<https://www.youtube.com/watch?v=-jP0r5tPMeE&list=PLBE756C00F124D7A2&index=12>

**Simply Cloning - Chapter 2 - PCR**

Table PCR mix

|  |  |
| --- | --- |
| 37.5 μl | ddH2O |
| 5 μl | 10x PCR buffer |
| 4 μl | dNTPs (10mM each) |
| 1 μl | Primer (forward), 10μM |
| 1 μl | Primer (reverse), 10 μM |
| 1 μl | DNA template |
| 0.5 μl | Polymerase |

1. 36 μl of double distilled water
2. 5 μl of 10x buffer
3. 4 μl of dNTP mix
4. 1 μl of forward primer
5. 1 μl of reverse primer
6. 1 μl of DNA template
7. 0.5 μl of Polymerase

Mix it up gently by pipetting up and down

Table PCR program

|  |  |
| --- | --- |
| 1. | 94 ͦC – 2 min |
| 2. | 94 ͦC – 30 sec |
| 3. | 55 ͦC – 30 sec |
| 4. | 72 ͦC – 1 min (1 min/kb) |
| 5. | Go back to 2. Repeat 29 times |
| 6. | 72 ͦC – 5 min |
| 7. | 4 ͦС – forever |

The new program: Bar

Lid: 100 ͦC

The reaction volume: 50 μl

1. Completely denature template
2. First step of the following cycles
3. Annealing step
4. Extension step
5. Repeat
6. To finish what is not completely amplified
7. In case come back tomorrow
8. End