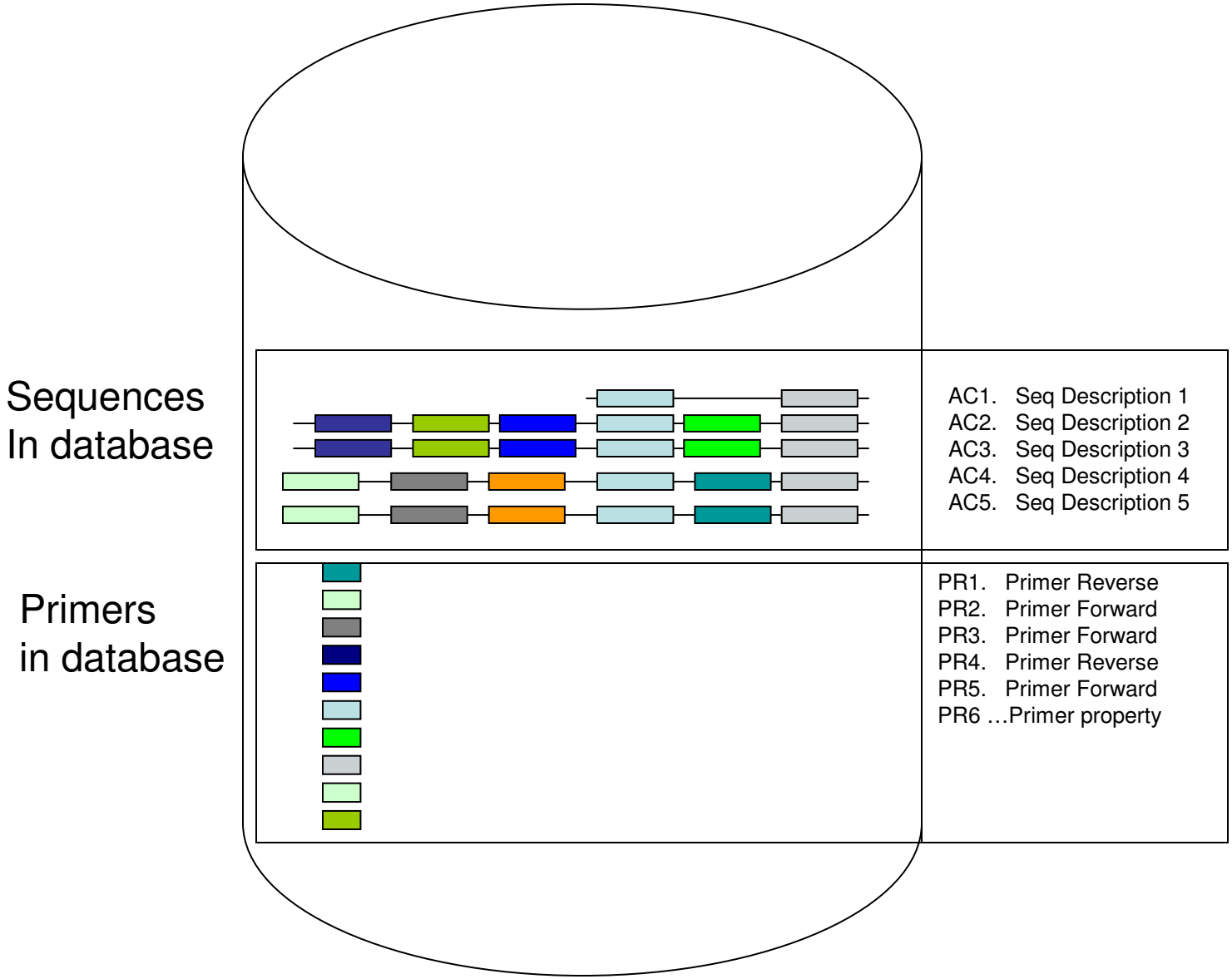

Sequences
In database
AC1. Seq Description 1
AC2. Seq Description 2
AC3. Seq Description 3
AC4. Seq Description 4
AC5. Seq Description 5
Primers
in database
PR1. Primer Reverse
PR2. Primer Forward
PR3. Primer Forward
PR4. Primer Reverse
PR5. Primer Forward
PR6 …Primer property

TERMINOLOGY

- **Orientation=** 5'>>>>>>>3'

- + **Strand=** always written 5' to 3'
  mode of single strand representation
- **Strand=** always written 3' to 5'
  Only shown when double strand representation

- **Denature=** Separating Strands

- **PCR=** Polymerase Chain Reaction

- **Primer=** Sub-sequence; typically 18-22 bases
  Forward= Always as the +strand (Note)
  Reverse= Always the –ve strand (Note composition)

Alignment: Seq 1: 5' ATATATATGGGGGGG 3'
Seq 2: 5' ATATATATCGCGCGC 3'

Complement: Seq 1: 5' ATATATATGGGGGGG 3'
3' TATATATACCCCCCC 5'

5’ --TAGCTATTTT ATGACCTATTACCCGGAATCACTTTTAAAAGG-3’
3’– ATCGATAAAATACTGGATAATGGGCCTTAGTGAAAATTTTCC- 5’

Piece of Genomic DNA representation

5’ --TAGCTATTTT ATGACCTATTACCCGGAATCACTTTTAAAAGG-3’

TACTGGAT <<<<<<< CTTAGTG Reverse

Denature

3’– ATCGATAAAATACTGGATAATGGGCCTTAGTGAAAATTTTCC- 5’

ATGACCTA >>>>>>>>> GAATCAC

Forward TACTGGAT >>>>

Forward ⟶ Synthesis
And Always on 3’
Reverse Priming direction

Note the issue with Primer representation
Especially the Reverse Primer

ATGACCTATTACCCGGAATCAC
TACTGGATAATGGGCCTTAGTG

PCR Product
-Note: It is a segment of genomic DNA

TACTGGAT

CTTAGTG

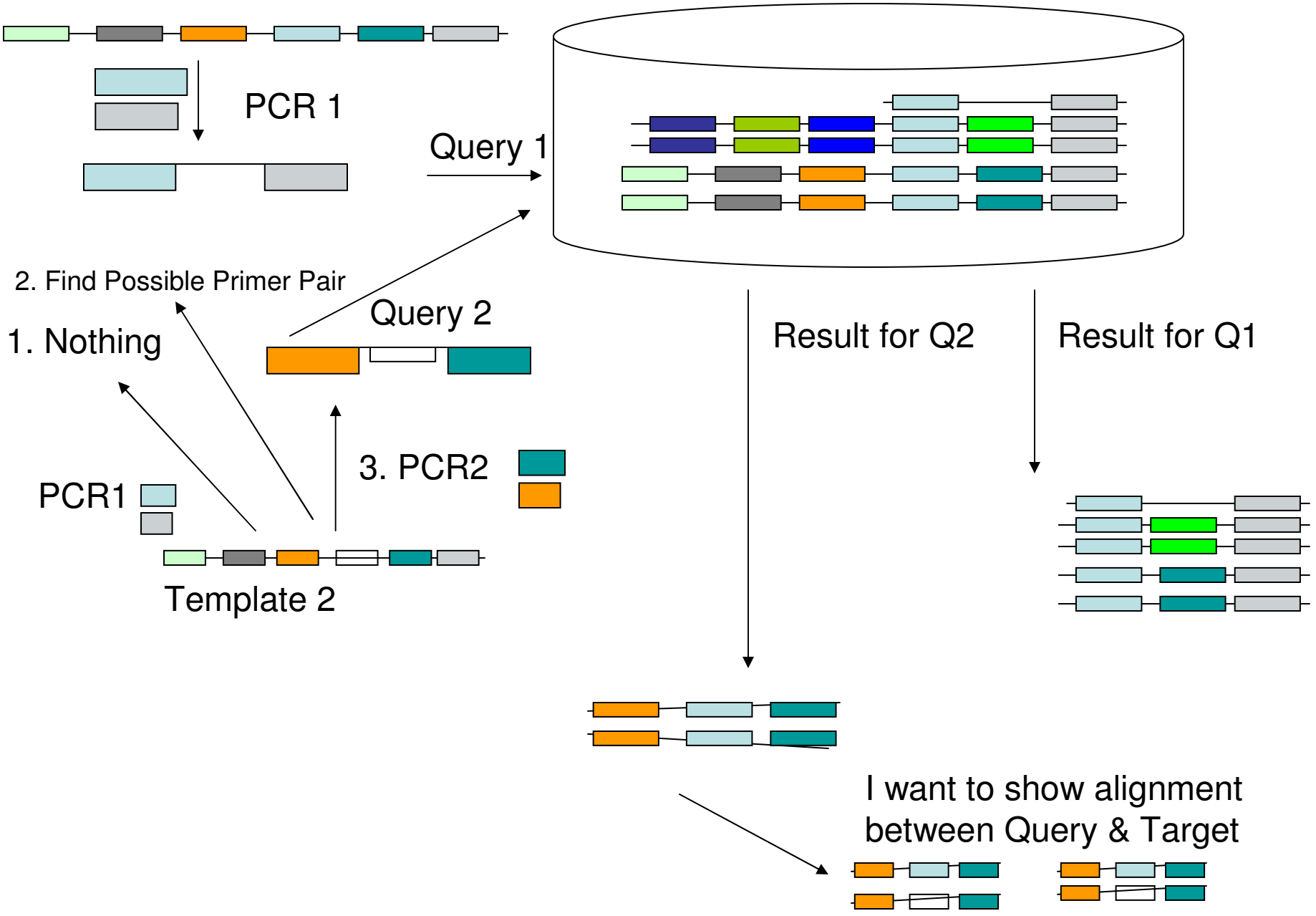
5’ GTGATTC 3’

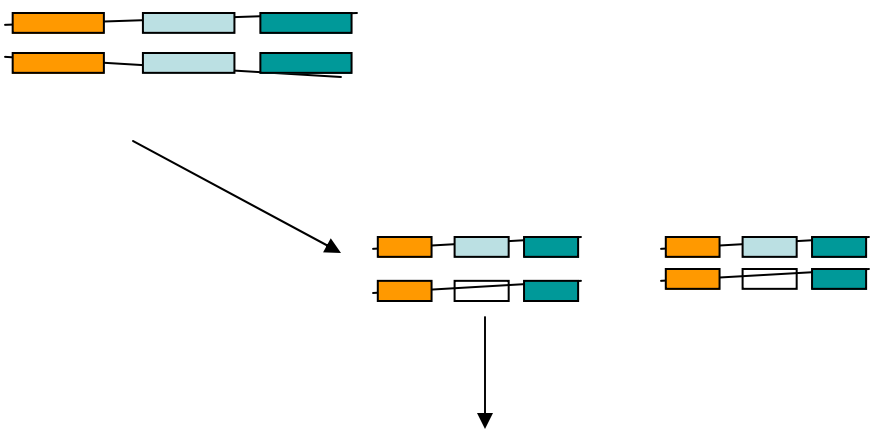
REVERSE

CTTAGTG

Complement

GAATCAC


PCR 1
Query 1
2. Find Possible Primer Pair
1. Nothing
Query 2
Result for Q2
Result for Q1
3. PCR2
PCR1
Template 2
I want to show alignment
between Query & Target



Aligning the sequences (Query and the one in database to show the reason why Primer set 1 did not work

CATGTTACTGGATGGGCCTA
CATGTTACTGGTCGGGCCTA

Summary:

•Find the next pair of primer from the database Flanking the primer set that did not work

•Query the database

•Show Alignment