BUFFER CHANGE

HindIII

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM MgCl2
1 mM Dithiothreitol
pH 7.9 @ 25°C

PstI

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM MgCl2
1 mM Dithiothreitol
pH 7.9 @ 25°C

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50 mM NaCl
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PstI

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM MgCl2
1 mM Dithiothreitol
pH 7.9 @ 25°C

Digest DNA first with enzyme HindIII, which cut in buffer 2

DNA	20 ul
Buffer 2 10x	5 ul
BSA 10x	5 ul
HindIII 20u/ul	1 ul
Water	21 ul
Tot	50 ul

Verify that HindIII completely digested DNA, running 5 ul on agarose gel

Digest the remaining 45 µl of DNA with the second enzyme PstI which cuts in buffer 3.

Do not load DNA on a column to purify it, but increase the volume of the reaction to 100 µl and add buffer*, NaCl and Tris buffer to convert the buffer 2 into buffer 3.

*Try two approaches:

1-add buffer 2
2-add buffer 3

?

A) ADD BUFFER 2

DNA digested in buffer 2	45 µl
Buffer 2 10x	µl
NaCl 1M	µl
Tris HCl 1M	µl
water	µl
BSA 10x	µl
Enzyme PstI 20u/ul µl	
Tot	100 µl

B) ADD BUFFER 3

DNA digested in buffer 2	45 µl
Buffer 3 10x	µl
NaCl 1M	µl
Tris HCl 1M	µl
water	µl
BSA 10x	µl
Enzyme PstI 20u/ul µl	
Tot	100 µl

1X NEBuffer 2:
50 mM NaCl
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1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM MgCl2
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Buffer 3		Buffer 2		What is missing?
100 mM NaCl 50 mM Tris-HCl	-	50 mM NaCl 10 mM Tris-HCl	=	

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50 mM NaCl
10 mM Tris-HCl
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pH 7.9 @ 25°C

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM MgCl2
1 mM Dithiothreitol
pH 7.9 @ 25°C

Buffer 3		Buffer 2		What is missing?
100 mM NaCl 50 mM Tris-HCl	-	50 mM NaCl 10 mM Tris-HCl	=	50 mM NaCl 40 mM Tris-HCl

Solution A:

1 - add buffer 2 to the new 55 ul

2 - transform total 100 ul (which are in buffer 2) in buffer 3*

Stock	**ul**	**final conc**
DNA digested in buffer 2	45 ul	

Solution A:

1 - add buffer 2 to the new 55 ul

2 - transform total 100 ul (which are in buffer 2) in buffer 3*

Stock	**ul**	**final conc**
DNA digested in buffer 2	45 ul	
Buffer 2 10x	5,5 ul	1x

Solution A:

1 - add buffer 2 to the new 55 ul

2 - transform total 100 ul (which are in buffer 2) in buffer 3*

Stock	**ul**	**final conc**
DNA digested in buffer 2	45 ul	
Buffer 2 10x	5,5 ul	1x
NaCl 1M		50 mM
Tris HCl 1M		40 mM
Water to 100ul		
BSA 10x		1x
Enzyme PstI20u/ul		20 u
Tot	100 ul	

- 1000mM:50mM = 20 = dilution factor
- 1000mM:40mM = 25 = dilution factor

Solution A:

1 - add buffer 2 to the new 55 ul

2 - transform total 100 ul (which are in buffer 2) in buffer 3*

Stock		**ul**	**final conc**
DNA digested in buffer 2		45 ul	
Buffer 2	10x	5,5 ul	1x
NaCl	1M	5 ul	50 mM
Tris HCl	1M	4 ul	40 mM
Water to 100ul		34 ul	
BSA	10x	5,5 ul	1x
Enzyme PstI	20u/ul	1 ul	20 u
Tot		100 ul	

* NaCl - 1000mM:50mM = 20 = dilution factor 100ul:20=5 ul

* Tris HCl - 1000mM:40mM = 25 = dilution factor 100ul:25=4 ul

Solution B:

1 - add buffer 3 to the new 55 ul

2 - transform the first 45 ul (which are in buffer 2) in buffer 3*

Stock	**ul**	**final conc**
DNA digested in buffer 2	45 ul	

Solution B:

1 - add buffer 3 to the new 55 ul

2 - transform the first 45 ul (which are in buffer 2) in buffer 3*

Stock	**ul**	**final conc**
DNA digested in buffer 2	45 ul	
Buffer 3 10x	5,5 ul	1x

Solution B:

1 - add buffer 3 to the new 55 ul

2 - transform the first 45 ul (which are in buffer 2) in buffer 3*

Stock	**ul**	**final conc**
DNA digested in buffer 2	45 ul	
Buffer 3 10x	5,5 ul	1x
NaCl 1M		50 mM
Tris HCL 1M		40 mM
Water to 100ul		
BSA 10x		1x
Enzyme PstI 20u/ul		20 u

Tot	100 ul	

*1000mM:50mM=20 = dilution factor

*1000mM:40mM=25 = dilution factor

Solution B:

1 - add buffer 3 to the new 55 ul

2 - transform the first 45 ul (which are in buffer 2) in buffer 3*

Stock	**ul**	**final conc**
DNA digested in buffer 2	45 ul	
Buffer 3 10x	5,5 ul	1x
NaCl 1M	2,25 ul	50 mM
Tris HCl 1M	1,8 ul	40 mM
Water to 100ul	38,95 ul	
BSA 10x	5,5 ul	1x
Enzyme PstI 20u/ul	1 ul	20 u

Tot	100 ul	

*NaCl - 1000mM:50mM=20 (dilution factor) 45ul:20=2,25 ul

*Tris HCl - 1000mM:40mM=25 (dilution factor) 45ul:25=1,8ul