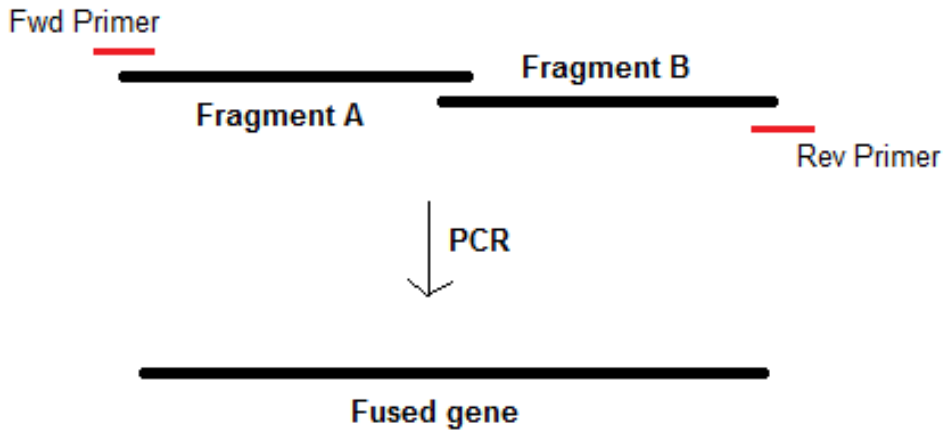
Splice by overlap extension

Protocol

Splice by overlap extension (SOE) is a simple method for fusing two gene fragments together. It is ideal for assembling gBlock fragments or introducing point mutations.

Assembling gBlock fragments

- A 25-30bp overlap with a GC content of roughly 50% between the two fragments is recommended.
- 200ng of each gBlock fragment is synthesized by IDT. Dissolve these fragments with 100uL of autoclaved water for a final concentration of 2ng/uL



PCR mixture

27uL of H_2O
10uL of 5x Phusion buffer
1uL dNTP
1uL DMSO
4uL Fragment A (8ng total)
4uL Fragment B (8ng total)
1uL Fwd Primer
1uL Rev Primer
1uL Phusion polymerase

50uL final volume

PCR Program:

95°C for 5 minutes

95°C for 30 seconds
60°C for 1 minute *
72°C for 1 minute (1 min/kb)
==>Repeat for 35 cycles

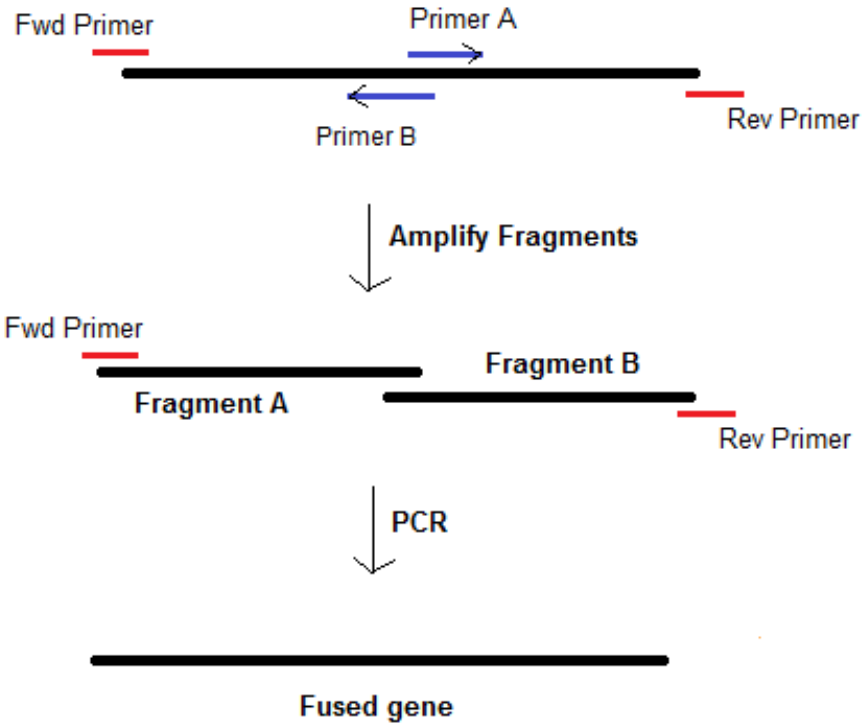
72°C for 10 minutes
4°C hold

* Annealing temperature should be adjusted according to the T_m of the primers.

Once the PCR is complete, run on a gel to confirm whether the genes have fused. Ligation and cloning can then commence.

Point Mutation by SOE

For a point mutation, two internal primers (40bp) are required. These internal primers should have an overlap of roughly 20bp. This overlap region is where the mutation of interest is introduced.



1. First, amplify the two fragments. Set up two PCR mixtures as follows:

Mixture A	Mixture B
34uL of H_2O	34uL of H_2O
10uL of 5x Phusion buffer	10uL of 5x Phusion buffer
1uL dNTP	1uL dNTP
1uL DMSO	1uL DMSO
1uL DNA template (50ng/uL)	1uL DNA template (50ng/uL)
1uL Fwd Primer	1uL Rev Primer
1uL Primer B	1uL Primer A
1uL Phusion polymerase	1uL Phusion polymerase
50uL final volume	50uL final volume

The same SOE PCR program listed above is used.

2. Run on a gel. Excise the two amplified fragments and purify the bands using the Omega Gel extraction kit.

3. Set up another PCR to fuse the two fragments. This time, no internal primers are used:

   PCR Mixture
   25uL of H_2O
   10uL of 5x Phusion buffer
   1uL dNTP
   1uL DMSO
   5uL Fragment A (gel purified product)
   5uL Fragment B (gel purified product)
   1uL Fwd Primer
   1uL Rev Primer
   1uL Phusion polymerase

   _____
   50uL final volume

   The same SOE PCR program listed above is used

4. Once the PCR is complete, run on a gel to confirm whether the genes have fused. Excise the band from the gel and clone into the desired backbone.