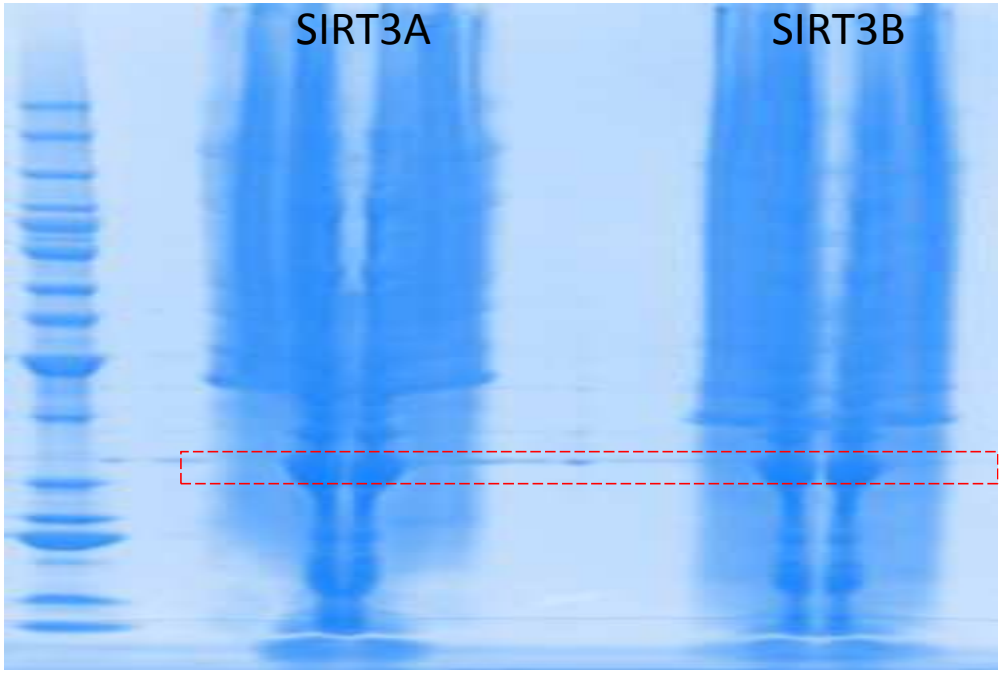
SIRT3 Purification

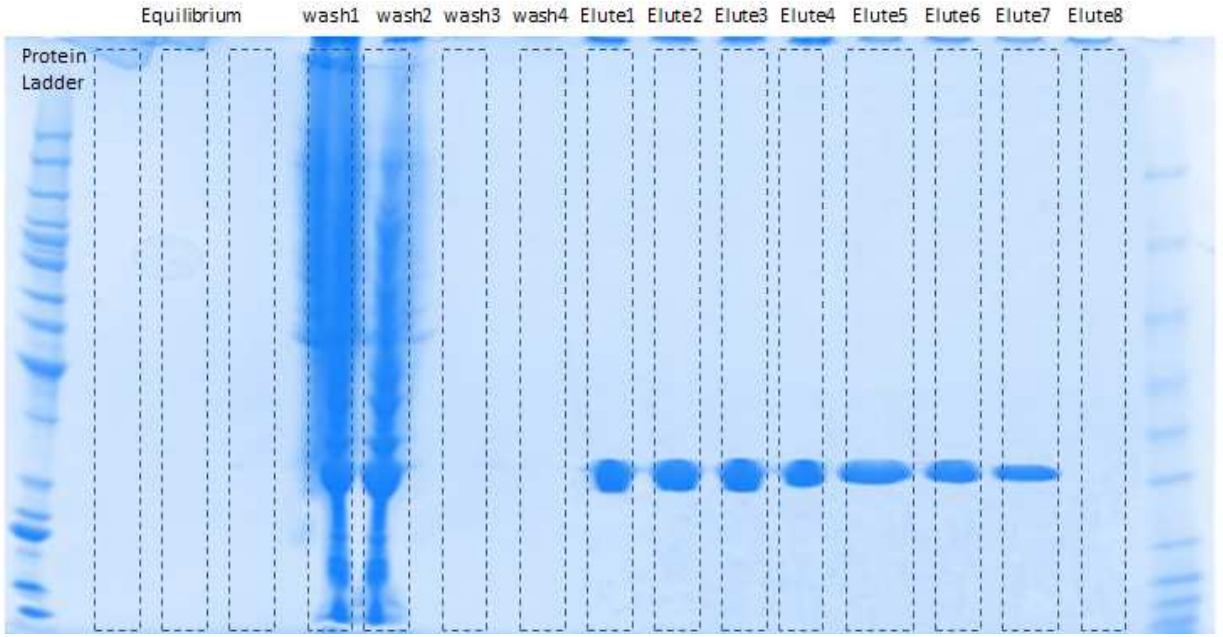
1. SIRT3 plasmids were transformed to BL21 cell.
2. Plate the above chemical transformation product on LB argar with Ampicillin (1:1000) at 37oC overnight.
3. Single colonies were detected and were cultured firstly in 500 ul LB broth/Amp till OD to 0.6.
4. Transfer 20 ul of above culture to 5mL LB broth/Amp overnight at 37oC with 250 rpm
5. Scale up for 100ml of cell culture.
6. Add IPTG for final concentration (2mM) at 37oC 250 rpm for 3 hours.
7. Centrifuge to get the cell pellet and stored at -80oC till use.
8. Add lysis buffer + bugbuster and sonication.
9. Centrifuge to obtain supernatant

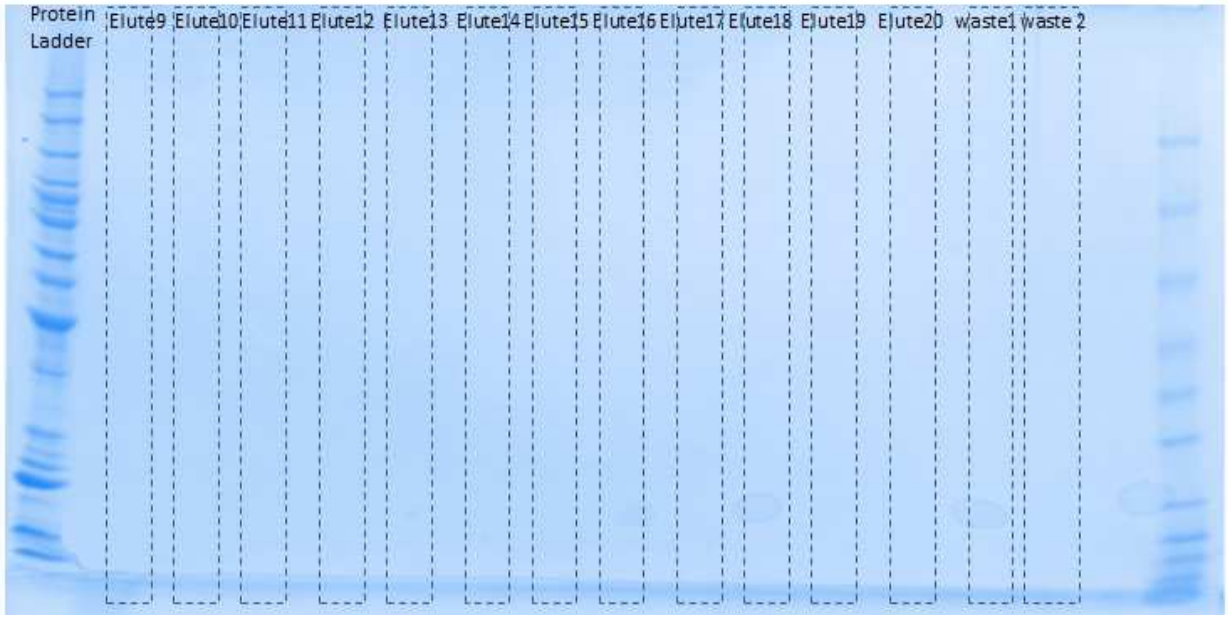
SIRT3 (102-399)
UniProt ID: Q9NTG7
Length: 298
Mass (Da): 32,700



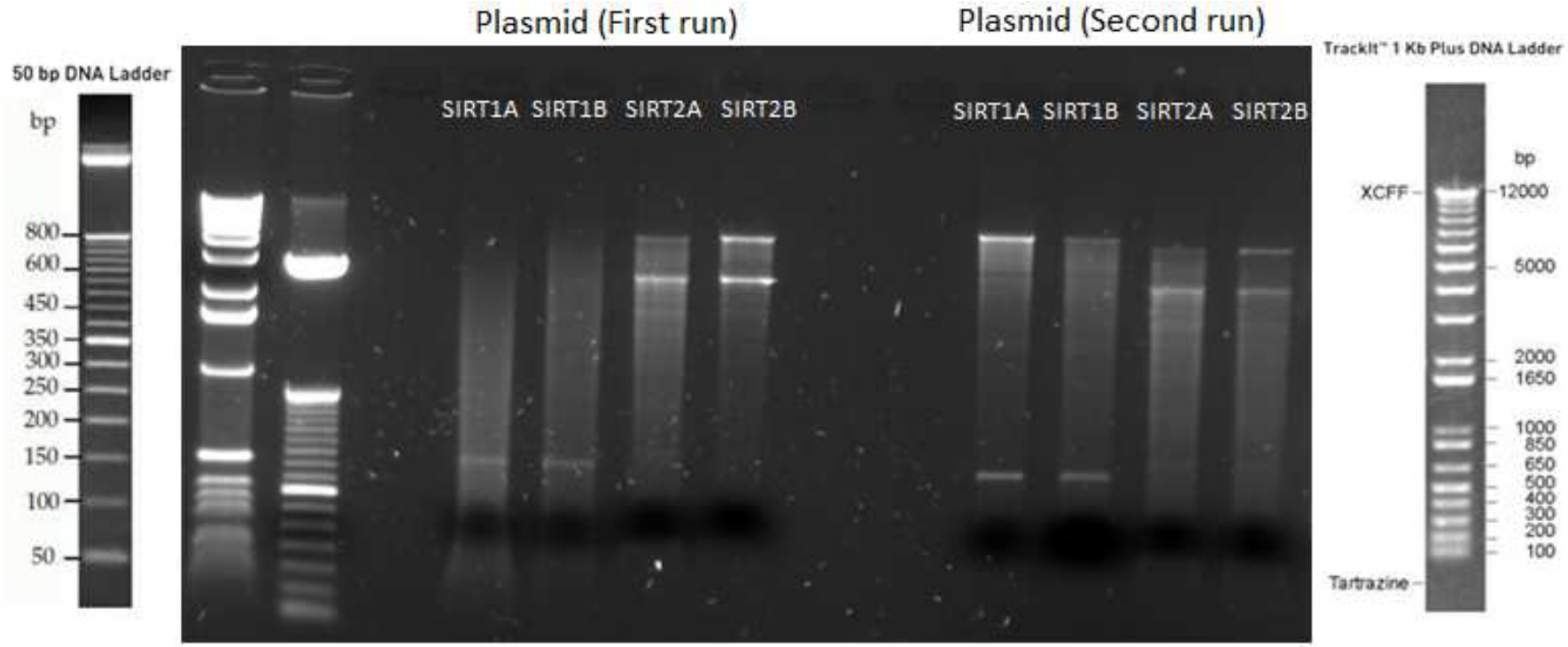
Under current condition, protein expression level is good.

10. AKTA first Ni-column purification
    - Equilibration 4.5 ml
    - Wash 20 column volume (20 ml,5ml /fraction)
    - Elution 10 cv (10 ml, 0.5 ml/fraction)
    - Waste
11. Run protein gel to check
    1. SIRT3 protein expression level
    2. Fraction content
        - First 2 wash (10 ml) are enough
        - First 7 elution (3.5ml) are good
    3. Modify AKTA program
        - Reduce wash volume to 10 CV
        - Reduce elution to 5 CV





- Raw sequencing data for SIRT1 and SIRT2 have been obtained from OriGene.
- The circular maps have been drew.
- The restriction sites have been obtained
  - SIRT1: Kpn1 and Not1
  - SIRT2: Ecor1 and Not1
- Double digestion was performed for SIRT1 and SIRT2



- Since different restriction enzymes have different working buffer, the double digest Finder from NEB web site was used to find right buffer.

SIRT1

Double Digest Finder

Use this tool to guide your reaction buffer selection when setting up double digests, a common timesaving procedure. Choosing the right buffers will help you to avoid star activity and loss of product. Learn more information about double digests, including how to set up a reaction.

Select 1st enzyme: KpnI

Select 2nd enzyme: NotI

Go

Enzyme	Cat #	Temp	Supplied NEBuffer	Supplements	% Activity in NEBuffer			
				SAM	1.1	2.1	3.1	CutSmart
KpnI	R0142	37°C	NEBuffer 1.1	no	100	75	10	50*
NotI	R0189	37°C	NEBuffer 3.1	no	10	50	100	25

SIRT2

Double Digest Finder

Use this tool to guide your reaction buffer selection when setting up double digests, a common timesaving procedure. Choosing the right buffers will help you to avoid star activity and loss of product. Learn more information about double digests, including how to set up a reaction.

Select 1st enzyme: EcoRI

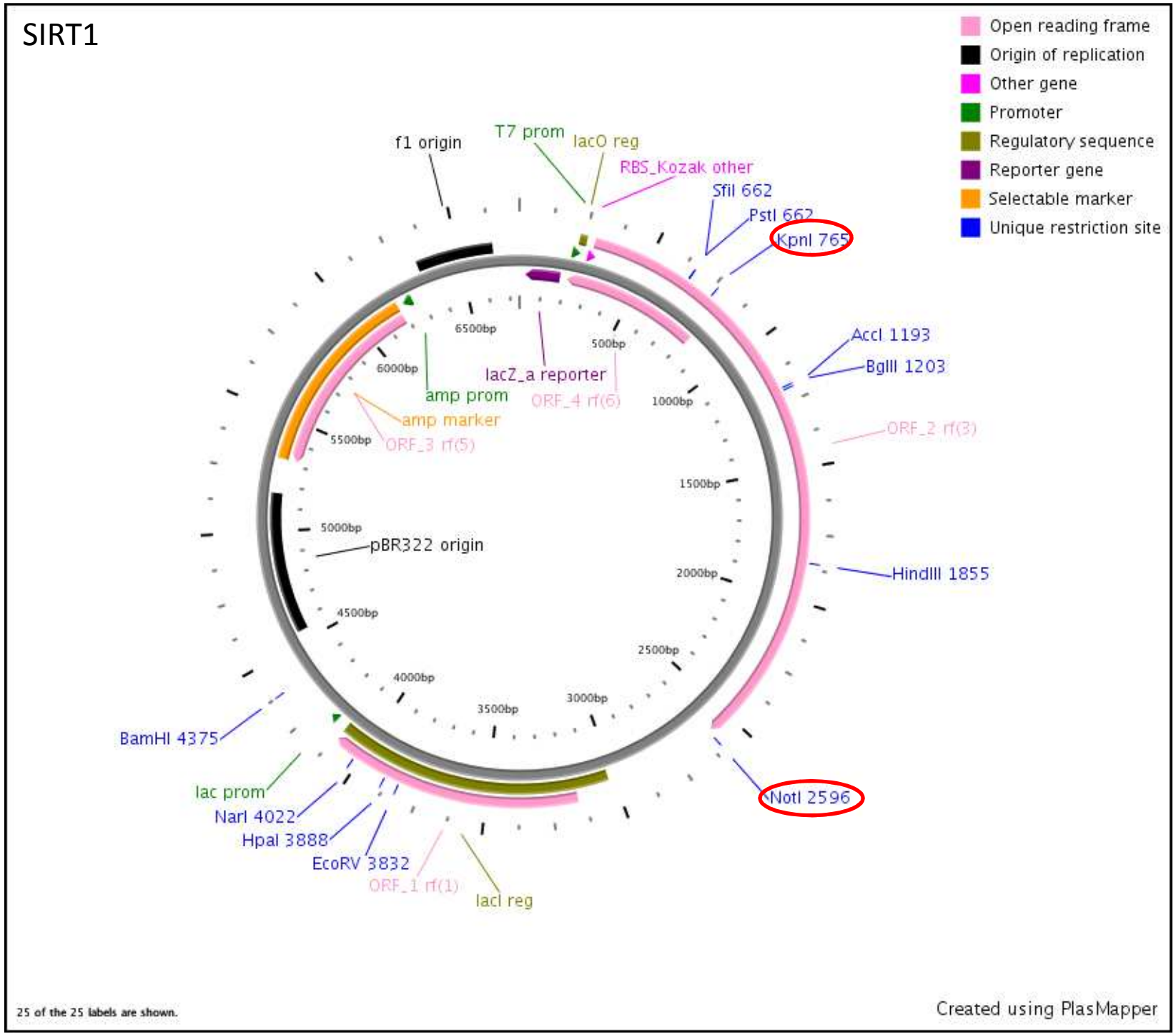
Select 2nd enzyme: NotI

Go

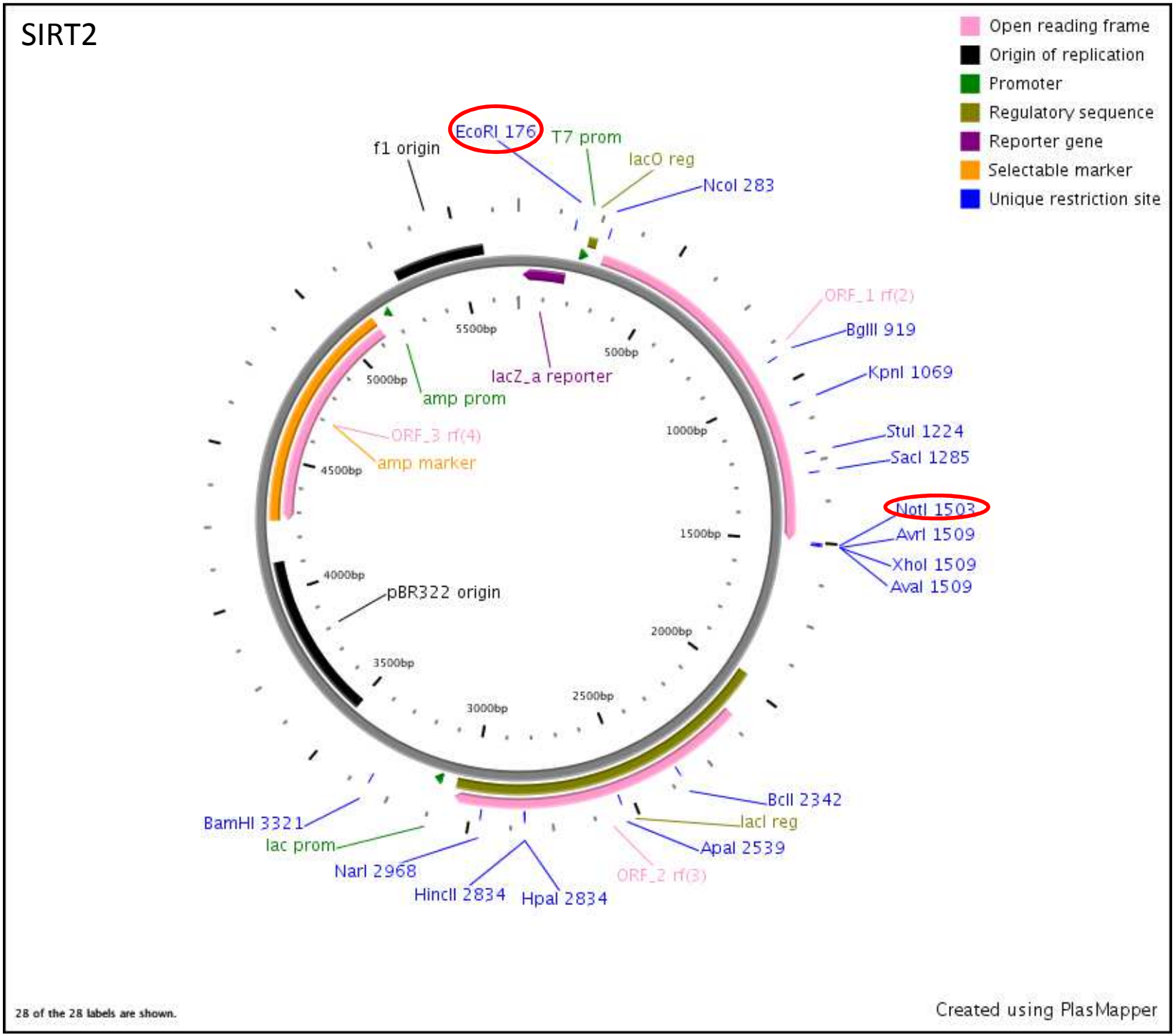
Enzyme	Cat #	Temp	Supplied NEBuffer	Supplements	% Activity in NEBuffer			
				SAM	1.1	2.1	3.1	CutSmart
EcoRI	R0101	37°C	NEBuffer EcoRI	no	25	100*	50	50*
NotI	R0189	37°C	NEBuffer 3.1	no	10	50	100	25

  - SIRT1 NEBuffer 2.1
  - SIRT2 NEBuffer 3.1

- New protein ladder was ordered.
- NEBuffer 2.1 was ordered.


SIRT1
Open reading frame
Origin of replication
Other gene
Promoter
Regulatory sequence
Reporter gene
Selectable marker
Unique restriction site
f1 origin
T7 prom
lacO reg
RBS_Kozak other
SfiI 662
PstI 662
KpnI 765
AccI 1193
BglII 1203
ORF_2 rf(3)
HindIII 1855
NotI 2596
BamHI 4375
lac prom
NarI 4022
HpaI 3888
EcoRV 3832
ORF_1 rf(1)
lacI reg
lacZ_a reporter
ORF_4 rf(6)
amp prom
amp marker
ORF_3 rf(5)
pBR322 origin
500bp
1000bp
1500bp
2000bp
2500bp
3000bp
3500bp
4000bp
4500bp
5000bp
5500bp
6000bp
6500bp
25 of the 25 labels are shown.
Created using PlasMapper

SIRT2


Open reading frame
Origin of replication
Promoter
Regulatory sequence
Reporter gene
Selectable marker
Unique restriction site
f1 origin
EcoRI 176
T7 prom
lacO reg
NcoI 283
ORF_1 rf(2)
BglII 919
KpnI 1069
StuI 1224
SacI 1285
NotI 1503
AvrI 1509
XhoI 1509
AvaI 1509
BclI 2342
lacI reg
ApaI 2539
ORF_2 rf(3)
HpaI 2834
HincII 2834
NarI 2968
lac prom
BamHI 3321
pBR322 origin
amp marker
ORF_3 rf(4)
amp prom
lacZ_a reporter
500bp
1000bp
1500bp
2000bp
2500bp
3000bp
3500bp
4000bp
4500bp
5000bp
5500bp
28 of the 28 labels are shown.
Created using PlasMapper