OVERLAP EXTENSION PCR (OE-PCR) FOR CONSTRUCTION OF CHIMERIC PROTEINS PROTOCOL

MATERIAL

- Q5 High Fidelity Polymerase (2X Master Mix) from NEB
- Plasmid Templates
- Extension Primers
- dH_2O
- PCR machine

STRATEGIES

- **1 Fragment**
  - Performing an OE-PCR with only one fragment means that we the extended insert sequence as primers for a whole plasmid amplification to integrate it into the target protein.
- **2 Fragments**
  - Performing an OE-PCR with two fragments corresponds to the linearization and extension of the target protein in the plasmid and the extension of the insert. In a second PCR, we use the extended insert again to circularize the plasmid via its flanking regions.
- **3 Fragments**
  - Performing an OE-PCR with three fragments, we extend the 5' part of the target protein, the 3' part of the target protein and the insert correspondingly with their matching flanking regions. In a second PCR reaction, the fragments are brought together in equimolar amounts and the full length "5'part-Insert-3'part" construct is amplified.

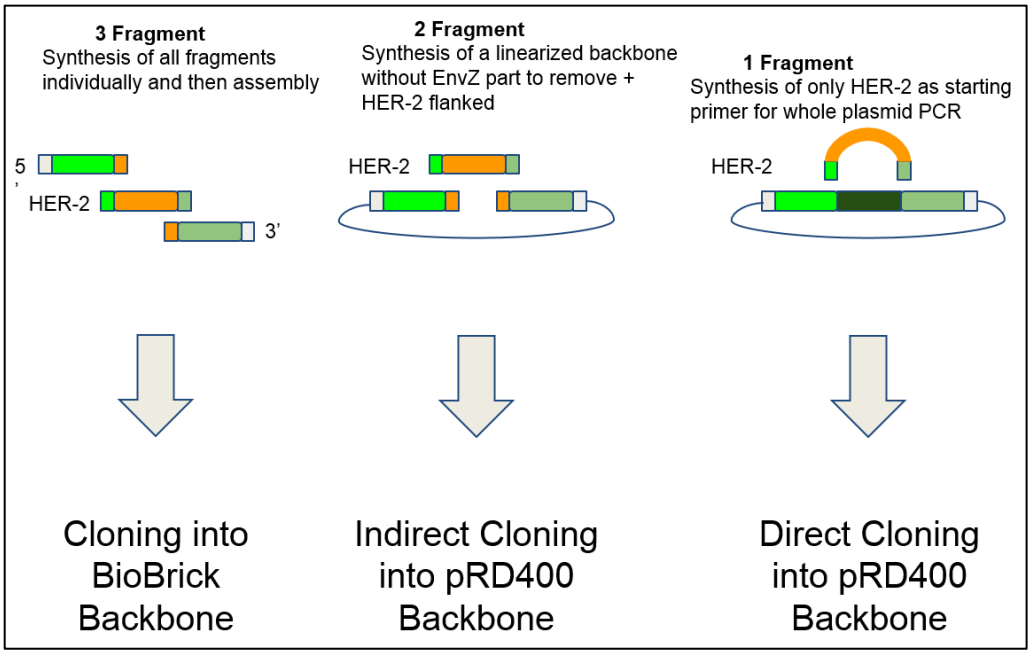

Figure 1: Schematic overview for OE-PCR in the example we used for constructing an Affibody-EnvZ Chimera

Primer Design:

For our experiment, we used the cloning program "Snapgene" to model our construct and to find appropriate primers. While constructing good primers for OE-PCR, we paid attention that the 3' of the primers ends preferably with one or two G or C bases. Furthermore, the constructed primer pairs had close melting temperatures in order to be used optimally in one PCR.

PROCEDURE

In our experiments, we mostly performed "1 Fragment OE-PCR" directly into a plasmid containing our target protein or "3 Fragment OE-PCR". Here we describe the general PCR mix conditions for the extension of single fragments:

Master Mix (for one PCR reaction):

Reagent	Quantities (1X)
2X Q5 Master Mix	25µL
Forward Primer (10µM)	2,5µL
Reverse Primer (10µM)	2,5µL
Template	2,5µL
dH_20	17,5µL

Cycles:

	98°C – 5 min	
30X	98°C – 30 sec	
	X °C – 30-45 sec	→ dependent on melting temperature of the primer pair
	72°C – X min	→ dependent on the size of the fragment
	72°C – 5-10 min	
	4°C – Hold	

During integration of the extended fragment into the circular plasmid template, you will not need any primers, so that your reaction mix will consist of:

25 µL 2X Q5 Master Mix
≈2,5 µL extended Insert
2,5 µL Plasmid template
25 µL dH_2O