# Site-directed mutagenesis PCR

**Date:** 2022-07-26
**Tags:** *3_Cloning 1_Wetlab demo*
**Created by:** Stefanie Brands 

(_Written by Stefanie Brands_)

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The goal of this experiment was to generate a mutagenesis library of the {ligand binding domain of the human farnesoid X receptor FXR|target sequence}. Each single residue of the LBD, which comprises residues {247-467|target residues} of the {FXRalpha2|:isoform:}, was mutated to alanine applying {site-directed mutagenesis|method}. For this, a {modified QuikChange|protocol} was applied. The wildtype FXR sequence was provided by {Dr. Jan Stindt, Klinik für Gastroenterologie, Hepatologie und Infektiologie, UKD|plasmid source} on {April 6, 2022|date of plasmid and strain receival} in {pno-Cherry|vector}.

{19.04.2022|date of experiment}

The PCR for generating the FXR variant {G002A|target mutation} was based on {pnoCherry::FXR alpha2 (as)|:template DNA:}. The wildtype sequence was amplified with the primers {ACCATGgcgTCAAAAATGAATCTCATTGAACATTCCC|forward primer} and {TTCATTTTTGAcgcCATGGTGGCGACCGGTGG|reverse primer}. The PCR product featured {5450|bp|product size}. The PCR mixture of {50|µL|total PCR volume} contained {0.5|□M|(primers)|PCR component} of both forward and reverse primers, {1|×|:Q5 buffer:|PCR component}, {20|ng|template DNA|PCR component}, {0.02|U/□L|Q5 polymerase|PCR component}, {0.2|mM|dNTP mix|PCR component} and {0.8|M|betaine monohydrate|PCR component}.

| Component | c (Stock) | | c (final) | | V [µL] | Mastermix | |
|---|---|---|---|---|---|---|---|
| for Primer | 5 | µM | 0,5 | µM | 5 | 15 | |
| rev Primer | 5 | µM | 0,5 | µM | 5 | 15 | |
| 5x Q5 Buffer | 5 | x | 1 | X | 10 | 30 | |
| Template | 10 | ng/µL | 20 | Ng | 2 | 6 | |
| dNTPs O5 | 10 | mM | 0.2 | mM | 1 | 3 | |
| Betaine | 4 | M | 0.8 | M | 10 | 30 | |
| ddH2O | | | | | 16.5 | 49.5 | |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SUM | | | | | 49.5 | 49.5 | x3 |
| Q5 Polymerase | 2 | U/µL | 0.02 | U/µL | 0.5 | 0.5 | per tube |
| Total | | | | | 50 | 50 | x3 |

After initialization for {30 s at 98 °C|initialization step}, {25|:PCR cycles:} of denaturation, {30 s at 98 °C|denaturation step}, annealing, {30 s at 55–65 °C|annealing step}, and elongation, {3 min at 72 °C|elongation step} were applied.

| Step | Time | | Temp | | |
|---|---|---|---|---|---|
| Initialization | 30 | s | 98 | °C | |
| Denaturation | 30 | s | 98 | °C | 25x |
| Annealing | 30 | s | 55-65 | °C | |
| Elongation | 3 | min | 72 | °C | |
| Storage | ∞ | | 4 | °C | |

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The success of the PCR was evaluated on a {1%|:agarose gel:}. A {5|µL|:PCR sample:} was mixed with {1|µL|:6x loading dye} and separated via {gel electrophoresis|method} at {100 V and 300 mA, (for) 30 min|electrophoresis parameters}. {GeneRuler 1 kb DNA Ladder|marker} was used as reference.

The PCR template was digested with {0.5|µL|:DpnI:|restriction enzyme} per 50 µL PCR for {1 h at 37 °C|incubation}.

The digested PCR was purified applying the {Macherey-Nagel|:PCR purification kit:}. The purified DNA was eluted with {30|µL|:$H_2O$:|elution volume} after {1 min at 70°C|incubation}. The DNA concentration

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was measured with an {Implen N60 Nanophotometer|device}. The purified PCR mix contained {272.2|ng/µL|:DNA:|concentration} and was diluted with $H_2O$ for further use to {87.7|ng/µL|DNA|dilution}.