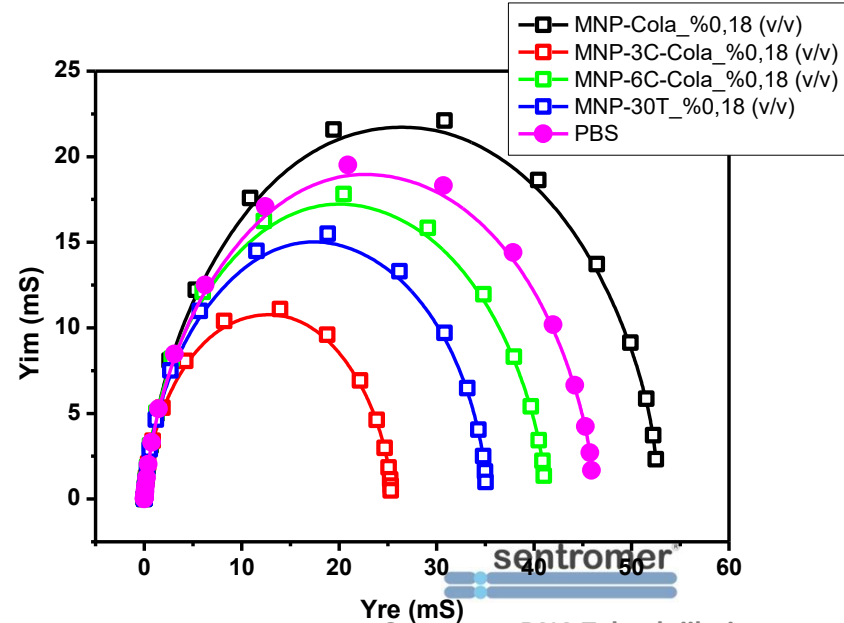
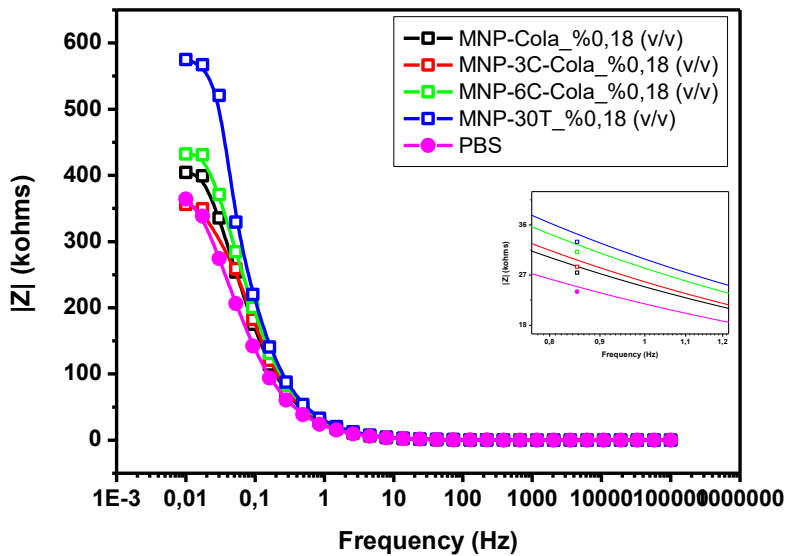
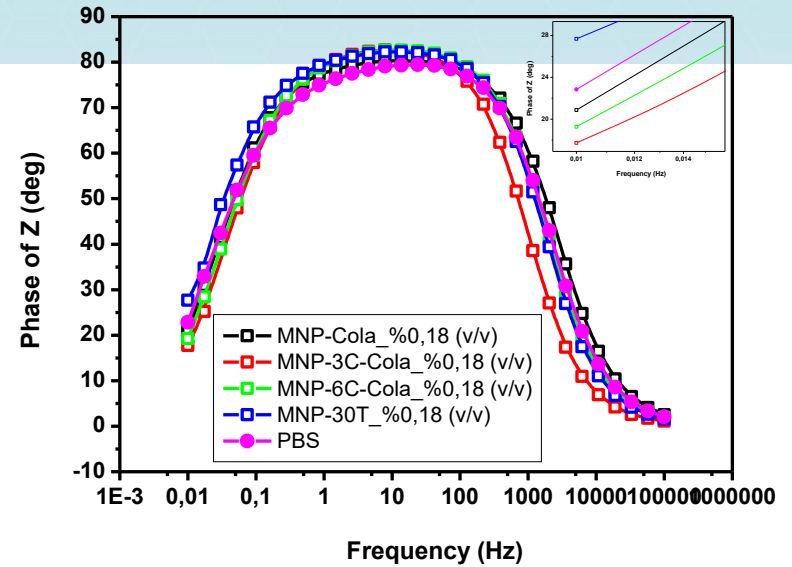
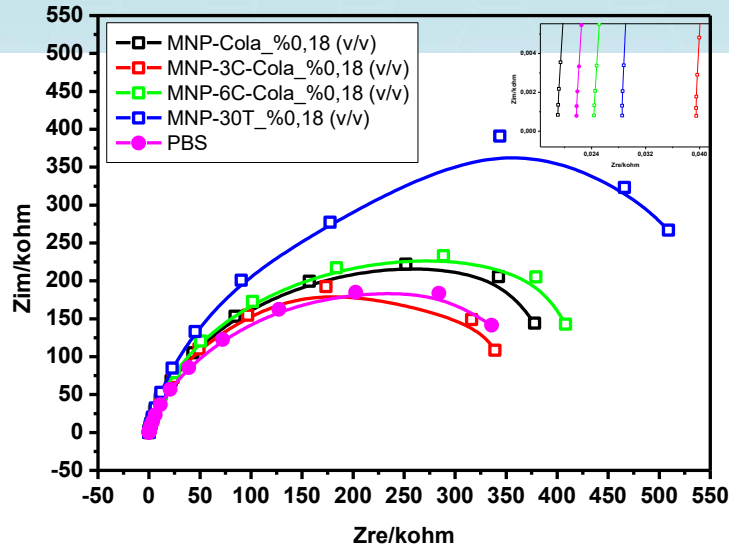
Guanin	1710 cm ⁻¹ 1527 cm ⁻¹	C-O gerilme titreşimi Molekül düzlem titreşimi
Timin	1663 cm ⁻¹	C=O gerilme titreşimi
Adenin	1610 cm ⁻¹	C=N gerilme titreşimi
Sitozin	1491 cm ⁻¹ 1527 cm ⁻¹	Molekül düzlem titreşimi



Sentromer DNA Teknolojileri

SKY-FOM009v001 14.12.2016 - 17/16

Aynı Yüzde Seyreltmedeki Oligo Bağlı MNP'lerin Empedans Ölçümleri

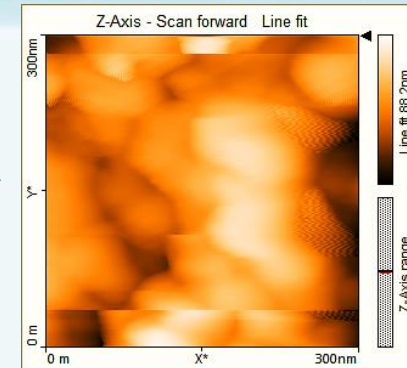
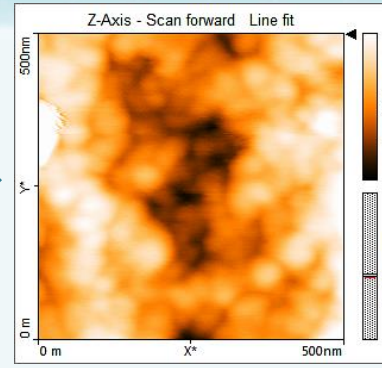
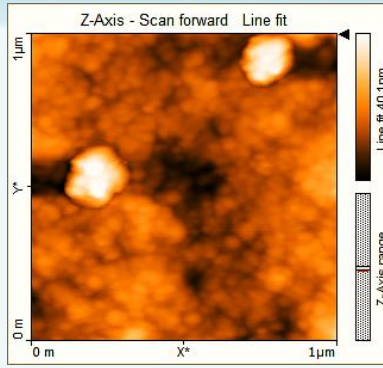


1 μm

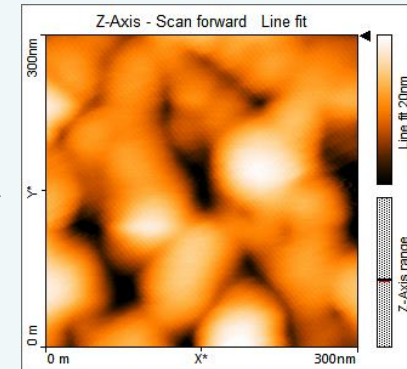
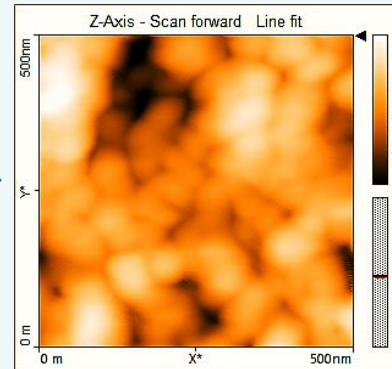
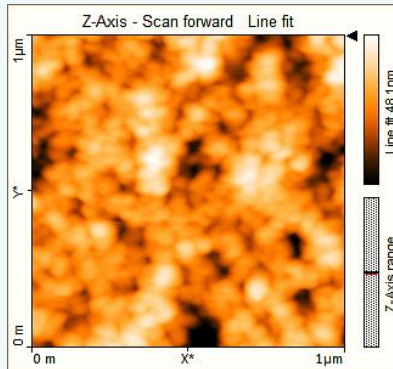
0,5 μm

0,3 μm

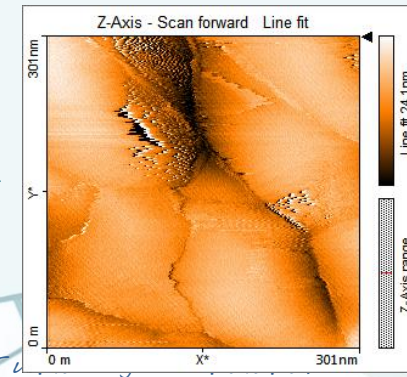
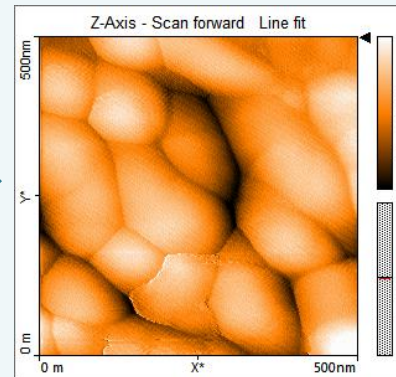
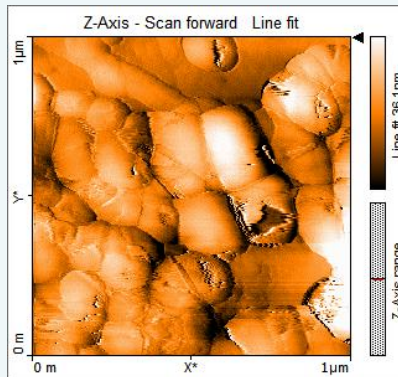
MNP (only)



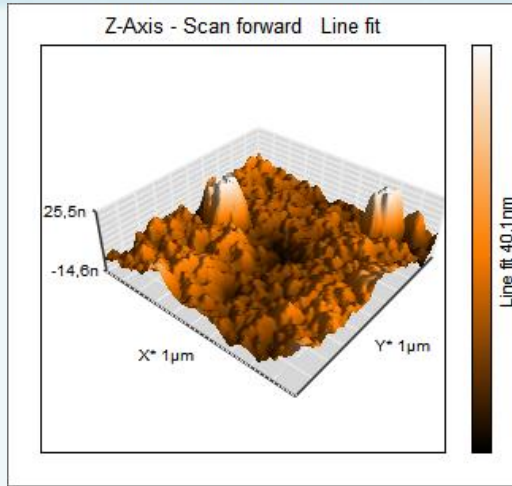
MNP-3C-Cola



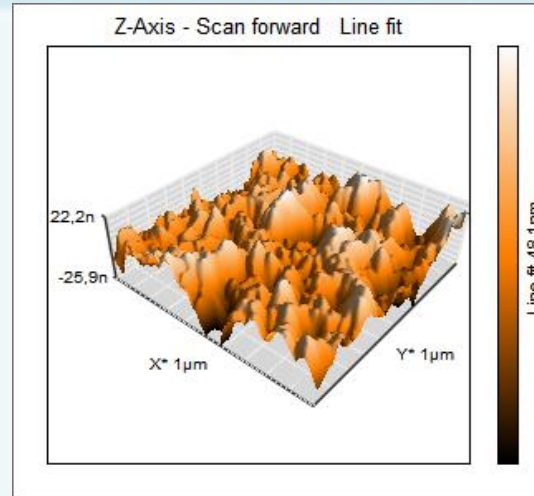
MNP-6C-Cola



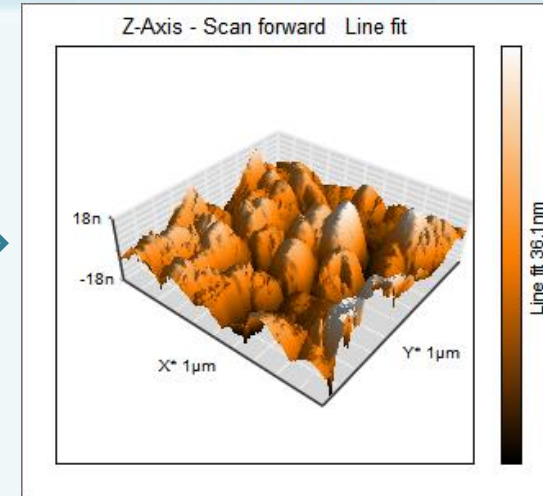
1 μm



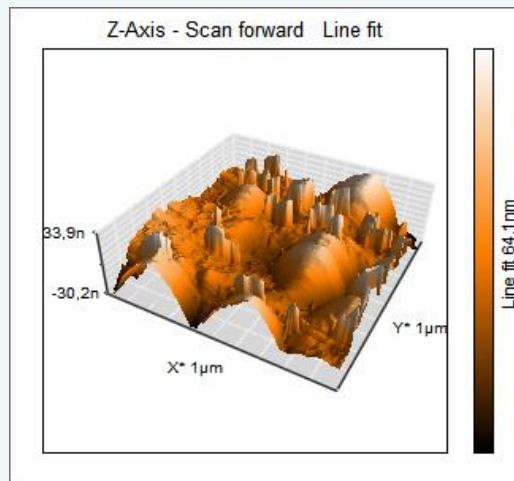
MNP (only)



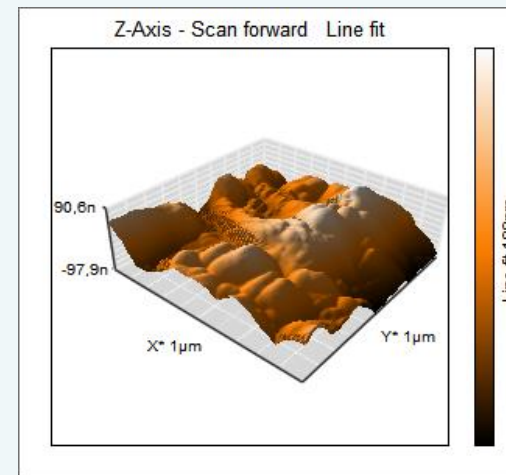
MNP-3C-Cola



MNP-6C-Cola

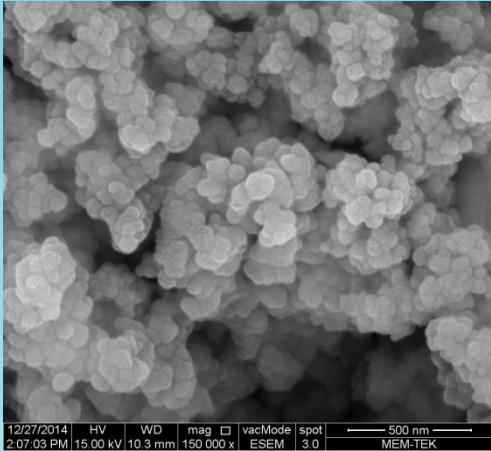


MNP-30T

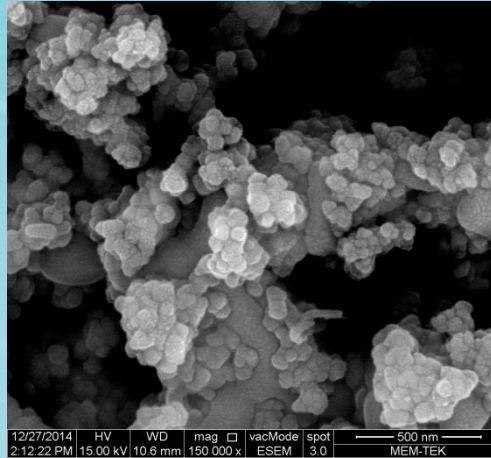


MNP-6C-ttr4

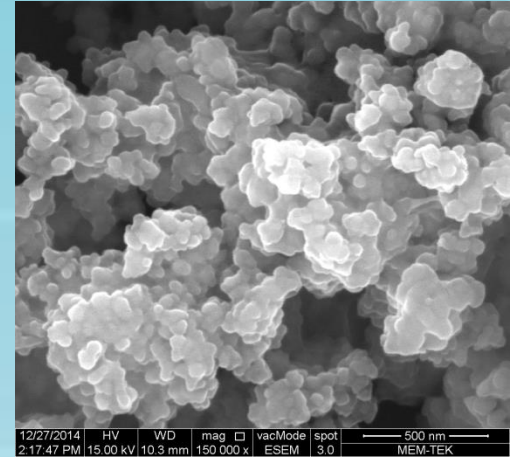
Tutku genetikdir.
Sentromer DNA Teknolojileri



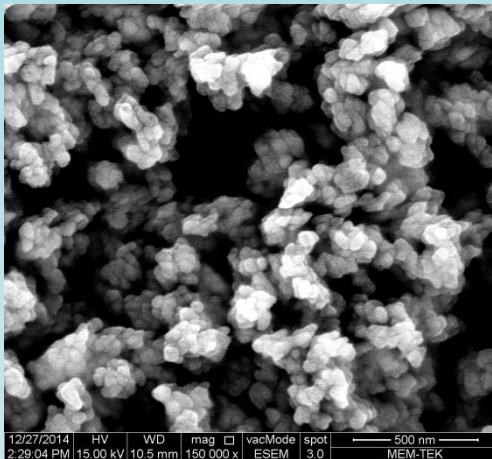
MNP (only)



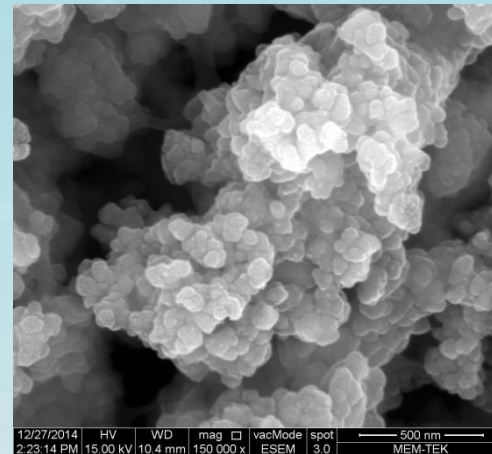
MNP-Cola



MNP-3C-Cola

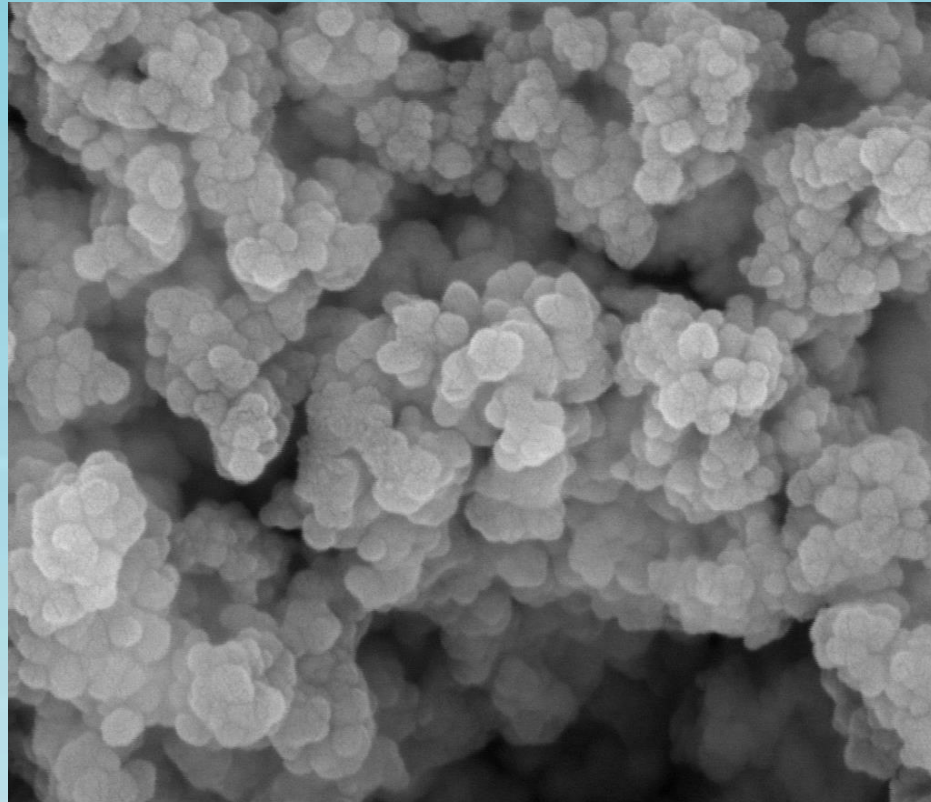


MNP-30T



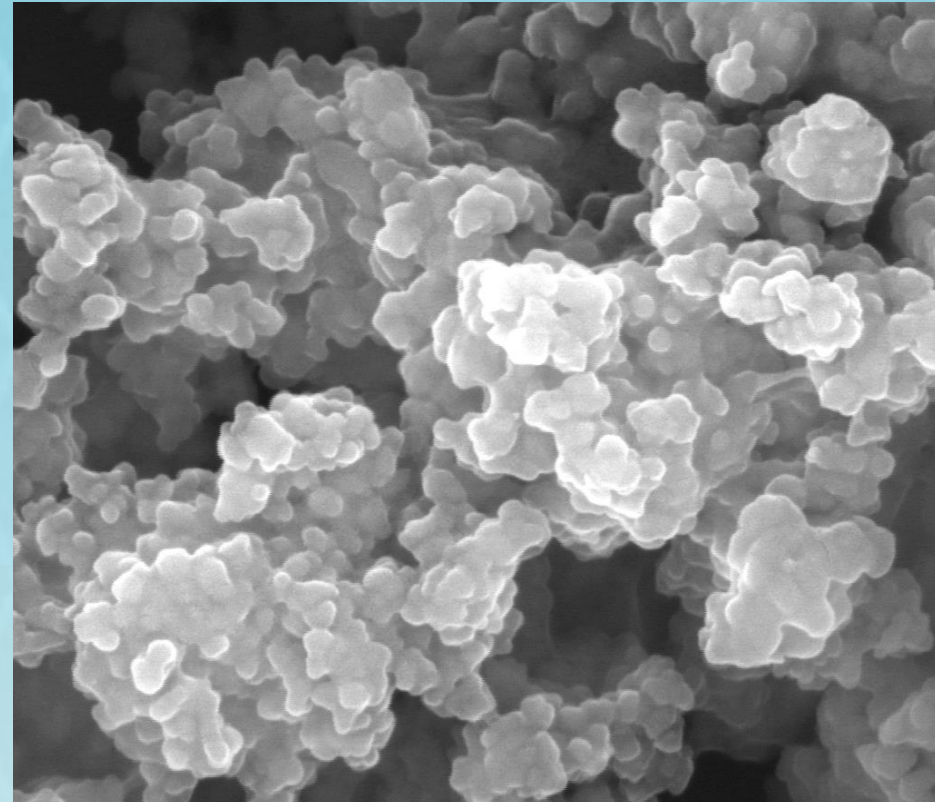
MNP-6C-Cola

SEM Görüntüleri_500nm ölçekte



12/27/2014 HV WD mag vacMode spot
2:07:03 PM 15.00 kV 10.3 mm 150 000 x ESEM 3.0
500 nm
MEM-TEK

MNP (only)



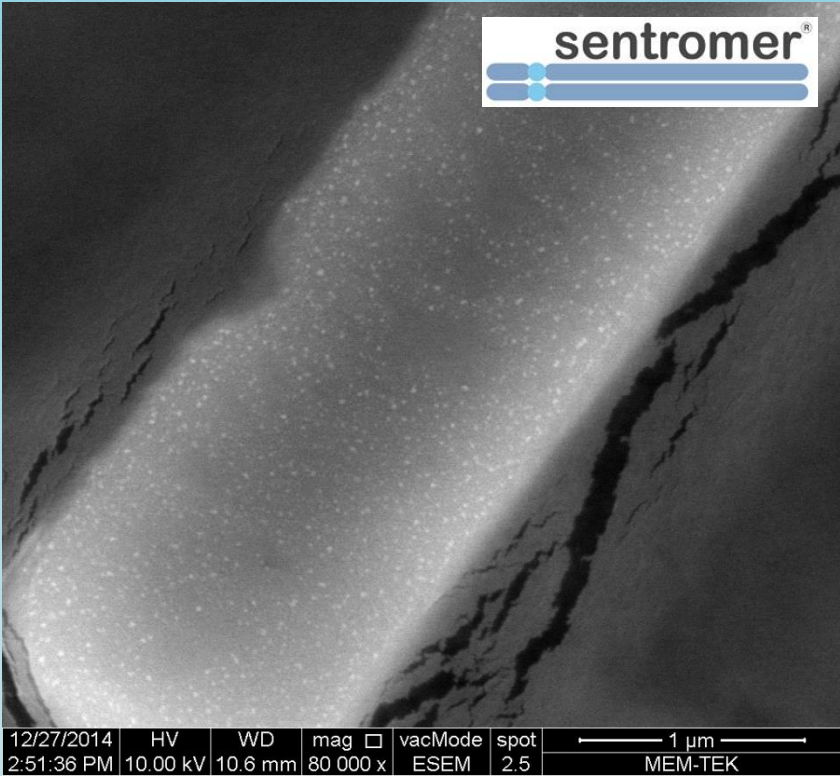
12/27/2014 HV WD mag vacMode spot
2:17:47 PM 15.00 kV 10.3 mm 150 000 x ESEM 3.0
500 nm
MEM-TEK

MNP-3C-Cola

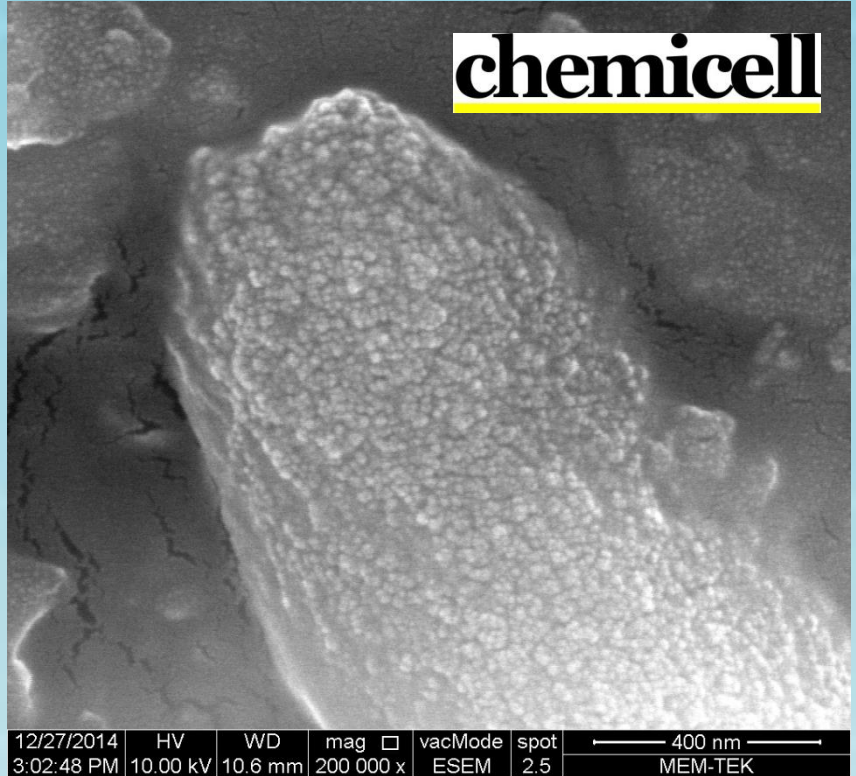


Biz sentezledik

Ticari, hazır alındı



ttr4 primeri



dT20mer primeri

SONUÇLAR/TARTIŞMA

- ✓ Çalışmada iki tür manyetik nanopartikülün kombine kullanımı ve LAMP aşamasıyla seçici ve hassas saptama yapılabileceği gösterilmiştir.
- ✓ Adaptör oligosuz doğrudan hedefe özgü MNP ile daha yüksek DNA eldesi gözlemlenmiştir.
- ✓ Amino modifiye oligoların MNP'ye bağlanması iyileştirilirse daha iyi sonuç alınabilir.

SentroPure™

DNA Ekstraksiyon ve Saflaştırma



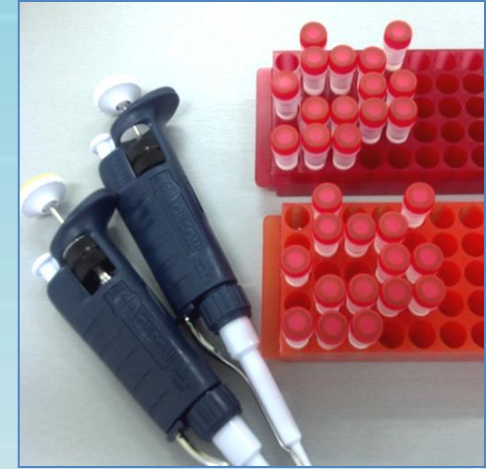
SentroPlex™

Real-time PCR



SentroPrime™

Oligo Sentezleme PCR ve Dizileme Primerleri



SENTROMER DNA TEKNOLOJİLERİ

PROFİL / ÜRÜNLER / HİZMETLER / İLETİŞİM



Sentromer DNA Teknolojileri, sipariş üzerine her türlü moleküler biyoloji çalışmalarında, tanı ve tedavide kullanılabilir kalitede sentetik oligonükleotit üretir ve zamanında teslim etmeyi taahhüt eder.

Kimyasal oligonükleotit sentezi, fosforamidit yöntemi takip edilerek rutin 0.2 umol ölçeğinde yapılır. Sentezlenen oligonükleotitler saflaştırılarak sentez kimyasallarından arındırılır ve liofilize olarak teslim edilir. Her sipariş eşliğinde gönderilen oligonükleotit raporu sentezlenen oligoların baz dizileri, moleküler ağırlıkları, miktar, OD ve sulandırma bilgilerini içerir.



SENSIS - SENTROMER ELEKTRONİK SİPARİŞ SİSTEMİ

Kullanıcı Adı

Parola

Sifremi unuttum / Yeni kullanıcı

GİRİŞ

OLİGO SAYISI	BAZ FİYATI	KARGO
1-6	0.65 EUR	7 TL
7-12	0.60 EUR	-
13-24	0.55 EUR	-
25-48	0.50 EUR	-

Oligo sayısı: Tek siparişte sentezletilen primer sayısı.

Baz fiyatı: 50 baza kadar 200 pmol



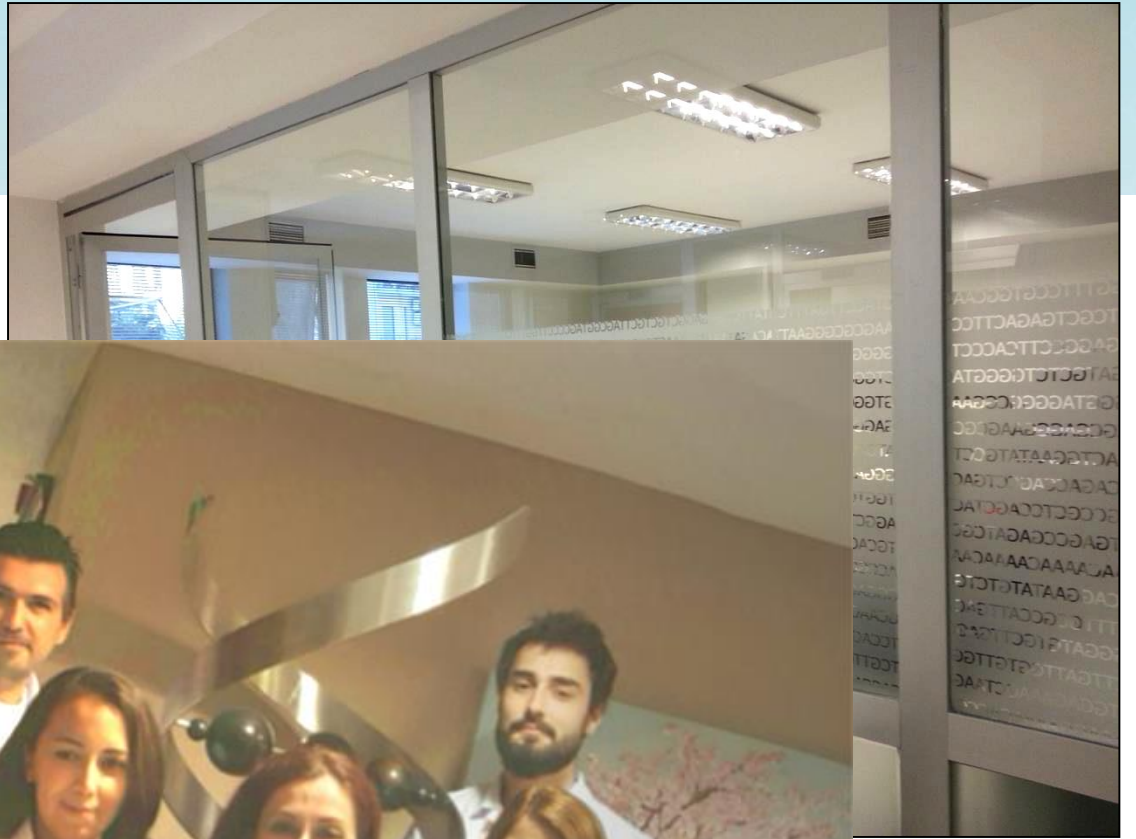
PAZARTESİ VE ÇARŞAMBA GÜNLERİ SAAT 17:00'DEN ÖNCE GİRİLEN SİPARİŞLER TAKİP EDEN GÜN SENTEZLENİR. STANDART OLIGOLARINIZ 3 İŞ GÜNÜNDE TESLİM EDİLİR.



Üretimiz ISO 9001:2008 ISO 13485:2012 kapsamındadır



Teşekkür ederiz!



Proje No: 1130145. TÜBİTAK-TEYDEB 1511 Öncelikli Alanlar Araştırma Teknoloji Geliştirme ve Yenilik Projeleri Programı ile desteklenmiş Sentromer DNA Teknolojileri tarafından yürütülmüştür.



Sentromer DNA Teknolojileri

SKY-FOM009v001 14.12.2016 - 26/16