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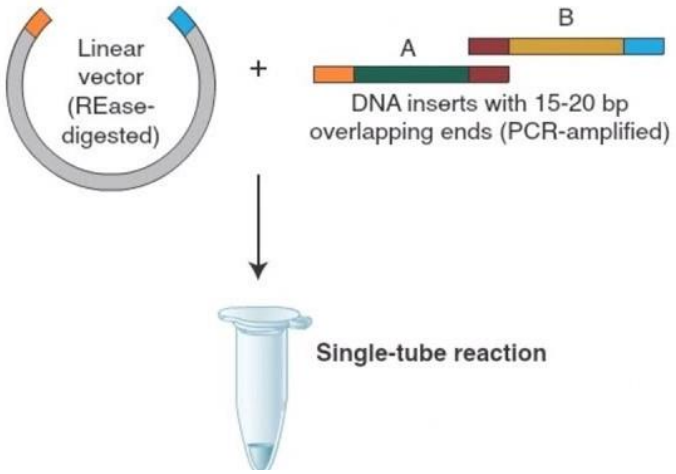
One Step Seamless Cloning Mix (CW3034)

Storage Condition: -20°C
Kit Components:

Component	CW3034S (20 rxns)	CW3034M (50 rxns)
2x Cloning MasterMix	100 µl	250 µl
PUC19 Vector, Linearized (20ng/µl)	10 µl	20 µl
500 bp Control Insert (20ng/µl)	10 µl	20 µl
RNase-Free Water	1 ml	1 ml



Optimal Quantities

We recommend a total of 0.02–0.5 pmols of DNA fragments when 1 or 2 fragments are being assembled into a vector and 0.2–1.0 pmoles of DNA fragments when 4–6 fragments are being assembled. Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, using the following formula:
pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)
50 ng of 5000 bp dsDNA is about 0.015 pmols.
50 ng of 500 bp dsDNA is about 0.15 pmols.

The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm or estimated from agarose gel electrophoresis followed by ethidium bromide staining.

Cloning Protocol

1. Set up the following reaction on ice:

	Recommended Amounts of Inserts Used for Assembly		
	1 Insert	2-5 Inserts	Positive Control**
Linear Vector (20-50 ng)	X µl 0.03 pmol	X µl 0.03 pmol	3 µl
Inserts	Y µl 0.06 pmol	Y µl 0.03 pmol/fragment	1 µl
Cloning Master Mix (2X)	5 µl	5 µl	5 µl
Deionized H_2O	5-X-Y µl	5-X-Y µl	1
Total Volume	10 µl***	10 µl***	10 µl

2. * Optimized cloning efficiency is 50-100 ng of vector with a 2-fold molar excess of each insert. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in cloning reaction should not exceed 20%.
   ** Control reagents are provided for 3 experiments.
   *** If greater numbers of fragments are assembled, additional Cloning MasterMix may be required.

3. Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 30 minutes when 4-6 fragments are being assembled. Following incubation, store samples on ice or at –20°C for subsequent transformation.