

Supplementary Table 1:

Primer sequences used in qPCR.

Primer	Sequence (5' to 3')
CYP1B1-AS1 Forward	ACACAAGAATCGGCACTGGT
CYP1B1-AS1 Reverse	GCCGCATGTTTCATCTGCAT
FOXO1 Forward	CCGGAGTTTAGCCAGTCCAA
FOXO1 Reverse	CACGCTCTTGACCATCCACT
NAE1 Forward	GGCCAAAGAGGGTCAAGGAA
NAE1 Reverse	AAATGGACTCTGGTGCCTGG
U6 Forward	CTCGCTTCGGCAGCACA
U6 Reverse	AACGCTTCACGAATTTGCGT
GAPDH Forward	CCGGGAAACTGTGGCGTGATGG
GAPDH Reverse	AGGTGGAGGAGTGGGTGTCGCTGTT

Supplementary Table 2:

CYP1B1-AS1 probe sequence used in FISH experiment.

Name	Sequence (5'-3')
NR-027252.1:	TGTTTCCTAGTTAGTCGCTTGAACTTCCAATGCCCTAT

Supplementary Table 3:

SiRNAs and negative control.

Name	Sequence (5' to 3')
FOXO1 siRNA1	CGGGCUGGAAGAAUCAAUTT
FOXO1 siRNA2	CCCUCGAACUAGCUCAAUTT
NAE1 siRNA	GGGUUGUGCUUUAGUCUGUTT
Negative control	UUCUCCGAACGUGUCACGUTT

Supplementary Table 4:

Primer sequences used in CHIP and dual-luciferase assay.

Primer	Sequence (5' to 3')
Promoter Forward	GTGACATTATTTATGAACCTGCTGC
Promoter Reverse	TGGGTGCTTCCTGCTGTTG

Supplementary Table 5:

Primer sequences used in RNA pull-down experiment.

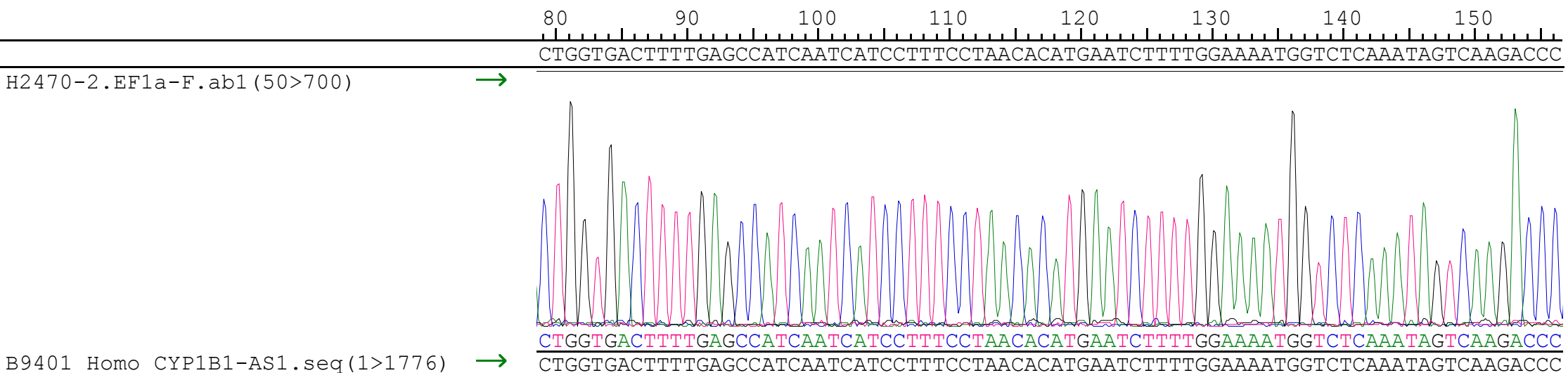
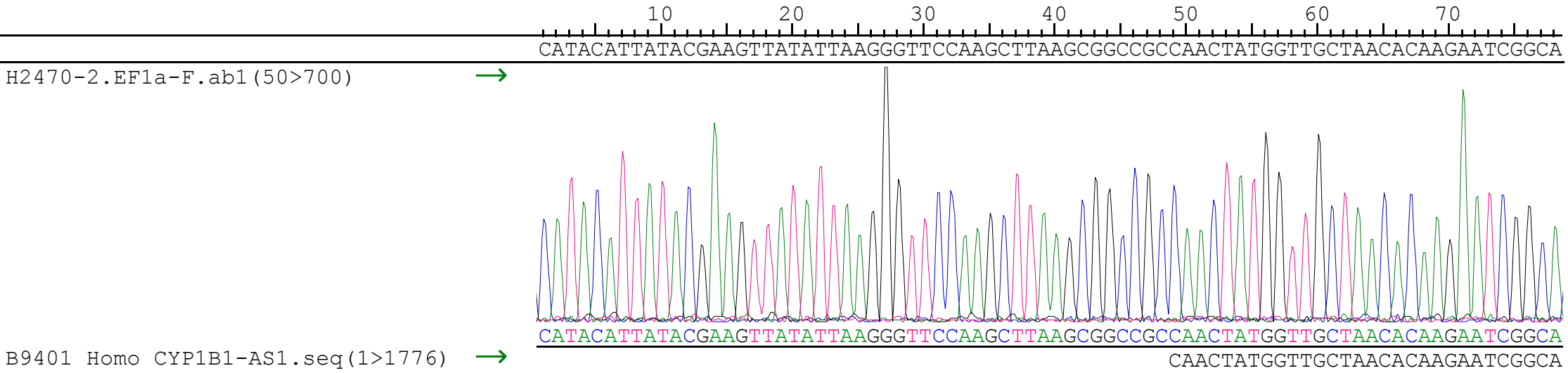
Primer	Sequence (5' to 3')
CYP1B1-AS1 sense Forward	taatacgaactcactatagggTGGTTGCTAACACAAGAATC G
CYP1B1-AS1 sense Reverse	AGTGAGTTATGGTCACCTTGGAATGG
CYP1B1-AS1 antisense Forward	taatacgaactcactatagggAGTGAGTTATGGTCACCTTG GAATGG
CYP1B1-AS1 antisense Reverse	TGGTTGCTAACACAAGAATCG

Supplementary Table 6:

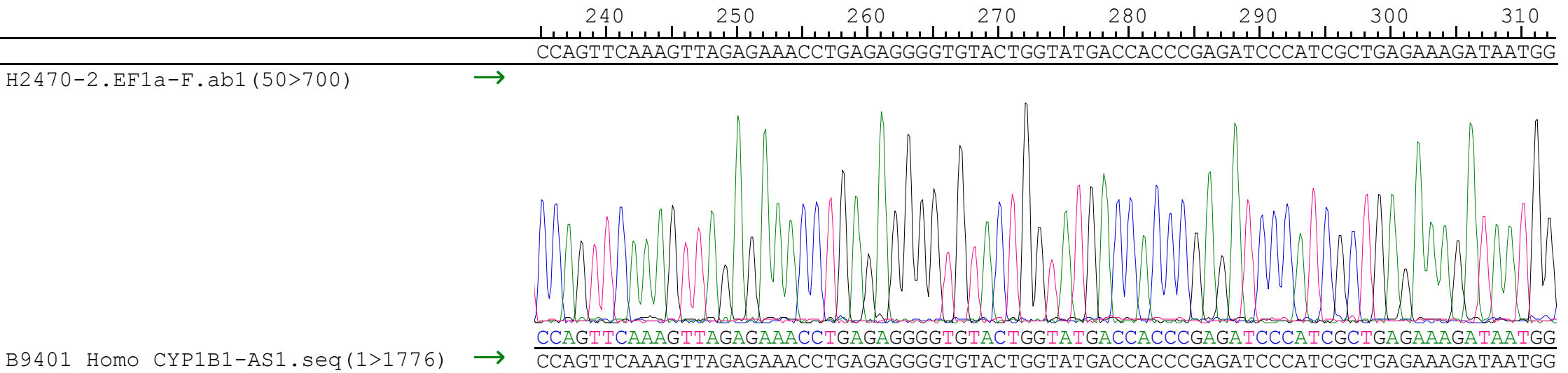
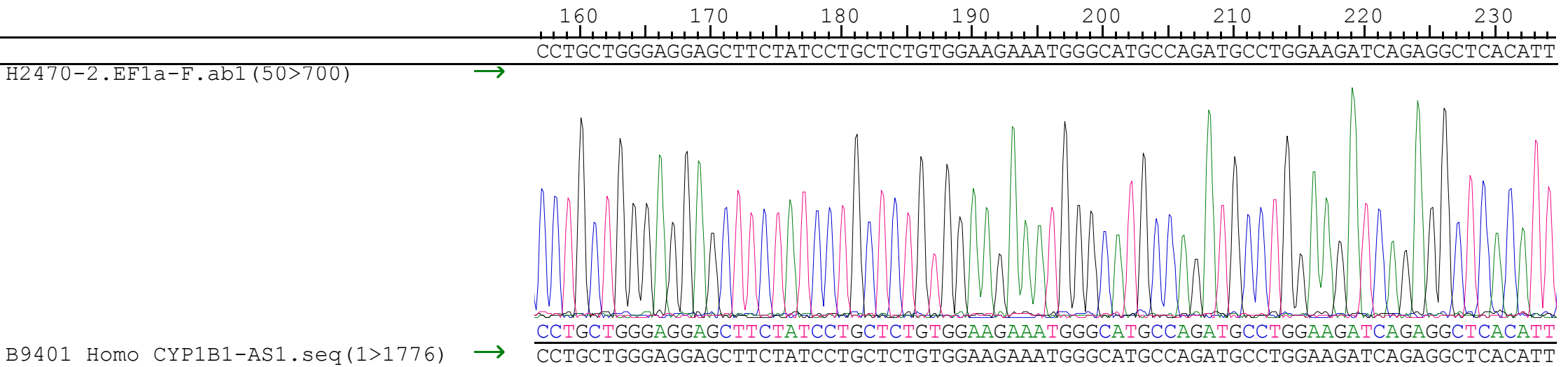
The antibodies used in this study.

Antibody	Specificity	Manufacturer	Code
anti-FOXO1	Rabbit monoclonal antibody	CST	2880
anti-NAE1	Rabbit monoclonal antibody	Abcam	ab187142
anti-p21	Rabbit monoclonal antibody	Abcam	Ab109520
anti-cyclin D1	Rabbit monoclonal antibody	Abcam	ab40754
anti-BCL2	Rabbit monoclonal antibody	Abcam	ab32124
anti-BAX	Rabbit monoclonal antibody	Abcam	ab32503
anti-NEDD8	Rabbit monoclonal antibody	Abcam	ab81264
anti-UBA3	Rabbit monoclonal antibody	Abcam	ab124728
anti-beta-actin	Rabbit polyclonal antibody	Abcam	ab8227

Project: Alignment of Homo CYP1B1-AS1.sqd Contig 1



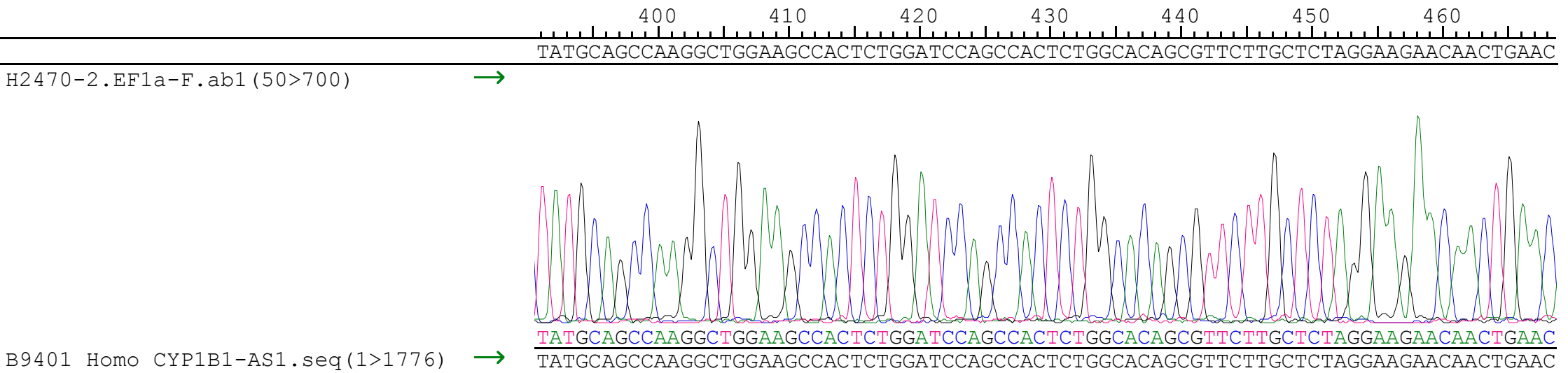
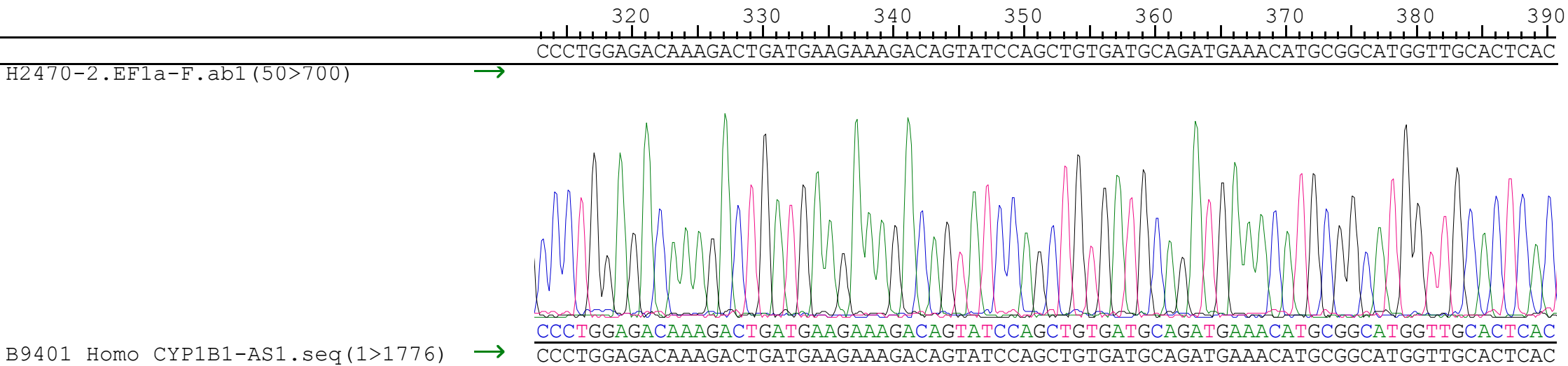
Project: Alignment of Homo CYP1B1-AS1.sqd Contig 1



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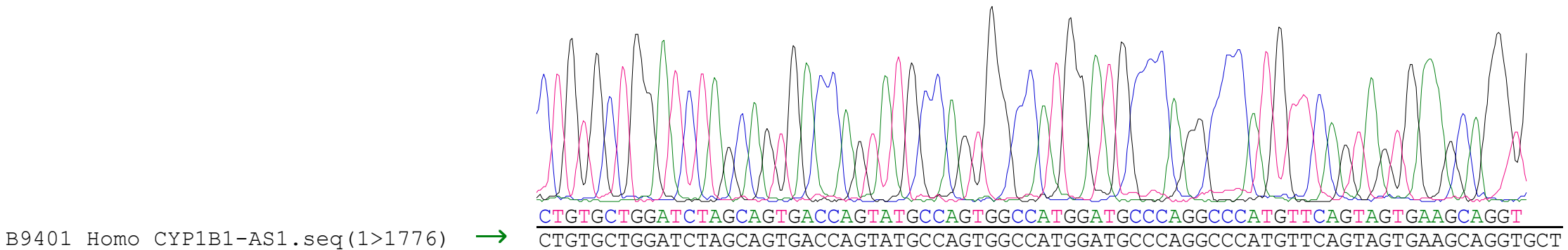
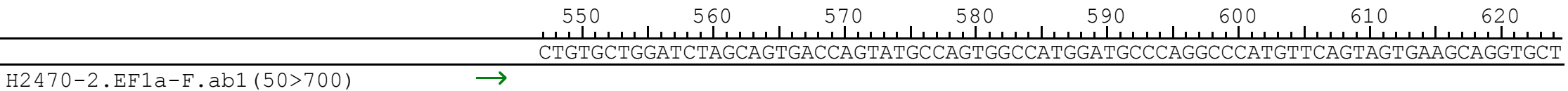
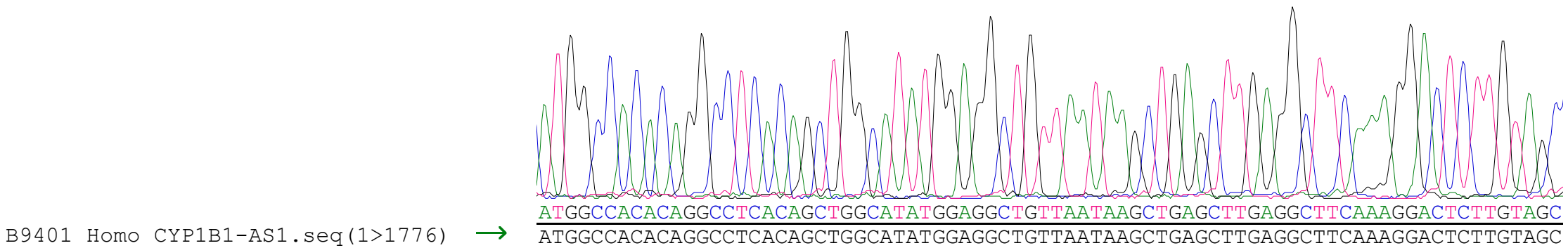
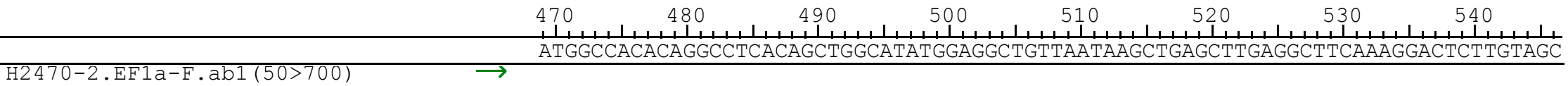
Project: Alignment of Homo CYP1B1-AS1.sqd Contig 1



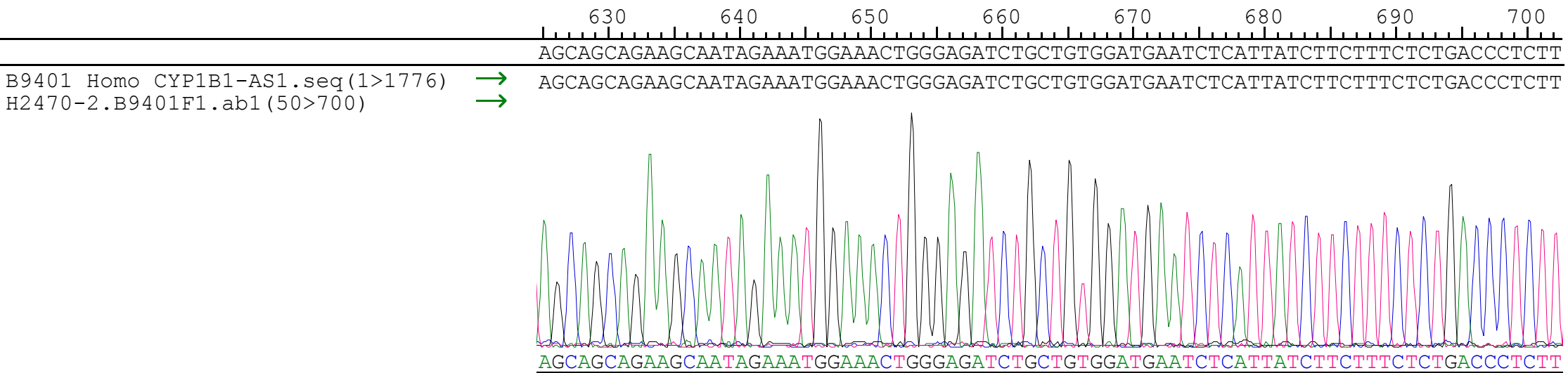
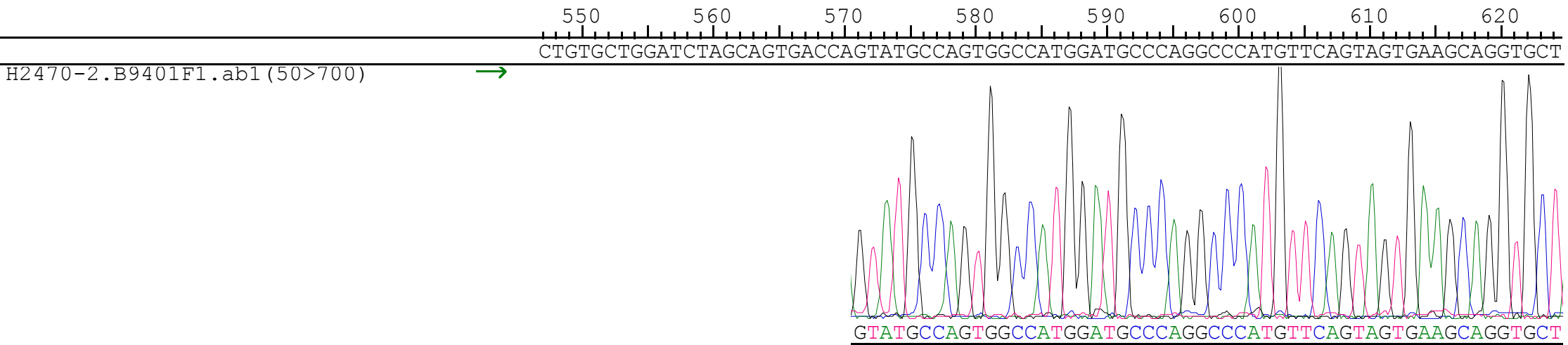
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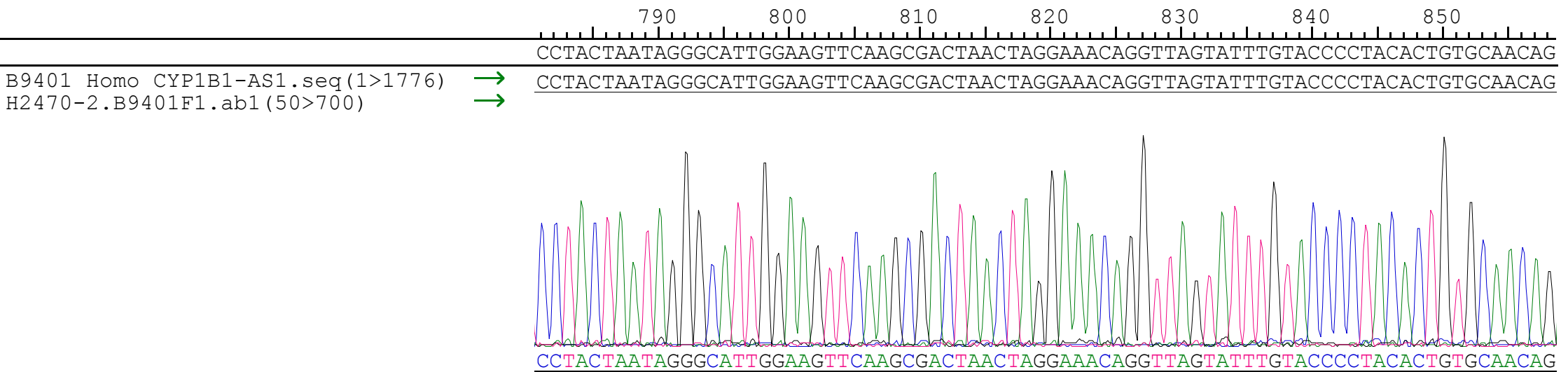
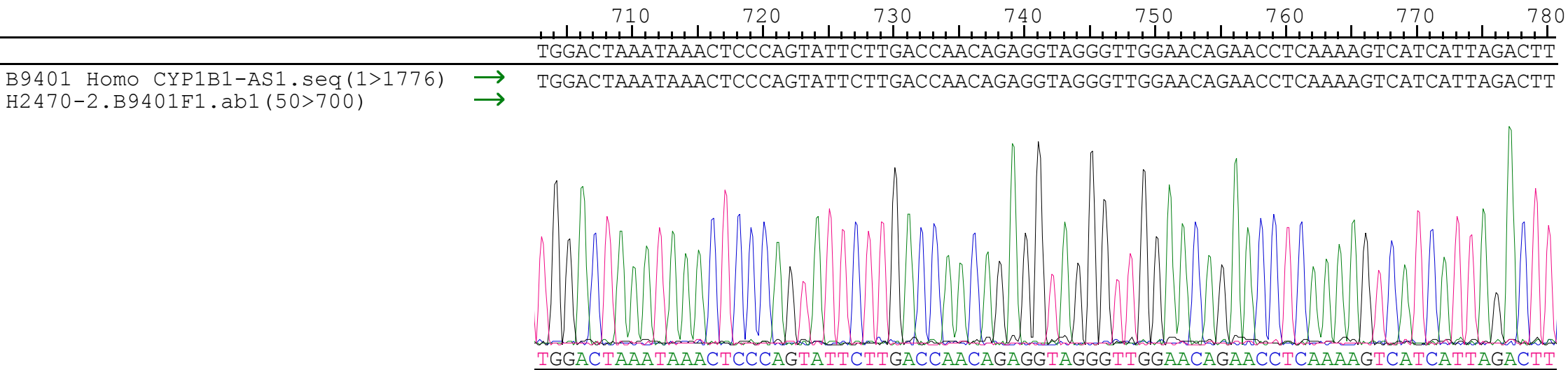
Project: Alignment of Homo CYP1B1-AS1.sqd Contig 1



Project: Alignment of Homo CYP1B1-AS1.sqd Contig 1

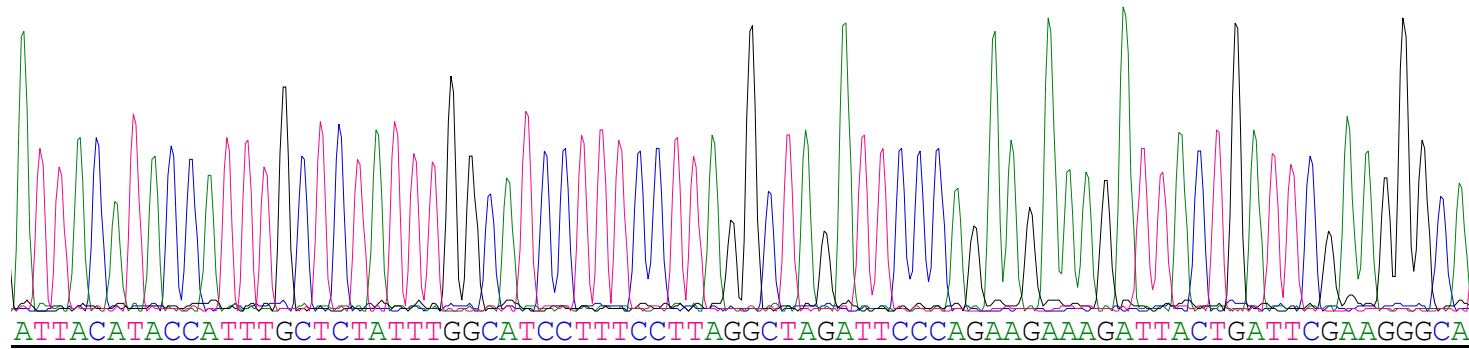


Project: Alignment of Homo CYP1B1-AS1.sq Contig 1

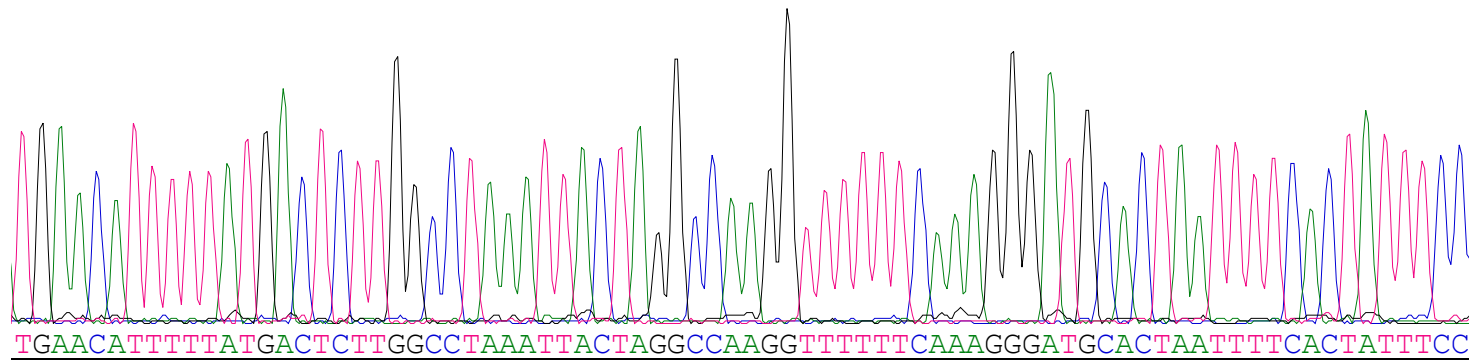


Project: Alignment of Homo CYP1B1-AS1.sq Contig 1

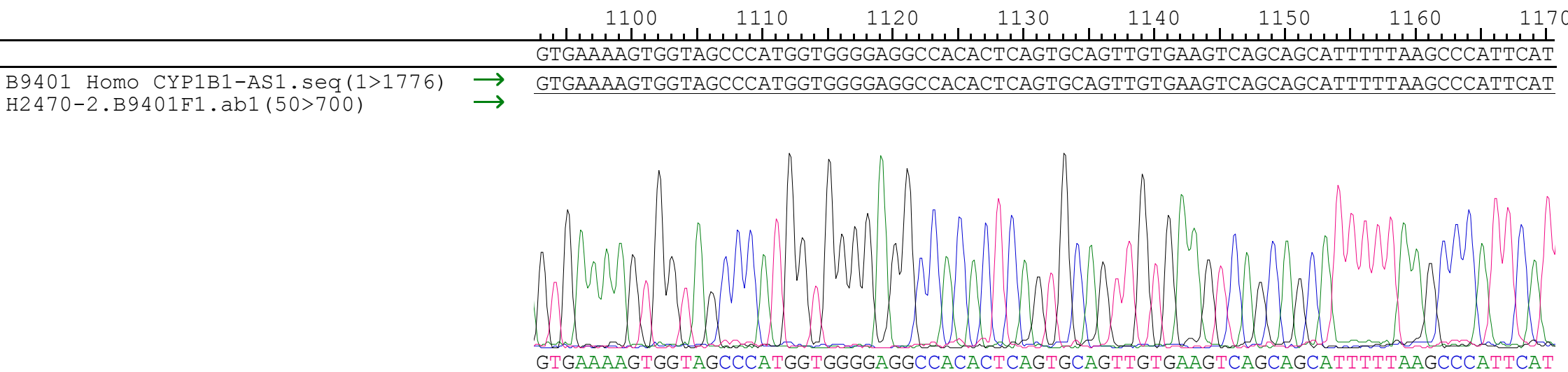
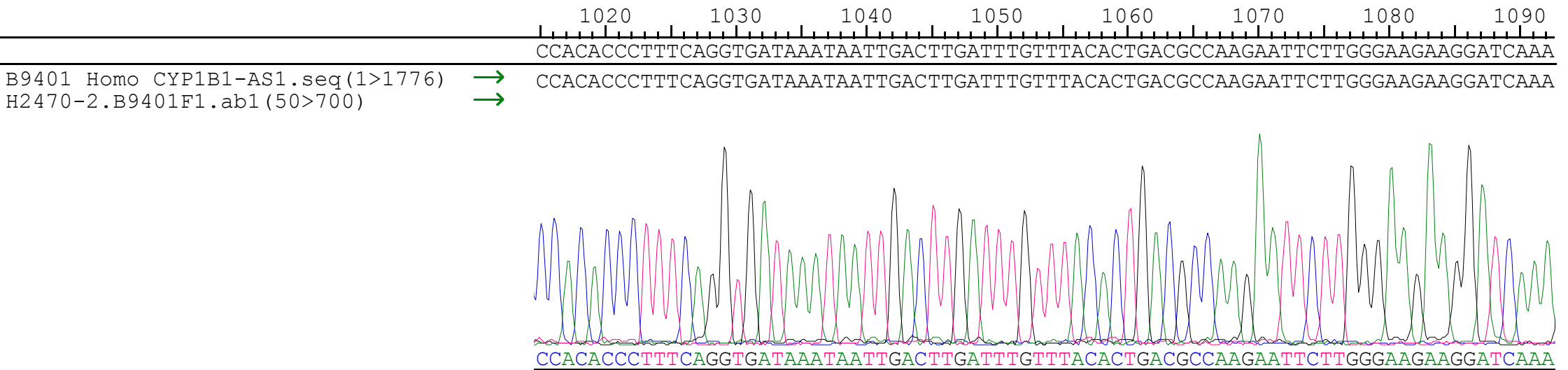
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ATTACATACCATTGCTCTATTTGGCATCCTTTCCCTTAGGCTAGATTCCCAGAAGAAAGATTACTGATTTCGAAGGGCA
B9401 Homo CYP1B1-AS1.seq (1>1776) → ATTACATACCATTGCTCTATTTGGCATCCTTTCCCTTAGGCTAGATTCCCAGAAGAAAGATTACTGATTTCGAAGGGCA
H2470-2.B9401F1.ab1 (50>700) →



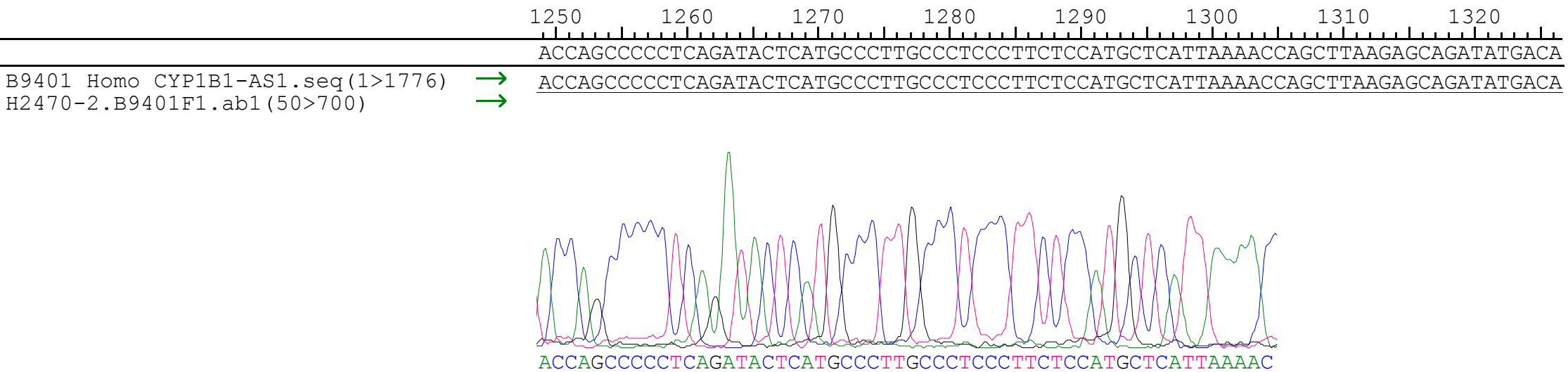
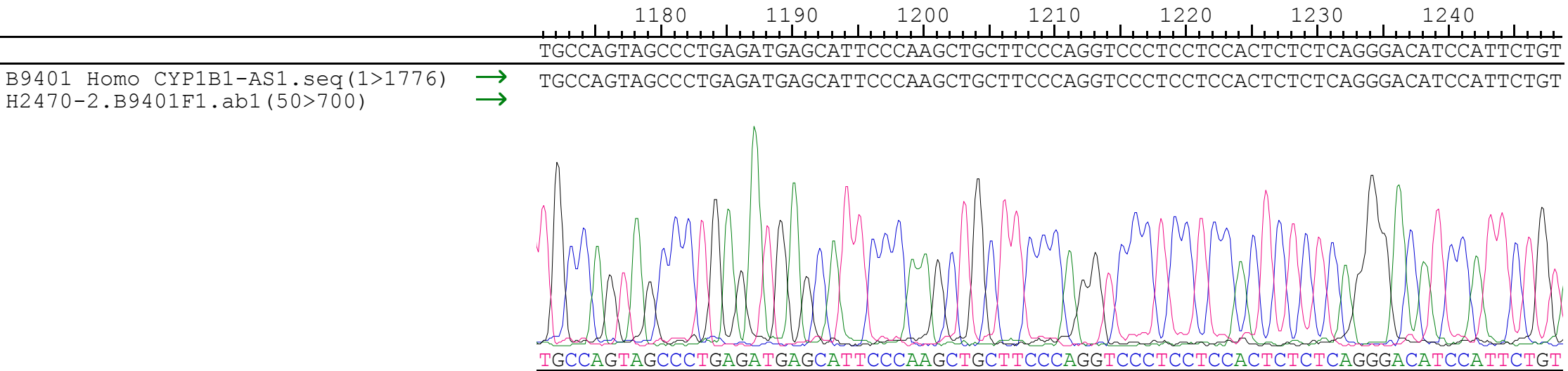
940 950 960 970 980 990 1000 1010
TGAACATTTTTATGACTCTTGGCCTAAATTACTAGGCCAAGGTTTTTTCAAAGGGATGCACTAATTTTCACTATTTCC
B9401 Homo CYP1B1-AS1.seq (1>1776) → TGAACATTTTTATGACTCTTGGCCTAAATTACTAGGCCAAGGTTTTTTCAAAGGGATGCACTAATTTTCACTATTTCC
H2470-2.B9401F1.ab1 (50>700) →



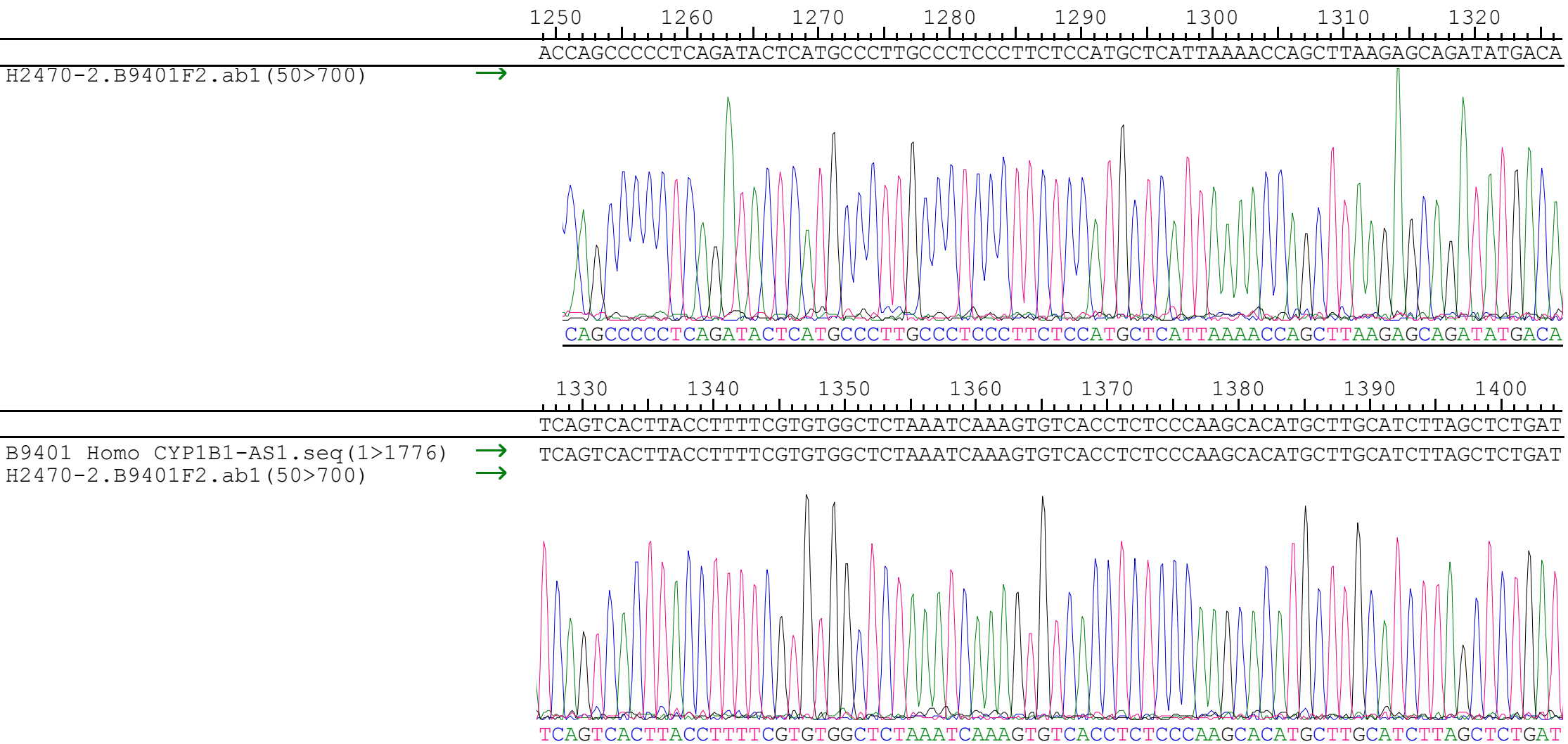
Project: Alignment of Homo CYP1B1-AS1.sq Contig 1



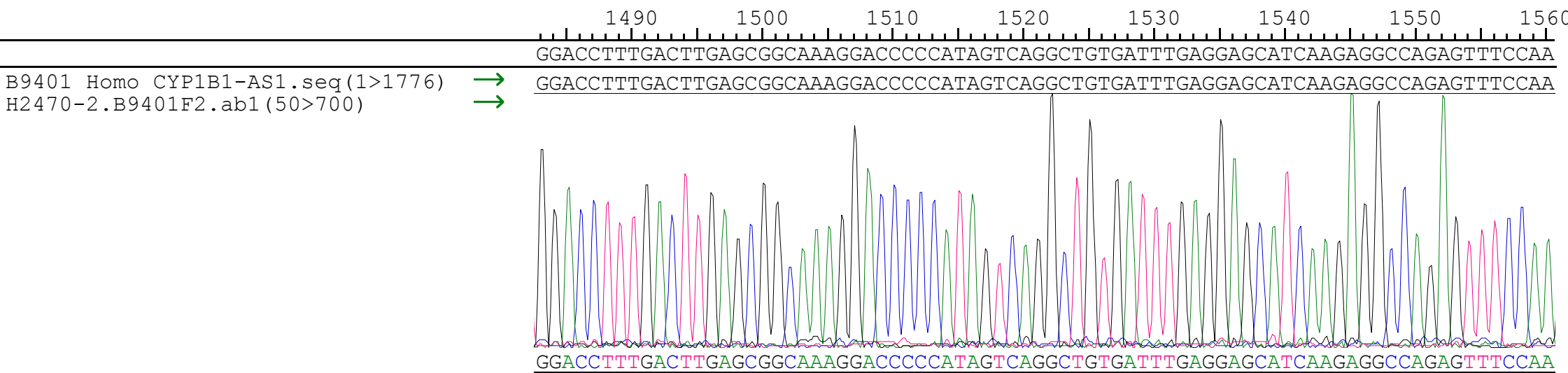
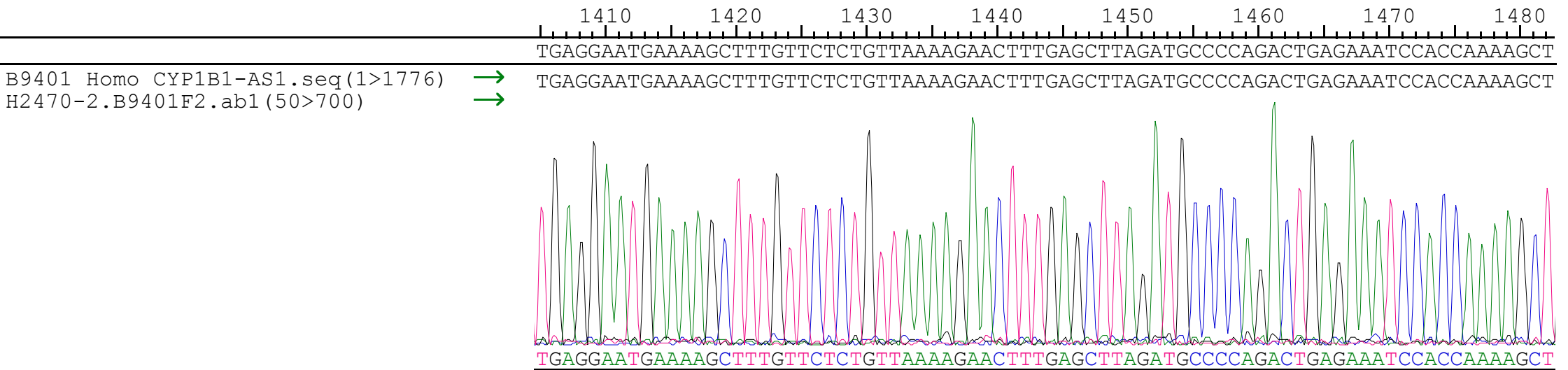
Project: Alignment of Homo CYP1B1-AS1.sq Contig 1



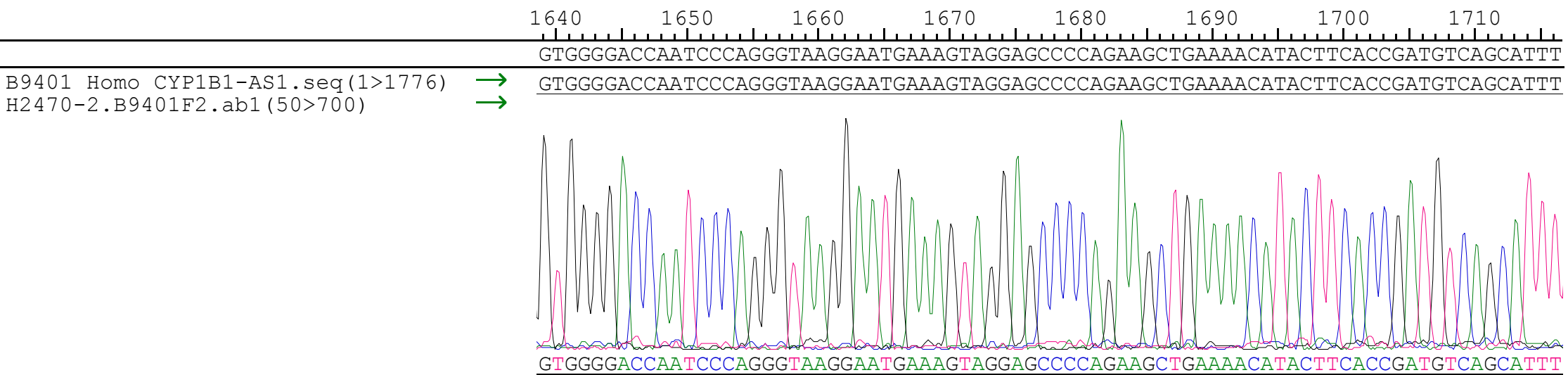
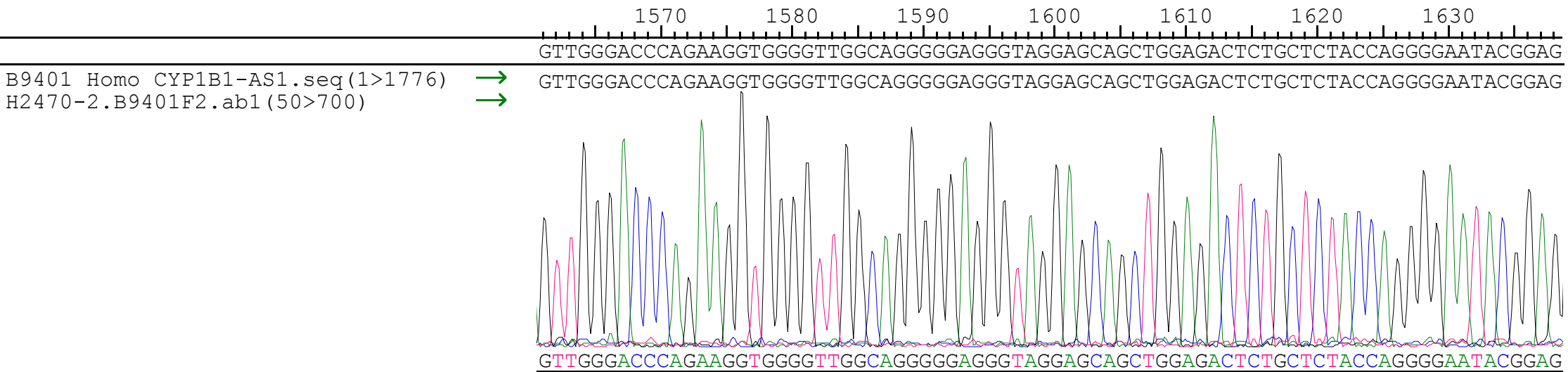
Project: Alignment of Homo CYP1B1-AS1.sqd Contig 1



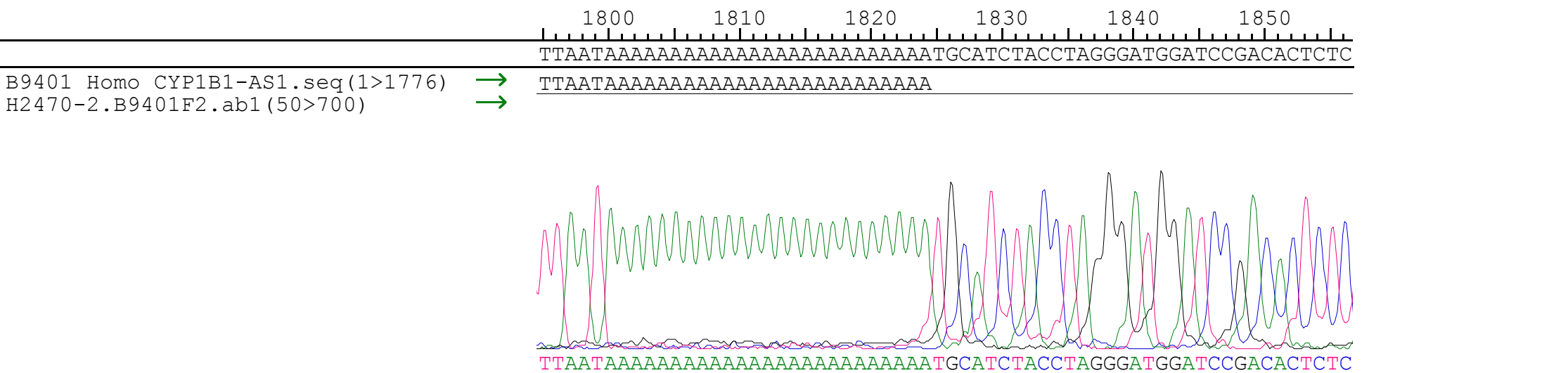
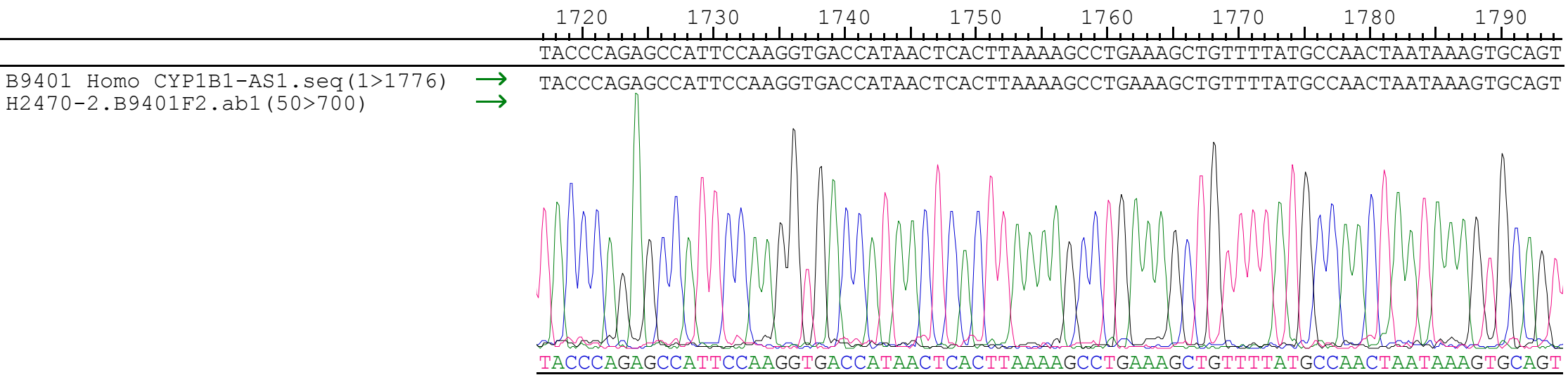
Project: Alignment of Homo CYP1B1-AS1.sq Contig 1



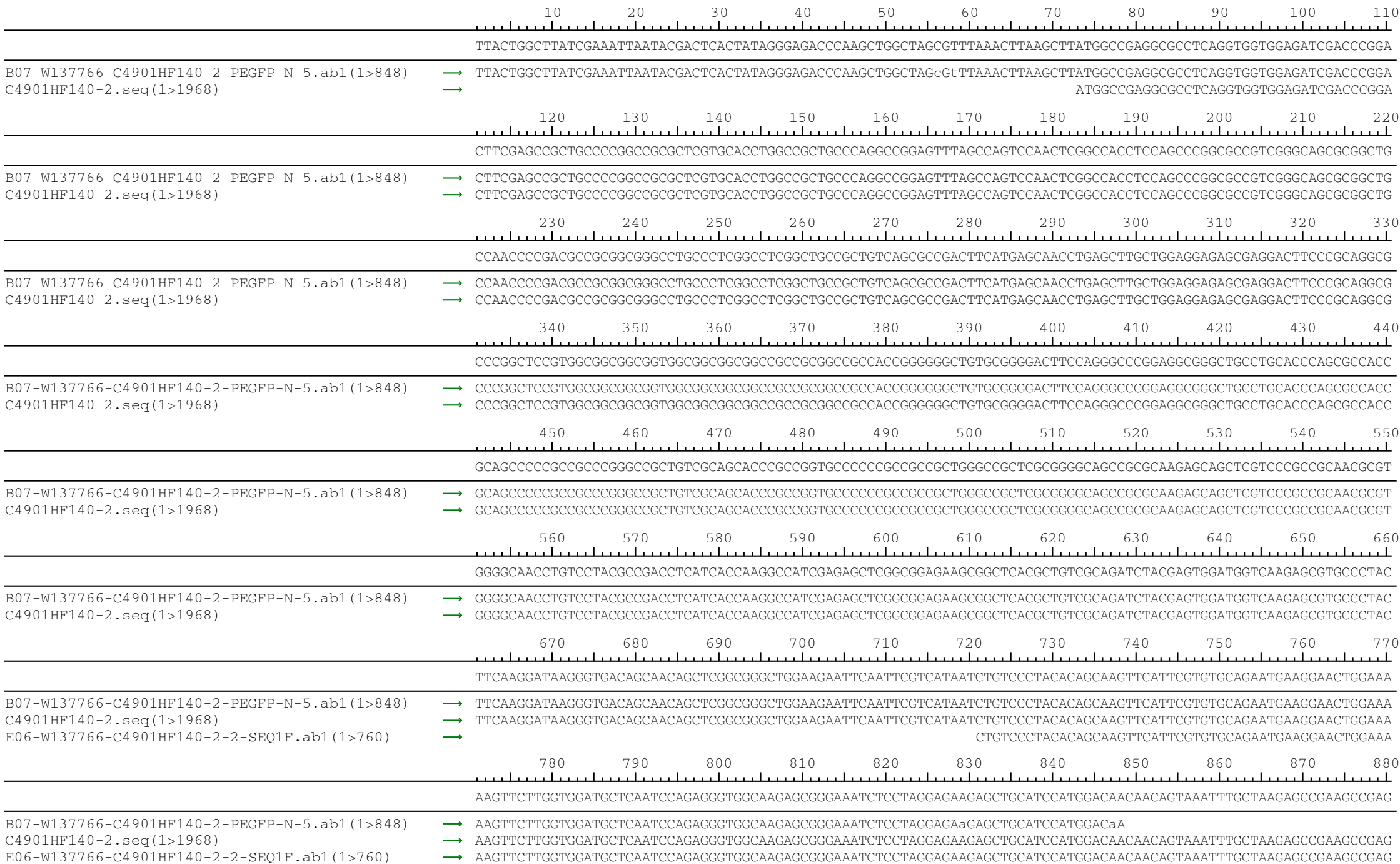
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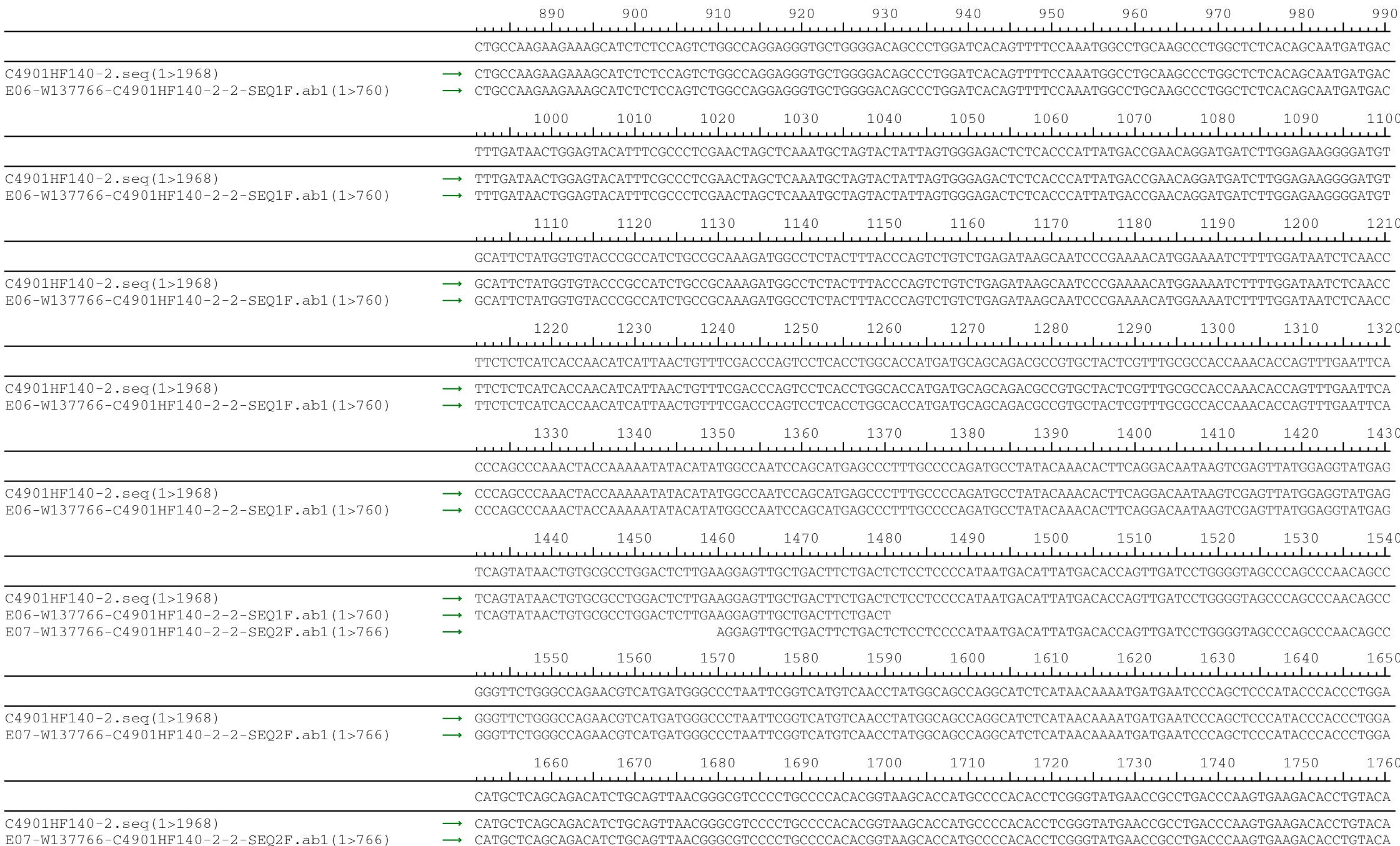
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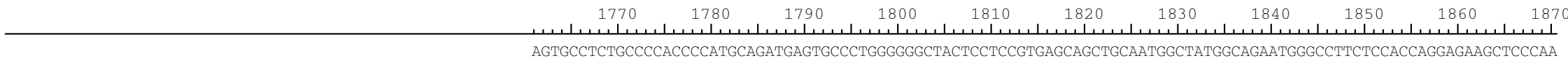
Supplementary Data 2. FOXO1 expression vector sequence.



Project: C4901HF140-2.SQD Contig 1

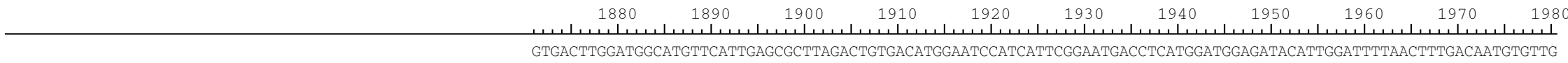


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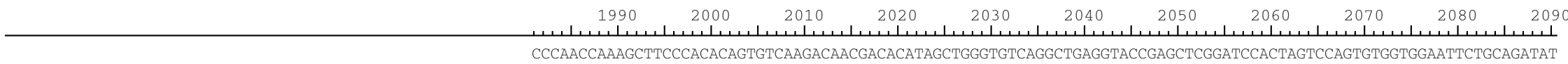
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C4901HF140-2.seq (1>1968) → AGTGCCTCTGCCCCACCCCATGCAGATGAGTGCCTTGGGGGGCTACTCCTCCGTGAGCAGCTGCAATGGCTATGGCAGAATGGGCCTTCTCCACCAGGAGAAGCTCCCAA
 E07-W137766-C4901HF140-2-2-SEQ2F.ab1 (1>766) → AGTGCCTCTGCCCCACCCCATGCAGATGAGTGCCTTGGGGGGCTACTCCTCCGTGAGCAGCTGCAATGGCTATGGCAGAATGGGCCTTCTCCACCAGGAGAAGCTCCCAA



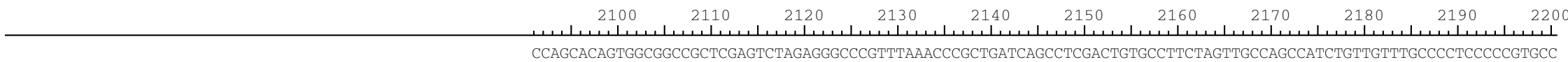
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C4901HF140-2.seq (1>1968) → GTGACTTGGATGGCATGTTTCATTGAGCGCTTAGACTGTGACATGGAATCCATCATTCGGAATGACCTCATGGATGGAGATACATTGGATTTTAACTTTGACAATGTGTTG
 E07-W137766-C4901HF140-2-2-SEQ2F.ab1 (1>766) → GTGACTTGGATGGCATGTTTCATTGAGCGCTTAGACTGTGACATGGAATCCATCATTCGGAATGACCTCATGGATGGAGATACATTGGATTTTAACTTTGACAATGTGTTG



CCCAACCAAAGCTTCCCACACAGTGTCAAGACAACGACACATAGCTGGGTGTCAGGCTGAGGTACCGAGCTCGGATCCACTAGTCCAGTGTGGTGGAAATCTGCAGATAT

C4901HF140-2.seq (1>1968) → CCCAACCAAAGCTTCCCACACAGTGTCAAGACAACGACACATAGCTGGGTGTCAGGCTGAGGTACCGAGCTCGGATCCACTAGTCCAGTGTGGTGGAAATCTGCAGATAT
 E07-W137766-C4901HF140-2-2-SEQ2F.ab1 (1>766) → CCCAACCAAAGCTTCCCACACAGTGTCAAGACAACGACACATAGCTGGGTGTCAGGCTGAGGTACCGAGCTCGGATCCACTAGTCCAGTGTGGTGGAAATCTGCAGATAT



CCAGCACAGTGGCGGCCGCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCC

E07-W137766-C4901HF140-2-2-SEQ2F.ab1 (1>766) → CCAGCACAGTGGCGGCCGCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCC



TTCCTTGACCCTGGAAGGTGCCACT

E07-W137766-C4901HF140-2-2-SEQ2F.ab1 (1>766) → TTCCTTGACCCTGGAAGGTGCCACT

Supplementary Fig.1 Expression characteristics of CYP1B1-AS1.

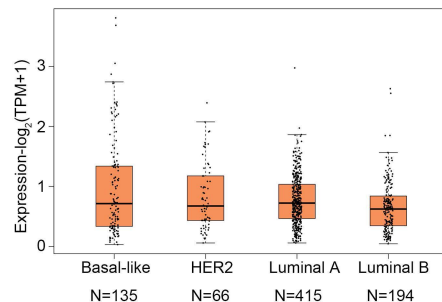
a

Protein coding potential

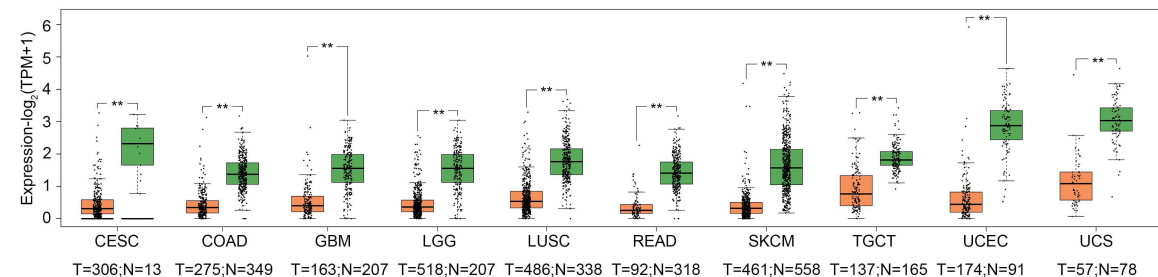
LNCipedia
version 5.2

Metric	Raw result	Interpretation
PRIDE reprocessing 2.0	0	non-coding
Lee translation initiation sites	0	non-coding
PhyloCSF score	-77.3697	non-coding
CPAT coding probability	16.57%	non-coding
Bazzini small ORFs	0	non-coding

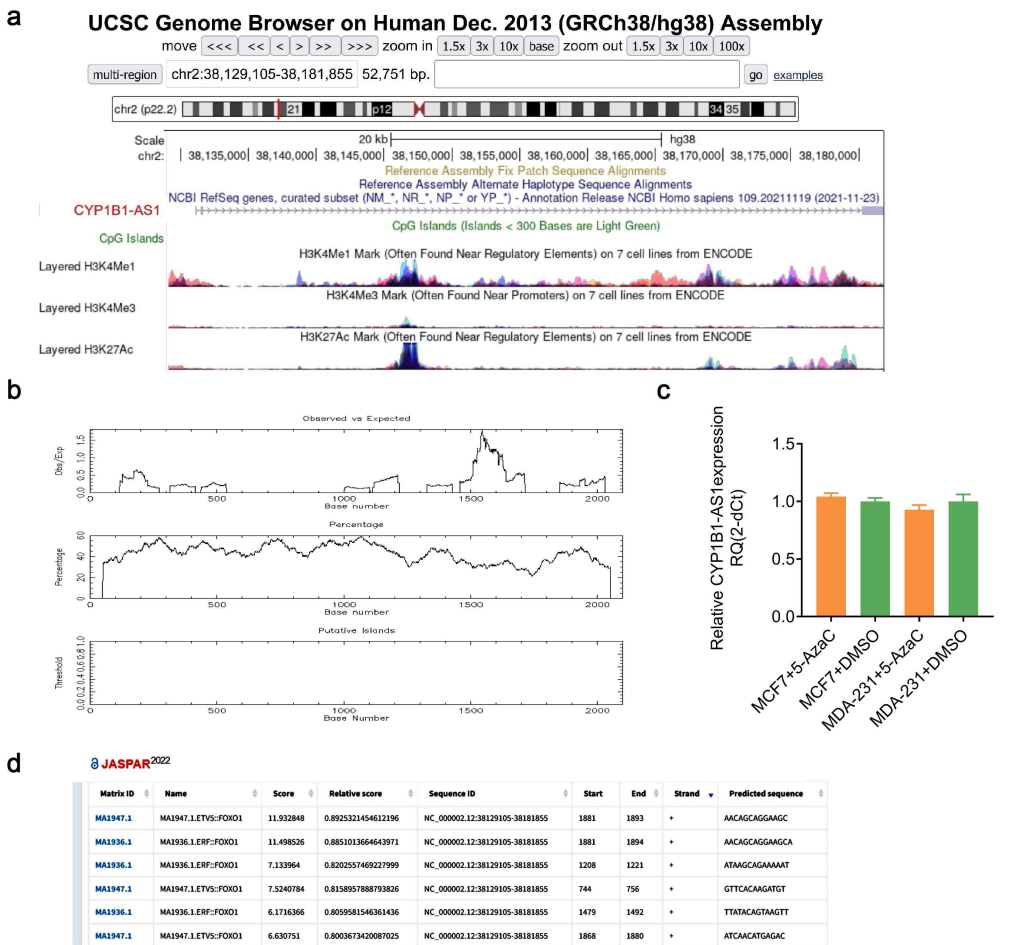
b



c



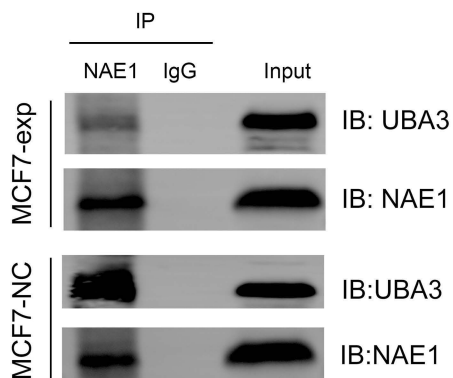
(a) The LNCipedia database shows the coding capacity and conserved features of CYP1B1-AS1. (b) TCGA data were used to analyze the expression of CYP1B1-AS1 across molecular subtypes of breast cancer. (c) TCGA data show that CYP1B1-AS1 is downregulated in various cancers. CESC, cervical and endocervical cancers. COAD, colon adenocarcinoma. GBM, Glioblastoma multiforme. LGG, brain lower grade glioma. LUSC, lung squamous cell carcinoma. READ, rectum adenocarcinoma. SKCM, skin cutaneous melanoma. TGTC, testicular germ cell tumor. UCEC, uterine corpus endometrial carcinoma. UCS, uterine carcinosarcoma. ** $P < 0.01$.



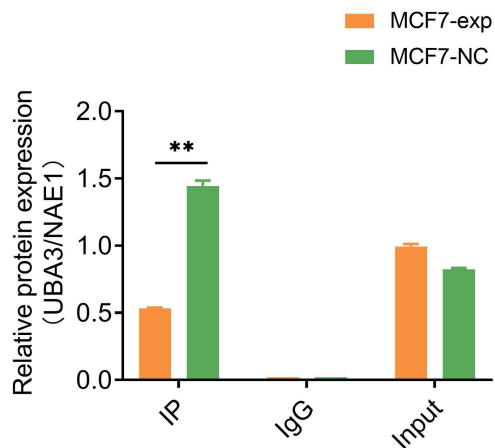
(a) Characterization of CYP1B1-AS1 gene and its promoter region provided by UCSC Genome Browser. (b) Methylation of the CYP1B1-AS1 promoter region predicted by EBI CpGPlot. (c) qPCR was used to detect changes in CYP1B1-AS1 after treatment of MCF7 and MDA-MB-231 cells with the demethylating drug 5-Azacytidine. 5-Azac, 5-Azacytidine. Solvent DMSO as blank control. Experiments were performed at least 3 times. Data are presented as mean \pm SEM and analyzed with a two-tailed *T* test. (d) JASPAR was used to analyze the binding sites of the transcription factor FOXO1 to the CYP1B1-AS1 promoter region.

Supplementary Fig.3 The results of the co-immunoprecipitation assay.

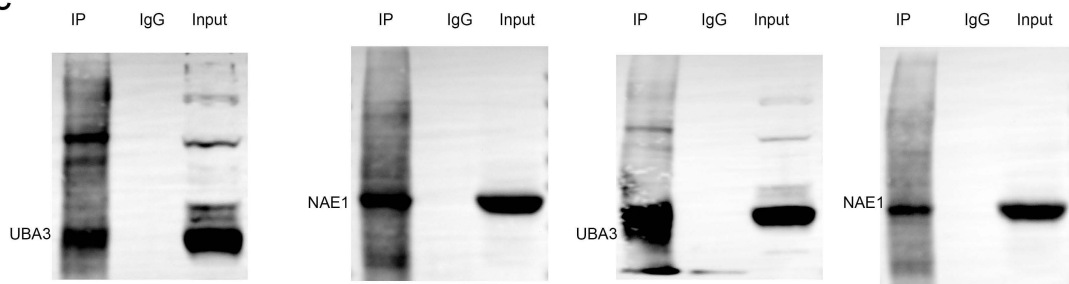
a



b



c



(a,b) Co-immunoprecipitation experiments were used to analyze changes in the amount of UBA3 protein bound to NAE1 in MCF7 cells after CYP1B1-AS1 upregulation.

** $P < 0.01$. (c) Raw Western blot data from co-immunoprecipitation experiments.

Supplementary Fig.4 Raw data of Western blot.

Fig.2f

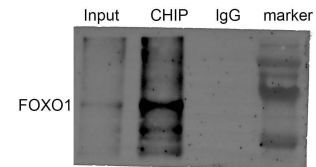


Fig.2i

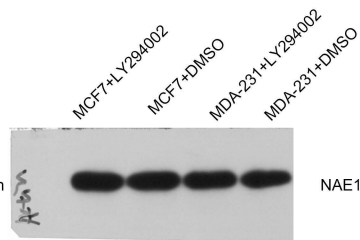
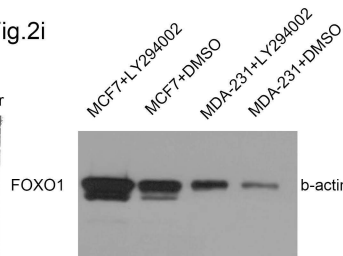


Fig.5e

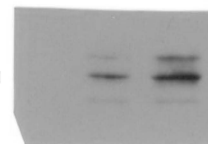


Fig.6a

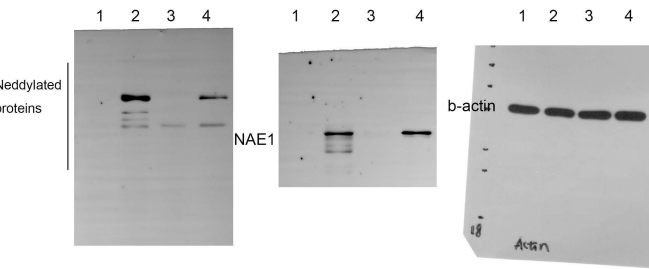


Fig.6c

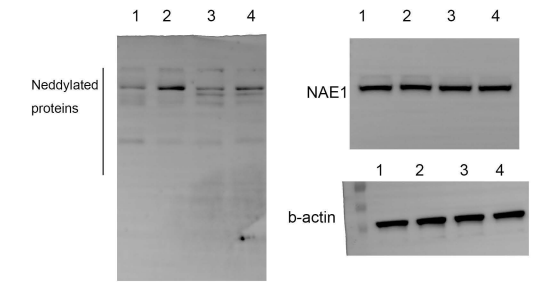
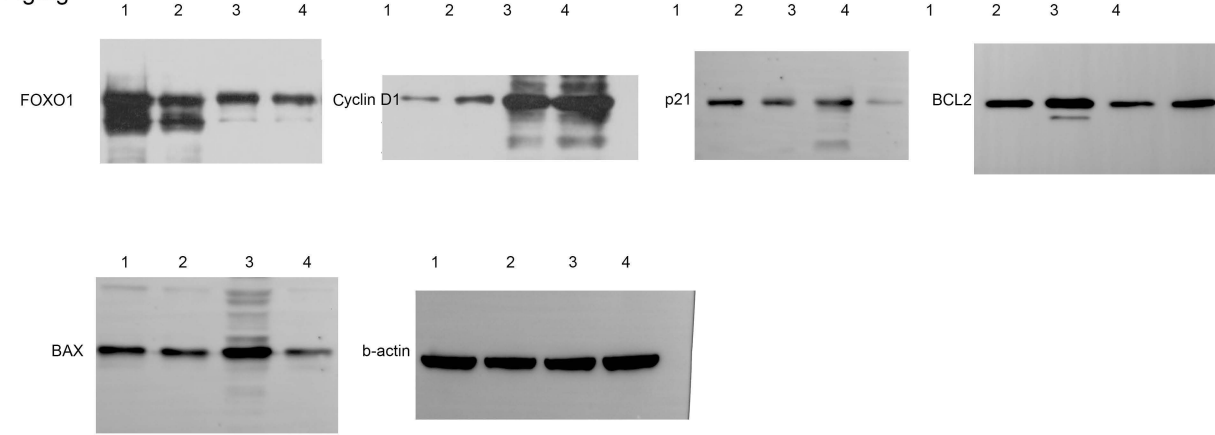


Fig.6g



1, MCF7-exp; 2, MCF7-NC; 3, MDA-231-exp; 4, MDA-231-NC.