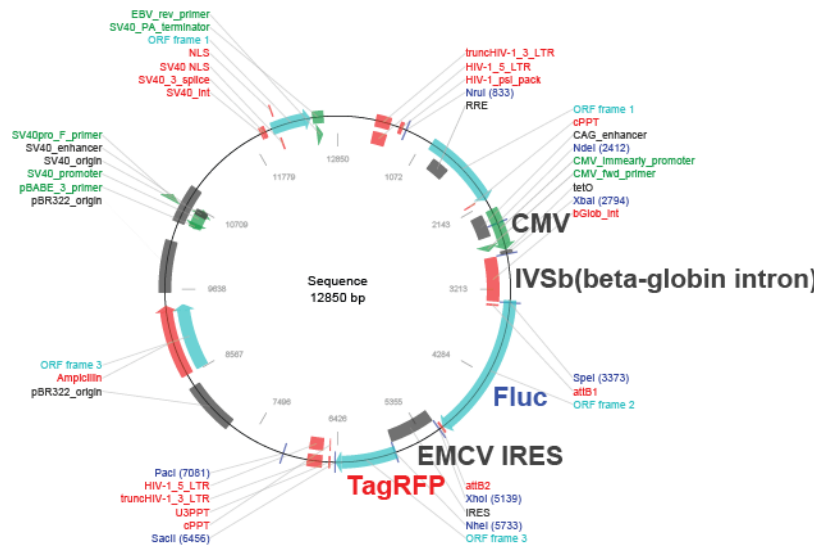


pTRIP.CMV.IVSB.Fluc[ISG].ires.TagRFP



Clone information

Description

Bicistronic lentiviral vector for expressing a gene of interest (firefly luciferase, in this example) in the first cistron with TagRFP in the second cistron. This plasmid is derived from the parental DEST vector pTRIP.CMV.IVSB.ires.TagRFP-DEST. It was generated by performing an LR reaction with LR Clonase II (Invitrogen) and pENTR221.Fluc. This plasmid is used to generate lentivirus stocks in conjunction with pCMV-gag-pol and pCMV.VSVg.

NOTE: All ISG expressing plasmids have the same architecture as this Fluc expressing construct. An ISG expressing version can be generated in any plasmid software package by deleting the ORF for Fluc and replacing with the ORF for the ISG of interest.

Location of features

CMV promoter: 2411-2794
 Beta globin intron: 2794-3373
 Fluc: 3437-5089
 EMCV IRES: 5147-5733
 TagRFP: 5739-6452

Propagation in *E. coli*

Recommended host strains: MDS42Rec (from Scarab Genomics) or DH5 α
 Resistance: ampicillin (100 μ g/ml)

Copy number: high

Lentivirus particle production

To generate lenti PP, three plasmids are needed: pTRIP, pCMV-VSVg, and pGag-pol.
For generating PP, The steps are as follows:

1. The day before transfection of DNA, split 293T and plate 400,000 cells/well on a 6 well plate that has been coated with poly-lysine.
2. The day of transfection, mix 6ul XtremeGene9 (Roche Cat# 06365809001) into 100ul Optimem (Invitrogen Cat# 51985-034) and let stand for 5 min...if there are many samples, a master mix can be made.
3. Aliquot DNAs into eppendorf tubes. The ratio of the plasmids for lentivirus production is:
pTRIP(GFP): 1.0ug
pCMV-VSVg: 0.2ug
pGag-pol: 0.8ug
4. Add 106 ul of the Optimem/XtremeGene9 mix to the DNA samples. Let this mix stand for at least 20 minutes at room temp.
5. During this 20min, change the media of the cells to DMEM (Invitrogen Cat#11995-065)/3%FCS/1%NEAA. Use 1.0ml media per well.
6. Add DNA mix to cells drop-wise and place back in incubator. Swirl plate to mix. 6 hours post-transfection, change media to 1.5 ml DMEM/3%FCS/1%NEAA.
7. 48 hours post-transfection, gently harvest the media from the cells. Add 1.5ml of fresh media (DMEM/3%FCS/1%NEAA) to the cells. Place the snap-cap tubes in a rack in the 4C fridge until the next day.
9. The next day (at 72 hours), repeat the harvesting as above. To each prep, add HEPES to a final concentration of 20mM and polybrene to final concentration of 4ug/ul.
10. Spin the 5ml tubes containing the supernatant at 1500rpm (520G) for 5 min in the table-top centrifuge to pellet cell debris. Alternatively, samples can be passed through a 0.45um syringe filter. Aliquot the supernatant into desired volume. Store at -80C.

References

A diverse range of gene products are effectors of the type I interferon antiviral response.

Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM.
Nature. 2011 Apr 28;472(7344):481-5

Projects

STRUCTURED AND OOPS PHP:

1. **Qatar Offer:** <http://www.qataroffer.com/>
2. **Smith and Franklin:** <http://www.smithandfranklin.com/>
3. **My Taxi Zone:** <http://www.mytaxizone.co.uk/>
4. **Xchanger Friend:** <http://www.xchangerfriend.com/>
5. **IFTMG:** <http://www.iftmg.org/>
Events Handling
6. **Rapid Removal:** <http://www.manvanremoval.com>
7. **Pakistan Car Rentals** <http://rentcars247.com/>
8. **Royal air Port Services** <http://www.gatwickparkinglot.co.uk/>
9. **Travel Cart** <http://travelcartuk.co.uk/>
10. **Pearson Limo** <http://115.186.163.165/projects/pearsonlimo/>
11. **Nexus Academic Publishers** <http://nexusacademicpublishers.com/>
12. **Kismat Connection:** <http://kismatkonnections.com/>
(Matrimonial Website, having chat module)
13. **The Gold For Ever:** <http://www.thegoldforever.com/>
is a Multi Level Marketing Website.
14. **EM Trust:** <http://www.emtrust.co.uk/>
15. **Thai Chat Love:** <http://www.hiconintl.com/dev/projects/thaichatlove/>
16. **Corretor:** <http://www.corretor.co.uk/>
Corretor is a brand new concept in selling used vehicles. You specify the price that you want to achieve for your car (we can help and advice based on current market prices) and then you leave the rest to us. We act as the broker between you "the seller" and potential buyers.
17. **Quality Clean:** <http://www.quality-clean.com/>
Quality Clean London are a large team of professional cleaners and tradesman with many years of experience behind us. Each individual is highly skilled in his/her field and will provide to you a top quality service to meet your needs.
18. **ARM Residential:** <http://www.armresidential.com/>
ARM Residential is a property letting and selling website.
19. **Logo Chair USA:** <http://logochairusa.com/>

20. **IT Linkers:** <http://www.itlinkers.com.au/>
IT Linkers is a young, dynamic & rapidly growing software house specializing in emerging web design and development, e-commerce solution, search engine optimization and data base design services. IT Linkers already have a large client base in UK, USA, Australia, Malaysia, Nigeria and Dubai. It planned to rapidly grow with the help of extensive experience and links in the European IT industry.
21. **Exchange House:** <http://www.xchangehouse.com/>
Xchange House is a professional e-currency exchanger and has provided services to more than 10000 clients through out the world for the last 7 years. Backed by rich experience, we know what our customers need speediness, reliability and lowest fees.
22. **2WomensHealth:** <http://www.2womenshealth.com/> (worldpay payment integration)
the personal website of London consultant gynecologist David A Viniker MD FRCOG (Worldpay payment integration)
23. **KS-International:** <http://www.ks-international.com/> (search module in client and back end)
K.S. INTERNATIONAL LTD is an internationally renowned supplier of Japanese truck, 4x4 and pick up parts for nearly two decades. Over the years we have established and specialized in ISUZU, MITSUBISHI and TOYOTA truck parts.
24. **Valid Coin:** <http://www.validcoin.com/>
Valid Coin is such like a payment gateway or State Bank, which accept payments in USD, Euro and Goldgrams.
25. **Sawyer Music Studio:** <http://www.sawyermusicstudio.com/>
Anwar Sawyer, Pianist and Vocalist, has performed as Marquese in La Traviata last year with Nyack College Opera. He just finished working on his first lead opera role as Gianni Schicchi in Gianni Schicchi in 2010. He has also performed the role of Schaunard 2008 in La Boheme and Dancarrion in Carmen in 2007.
26. **FIX MY THINKING:** <http://fixmythinking.com/>
27. **Employee Portal:** <http://work.thanavalley.com/EmployeePortal/>
28. **Living Sofism:** <http://www.livingsufism.org/>
29. **Almadina:** <http://115.186.163.165/projects/almadina/>
30. **Life Biological, Research and diagnostics Products;** <http://115.186.163.165/projects/biological/>
31. **MY Taxi Zone:** <http://www.mytaxizone.com>
32. **Eite business:** <http://www.eliteba.ca>
33. **Trade Inn:** <http://115.186.163.165/projects/tradeinn/>

SHOPPING CARTS:

34. **Bee My Guy:** <http://www.beemyguy.com/>
Beemyguy is a new dating website with new ideas a site where guys or girls wanting to meet beautiful girl just search for that perfect date get their mobile number e-mail address and make contact
35. **Mahatma Gandhi:** <http://www.m-gandhi.co.uk/> (having ajax based shopping cart)
Mahatma Gandhi has been providing quality Indian food since 1982 and being the first Indian takeaway in Essex have built up a respectable reputation for serving the finest Indian food over the many years that we have been open.
36. **A1 Gift Cards** <http://a1giftcards.com/>

37. **Weald Place Farm Bees:** <http://wealdplacefarmbees.co.uk/>
Weald Place Farm Bees is a bees and honey collection farm. Where Hives are collecting for their employers. Including selling of honey, Educating their people about how to keep Hives more protective.
38. **Beauty Crackers:** <http://work.thanavalley.com/beauty/>
Beautycrackers is an exciting new way for you to indulge in your passionate for beauty products. Each month, members of Beautycrackers receive a selection of quality samples allowing them to “test-drive” products in the comfort of their home. We select the very best and give you the opportunity to find new favourites without the expense of buying full-sized products that you may not like.
39. **LyricPeddler:** <https://www.lyricpeddler.com/>
40. **Eazy To Buy:** <http://websolutionlive.com/developer/eazy2buy/>
41. **E-Business Bazar:** <http://115.186.163.165/projects/e-businessbazar/>
42. **Electric Power:** <http://www.electpower.com/>

JQUERY BASED SHOPPING CARTS:

43. **CCShop TV:** <http://www.ccshop.tv/> , <http://www.ccsellz.com/> , <http://www.cvvbank.com/>,
CC Shop is a Credit cards website where a bunch of CCs, Dumps and Bank Logins are available.
44. **BigBuyerShop:** <http://www.bigbuyershop.com/> , <http://www.usellz.com/>
BigBuyerShop is a Credit cards website where a bunch of CCs, Dumps and Bank Logins are available.
45. **Estampitas:** <http://www.inventive360.com/proyectos/estampitas/>

INTERSPIRE:

46. **Fire Place Store Online:** <http://www.fireplacestoreonline.com/> (checkout process)

SMARTY:

47. **B&A Dairies:** <http://www.badairies.co.uk/> (all payment modules)
B&A Dairies are Coventry's leading doorstep delivery service. We deliver milk, bread, eggs and many other products straight to your door.

OS COMMERCE & WRODPRESS:

48. <http://www.natural-herbal-remedy-supplements.com/> and <http://www.gogreenremedy.com/>

Menu customization on both sites through php curl.

JOOMLA: <http://www.thanavalley.com>

Updates customer information (Admin)

- <Newsletter></Newsletter> should have true/false in it

In Orders XML of Customers adds a new record (User Section)

- Set 0 value of Thursday if it as no value
- Set ordered value to 0 by default

In Customer updated Order (User Section) and Customer updated Order (User Section) 2 and Customer Deletes Order (User Section)

- Set ordered value to 0 by default
- Set WCDate value in file
- Set startdate and enddate value

In Admin adds a new Product (Admin Section) and Admin updates existing Product (Admin Section)

Set purchase cost value

Emerging Topics in Software Engineering

MS/ MPHIL

Model Paper 2

Spring Semester 2013

Attempt any seven Questions
Three Hours

Time:

Q1. What is Requirement Engineering? Discuss the two requirement gathering methods: Brainstorming and Storyboarding.

Q2. A problem is like a tree and software engineer has to touch every single branch and leave of that tree. Illustrate problem analysis based upon this statement.

Q3. Define the Goal of a Project. How is it different from a Project's Scope.

Q4. Why building an 'as simple as possible model' is like half the job done? Define and explain a Business Model.

Q5. The iterative approach model is a hybrid of the waterfall and spiral models. Decouple the lifecycle phases from the software activities that take place in each phase. Briefly touch upon each step.

Q6. The future Software Engineers will be more ethically conscious. Discuss.

Q7. Why we should use the title 'Software Management' instead of 'Software Engineering'. Give your answer in the light of emerging topics and future trends.

Q8. There is a difference between client, customer and user. Explain your answer by discussing the stakeholders in general in project management.

Q9. Discuss the following Software Quality Assurance testing methods.

1. Black box testing
2. White box testing(Check efficiency of the software)

Q10. Give a brief on future job prospects in Requirement Engineering.

Emerging Topics in Software Engineering

MS/ MPHIL

Model Paper

Spring Semester 2013

Attempt any seven Questions
Three Hours

Time:

Q1. What is Requirement Engineering? Discuss the two requirement gathering methods: Interviewing and Prototyping.

Q2. Why molding words of the customer into technical language is so tough in software engineering? Cite a rocky problem to support your answer.

Q3. Define the Scope of a Project. How is it different from a Project's Goal.

Q4. SRS will soon be overtaken by VISION documents. Discuss.

Q5. Write a short comparative note on the functional and non- functional requirements.

Q6. Software Engineer likes to give answer in YES and NO. Soon this is going to change. Discuss.

Q7. Why should we use the title 'Software Management' instead of 'Software Engineering'. Give your answer with reference to managing the customer.

Q8. Write a comprehensive note on balancing Cost and Time factors in a successful project.

Q9. Discuss the following Software Quality Assurance testing methods.

1. Component Testing
2. Unit Testing

Q10. Give a brief on future job prospects in Requirement Engineering.