Preparation of Penn State DNA ladders from pPSU1 and pPSU2 plasmids

1. analytical digest (each plasmid at ~0.1 μg/μl):

water	34 μl
10x NEBuffer 3.1	5 μl
1 μg/μl pPSU1	5 μl
1 μg/μl pPSU2	4 μl
20 units/μl EcoRV	2 μl
	50 μl

water	33 μl
10x NEBuffer 3.1	5 μl
1 μg/μl pPSU1	5 μl
1 μg/μl pPSU2	5 μl
20 units/μl PstI	2 μl
	50 μl

water	17 μl
10x NEBuffer 3.1	2.5 μl
1 μg/μl pPSU1	2.5 μl
1 μg/μl pPSU2	2 μl
10 units/μl NcoI	1 μl
	25 μl

digest at 37°C for 2 hours, check 1 μl of digest on 1% agarose gel

2. preparative digest (each plasmid at ~0.25 μg/μl):

water	80 μl
10x NEBuffer 3.1	20 μl
1 μg/μl pPSU1	50 μl
1 μg/μl pPSU2	40 μl
20 units/μl EcoRV	10 μl
	200 μl

water	70 μl
10x NEBuffer 3.1	20 μl
1 μg/μl pPSU1	50 μl
1 μg/μl pPSU2	50 μl
20 units/μl PstI	10 μl
	200 μl

water	40 μl
10x NEBuffer 3.1	10 μl
1 μg/μl pPSU1	25 μl
1 μg/μl pPSU2	20 μl
20 units/μl NcoI	5 μl
	100 μl

digest at 37°C overnight, check 0.5 μl of digest on 1% agarose gel

3. dilution of preparative EcoRV digest for ~20 ng/μl 1 kb ladder working stock (-NcoI digest):

10 mM Tris-Cl pH 8.0, 0.1 mM EDTA	1.98 ml
EcoRV digest containing ~0.25 μg/μl pPSU1 and ~0.25 μg/μl pPSU2	0.1 ml
6x gel loading buffer	0.42 ml
	2.5 ml

4. dilution of preparative EcoRV digest for ~30 ng/μl 1 kb ladder working stock (+NcoI digest):

10 mM Tris-Cl pH 8.0, 0.1 mM EDTA	1.93 ml
EcoRV digest containing ~0.25 μg/μl pPSU1 and ~0.25 μg/μl pPSU2	0.1 ml
NcoI digest containing ~0.25 μg/μl pPSU1 and ~0.25 μg/μl pPSU2	0.05 ml
6x gel loading buffer	0.42 ml
	2.5 ml

5. dilution of preparative PstI digest for ~20 ng/μl 100 bp ladder working stock:

10 mM Tris-Cl pH 8.0, 0.1 mM EDTA	1.98 ml
PstI digest containing ~0.25 μg/μl pPSU1 and ~0.25 μg/μl pPSU2	0.1 ml
6x gel loading buffer	0.42 ml
	2.5 ml

Notes

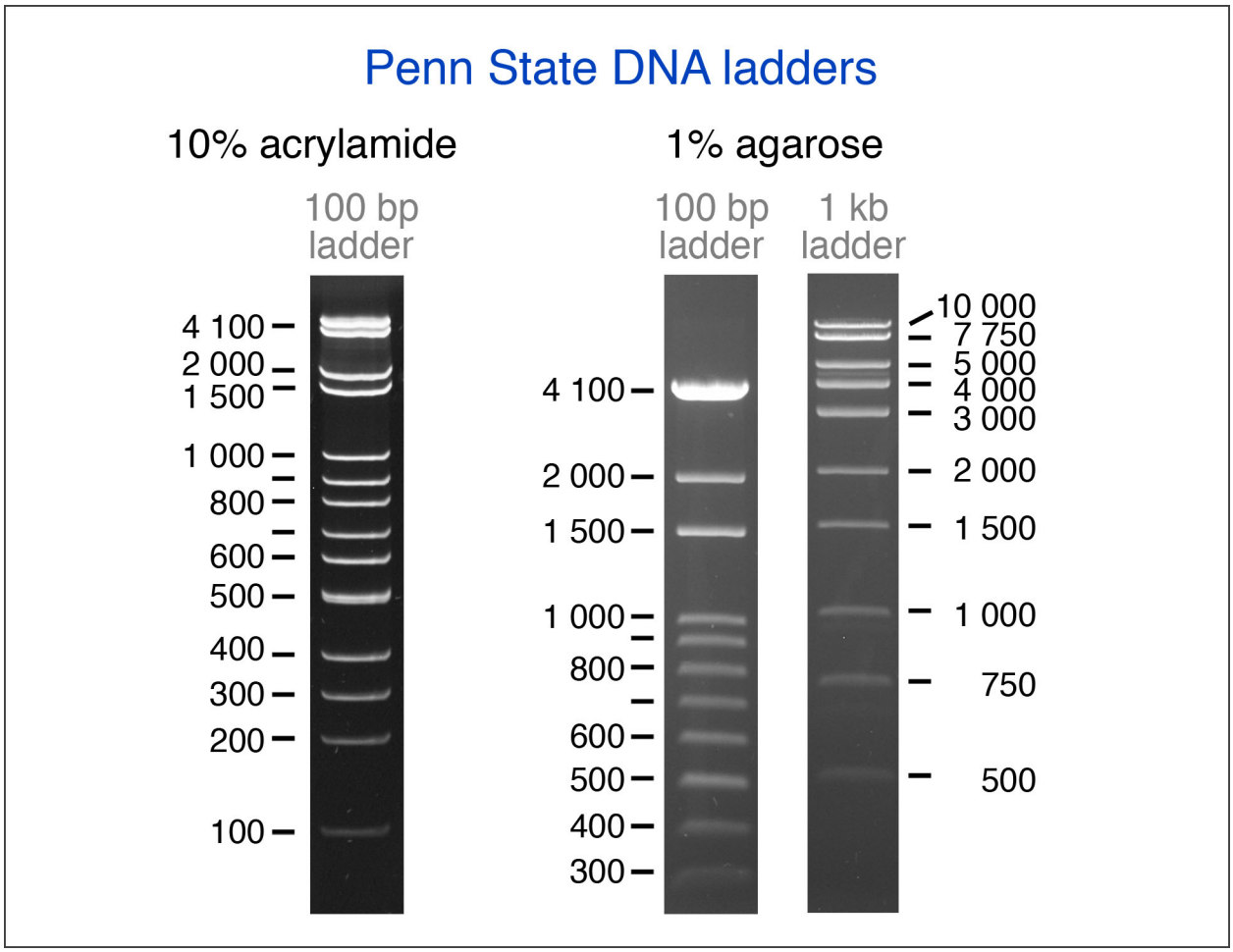
1. Separate restriction digestions of pPSU1 and pPSU2 are recommended if the concentrations of the DNA concentrations of the two plasmids are not well determined, as might be the case if RNA contamination is present.

2. The amount of restriction enzyme provided is a guide. More or less restriction enzyme may be necessary depending on the quality of the plasmid prep and the source of the restriction enzyme.

3. The suggested working stock of ~20 ng/μl is sufficiently concentrated for our needs, but you may wish to use a higher or lower concentration.

4. We use 10-20 μl of the 1 kb ladders on agarose gels, and 5 μl of the 100 bp ladder on polyacrylamide gels.

5. Gels for the Penn State ladders are provided on p. 3 of this document.



The figure above can be printed, cut along the border and inserted into a 5 inch x 7 inch photo holder.