

Alignment

In this file we show an alignment of the sequencing results with the full-length otoferlin sequences of both dual AAV CDS and wild-type variant 4. See below a detailed description of all the sequences.

Full-length sequences:

- **DualAAV mOtof CDS** – full-length mouse otoferlin sequence used to clone the AAV constructs
- **WT mOtof var4 CDS** – full-length otoferlin variant 4 (NM_001313767.1), present in our wild-type mice

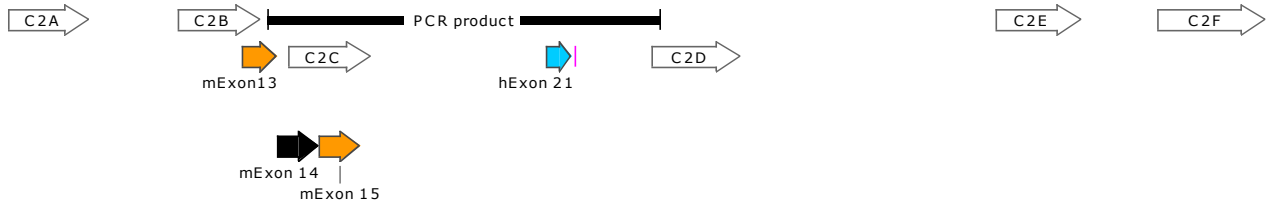
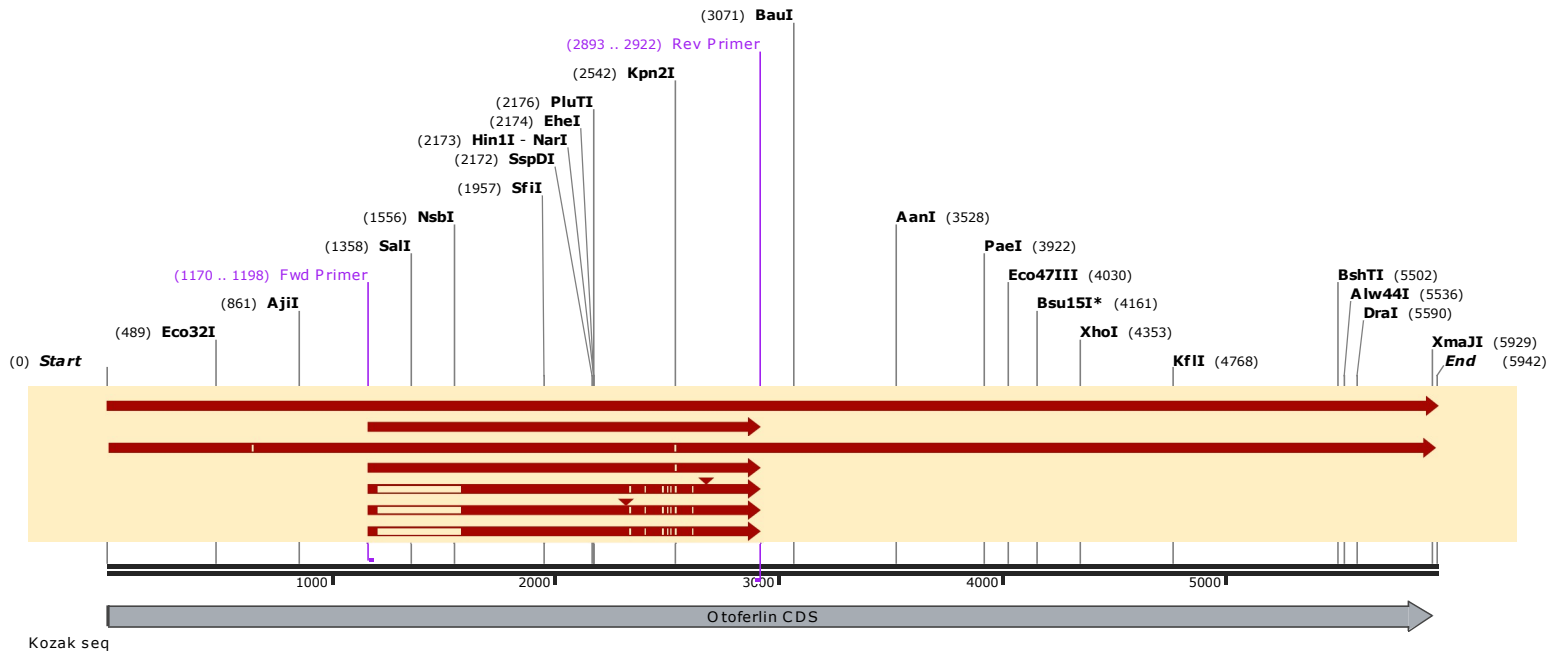
Sequencing results:

- **(*) DualAAV-TS (1753 bp)** - representative sequencing result of band * (1753 bp) for the injected ear of *Otof*^{-/-} mice in figure EV4, B
- **(*) WT (1753 bp)** - representative sequencing result of band * (1753 bp) for the control ear of wild-type mice in figure EV4, B
- **(c) Otof^{-/-} (1679 bp)** – representative sequencing result of band c (1679 bp) in figure EV4, B
- **(b) Otof^{-/-} (1480 bp)** – representative sequencing result of band b (1480 bp) in figure EV4, B
- **(a) Otof^{-/-} (1379 bp)** – representative sequencing result of band a (1379 bp) in figure EV4, B

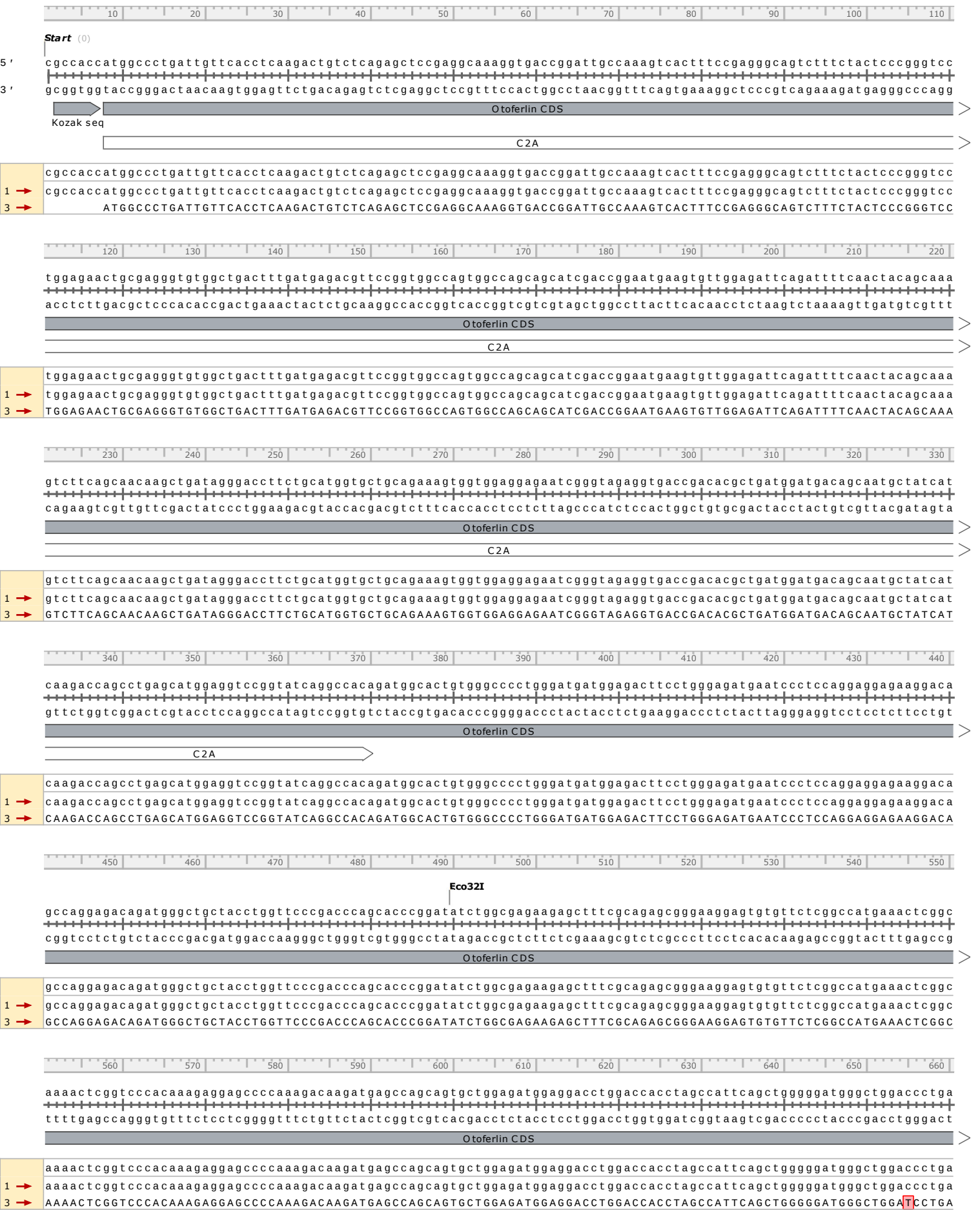
The 101 bp additional sequence in b (Seq 6 in alignment) is the intron between Exon 20 and 21.

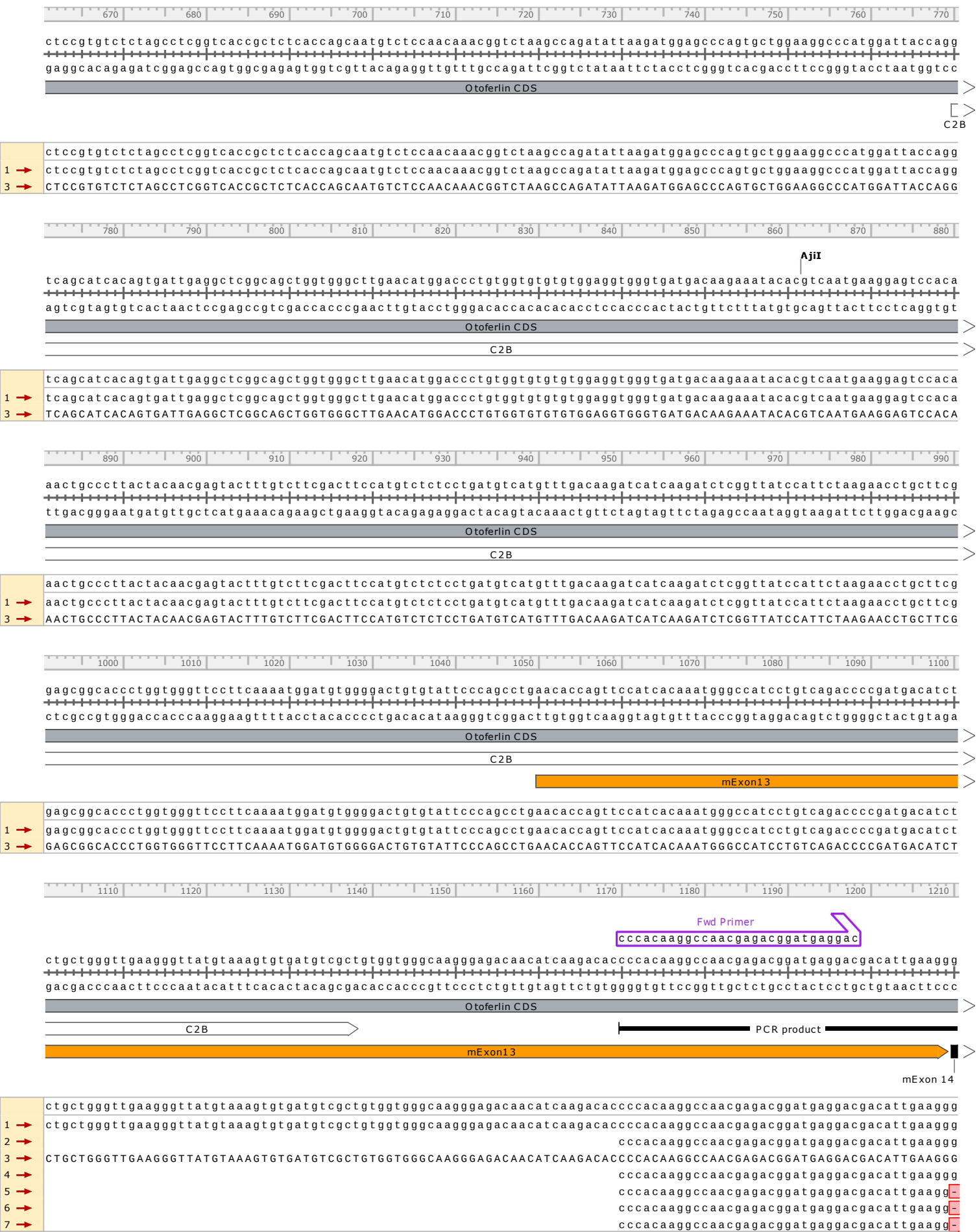
The 300 bp additional sequence in c (Seq 5 in alignment) is the intron between Exon 23 and 24.

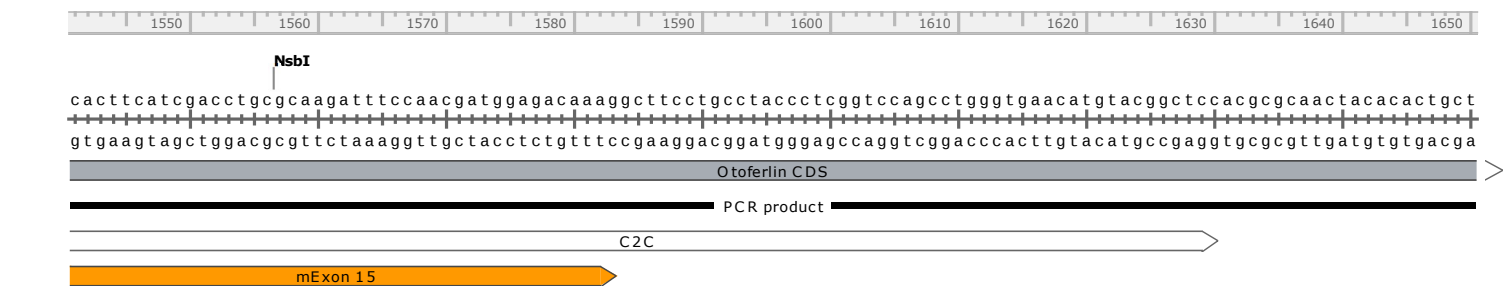
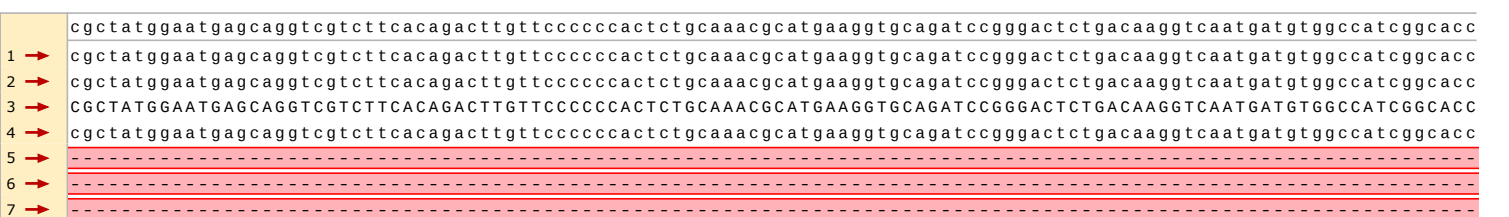
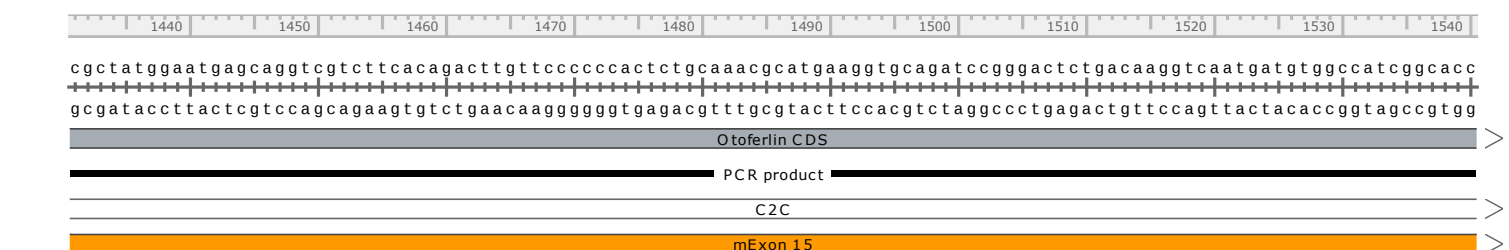
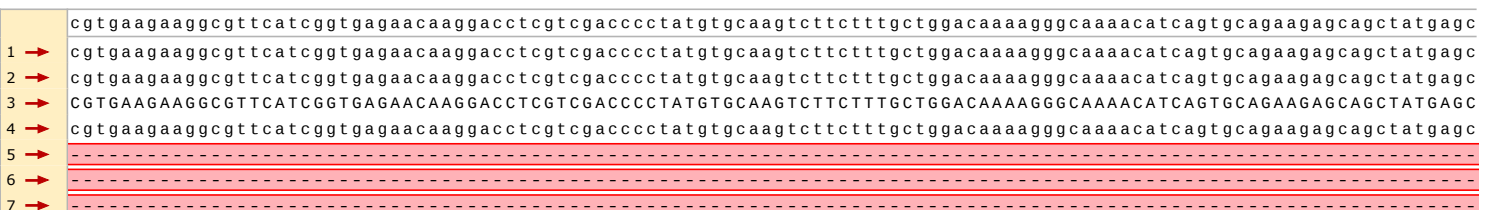
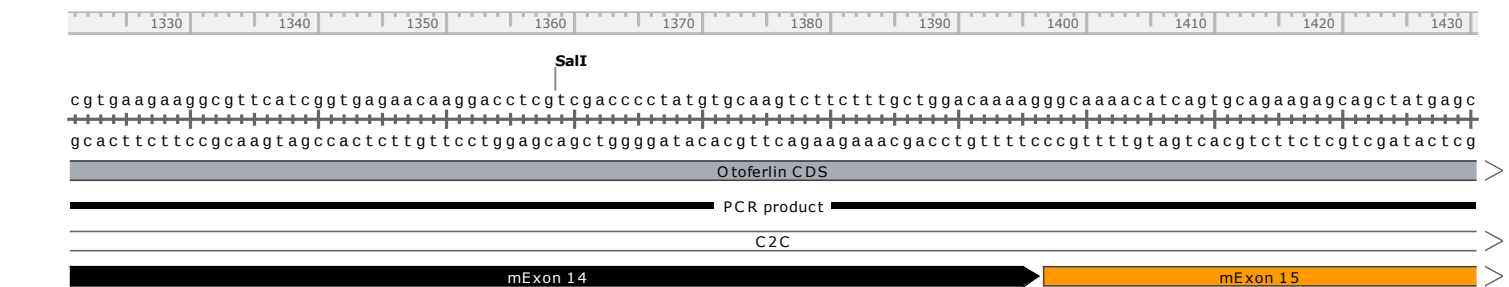
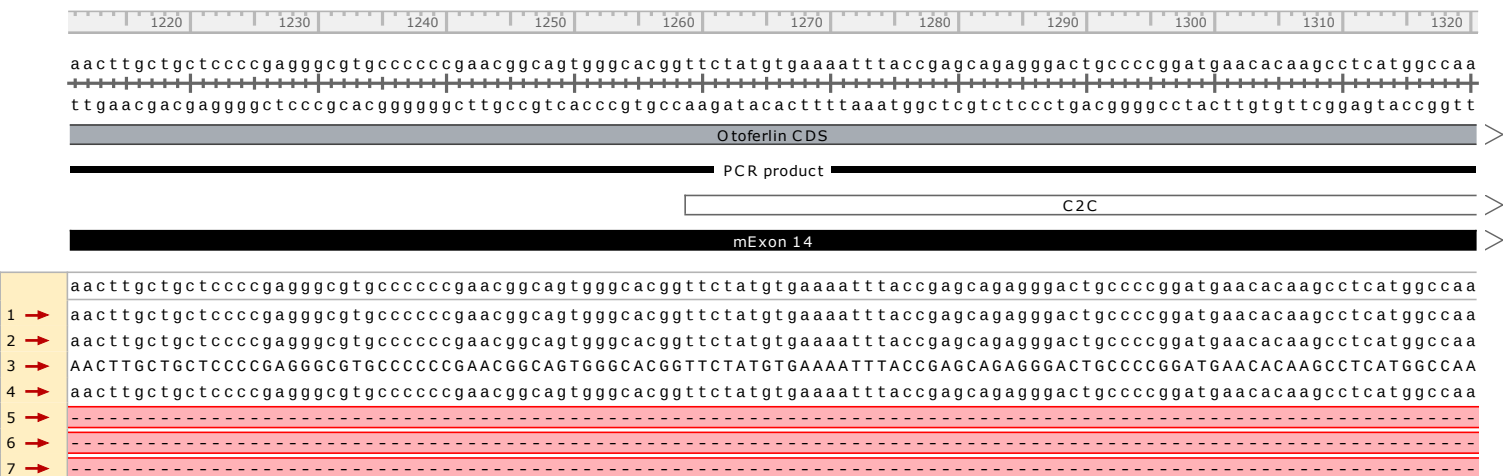
bp DNA linear bp DNA circular bp DNA circular bp DNA circular

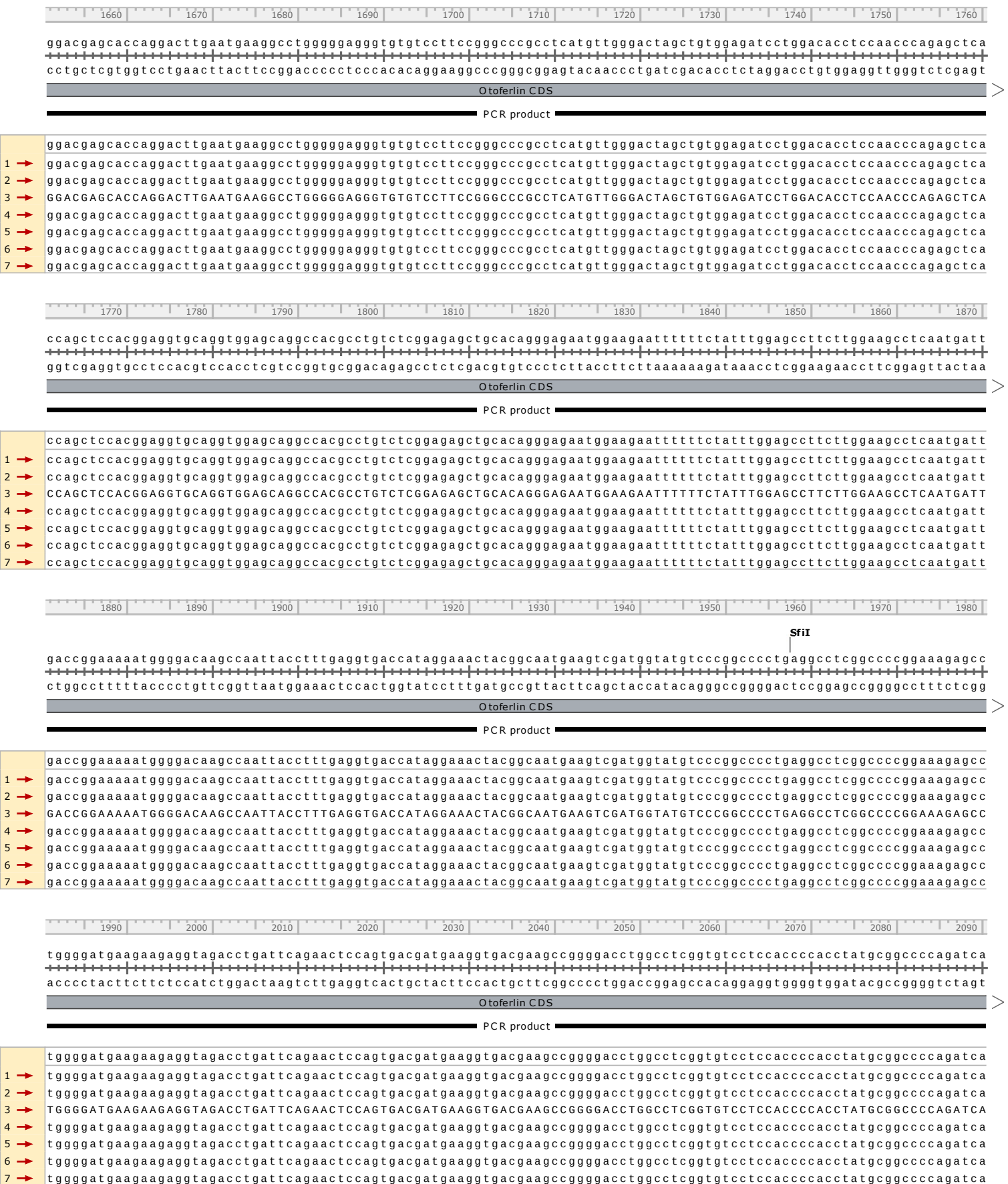


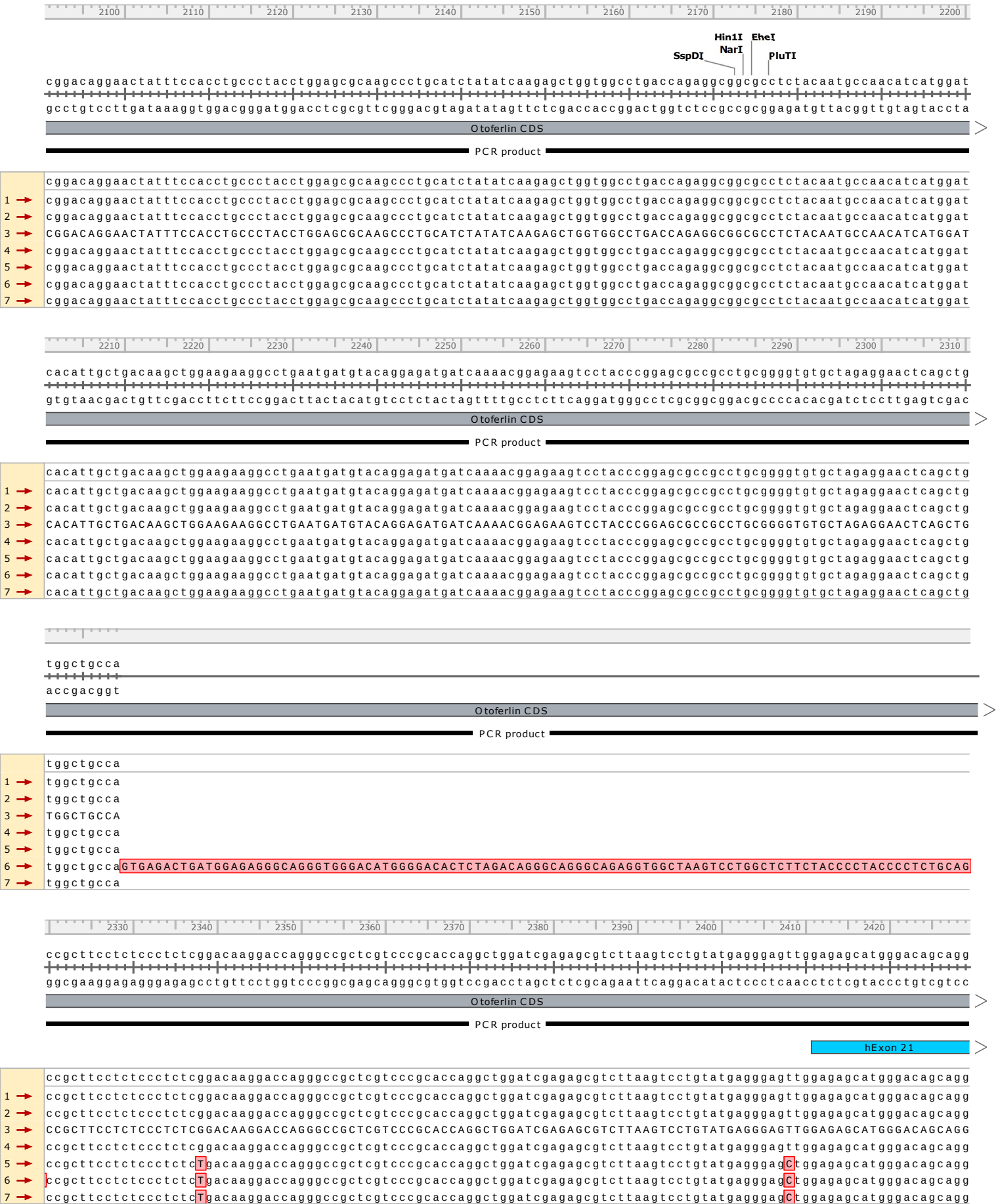
Alignment
5942 bp

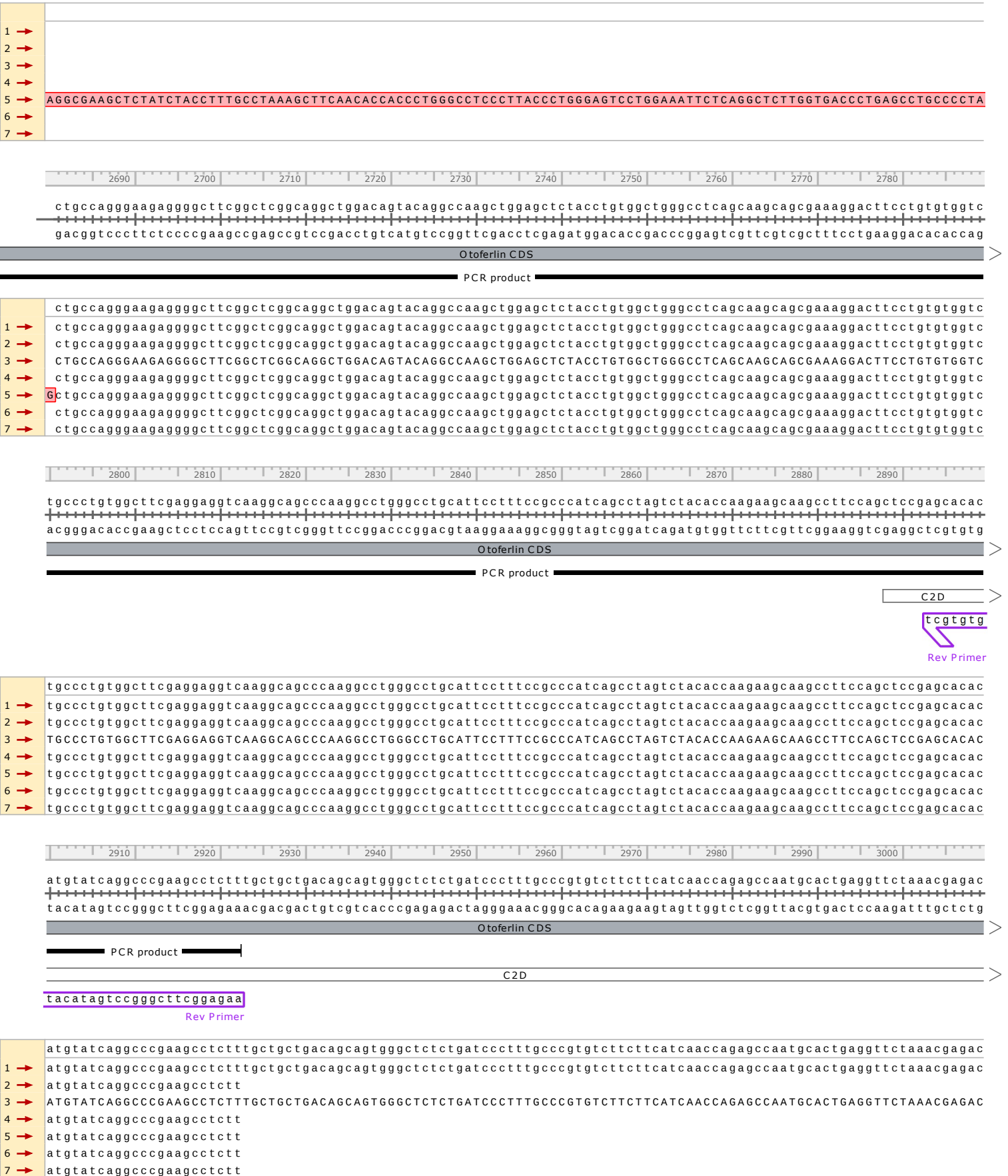


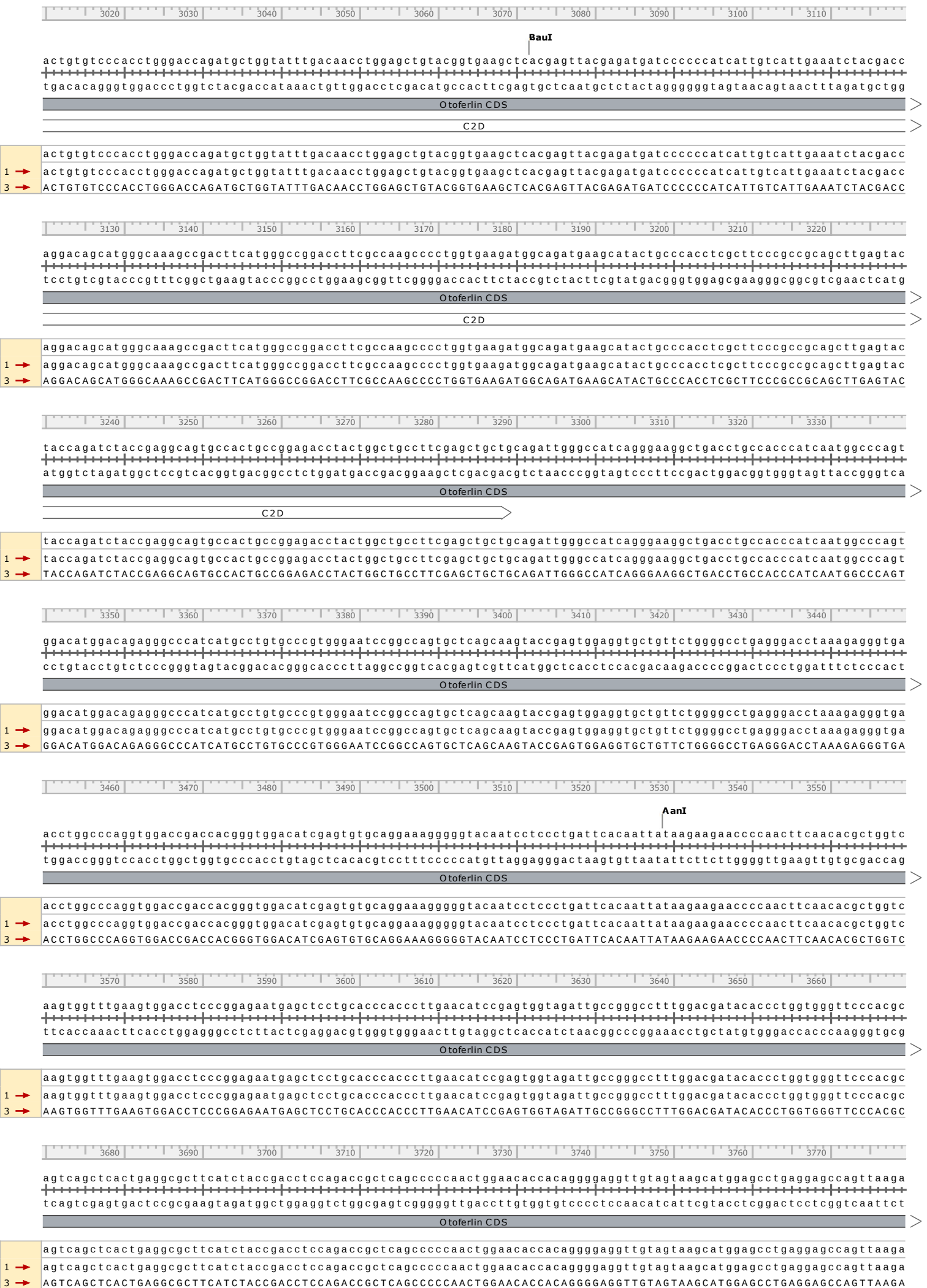


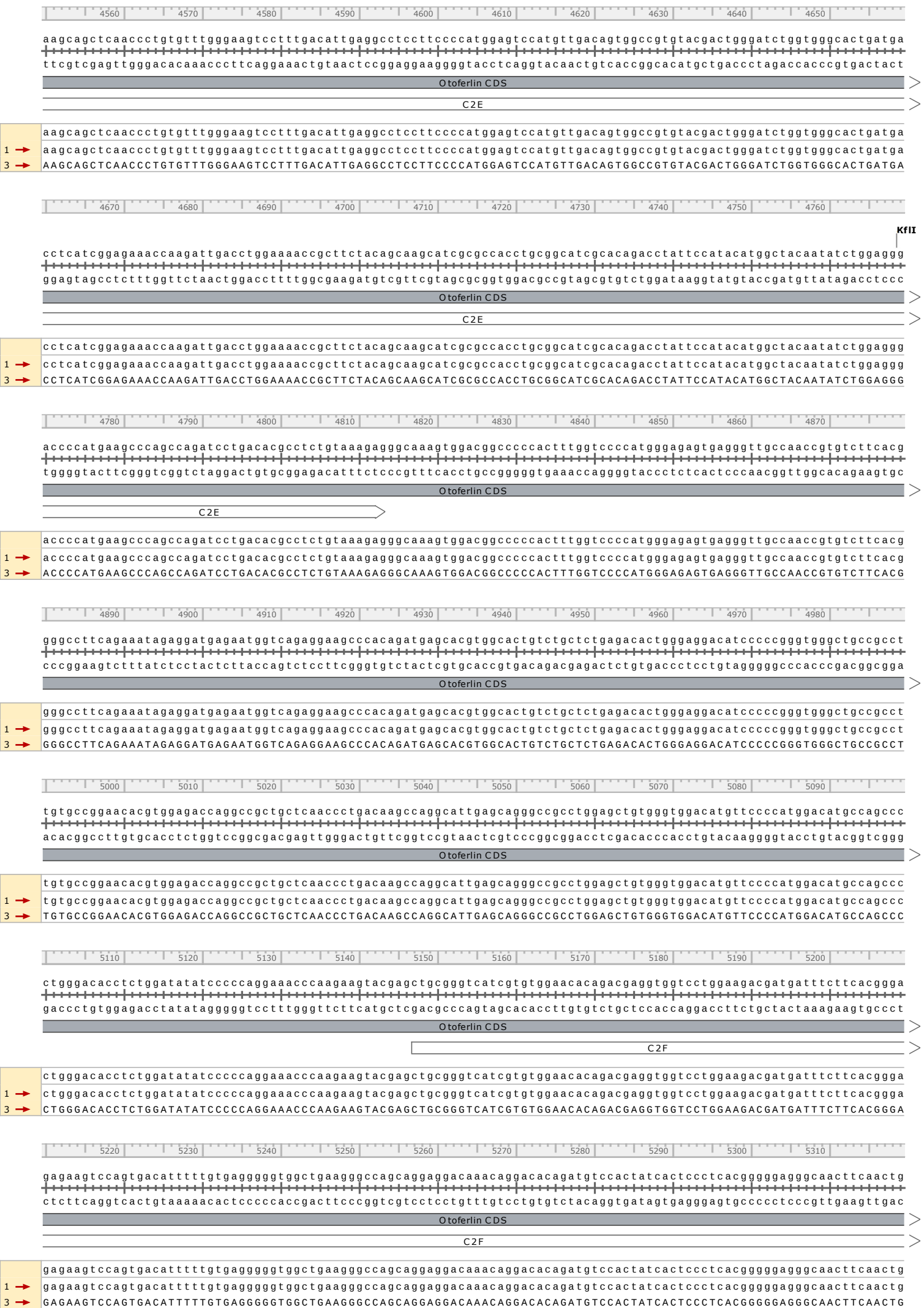


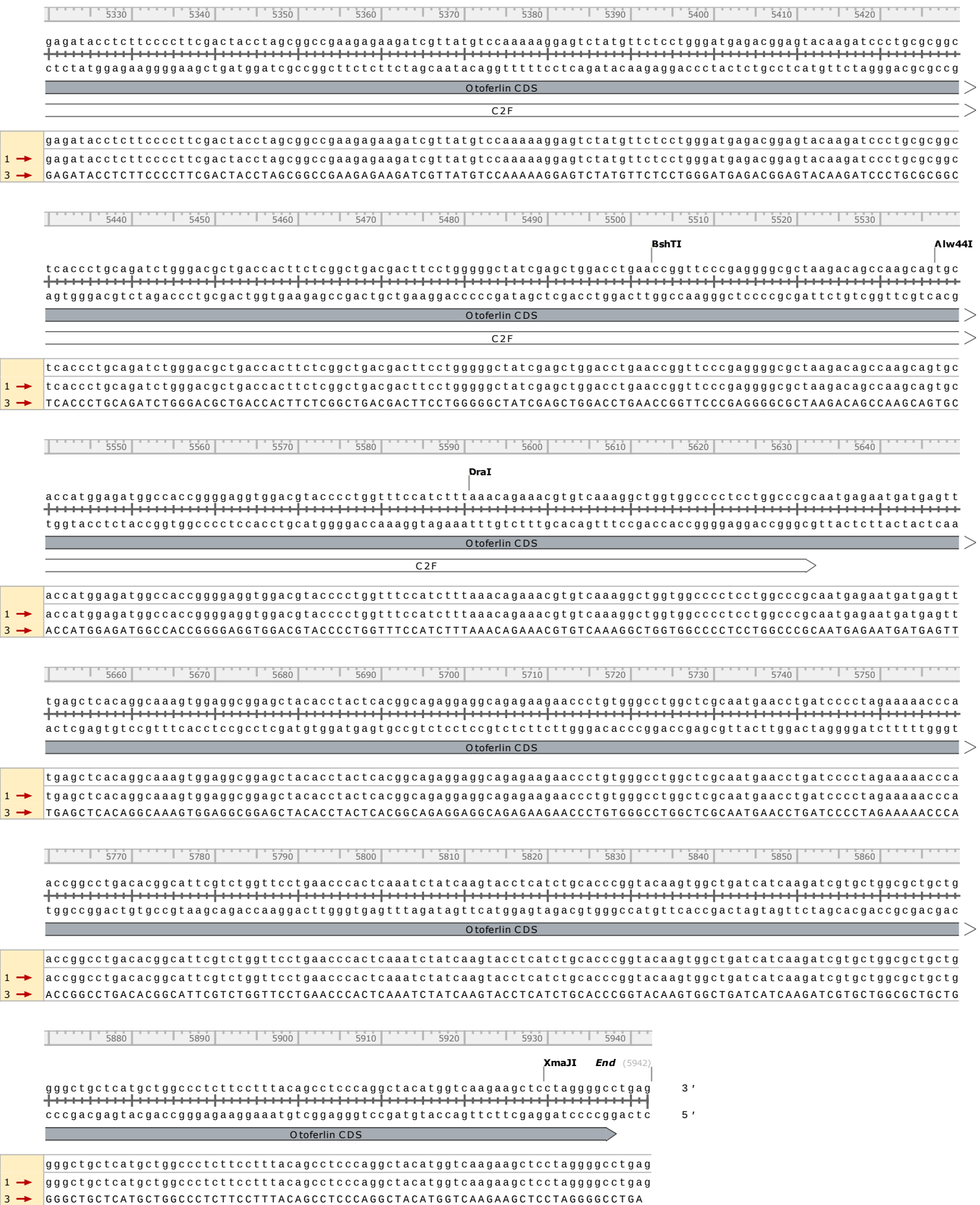












Original Sequence:

- 1: DualAAV mOtofCDS ➡
5942 bases
1 .. 5942
- 2: (*) DualAAV-TS (1753 bp) ➡
1753 bases
1 .. 1753
- 3: WT mOtof var4 CDS (NM_001313767.1) ➡
5934 bases
1 .. 5934 (2 mismatches)
- 4: (*) WT (1753 bp) ➡
1753 bases
1 .. 1753 (1 mismatch)
- 5: (c) Otof-/- (1679 bp) ➡
1679 bases
1 .. 1679 (7 mismatches, 2 gaps)
- 6: (b) Otof-/- (1480 bp) ➡
1480 bases
1 .. 1480 (7 mismatches, 2 gaps)
- 7: (a) Otof-/- (1379 bp) ➡
1379 bases
1 .. 1379 (7 mismatches, 1 gap)