FusionPCR (USE Primestar!)

1st round PCR **without** primers (25 µl):

Components	**Amounts (µl)**	**Final []**	**Cycle (15x)**		
ddH_2O	25-(8.25+DNA) µl		Initial denaturation	95°C	3 min
5x PS buffer	5 µl	1x	**Denaturation**	**98°C**	**10 sec**
2.5 mM dNTPs	3 µl	300 µM	**Annealing T**	**58°C**	**15 sec**
(If dNTPs 10 mM)	(0.75 µl)	(300 µM)	**Extension****	**72°C**	**1min/kb**
Template DNA	*		Final extension	72°C	10 min
Polymerase PS	0.25 µl	-	Forever	15°C	-

*DNA template: Mix the purified fragments with a molar ratio at **1:3:5:7:XX:7:5:3:1**. The molar of DNA fragment increases from termini to middle with a arithmetic of 2. The amount of terminal DNA is 50-100 ng/kb (0.05 ng/bp).
For example: if you have three fragments, say promoter-TF-terminator then you should have 1:3:1;

Promoter 0.05 ng/bp (1x)
TF 0.15 ng/bp (3x)
Terminator 0.05 ng/bp (1x)

By multiplying with the length (bp) of your fragments you get the amount you need (ng). Divide this with the concentration of your fragments and you get the volume (µl). Total volume of your fragments will then correspond to *. The reason for why these ratios need to be considered is to minimize the risk of unspecific bands. You want to have more of the middle fragments to minimize the risk of unspecific bands.

** Extension depends on the **longest** fragment.

2nd round PCR **with** primers (50 µl):

Components	**Amounts (µl)**	**Final []**	**Cycle (35x)**		
ddH_2O	30.5 µl		Initial denaturation	95°C	3 min
5x PS buffer	10 µl	1x	**Denaturation**	**98°C**	**10 sec**
2.5 mM dNTPs	4 µl	200 µM	**Annealing T**	**58°C**	**15 sec**
(If dNTPs 10 mM)	(1 µl)	(200 µM)	**Extension**^^^	**72°C**	**1min/kb**
^10 µM $primer_{forward}$	1.5 µl	300 µM	Final extension	72°C	10 min
^10 µM $primer_{reverse}$	1.5 µl	300 µM	Forever	15°C	-
Template DNA	^^				
Polymerase PS	0.5 µl	-			

^ Add forward primer for the **first** fragment and reverse primer for the **last** fragment.
^^ **2 µl** from the unpurified PCR products from the 1st round.
^^^ Depends on the **entire** fragment.