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# LAMP PRIMER DESIGN FOR MONITORING GENE EXPRESSION OF TUMOR MARKERS IN 3D CANCER CULTURES

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**Introduction:** Cancer is one of the deadliest diseases, and its early detection and timely treatment are extremely important. The use of 3D cultures (spheroids) is the most relevant approach in cancer research because it mimics the *in vivo* environment of cells. Monitoring the tumor marker expression with different diagnostic methods is very important in determining specific therapy. Loop-mediated isothermal amplification (LAMP) is an innovative molecular tool that has a wide application, including the field of cancer diagnostics.

**Aim:** The study aimed to establish 3D cultures of different cancer cell lines, as well as the development and *in silico* analysis of LAMP primers' specificity for selected tumor markers.

**Material and Methods:** The human cell lines used for establishing spheroids are: HDF (dermal fibroblasts-control), U-87 (glioblastoma), Caco-2 (colorectal adenocarcinoma), and A549 (lung cancer). Spheroids were formed by seeding 100.000 cells/well in BIOFLAT™ ultra-low attachment plates. Then, the plates were centrifuged at 10min/1250rpm and incubated for seven days at 37°C and 5% CO<sub>2</sub>. The formation and morphology of the spheroids were monitored by microscopic methods for seven days. LAMP primers were designed for the *GAL3*, *SOX2*, *MMP2*, and *MMP9* tumor markers using the PrimerExplorerV5 software. By inserting the target sequences into the software, primer candidates for the target genes were generated, where their further selection was performed based on the following parameters: 1. the highest ΔG value for dimerization and 2. selection of primers with ΔG ends greater than -4. BioEdit software was used for *in silico* analysis of primer specificity.

**Results:** Successful cultivation, survival, and significant growth of spheroids of all tested cell lines during incubation were noticed by microscopic methods. *In silico* analysis of the designed LAMP primers showed their high specificity.

**Conclusion:** The described results indicate a great possibility of applying sophisticated cellular and molecular biology techniques in diagnostics and monitoring cancer treatment.

**Keywords:** spheroids, LAMP, cancer, tumor marker

# DIZAJN LAMP PRAJMERA ZA PRAĆENJE GENSKE EKSPRESIJE TUMOR MARKERA U 3D KULTURAMA KANCERA

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dr Ivana Gadjanski,  
viši naučni saradnik

**Autori:**

Teodora Knežić  
Mila Djisalov



# UVOD

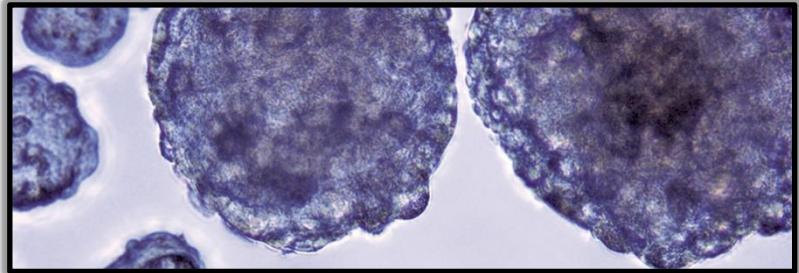
## 3D kulture kancerskih ćelija (sferoidi)

Upotreba tumorskih sferoida predstavlja jedan od najrelevantnijih pristupa u ispitivanjima raka.

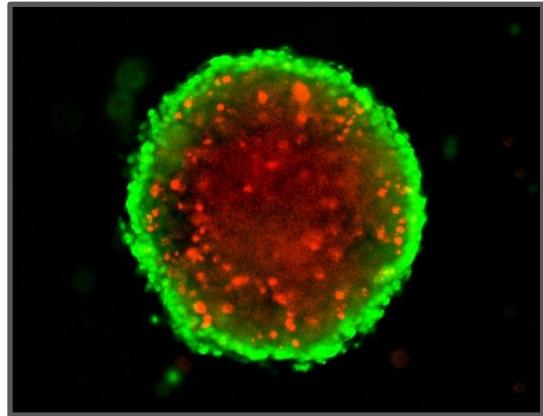
- Imitiraju biologiju *in vivo* „čvrstih“ tumora
- Laki za manipulaciju
- Smanjuju troškove ispitivanja i potrebu za model životinja
- Omogućavaju ponovljivost rezultata
- Lako se integrišu u različite skrining uređaje i snimaju naprednim tehnikama



Slika 1. Metastaze raka (Izvor: [Internet 1](#))



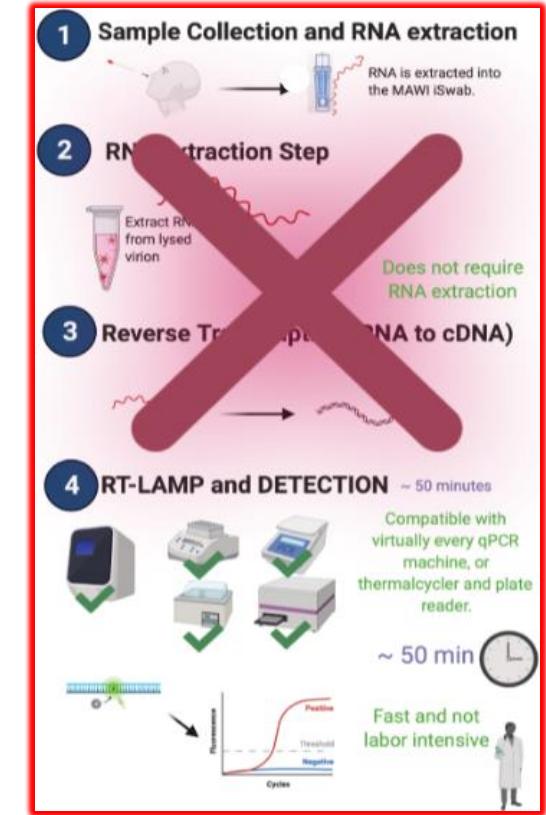
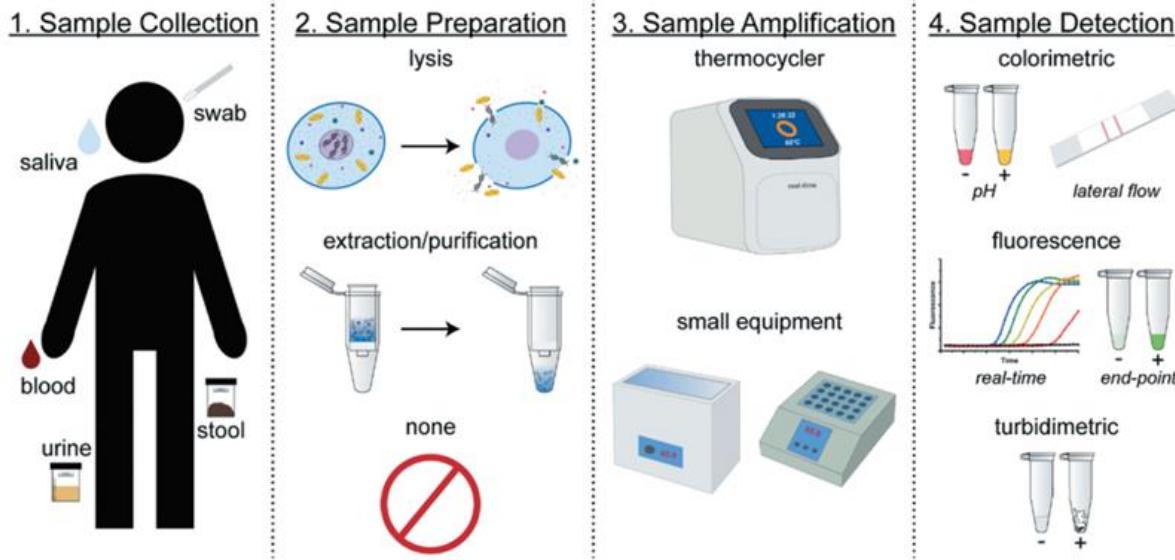
Slika 2. Tumorski sferoidi (Izvor: [Internet 2](#))



Slika 3. Tumorski sferoid – fluorescentna mikroskopija  
(Izvor: [Internet 3](#))

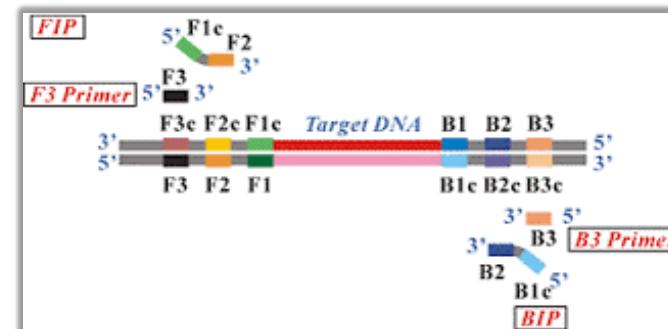
# Petljom-posredovana izotermalna metoda amplifikacije nukleinskih kiselina (LAMP)

## Molecular Diagnostics Based on LAMP

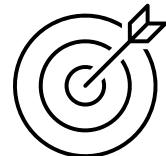


### Prednosti LAMP metode:

- Brza i jednostavna metoda pogodna za praćenje genske ekspresije
- Visoka specifičnost** – koristi se šest prajmera za prepoznavanje osam regiona ciljne sekvene
- Izotermalni uslovi reakcije** – nema potrebe za cikličnim termostatom
- Visoka otpornost na inhibitore reakcije
- Veliki potencijal za integraciju u biosenzore u cilju brze detekcije



## CILJ STUDIJE

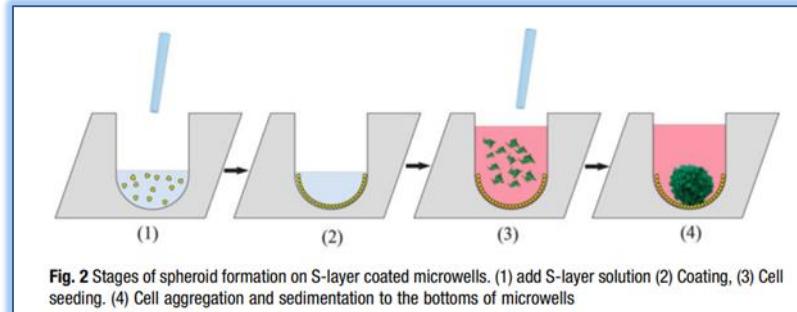


**Uspostavljanje 3D kultura različitih ćelijskih linija kancera, kao i razvoj i *in silico* analiza specifičnosti LAMP prajmera za odabране tumor markere.**

# Uspostavljanje 3D kultura različitih ćelijskih linija kancera



Slika 4. BIOFLAT™ kultivacione ploče sa niskim stepenom prijanjanja



Slika 5. Proces formiranja sferoida u bunarićima sa niskim stepenom prijanjanja

## Koraci:

**Zasejavanje** 100 000 ćelija po bunariću u BIOFLAT™ kultivacione ploče sa niskim stepenom prijanjanja



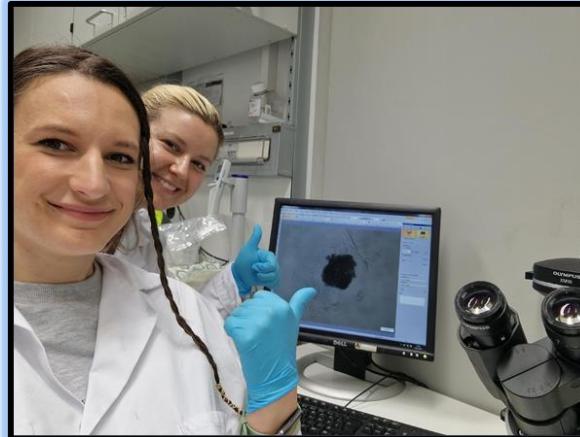
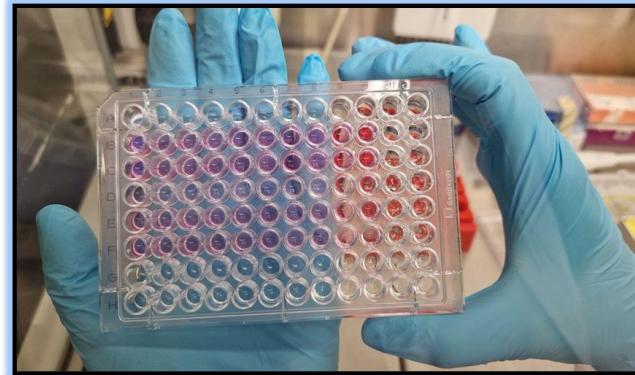
**Centrifugiranje** ploča 10 minuta na 1250 rpm



**Inkubacija** tokom sedam dana na 37 °C i 5% CO<sub>2</sub>



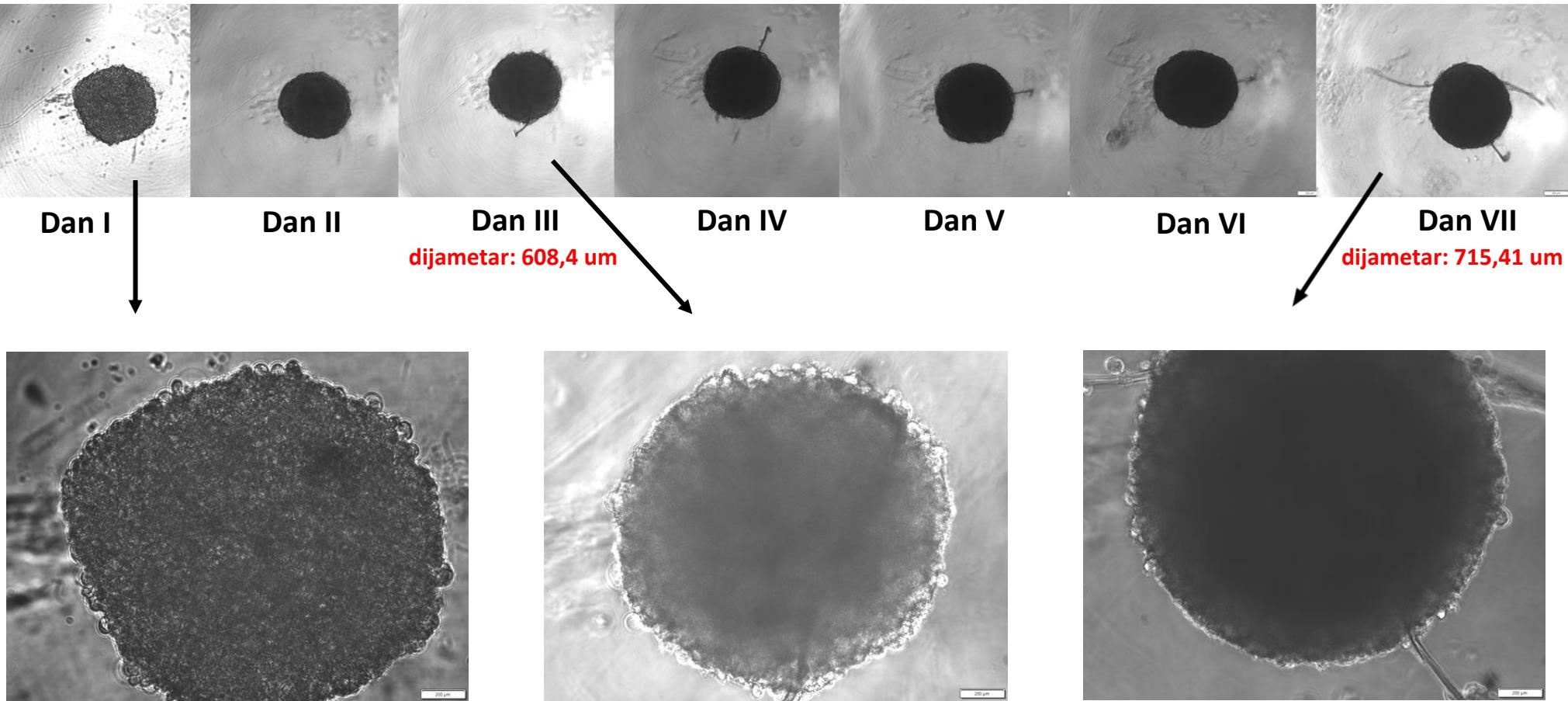
**Praćenje** formiranja i morfologije sferoida tokom sedam dana mikroskopskim metodama



# Praćenje formiranja sferoida mikroskopskim metodama

## U-87 ćelije

Uvećanje 4X



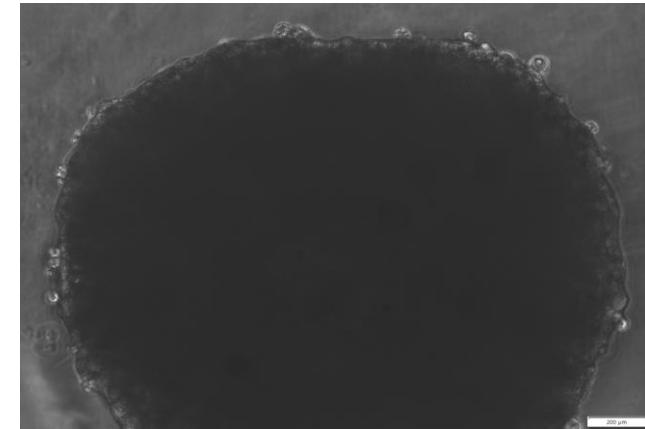
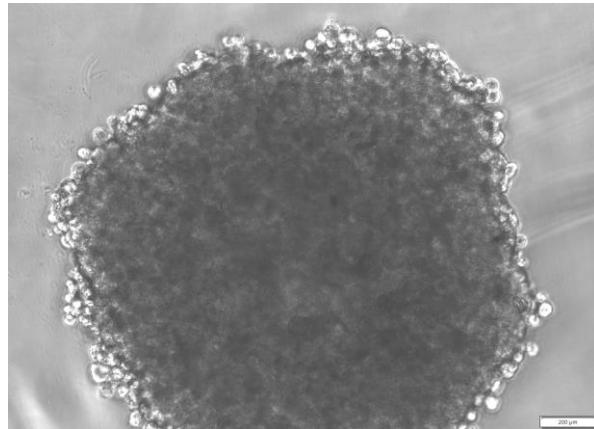
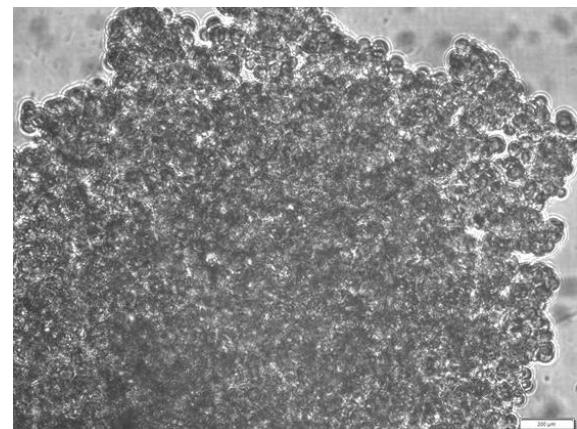
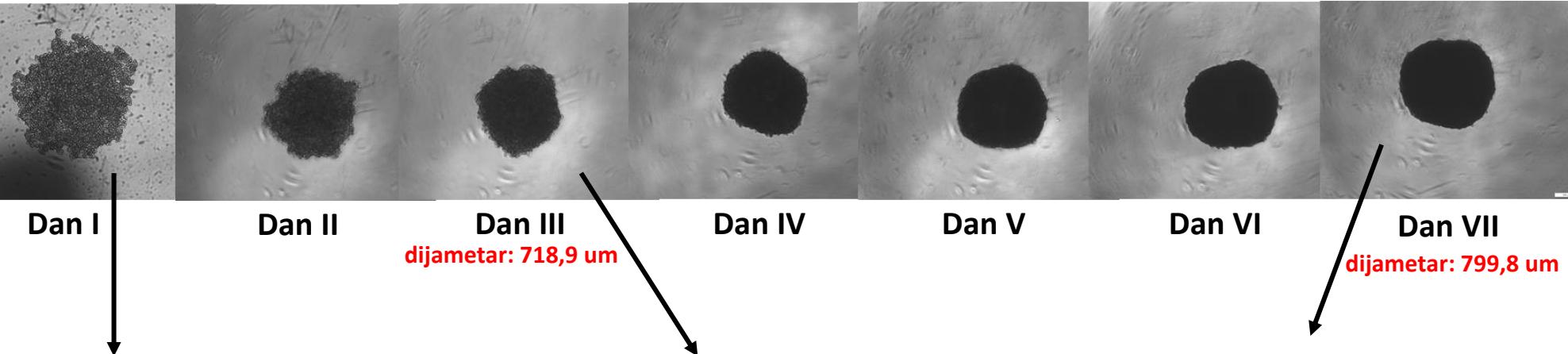
Uvećanje 10X

Primećeno je **povećanje** dijametra sferoida tokom sedmodnevne kultivacije, kao i formiranje nekrotičnog jezgra nakon tri dana.

# Praćenje formiranja sferoida mikroskopskim metodama

## A549 ćelije

Uvećanje 4X



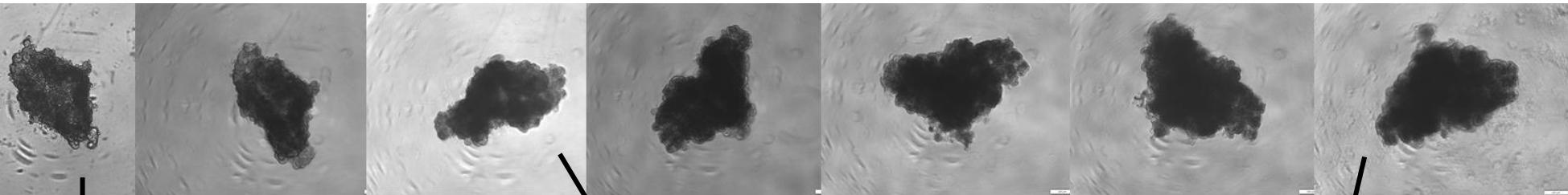
Uvećanje 10X

Primećeno je **povećanje** dijametra sferoida tokom sedmodnevne kultivacije, kao i formiranje nekrotičnog jezgra nakon tri dana.

# Praćenje formiranja sferoida mikroskopskim metodama

## Caco-2 ćelije

Uvećanje 4X



Dan I

Dan II

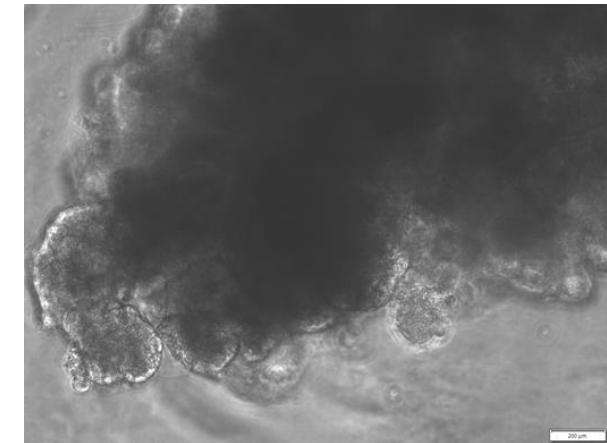
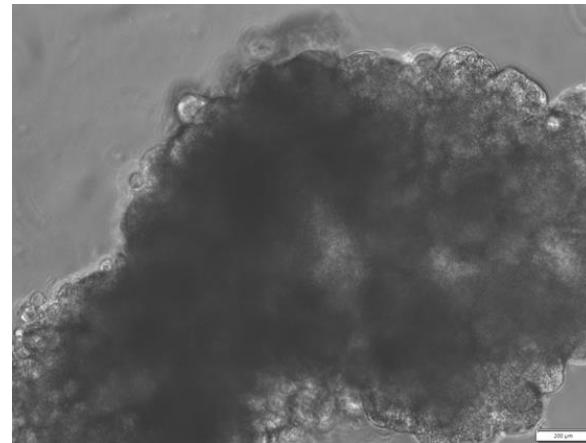
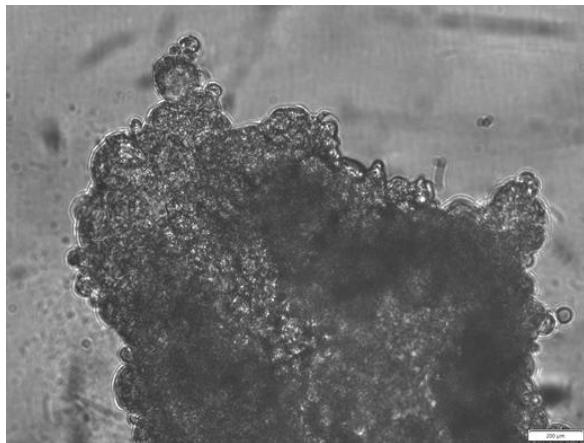
Dan III  
dijametar: 787 um

Dan IV

Dan V

Dan VI

Dan VII  
dijametar: 1330 um



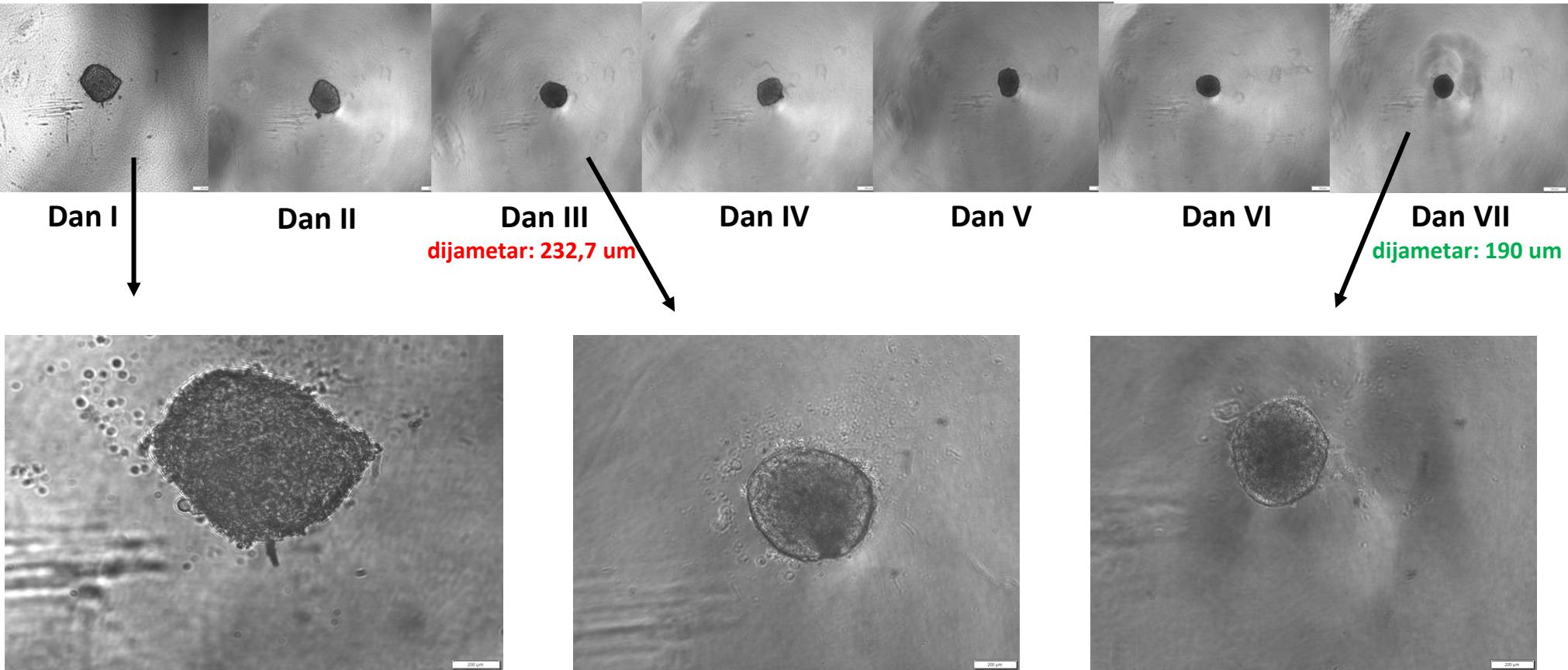
Uvećanje 10X

Primećeno je **povećanje** dijametra sferoida tokom sedmodnevne kultivacije, kao i formiranje nekrotičnog jezgra već nakon jednog dana.

# Praćenje formiranja sferoida mikroskopskim metodama

## HDF ćelije (kontrolna linija)

Uvećanje 4X



Uvećanje 10X

Primećeno je **smanjenje** dijametra sferoida tokom sedmodnevne kultivacije, kao i formiranje nekrotičnog jezgra nakon tri dana.

# Dizajn LAMP prajmera za odabrane tumor markere

| Ciljni gen  | Detalji   |
|---|---|
| Galektin-3 ( <i>Gal-3</i> )                                 | <p><b>Uključen u:</b></p> <ul style="list-style-type: none"><li>• Rast i progresiju tumora</li><li>• Anti-apoptozu</li><li>• Adheziju, angiogenezu</li></ul> <p><b>Dokazana je njegova prekomerna ekspresija u raznim tumorima.</b></p> |
| Sex-determining region Y-related HMG box 2 ( <i>SOX-2</i> ) | <ul style="list-style-type: none"><li>• Onkogen</li></ul> <p><b>Prekomerna ekspresija SOX2 je povezana sa malom stopom preživljavanja obolelih od raka.</b></p>   |
| Matriks metaloproteinaza 2 ( <i>MMP-2</i> )                 | <ul style="list-style-type: none"><li>• MMP2 promoviše rast tumora degradiranjem matriksa i promovisanjem angiogeneze.</li></ul> <p><b>Ekspresija MMP2 je značajno viša u tkivu glioblastoma u poređenju sa zdravim tkivom.</b></p>     |
| Matriks metaloproteinaza 9 ( <i>MMP9</i> )                  | <ul style="list-style-type: none"><li>• MMP9 promoviše rast tumora degradiranjem matriksa i promovisanjem angiogeneze.</li></ul> <p><b>Ekspresija MMP9 je značajno viša u tkivu glioblastoma u poređenju sa zdravim tkivom.</b></p>     |
| <i>IPO8</i> – referentni gen                                | Jedan od najstabilnijih referentnih gena koji se koriste za analizu genske ekspresije u istraživanjima raka.  |

# Dizajn LAMP prajmera za odabrane tumor markere

## Dizajn LAMP prajmera pomoću onlajn softvera PrimerExplorer V5

LAMP primer designing software

**PrimerExplorer** Loop-mediated Isothermal Amplification **HOME**

**PrimerExplorer >>**  
Features and operating environment for the LAMP primer designing software [PrimerExplorer]

**Queries >>**  
For inquiries, please contact us from the inquiry form of our corporate website.

**Links**

**Eiken GENOME SITE >>**

**EIKEN CHEMICAL CO.,LTD. >>**

**荣研专利LAMP中国总代理  
北京蓝谱生物科技有限公司**

**PrimerExplorer is a primer designing software specifically for LAMP, a novel gene amplification method.**

**PrimerExplorer V4** Please click for software information

**PrimerExplorer V5** Please click for software information

**Caution upon usage**

**Java Runtime Environment (JRE)** is required for PrimerExplorer. Please download the JRE from the following site and install it. ([V4-Operating Environment](#))

For V4: [Java Runtime Environment\(JRE\) 1.6.0\\_33](#)

**[Caution]**  
Fire Wall or similar software might interfere and block the Java program from correctly transferring data.  
The Fire Wall settings might restrict the operation of Applet. In this case, please consult your LAN administrator.

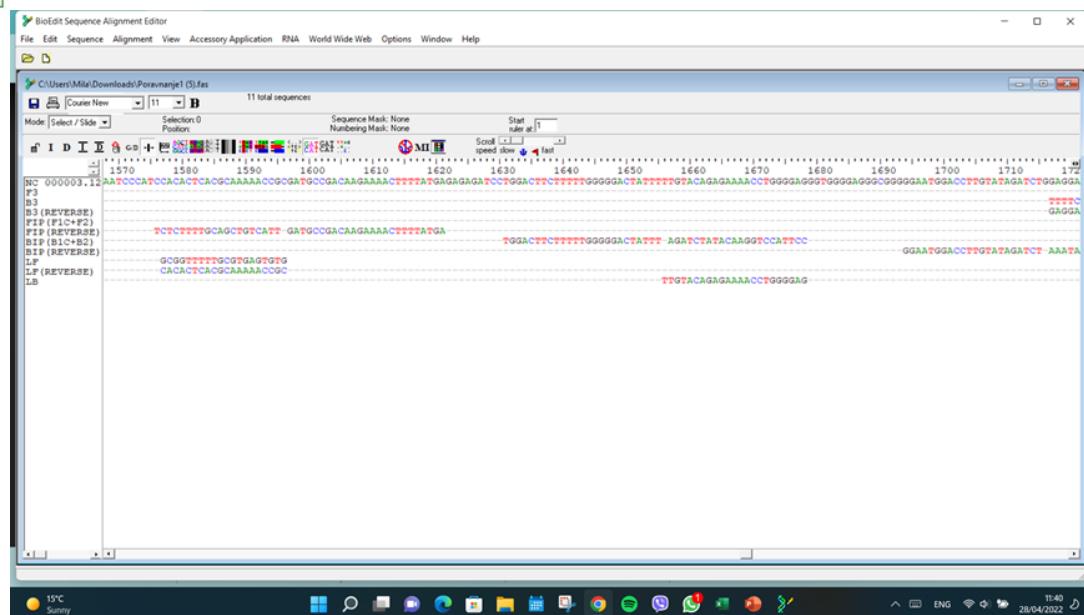
**Notes**

Ubacivanjem ciljnih sekvenci u softver generisali su se prajmer kandidati za ciljne gene.

Njihova dalja selekcija se vršila na osnovu sledećih parametara:

- najveća  $\Delta G$  vrednost za dimerizaciju
- $\Delta G$  krajevi veći od -4

## In silico analiza specifičnosti prajmera pomoću BioEdit softvera



# Dizajn LAMP prajmera za odabrane tumor markere

| IPO8 primers                     |   |       |              |                    |
|----------------------------------|---|-------|--------------|--------------------|
| Primer candidate 1 for IPO8 gene |   |       |              |                    |
| name (label)                     | sequence (5'-3')                                  | scale | purification | Physical Condition |
| IPO8PC1 - F3                     | AATTCCAAAGTGTATTGGGATA                            | 50 mM | Desalted     | Dried              |
| IPO8PC1 - B3                     | CTACAAACCAAATGTACAAC                              | 50 mM | Desalted     | Dried              |
| IPO8PC1 - FIP(F1c+F2)            | GTCATGGTGGCACAGTAAGAT-AGCTATTATC<br>TAGTGCTTCACA  | 50 mM | Desalted     | Dried              |
| IPO8PC1 - BIP(B1c+B2)            | AGCACAGGTAATGGTACTGT-GGACCATGA<br>GCAAGTTCT       | 50 mM | Desalted     | Dried              |
| IPO8PC1 - LB                     | GAAGCAGAAAACAAAGTTCAGGTG                          | 50 mM | Desalted     | Dried              |
| Primer candidate 2 for IPO8 gene |   |       |              |                    |
| name (label)                     | sequence (5'-3')                                  | scale | purification | Physical Condition |
| IPO8PC2 - F3                     | TGAAGTGTGACTGTCTAAAG                              | 50 mM | Desalted     | Dried              |
| IPO8PC2 - B3                     | CACCACTGCATTATCTTGG                               | 50 mM | Desalted     | Dried              |
| IPO8PC2 - FIP(F1c+F2)            | CAAACCCACCTAGGTTCTATT-CTGTTGGAA<br>TTGACTTTAAAAGC | 50 mM | Desalted     | Dried              |
| IPO8PC2 - BIP(B1c+B2)            | GAGTAGGTATAAATGCAAGGATCCC-GTTAAC<br>CCAACCTCTC    | 50 mM | Desalted     | Dried              |
| IPO8PC2 - LF                     | CCTTCAAGGATTCCAACACGCA                            | 50 mM | Desalted     | Dried              |
| IPO8PC2 - LB                     | AAAAAGGGAGAGTGAAGACACTGCC                         | 50 mM | Desalted     | Dried              |

| MMP9 primers                     |  |       |              |                    |
|----------------------------------|--|-------|--------------|--------------------|
| Primer candidate 1 for MMP9 gene |  |       |              |                    |
| name (label)                     | sequence (5'-3')                               | scale | purification | Physical Condition |
| MMP9PC1 - F3                     | CCCGACACAGACAAGATCC                            | 50 mM | Desalted     | Dried              |
| MMP9PC1 - B3                     | CACAGTGCCTGGCACATT                             | 50 mM | Desalted     | Dried              |
| MMP9PC1 - FIP(F1c+F2)            | TGGGTCCTGGCTTGGTGCAT-CAGAGGAG<br>AGGCCTCT      | 50 mM | Desalted     | Dried              |
| MMP9PC1 - BIP(B1c+B2)            | CTCCTCAGTACAGGACGGCAG-CGGCACTT<br>GATGACTGTTGT | 50 mM | Desalted     | Dried              |
| MMP9PC1 - LB                     | GTGGTTGTATGAAAGCTAGT                           | 50 mM | Desalted     | Dried              |

| SOX2 primers                     |   |       |              |                    |
|----------------------------------|---|-------|--------------|--------------------|
| Primer candidate 1 for SOX2 gene |   |       |              |                    |
| name (label)                     | sequence (5'-3')                                | scale | purification | Physical Condition |
| SOX2PC1 - F3                     | AAATCCCATACCCACAG                               | 50 mM | Desalted     | Dried              |
| SOX2PC1 - B3                     | TTTCCTGAGTTCTTCTTCCTC                           | 50 mM | Desalted     | Dried              |
| SOX2PC1 - FIP(F1c+F2)            | TCATAAAAGTTCTTGTGGCATC-AATGACA<br>GCTGAAAAGAGA  | 50 mM | Desalted     | Dried              |
| SOX2PC1 - BIP(B1c+B2)            | TGGACTCTTTGGGGACTATT-AGATCT<br>ATACAAGGTCCATTTC | 50 mM | Desalted     | Dried              |
| SOX2PC1 - LF                     | GCGGTTTGTGAGTGTG                                | 50 mM | Desalted     | Dried              |
| SOX2PC1 - LB                     | TTGTACAGAGAAAACCTGGGGAG                         | 50 mM | Desalted     | Dried              |

| Gal3 primers                     |  |       |              |                    |
|----------------------------------|--|-------|--------------|--------------------|
| Primer candidate 1 for Gal3 gene |  |       |              |                    |
| name (label)                     | sequence (5'-3')                                 | scale | purification | Physical Condition |
| Gal3PC1 - F3                     | GAGGAATCCTTTTCGTGTT                              | 50 mM | Desalted     | Dried              |
| Gal3PC1 - B3                     | CTCCGCTACTAAGGAGGC                               | 50 mM | Desalted     | Dried              |
| Gal3PC1 - FIP(F1c+F2)            | GACAGTGTGAGACCTGCTC-TTTAGGTGTT<br>TAGACCATATCAGA | 50 mM | Desalted     | Dried              |
| Gal3PC1 - BIP(B1c+B2)            | ACTCAGGCTGGAGTACAGTG-AAGTGGGAG<br>ATCGTTG        | 50 mM | Desalted     | Dried              |
| Gal3PC1 - LB                     | CATGGTCACAGCTATTGCGAC                            | 50 mM | Desalted     | Dried              |
| Primer candidate 2 for Gal3 gene |  |       |              |                    |
| name (label)                     | sequence (5'-3')                                 | scale | purification | Physical Condition |
| Gal3PC2 - F3                     | TTCAGGTCAGGGAGTC                                 | 50 mM | Desalted     | Dried              |
| Gal3PC2 - B3                     | AGCCTCTCCCATTTCTCC                               | 50 mM | Desalted     | Dried              |
| Gal3PC2 - FIP(F1c+F2)            | TCCGAGTCTGGAAACCCG-GGAGGCTGGGA<br>CTTAGGTT       | 50 mM | Desalted     | Dried              |
| Gal3PC2 - BIP(B1c+B2)            | ACTCTGGAAATTGGGTGCTGC-TCCTAGAGAC<br>AGCCTGAGTC   | 50 mM | Desalted     | Dried              |
| Gal3PC2 - LF                     | TGGCGAACCTGAAGGTAAGG                             | 50 mM | Desalted     | Dried              |
| Gal3PC2 - LB                     | TGAGGACACCTGGACCTAG                              | 50 mM | Desalted     | Dried              |

| MMP2 primers                     |   |       |              |                    |
|----------------------------------|---|-------|--------------|--------------------|
| Primer candidate 1 for MMP2 gene |   |       |              |                    |
| name (label)                     | sequence (5'-3')                                  | scale | purification | Physical Condition |
| MMP2PC1 - F3                     | ATATGGATGTTGGGAACT                                | 50 mM | Desalted     | Dried              |
| MMP2PC1 - B3                     | ATAACCAAATTGGGCTG                                 | 50 mM | Desalted     | Dried              |
| MMP2PC1 - FIP(F1c+F2)            | CATTACTACACTTGTCTGTG-AGCTAGGCT<br>GAACAATACAAAC   | 50 mM | Desalted     | Dried              |
| MMP2PC1 - BIP(B1c+B2)            | TTGTTTGTAGATGGGTATTGGGA-AAACCA<br>ATTAGCTACCAATCA | 50 mM | Desalted     | Dried              |
| MMP2PC1 - LB                     | TGGGAGAACGGACGTGA                                 | 50 mM | Desalted     | Dried              |
| Primer candidate 2 for MMP2 gene |   |       |              |                    |
| name (label)                     | sequence (5'-3')                                  | scale | purification | Physical Condition |
| MMP2PC2 - F3                     | GGCCTTAATCAAATAGGAGA                              | 50 mM | Desalted     | Dried              |
| MMP2PC2 - B3                     | TTCTGTCGCAATTCG                                   | 50 mM | Desalted     | Dried              |
| MMP2PC2 - FIP(F1c+F2)            | CTCTCTAACGCTCTAAACATCA-GTTAAGAC<br>AAACAGACACTG   | 50 mM | Desalted     | Dried              |
| MMP2PC2 - BIP(B1c+B2)            | ACAATATGGATGGAGGTAGTCAGG-GGAGCT<br>GAGAAACATCTC   | 50 mM | Desalted     | Dried              |
| MMP2PC2 - LB                     | GGTAGAGGGAGAGAACAGGGTAG                           | 50 mM | Desalted     | Dried              |
| Primer candidate 3 for MMP2 gene |   |       |              |                    |
| name (label)                     | sequence (5'-3')                                  | scale | purification | Physical Condition |
| MMP2PC3 - F3                     | GCCTCTAATCAAATAGGAGTT                             | 50 mM | Desalted     | Dried              |
| MMP2PC3 - B3                     | GGATCAAAGGAAGGTTGGA                               | 50 mM | Desalted     | Dried              |
| MMP2PC3 - FIP(F1c+F2)            | GTGTTTCCCTCTAACGCTCTC-ACAGACACT<br>ACTGTTCAG      | 50 mM | Desalted     | Dried              |
| MMP2PC3 - BIP(B1c+B2)            | GTAGAGGGAGAGAACAGGGTAG-AGAGTT<br>CTGTCGCAATC      | 50 mM | Desalted     | Dried              |
| MMP2PC3 - LB                     | GAGGATGTTCTCAGCTCCAGCA                            | 50 mM | Desalted     | Dried              |



## ZAKLJUČAK

- **Mikroskopskim metodama ustanovljena je uspešna kultivacija sferoida svih odabranih ćelijskih linija tokom sedam dana;**
- ***In silico* analiza dizajniranih LAMP prajmera pokazala je njihovu visoku specifičnost;**
- **Opisani rezultati ukazuju na veliku mogućnost primene sofisticiranih tehnika ćelijske i molekularne biologije u oblasti dijagnostike i praćenja tretmana raka.**



**HVALA NA PAŽNJI!**