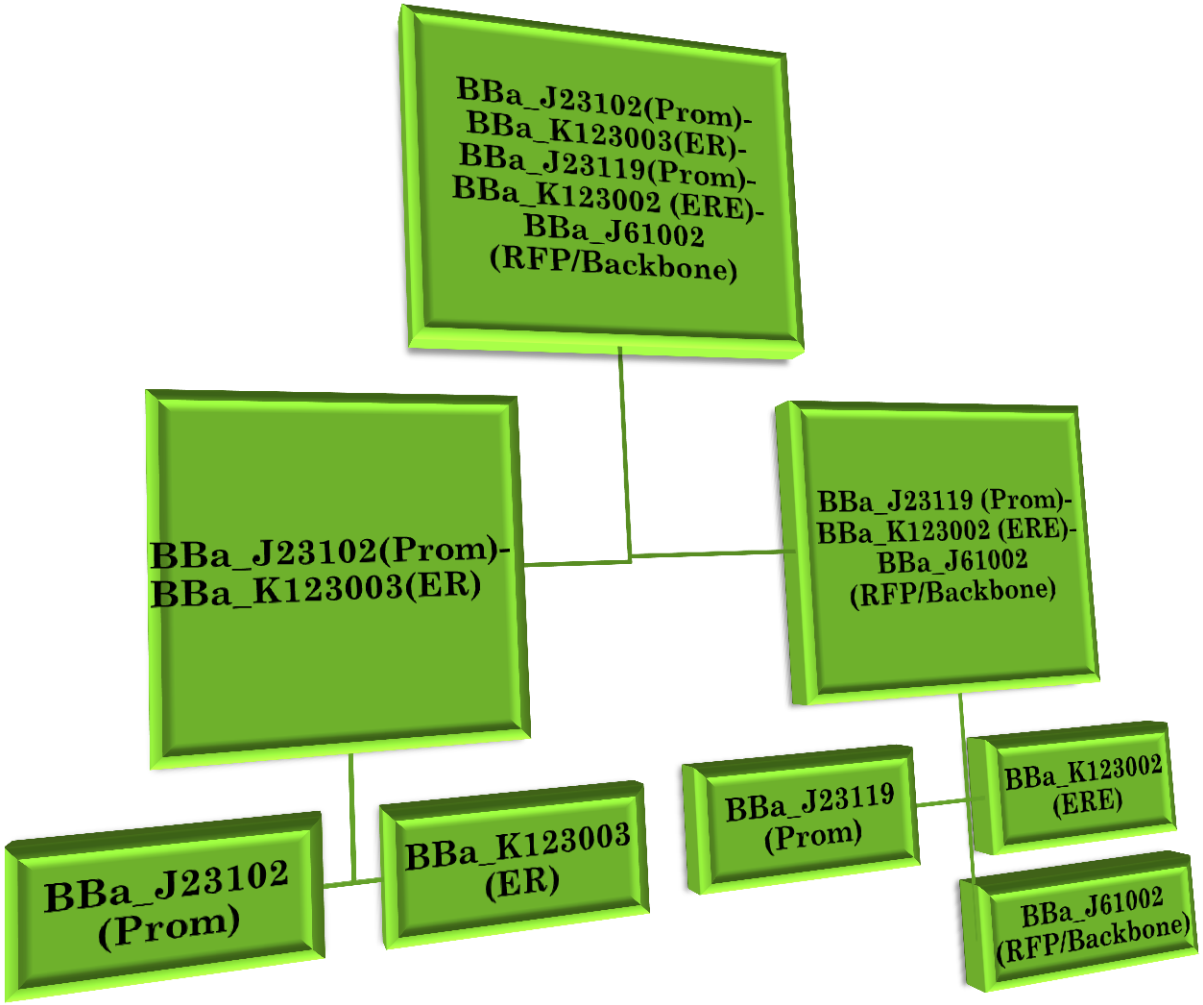
Estrogen Detection By Luminance

Kyle & Josh

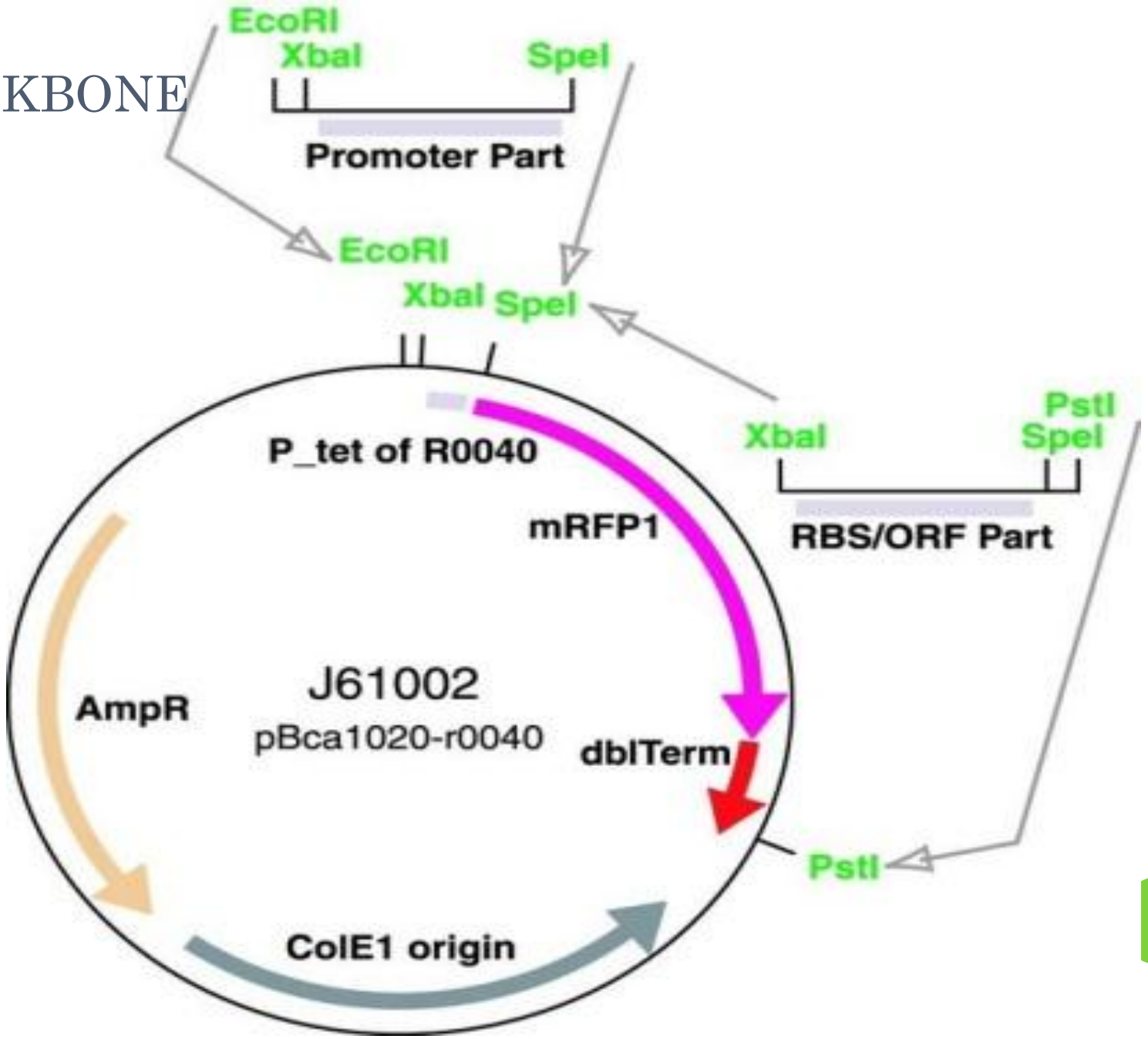
Why

- Uncontrolled levels of estrogen in waste water treatment plants is a growing problem.
- The high levels of estrogen have been found to cause feminization of fish that are exposed to it.
- More studies show that their also harmful to humans.

Assembly


BBa_J23102(Prom)-
BBa_K123003(ER)-
BBa_J23119(Prom)-
BBa_K123002 (ERE)-
BBa_J61002
(RFP/Backbone)
BBa_J23102(Prom)-
BBa_K123003(ER)
BBa_J23119 (Prom)-
BBa_K123002 (ERE)-
BBa_J61002
(RFP/Backbone)
BBa_J23102
(Prom)
BBa_K123003
(ER)
BBa_J23119
(Prom)
BBa_K123002
(ERE)
BBa_J61002
(RFP/Backbone)

BACKBONE


EcoRI
XbaI
SpeI
Promoter Part
EcoRI
XbaI
SpeI
PstI
XbaI
SpeI
RBS/ORF Part
P_tet of R0040
mRFP1
J61002
pBca1020-r0040
AmpR
dblTerm
PstI
ColE1 origin

Goals of Project

- Attach parts
  - BBa_J61002, BBa_J23102, BBa_K123003, BBa_J23119, BBa_K123002, BBa_J61002
  - Show that parts are attached in the correct order
  - And are actually present in final assembly
- Devise a testing method to show that:
  - Parts work for detecting estrogen in water.

Step 1

- We at first decided to work with exclusively BioBrick's parts
  - BBa_E0240 (GFP)
  - BBa_E0840 (GFP)
  - BBa_K123003(ER)
  - BBa_K123002 (ERE)
- We resuspended these from the BioBricks library and transformed all of them into competent cells.

Step 2

- We prepared and stored our DNA using the Genejet plasmid mini prep kit.
- We also resuspended the part ERE from the 2009 and again from the 2010 kit plates and plated them.
  - Again this yielded nothing. Our controls worked but neither grew.

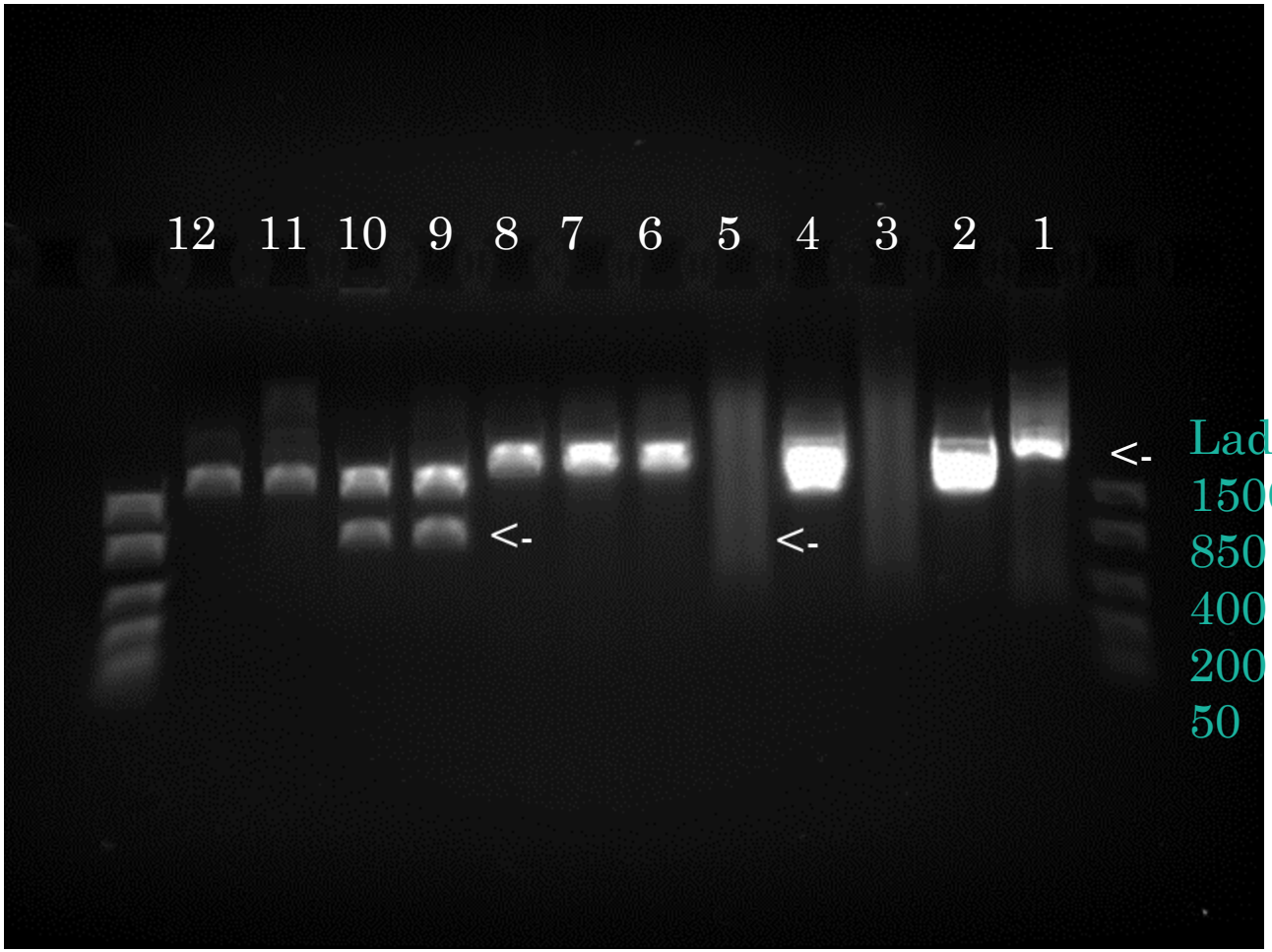
Step 3

- We digested all the parts: BBa_E0240 (GFP),BBa_E0840 (GFP), and BBa_K123003(ER) with Ecori and Spel.
  - To release our target DNA

Results from Plated BioBrick Parts

BioBrick Number	BBa_123003	BBa_K123003	BBa_K123002	BBa_K123002	BBa_E0840	BBa_E0840	BBa_E0240	BBa_E0240	Water w/ Ampicillin	Water w/o Ampicillin	PBluescript
DNA(µl)	1	5	1	5	1	5	1	5	0	0	0
Competent Cells (µl)	40	40	40	40	40	40	40	40	40	40	40
Number of Colonies	25	400	0	0	0	91	10	19	0	9600	4800
Colony Number assigned	3,4	1,2	-	-	-	5,8	9,10	11,12	-	-	-

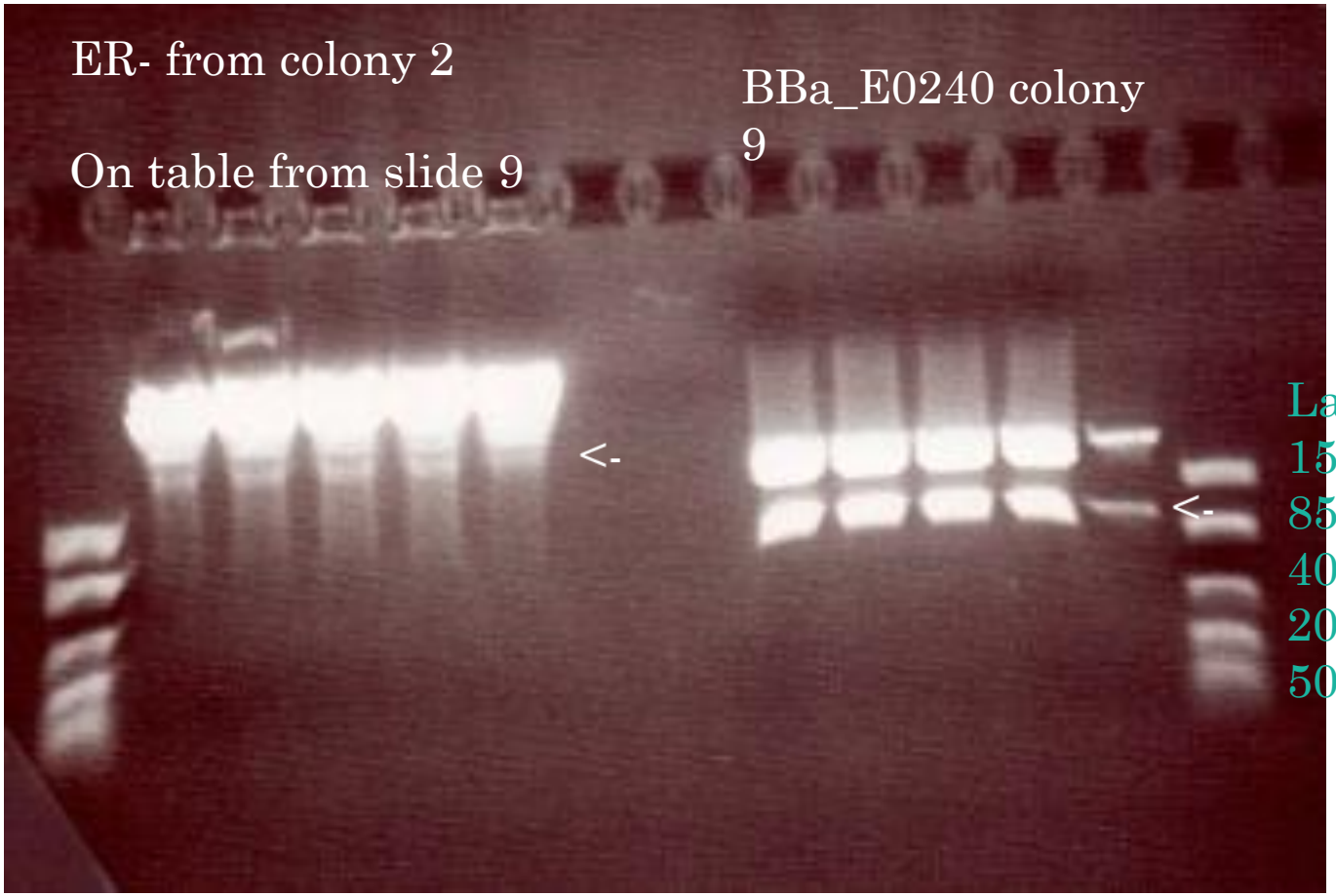
Results



Step 4

- We digested part ER (well 2) with Spel and Ptsl enzymes
- We digested part GFP 240 (well 9) with Xbal and Ptsl enzymes

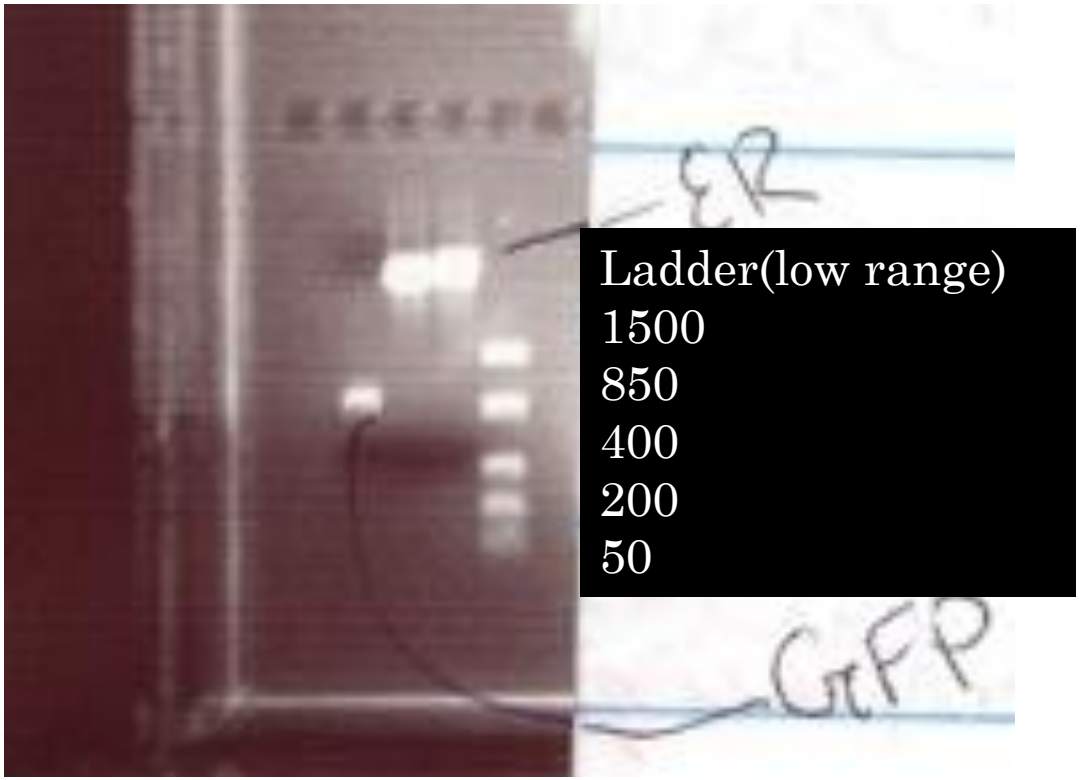
Results from Digests



Gel extraction of ER and GFP

- We have both our parts DNA (ER with plasmid attached and our GFP DNA) mixed with gel so we want to just have DNA by the end of this extraction.
  - ER was digested with Spel and Ptsl.
  - And GFP 240 was digested with Xbal and Ptsl
- We used the Genejet Gel Extraction Kit to do this. We then ran a gel to make sure that our DNA was pure.

Results from Gel extraction of ER and GFP 240

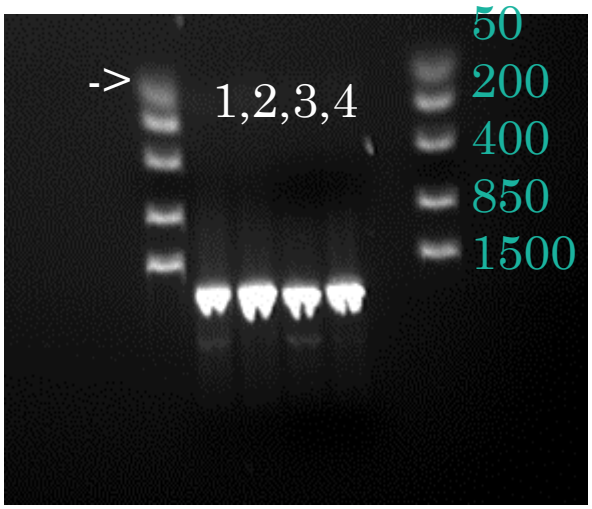


Working with new parts!

- We worked with parts,
  - BBa_J23119 This part contains a promoter, GFP and Term, standard backbone
  - BBa_J23102 This part contains a promoter, GFP and Term
  - BBa_J23100 This part contains a promoter, GFP and Term
  - BBa_J04450 RFP

BBA_J23119 DNA PREPARATION DIGESTION

- Because the other parts glowed so distinctly pink, revealing that that their RFP's were working.



Oligos

- We had an oligos made of part (ERE) BBa_K123002 with Xbal and Pstl ends so we could later attach to the rest of our parts.

- We wanted to put the ERE oligos with parts:
  - BBa_J23119 (digested with Erori and Spel)
  - BBa_J23102 (digested with Ecori and Spel)
  - BBa_K123003 (digested with Xbal and Pstl)

Ligating BBa_J23119 and our Oligos together

10X Buffer	1µL	1µL	1µL
Oligos	1µL	3µL	5µL
BBa_J23119	5µL	3µL	1µL
T4 DNA Ligase	.4µL	.4µL	.4µL
H_2O	2.6µL	2.6µL	2.6µL

Ligated Part (ere and BBa_J23119) results

Tube #	Tube 1	Tube 1	Tube 1	Tube 2	Tube 2	Tube 2	Tube 3	Tube3	Tube 3	Tube 4	Tube 4	Tube 4	Control w/Amp	Control w/o Amp	Pbluescript
Conc. of BBa_J23119	1	3	5	1	3	5	1	3	5	1	3	5	0	0	0
Conc. of Oligos	5	3	1	5	3	1	5	3	1	5	3	1	0	0	0
# of Colonies	1	0	0	30	19	0	0	0	6	4	13	240	0	2880	2096
Colony #	1	-	-	2,3	4,5	-	-	-	6,7	8,9	10,11	12,13	-	-	-

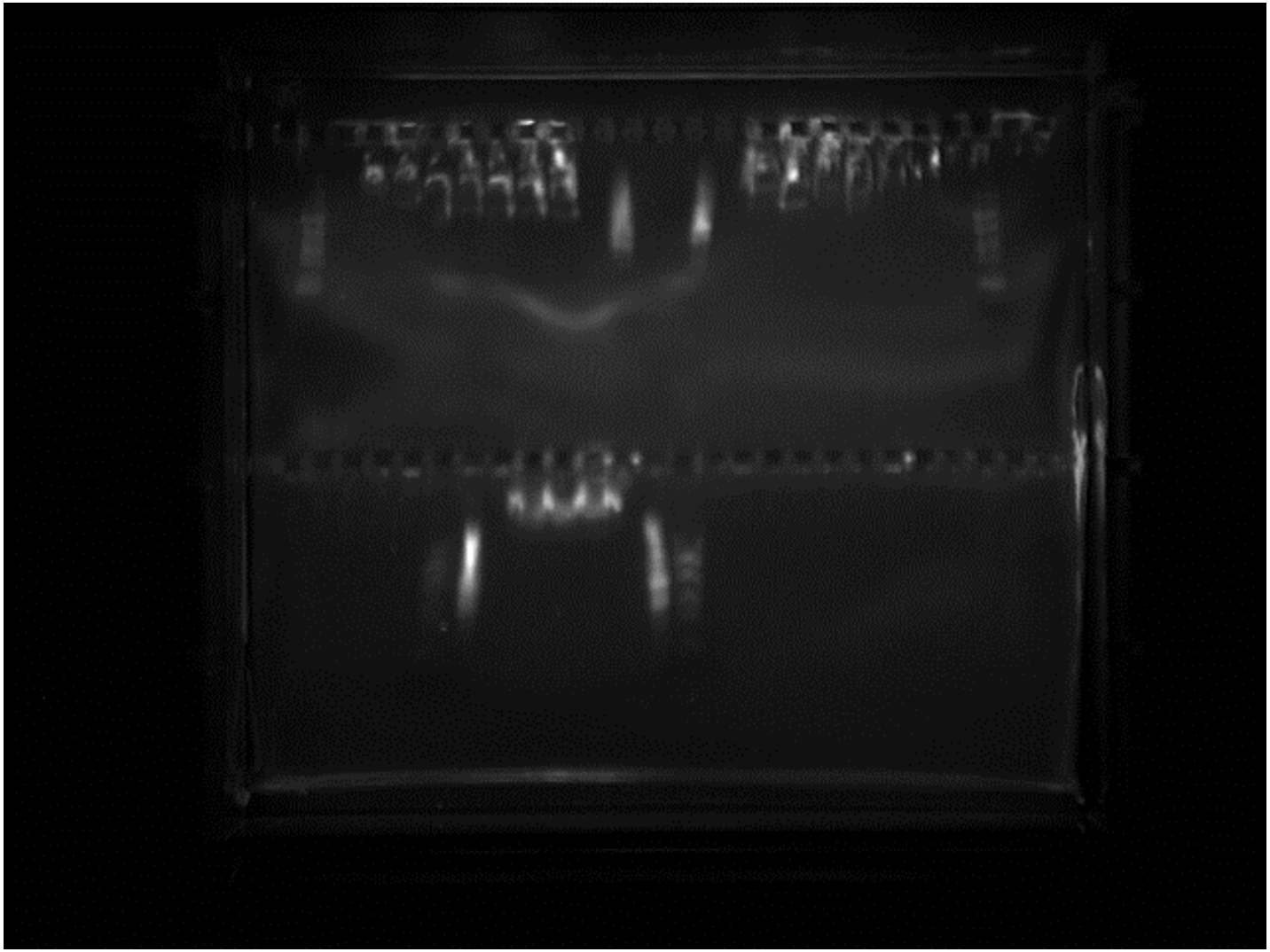
The ligated part

- We digest the ligated part with Ecori and Spel.
  - The part should have been about 80 to 90bp

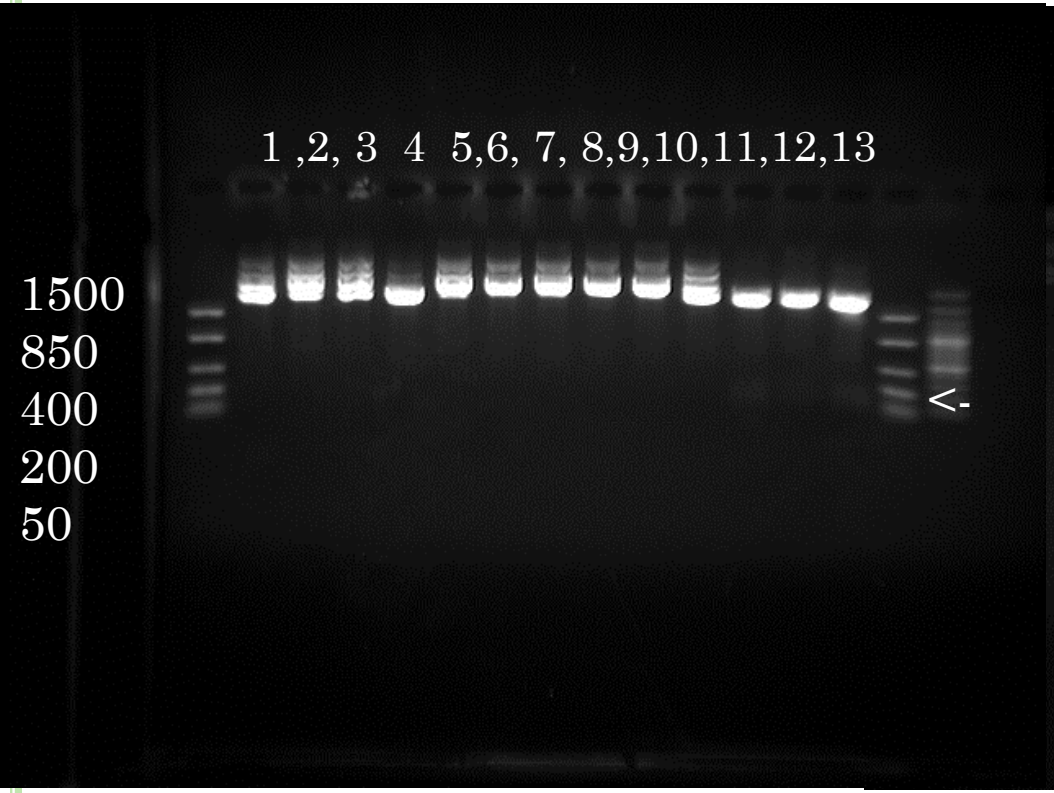
We think we ran this gel for too long and that is why we cant even see the ladders.

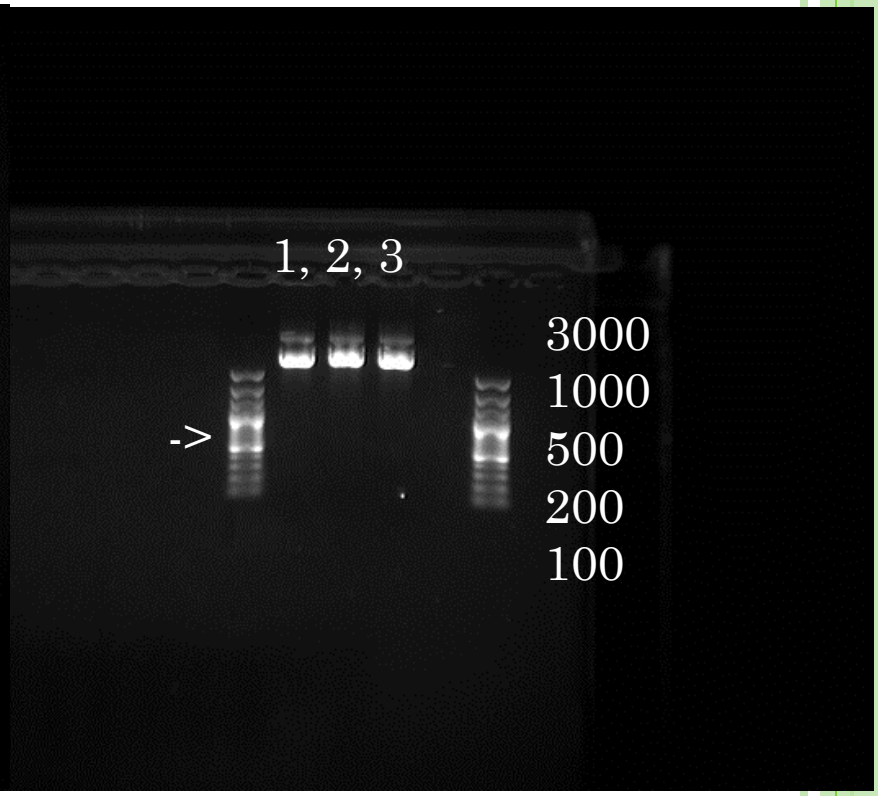
Digestions of our ligated part and part BBa_J23102

- We want to see if we can cut the promoter out of part BBa_J23102, we digested this part with Ecori and Xbal
  - Its about 35bp
- We also had to run another gel on our ligated part. We digested it with Ecori and Spel, again.
  - This time we modified the procedure and replaced the H_2O with DNA, instead of 7µl we had 12µl.



Reran Gel

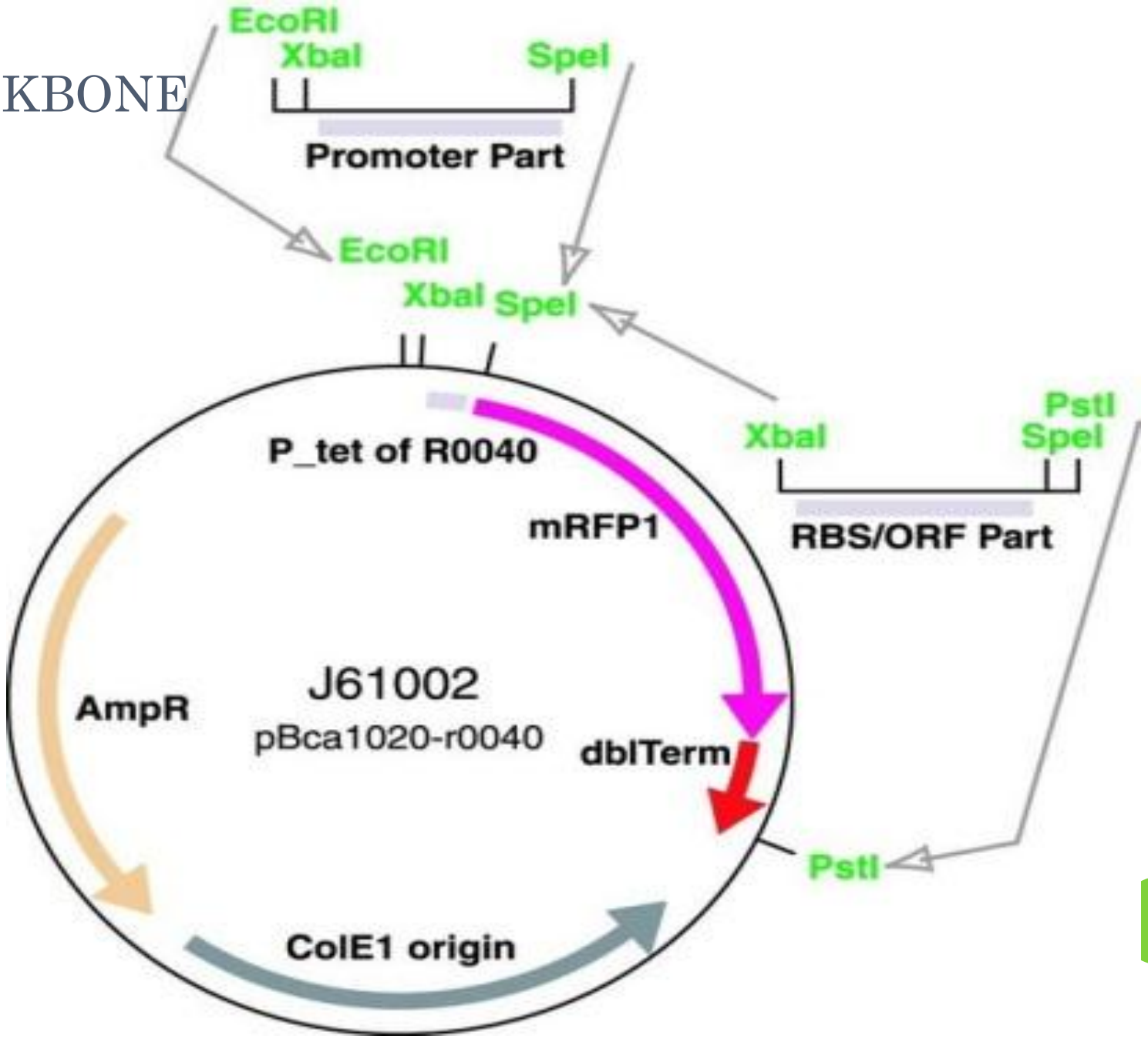

1 ,2, 3 4 5,6, 7, 8,9,10,11,12,13
1500
850
400
200
50
<-


1, 2, 3
->
3000
1000
500
200
100

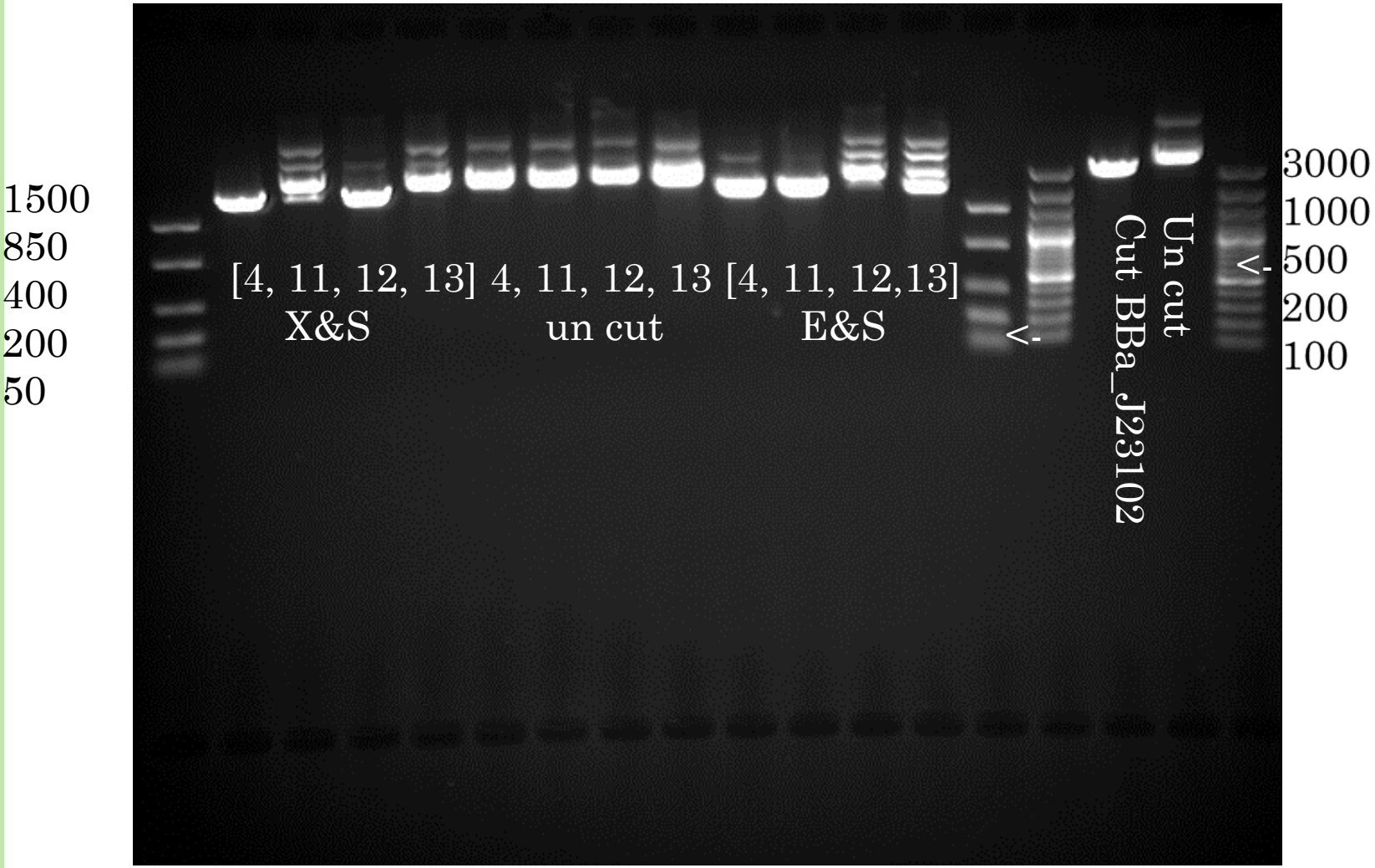
Reexamine

- We reexamined or part BBa_J23102 and realized we needed to cut it with Spel to find out if there is a scar site or not.
- We wanted to be definitive about whether or not the Ecori enzyme was working so we re ran the gel for our ligated part with the enzymes Ecori & Spel in one set and the other with Xbal & Spel
- We also sent the ligated part out to be sequenced along with our ER (BBa_K123003)

BACKBONE


EcoRI
XbaI
SpeI
Promoter Part
EcoRI
XbaI
SpeI
PstI
XbaI
SpeI
RBS/ORF Part
P_tet of R0040
mRFP1
J61002
pBca1020-r0040
AmpR
dblTerm
PstI
ColE1 origin

Results From Ecori Check



Sequencing

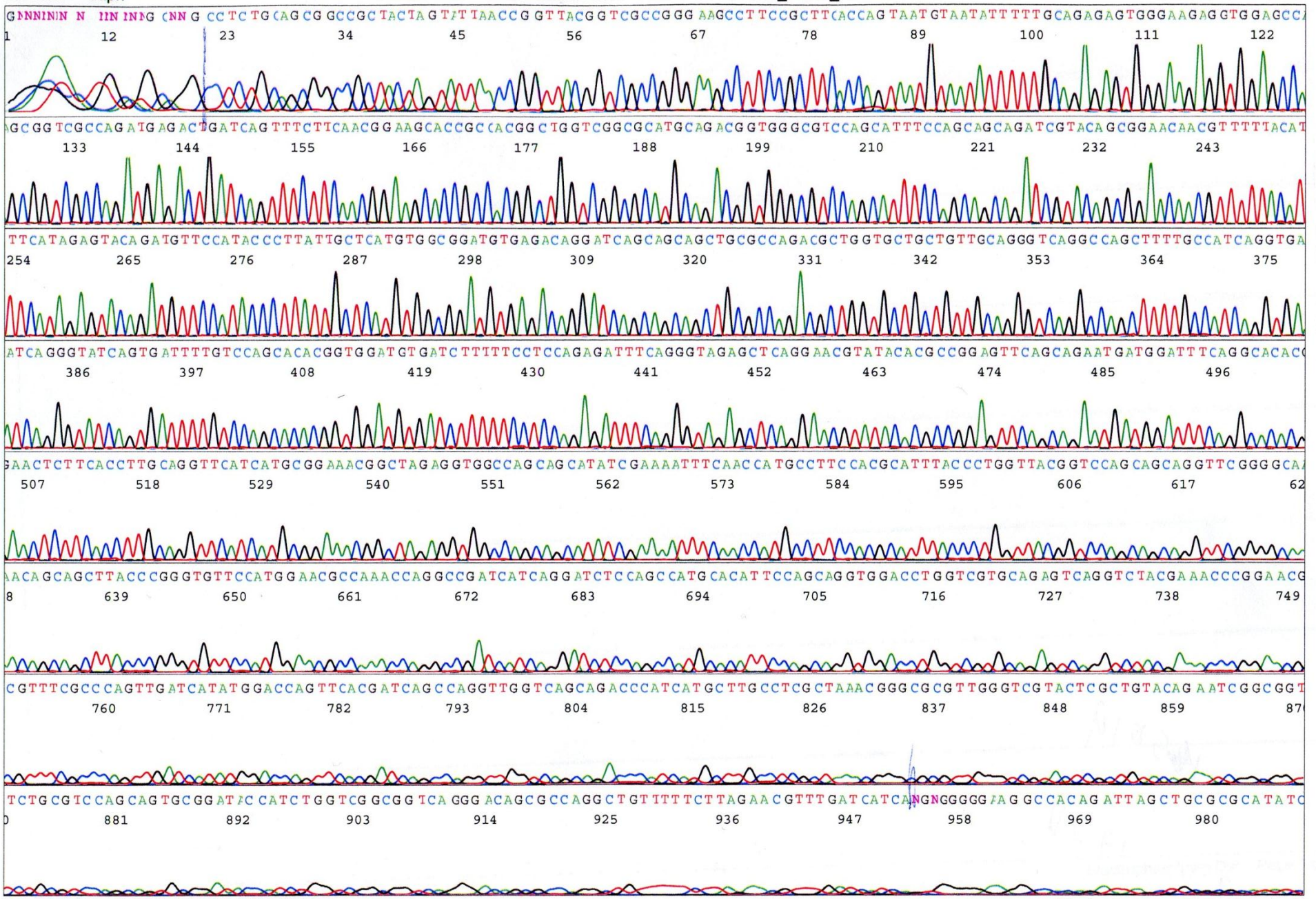
- The sequencing for our parts showed that our ligated part contained no DNA
- BBa_K123003 (ER) was sequenced and we compared it to the NCBI wed site
  - The sequence was a 95% match to the Homo sapiens estrogen receptor isoform 1
  - The 5% difference can be accounted for since after 950 Bps the reaction had ran through and also didn't start till 26 Bps into the sequence because of the primers used in the processes
  - Its expectance value was 1·10 ^-159

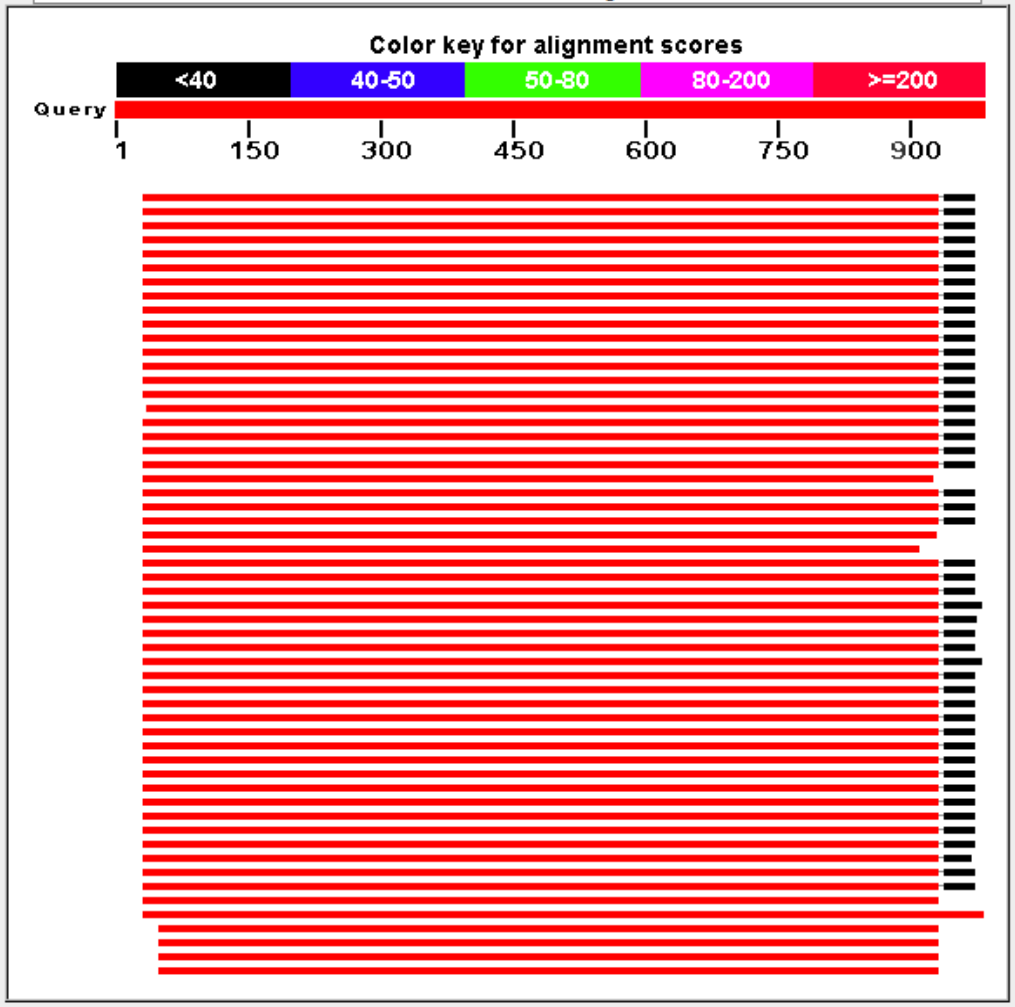
Biosystems

S/N G:170 A:200 T:146 C:226
KB.bcp
KB 1.1.1 Cap:7

ER_R-1_674622
KB_3730_POP7_BDTv3.mob
Pts 1757 to 16537 Pk1 Loc:1756
Version 5.1.1 HiSQV Bases: 1015 Axel Schwekendiek_73532_Run ID: 9003

Nov 23,2010 12:32AM, CST
Nov 23,2010 12:55AM, CST
Spacing:14.19 Pts/Panel1500
Plate Name: OC9003





criptions

nd for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AAX42995.1	estrogen receptor 1 [synthetic construct]	558	590	95%	1e-159	100%	
NP_000116.2	estrogen receptor isoform 1 [Homo sapiens] >ref|NP_001116212.1| e	558	590	95%	1e-159	100%	GM
AAD52984.1	estrogen receptor alpha [Homo sapiens]	558	590	95%	1e-159	100%	G
BAF85708.1	unnamed protein product [Homo sapiens]	556	588	95%	4e-159	100%	G

Accession	Description	Max score	Total score	Query coverage	E value
8877		1088	1088	96%	0.0

gnments

Select All Get selected sequences

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>lcl|8877
Length=1849

 Score = 1088 bits (589),  Expect = 0.0
 Identities = 838/958 (88%), Gaps = 20/958 (2%)
 Strand=Plus/Minus

Query  26    TTAACCGGTTACGGTCGCCGGGAAGCCTTCCGCTTCACCAGTAATGTAATATTTTTGCAG  85
             ||||||||| ||||||||||||| |||||||||| || || ||||| |||||||| |
Sbjct  1849  TTAACCGGTAACGGTCGCCGGGAAACCTTCCGCTTCGCCGGTGATGTAGTATTTTTGTAA  1790

Query  86    AGAGTGGGAAGAGGTGGAGCCAGCGGTCGCCAGATGAGACTGATCAGTTTCTTCAACGGA  145
             |||||| |||||||| || || |||||||||||| |||||||| || ||||||||||| ||
Sbjct  1789  AGAGTGAGAAGAGGTAGAACCCGCGGTCGCCAGGTGAGACTGGTCGGTTTCTTCAACAGA  1730

Query  146   AGCACCGCCACG-GCTGGTCGGCGCATGCAGACGGTGGGCGTCCAGCATTTCCAGCAGCA  204
              ||||| ||||| |  ||||||||| |||||||||| |||||||||||||||||||||||
Sbjct  1729  CGCACCACCACGAG-AGGTCGGCGCGTGCAGACGGTGCGCGTCCAGCATTTCCAGCAGCA  1671

Query  205   GATCGTACAGCGGAACAACGTTTTTACATTTCATAGAGTACAGATGTTCCATACCCTTAT  264
             | |||||||||||||||||||||| |||||||||||||||| |||||||||| || |
Sbjct  1670  GGTCGTACAGCGGAACAACGTTTTTGCATTTCATAGAGTACAGGTGTTCCATACCTTTGT  1611

Query  265   T-GCTCATGTGGCGGATGTGAGACAGGATCAGCAGCAGCTGCGCCAGACGCTGGTGCTGC  323
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Query  324   TGTTGCAGGGTCAGGCCAGCTTTTGCCATCAGGTGAATCAGGGTATCAGTGATTTTGTCC  383
             ||||| | |||||| || ||||| |||||||||| |||||||| || ||||||||||||
Sbjct  1551  TGTTGTAAGGTCAGACCCGCTTTCGCCATCAGGTGGATCAGGGTGTCGGTGATTTTGTCC  1492

Query  384   AGCACACGGTGGATGTGATCTTTTTCCTCCAGAGATTTCAGGGTAGA-GCTCAGGAACGT  442
             || |||||||||||||| |||||||| |||||||||||||||||||| |  |||||| ||
Sbjct  1491  AGAACACGGTGGATGTGGTCTTTTTCTTCCAGAGATTTCAGGGTAGAAGA-CAGGAAGGT  1433

Query  443   ATACACGCCGGAGTTCAGCAGAATGATGGATTTCAGGCACACGAACTCTTCACCTTGCAG  502
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Summery

- We ended up changing our GFP (BBa_E0240) because we found one that had a promoter and terminator with it.
  - Parts BBa_J23102, BBa_J23100, BBa_J23119
- Our ligated part (ERE, BBa_J23119) contained no DNA determined by sequencing and 3 gels.
- We were unable to start assembling our second part containing the ER (BBa_K123003) and BBa_J23102.
- We ran 12 gels total and had 37 mini preps.