**Designing Cycling Conditions**

1. Use the rough calculation to approximate the Tms of your primers.

2°C x (A + T) + 4°C x (G + C)

Example: rbcLa \_F ATGTCACCACAAACAGAGACTAAAGC

A + T = 15 15 x 2 = 30

G + C = 11 11 x 4 = 44

30 + 44 = 74

1. Design cycling conditions for your primer set.

Initial cycle 95°C for 5 minutes

30x 95°C for 30 seconds

5°C below lowest primer Tm for 30 to 60 seconds

72°C for 30 – 60 seconds depending on length of amplicon

Final extension 72°C for 5 – 15 minutes

Hold 4 - 12°C

1. Look in the article “DNA barcodes for everyday life” and write down the cycling conditions reported in the article.

Table 5 shows cycling conditions for each primer set. Below are conditions for ITS.

Initial cycle 95°C for 4 minutes

30x 94°C for 10 seconds

50°C for 5 seconds

72°C for 60 seconds

Final extension 72°C for 10 minutes

Hold 4 - 12°C

**PCR Reactions**

1. Look in the article “DNA barcodes for everyday life” and fill in the table for the PCR reaction components reported in the article.

25 µL reactions

|  |  |  |
| --- | --- | --- |
| **Component** | **Volume** | **Concentration** |
| DNA | 2 µL | Varying concentrations |
| Water | 17.5 µL |  |
| 10x PCR buffer | 2.5 µL | 1x |
| 50 mM MgCl2 | 1 µL | 2 mM |
| 10 mM dNTP mix | 0.5 µL | 0.2 mM |
| 10 mM forward primer | 0.5 µL | 0.2 mM |
| 10 mM reverse primer | 0.5 µL | 0.2 mM |
| 5 U/ µL Taq polymerase | 0.5 µL | 0.1 U/ µL |

For most calculations you can use C1V1 = C2V2

Where you see an x in the concentration as in 10x, it means that the concentration is 10 times what is needed in the final reaction. So it needs to be diluted 10 fold.

PCR buffer:

C1 = 10x V1 = 2.5 µL V2 = 25 µL

10x \* 2.5 µL = C2 \* 25 µL

C2 = 1x

dNTP mix

C1 = 10 mM V1 = 0.5 µL V2 = 25 µL

10 mM \* 0.5 µL = C2 \* 25 µL

C2 = 0.2 mM

**PCR Reactions**

**For TopTaq DNA Polymerase**

**(the more complicated calculation)**

20 μL reaction volumes

2 μL template

1 μL primer mix (10 μM F and R primers together)

Calculations based upon information on page 15 of TopTaq PCR Handbook.

<https://www.qiagen.com/us/resources/resourcedetail?id=7a9e9a75-5f2c-454a-8a75-cff0d3a59fbe&lang=en>

|  |  |
| --- | --- |
| **Component** | **Volume** |
| Template | 2 |
| 10 mM Primers | 1 |
| **TopTaq 10x Buffer** | 2 |
| 10 mM dNTPs | 1 |
| TopTaq DNA Polymerase | 0.25 |
| 10x Coral Dye | 2 |
| 10 mM MgCl2 to bring to 1.5 MgCl2  Water | 0  11.75 |
| 10 mM MgCl2 to bring to 2.5 MgCl2  Water | 2  9.75 |
| 10 mM MgCl2 to bring to 4.0 MgCl2  Water | 5  6.75 |

Reactions were set up with either 1.5 mM, 2.5 mM or 4.0 mM MgCl2. The 1x Taq buffer is already at 1.5 mM. No additional MgCl2 needs to be added to the 1.5 mM reaction. For the 2.5 mM we need to add 1.0 mM and for the 4.0 mM we need to add 2.5 mM.

For 4.0 mM MgCl2 reaction:

C1 = 10 mM C2 = 2.5 mM V2 = 20 µL

10 mM \* V1 = 2.5 mM \* 20 µL

V1 = 5 µL

The volume of water listed for each MgCl2 concentration is the amount needed to bring the reaction volume to 20 µL.

**PCR Reactions**

**For HotStarTaq Plus 2x Mastger Mix**

20 μL reaction volumes

2 μL template

1 μL primer mix (10 μM F and R primers together)

Calculations based upon information on page 29 of HotStarTaq Plus PCR Handbook.

<https://www.qiagen.com/us/resources/resourcedetail?id=53e71936-cf64-4a9b-a9d2-fccee4868aa7&lang=en>

|  |  |
| --- | --- |
| **Component** | **Volume** |
| Template | 2 |
| Primers | 1 |
| **HotStarTaq MM** | 10 |
| Coral Dye | 2 |
| 10 mM MgCl2 to bring to 1.5 MgCl2  Water | 0  5 |
| 10 mM MgCl2 to bring to 2.5 MgCl2  Water | 2  3 |
| 10 mM MgCl2 to bring to 4 MgCl2  Water | 5  0 |

Typically we would set up a master mixture that has n+1 volumes where n = the number of reactions that we are setting up. For example if you wanted to set up 3 reactions, you would make up a master mixture of 3+1 = 4 volumes.

|  |  |  |
| --- | --- | --- |
| **Component** | **Volume for 1 rxn** | **N+1** |
| Template | 2 |  |
| Primers | 1 | 4 |
| **HotStarTaq MM** | 10 | 40 |
| Coral Dye | 2 | 8 |
| 10 mM MgCl2 to bring to 2.5 MgCl2 | 2 | 8 |
| Water | 3 | 12 |

Label your PCR tubes, add 2 µL template to each tube and then add 18 µL of the master mixture to each tube. Making the excess volume of master mixture allows for pipetting errors.

**PCR Reactions**

**For TopTaq 2x Master Mix**

20 μL reaction volumes

2 μL template

1 μL primer mix (10 μM F and R primers together)

Calculations based upon information on page 24 of TopTaq PCR Handbook.

<https://www.qiagen.com/us/resources/resourcedetail?id=7a9e9a75-5f2c-454a-8a75-cff0d3a59fbe&lang=en>

|  |  |
| --- | --- |
| **Component** | **Volume** |
| Template | 2 |
| Primers | 1 |
| **TopTaq MM** | 10 |
| Coral Dye | 2 |
| 10 mM MgCl2 to bring to 1.5 MgCl2  Water | 0  5 |
| 10 mM MgCl2 to bring to 2.5 MgCl2  Water | 2  3 |
| 10 mM MgCl2 to bring to 4 MgCl2  Water | 5  0 |