Preparation of Penn State DNA ladders from pPSU1 and pPSU2 plasmids

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

water	35.5 μl
10x NEBuffer 3.1	5 μl
1 μg/μl pPSU1	5 μl
1 μg/μl pPSU2	4 μl
20 units/μl EcoRV	0.5 μl
	50 μl

water	35 μl
10x NEBuffer 3.1	5 μl
1 μg/μl pPSU1	5 μl
1 μg/μl pPSU2	4 μl
20 units/μl PstI	1 μl
	50 μl

water	17.5 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	0.5 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	89 μl
10x NEBuffer 3.1	20 μl
1 μg/μl pPSU1	50 μl
1 μg/μl pPSU2	40 μl
20 units/μl EcoRV	1 μl
	200 μl

water	85 μl
10x NEBuffer 3.1	20 μl
1 μg/μl pPSU1	50 μl
1 μg/μl pPSU2	40 μl
20 units/μl PstI	5 μl
	200 μl

water	44.5 μl
10x NEBuffer 3.1	10 μl
1 μg/μl pPSU1	25 μl
1 μg/μl pPSU2	20 μl
10 units/μl NcoI	0.5 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 ~0.20 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 ~0.20 μg/μl pPSU2	0.1 ml
NcoI digest containing ~0.25 μg/μl pPSU1 and ~0.20 μg/μl pPSU2	0.05 ml
6x gel loading buffer	0.42 ml
	2.5 ml

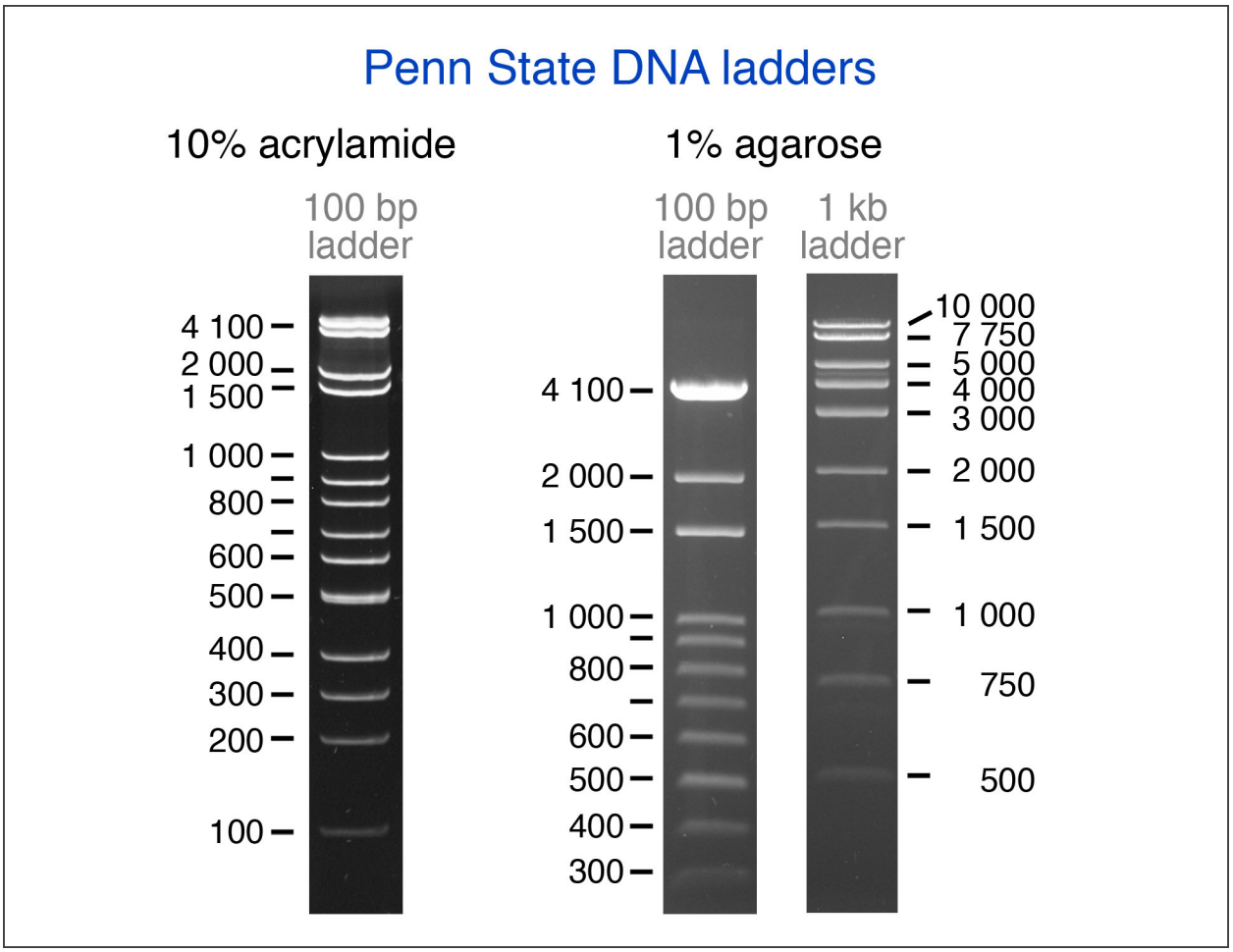
5. dilution of preparative PstI digest for ~20 ng/μl 100 bp ladder working stock:

10 mM Tris-Cl pH 8.0, 0.1 mM EDTA	1.98 ml
PstI digest containing ~0.25 μg/μl pPSU1 and ~0.20 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 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 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.

PstI			**EcoRV**			**NcoI**
pPSU1	**pPSU2**	**pPSU1 & pPSU2**	**pPSU1**	**pPSU2**	**pPSU1 & pPSU2**	**pPSU1 & pPSU2**
4 100	4 100	2x 4 100	5 000	4 000	5 000	10 000
2 000	1 500	2 000	2 000	3 000	4 000	7 750
1 000	600	1 500	1 500	750	3 000	
900	500	1 000	1 000		2 000	
800	400	900	500		1 500	
700	300	800			1 000	
500	200	700			750	
	100	600			500	
	50	2x 500				
		400				
		300				
		200				
		100				
		50				



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