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June 2021

Sunday, June 6, 2021

To Do:

1. Count plates
2. Diagnostic digests

Results and Data:

When Marisa digested pKR14, pKR15, and pKR16 with NdeI/BamHI, we expected bands of ~260 bp. Hannah sequenced these plasmids after she made them and both cut site are in the region she sequenced, so I didn't expect any issues. However, the digest yielded 2 bands of high molecular weight (poorly resolved). Note that the size of the plasmid is about 7,700 bp. So the sizes could be consistent with a single cut of some plasmid and some uncut. But one digest performed with pKR14 went overnight (see Marisa's results from 6/2/21)- unlikely to be uncut if it went overnight. Are there two populations of plasmid? Would be surprising if all three had a contaminating plasmid.

Perform some diagnostic digests:

Digest	Plasmid	Enzyme 1	Enzyme 2	Bands	Logic
1	pKR14	NdeI	BamHI-HF	7450, 262	Can I replicate Marisa's digest?
2	pKR14	KpnI-HF	BamHI-HF	7109, 339, 264	Can I cut out rpsU1 and is it the right size?
3	pKR14	NdeI	EcoRV-HF	7418, 294	Can I cut out rpsU1 with NdeI and EcoRV? Is NdeI working?
4	pKR21	NdeI	BamHI-HF	7449, 1576	Does this work on another pF plasmid?
5	pKR21	NdeI	EcoRV-HF	7417, 1608	Mistake- meant to use NotI-HF and BamHI-HF
6	pKL85	NdeI	BamHI-HF	7944, 351	Is it this combination of enzymes or the plasmid??
7	pKL85	NdeI	EcoRV-HF	4334, 3784, 177	Is it NdeI?

pKR14 pF-nat-*rpsU1* is the plasmid we had been trying to clone with.
pKR21 pF-nat-FTL-1251 is the plasmid we can try cloning with (and an alternate strategy that uses a NotI site)
pKL85 is a pEX plasmid (not a pF plasmid at all, to see if something odd happened with all the pF plasmids)

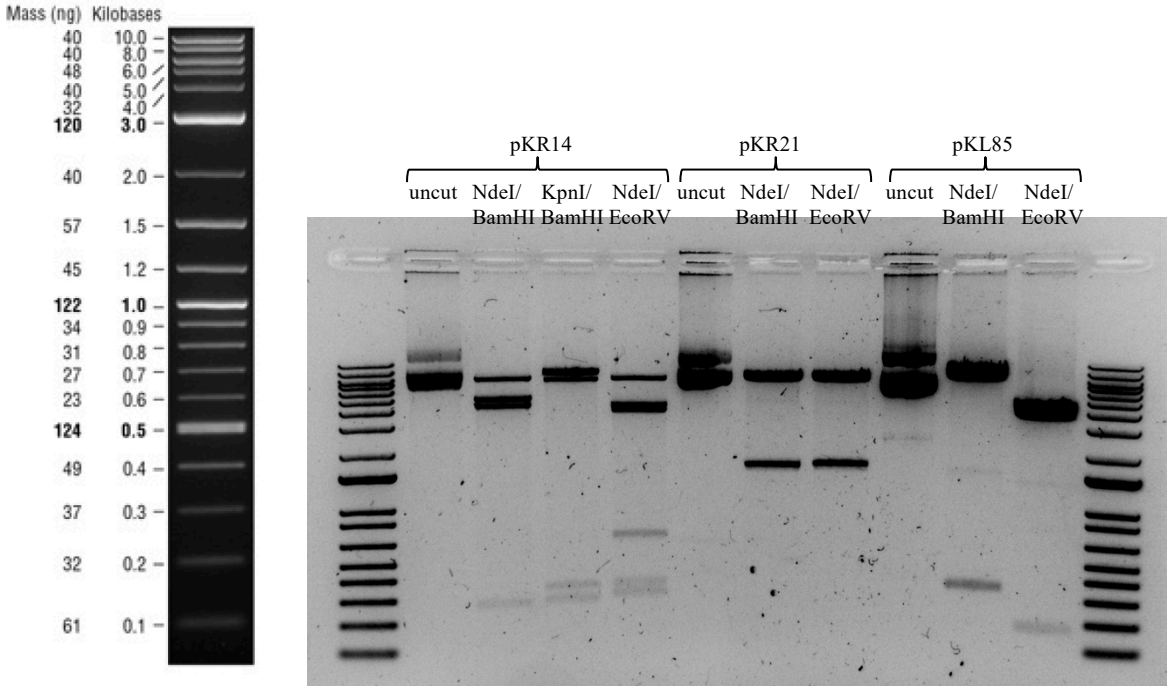
Master mix:

Number of rxns		8
Water	15	120
10x CSB	2	16
Plasmid	2	indiv
Enzyme 1	0.5	indiv
Enzyme 2	0.5	indiv
Total Vol	20	

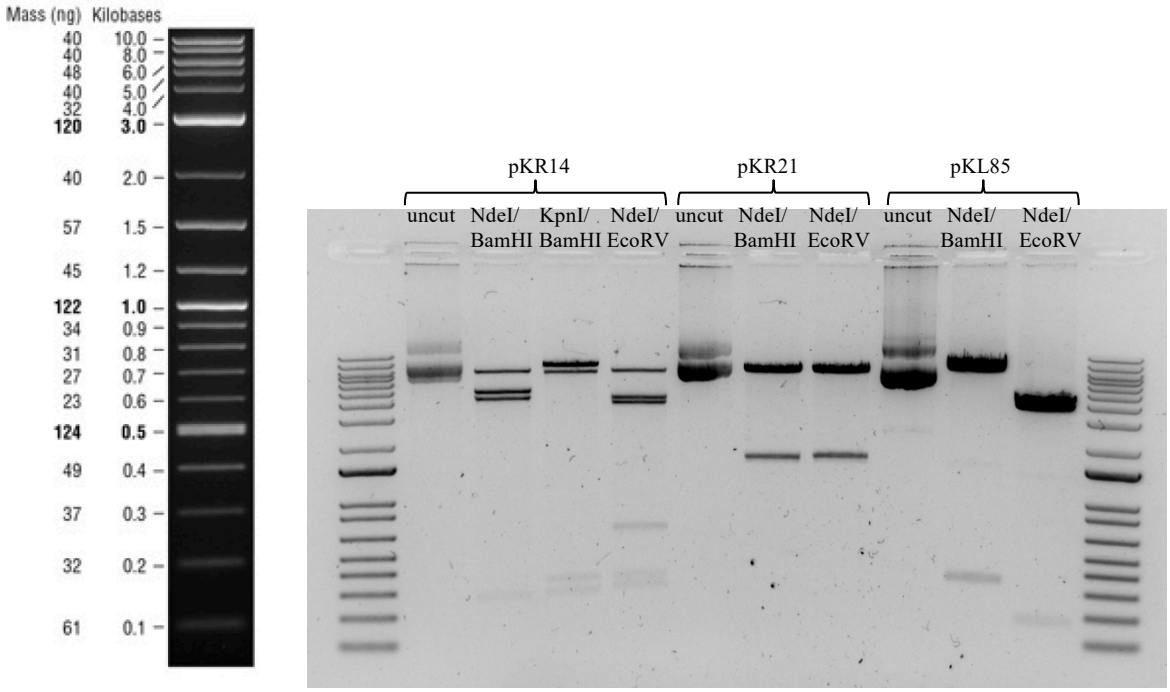
Aliquot 17ul water plus cutsmart buffer, add plasmid and enzymes individually. Incubate 37°C for ~50'

Run gel. Have ladder on both ends and load 2uL of uncut plasmid before each digest:

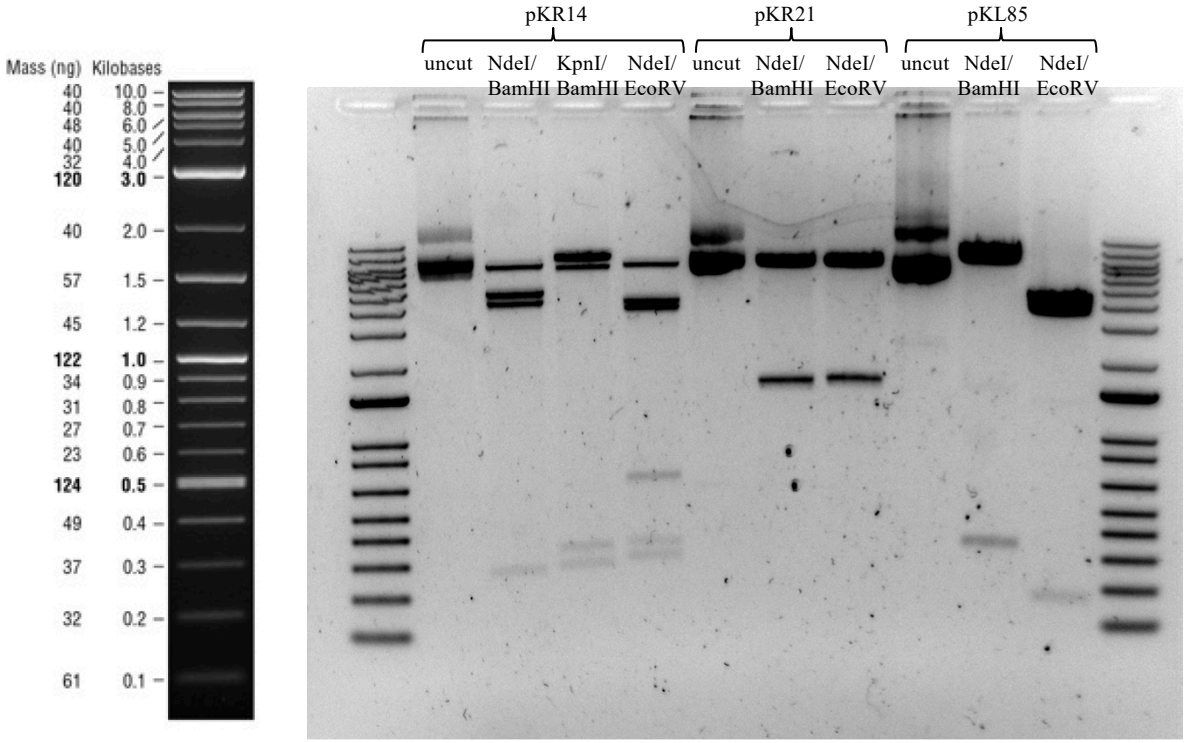
Lane	Contents	Lane	Contents
1	ladder (thermo 1kb plus)	7	Digest 4: pKR21 NdeI/BamHI
2	pKR14 uncut	8	Digest 5: pKR21 NdeI/EcoRV
3	Digest 1: pKR14 NdeI/BamHI	9	pKL85 uncut
4	Digest 2: pKR14 KpnI/BamHI	10	Digest 6: pKL85 NdeI/BamHI
5	Digest 3: pKR14 NdeI/EcoRV	11	Digest 7: pKL85 NdeI/EcoRV
6	pKR21 uncut	12	ladder (thermo)

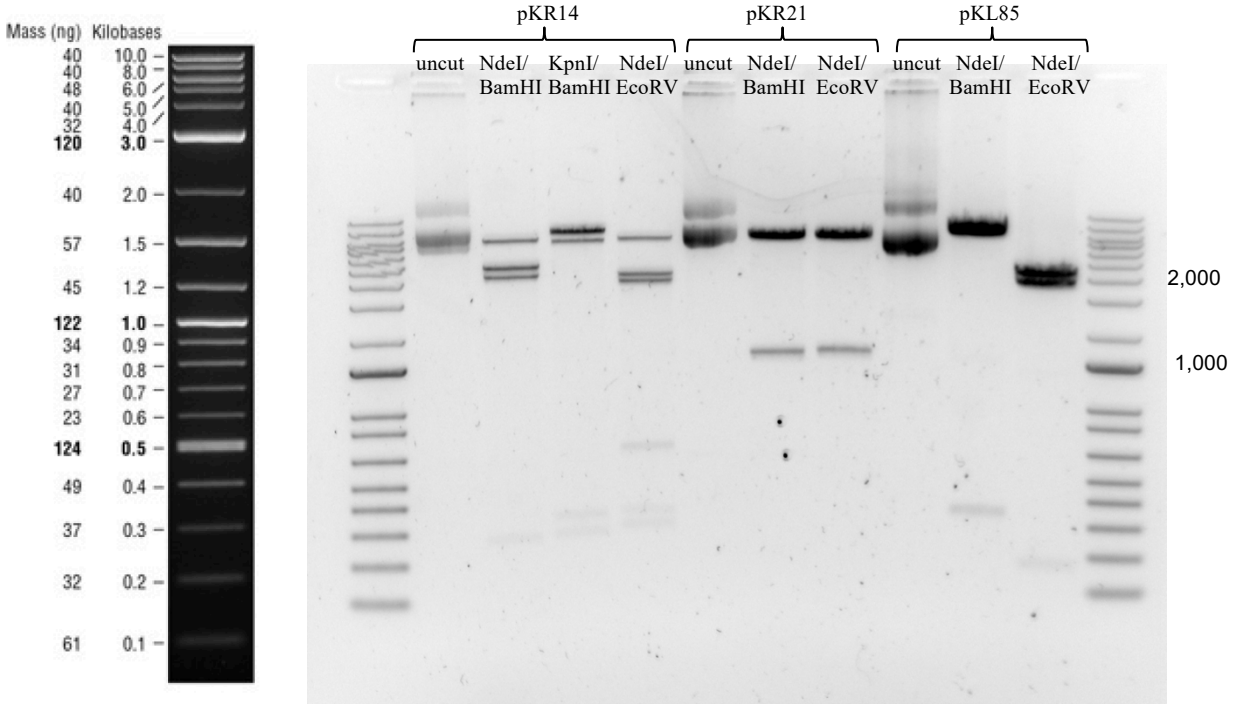


In this first image, you can see the lower MW bands well. In the second image, you can see the individual higher bands more clearly.



Conclusions- there's something off about pKR14. I can see the band corresponding to *rpsU1*, but there are two extra bands. Looks like an extra NdeI cut site? Or another plasmid is present- need to actually figure out what size those bands are. Run longer to see (although I don't have much time, so can't be too much longer! ☹)





Is pKR14 contaminated with a pEX plasmid?

Future To-Do

Move 1° LVS pKR10-1 into strain box

Bibliography

Chalabaev, S., Anderson, C., Onderdonk, A., Kasper, D. (2011). **Sensitivity of Francisella tularensis to ultrapure water and deoxycholate: implications for bacterial intracellular growth assay in macrophages.** Journal of microbiological methods 85(3), 230 - 232. https://dx.doi.org/10.1016/j.mimet.2011.03.006

Hoang, K., Fitch, J., White, P., Mohapatra, N., Gunn, J. (2020). **The sensor kinase QseC regulates the unlinked PmrA response regulator and downstream gene expression in Francisella** Journal of Bacteriology https://dx.doi.org/10.1128/jb.00321-20