DESCRIPTION

The Harmony Cloning Kit is designed for efficient cloning of one or more DNA fragments into any stable vector. PCR amplified DNA fragments designed with 15 base pairs of homology to the vector are fused together by homologous recombination. This process is quick and easy as reactions take place in the provided tube on ice and then transformed directly into competent bacterial cells. This allows for seamless constructs with correct orientation and has the flexibility to modify, add or remove restriction sites if needed. The Harmony Cloning Kit can be stored at -20ºC for up to 3 months.

PRODUCT ADVANTAGES

- Flexible. Clone any insert into any stable vector.
- Simple. Incubate all reactions on ice and perform bacterial transformation in one tube.
- Efficient. Fuse multiple DNA fragments with any vector in one reaction.
- Seamless. Get final constructs with desired restriction sites and no extra bases.

KIT CONTENTS

Kit Contents	Quantity	Preparation and Storage Notes
Ready-to-use Harmony mix	1 µL x 100 tubes	Store at -20ºC
Control Insert and Control Vector		

NOTE

Not compatible with highly unstable vectors.

REAGENTS REQUIRED BUT NOT PROVIDED

- DNA polymerase (for PCR amplification)
- Chemically competent cells
- SOC medium.

Product No.	Size	Price
M5100	1 Kit (100 reactions)	$280.00

PREPARATION INSTRUCTIONS

1. Keep Ready-to-use Harmony mix at -20ºC
2. Insert preparation: Design PCR primers to contain at least 15 base pairs of homology with the linearized destination vector (See PCR Primer Design section below). Amplify PCR fragments and confirm size on an agarose gel. Cut out band and purify from agarose gel by spin-column purification.
3. Vector preparation: Linearize vector by restriction enzyme digest. Cut out band and purify from agarose gel by spin-column purification.

PROTOCOL

1. Set up Harmony cloning reaction

X uL	Insert (3:1 molar ratio insert to vector)
X uL	Vector (50-100 ng)
1 uL	Ready-to-use Harmony mix
5-10 uL	Total volume

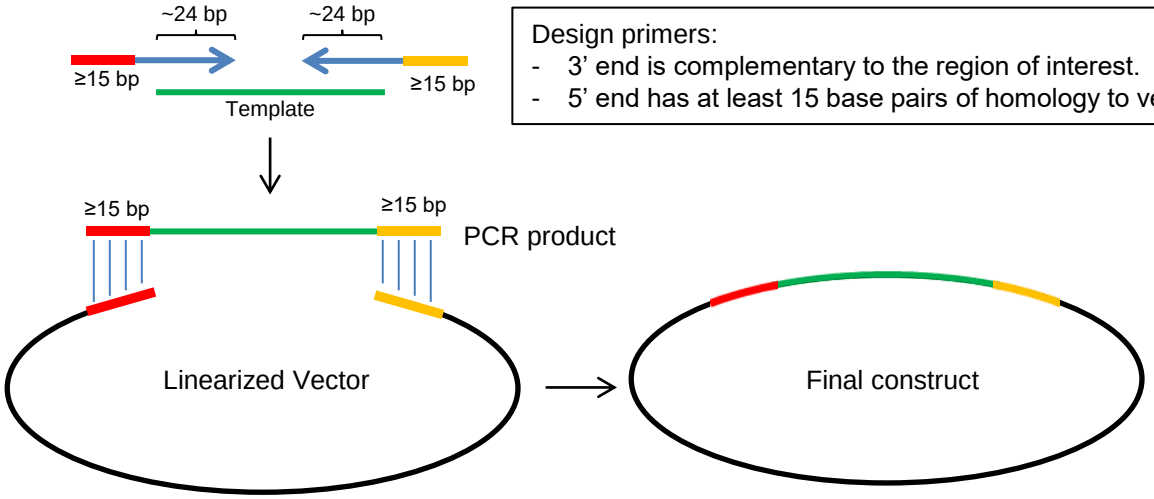
2. Incubate reaction on ice for 30 minutes

   Note: Depending on the length of the insert, and number of inserts, it may be necessary to increase the incubation time.

3. Transformation

   Add chemically competent cells directly to Harmony cloning mixture. Follow transformation protocol for specific

PCR PRIMER DESIGN



Design primers:
- 3’ end is complementary to the region of interest.
- 5’ end has at least 15 base pairs of homology to vector.

MOLAR RATIO CALCULATION

$$\frac{\text{ng of vector} \quad X \quad \text{kb size of insert}}{\text{kb size of insert}} \quad X \quad \text{Molar ratio} \quad \frac{\text{Insert}}{\text{Vector}} \quad = \quad \text{ng of insert}$$