

Genetic heterogeneity for *SMARCB1*, *H3F3A* and *BRAF* in a malignant childhood brain tumour: genetic-pathological correlation

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Short title: Genetic intra-tumoural heterogeneity in an a malignant paediatric brain tumour

Intra-tumour heterogeneity is an important diagnostic, therapeutic and prognostic challenge. Its extent and mechanism in brain tumours is incompletely understood[1]. We describe a malignant tumour with unique pathological and genetic features. Most notably the tumour contained mutations in the *SMARCB1* gene (typically associated with Atypical Teratoid/Rhabdoid Tumours[2]), the *H3F3A* gene (typically associated with high grade glioma in children[3]) and the *BRAF* gene. Furthermore, there was marked heterogeneity in mutation load between different parts of the tumour. This heterogeneity has implications both for the evolution of the tumour and for its diagnosis.

A previously healthy 14-year-old girl presented with acute onset of headache, vomiting, blurred vision, olfactory and gustatory hallucinations, and flashes of past experience. MRI scan of the brain showed a localized heterogeneous haemorrhagic lesion in the left mesial

temporal region (Figure 1). She underwent subtotal tumour resection and was then treated with cranio-spinal irradiation. However, residual tumour persisted through treatment and she had cytological evidence of CSF dissemination. She died three months after presentation.

The tumour (Figure 2) was of high cellularity and consisted of large cells with eosinophilic cytoplasm and large vesicular nuclei. Some cells had prominent nucleoli. There were frequent mitotic figures and apoptotic bodies, and moderate to severe nuclear pleomorphism. Some areas resembled a glioblastoma by virtue of areas of pseudo-palisading necrosis. There was no microvascular proliferation, no Rosenthal fibres, and no eosinophilic granular bodies. In some areas, there was a myxoid stroma, vascular reaction and tumour reticulin deposition. Immunostaining was positive for vimentin and showed patchy reactivity for desmin, EMA, synaptophysin, CD34, but was negative for SMA, neurofilament and cytokeratin. There were a few foci of GFAP-positive tumour cells throughout the tumour but most tumour cells were negative. The Ki67 labelling index was very high. There was a mixture of parts of the tumour where many of the tumour cells retained INI-1 immunoreactivity ('region 1') and other parts of the tumour where most of the tumour cells showed loss of INI1 immunoreactivity ('region 2').

Genetic heterogeneity was demonstrated by sequencing of the coding regions of *SMARCB1* gene, exon 15 of the *BRAF* gene and exon 1 of the *HIST1H3B* and *H3F3A* genes from the 2 regions of the tumour. *SMARCB1* gene copy number was demonstrated using MLPA.

A 2bp duplication was detected in exon 5 of the *SMARCB1* gene, leading to a frameshift mutation c.560_561dupCC (Figure 3). This results in a predicted truncated protein p.Ile189Profs*21. We examined the mutation in two regions of the tumour; one of which showed residual INI1 staining with only focal loss ('region 1') and in one of which most cells were negative for INI-1 ('region 2'). The mutation was detected reproducibly at high levels in region 2 but only at low levels in region 1. MLPA using *SMARCB1* Kit P258 (MRC Holland, Amsterdam, the Netherlands) demonstrated loss of heterozygosity (LOH) for the entire *SMARCB1* gene and several additional genes within the 22q11 region only in region 1 but not region 2. However, it should be noted that the limit of detection with MLPA is high which may have masked the extent of LOH in these heterogeneous samples.

A c.1799T>A mutation was detected in exon 15 of the *BRAF* gene in both areas, resulting in the amino acid change p.Val600Glu. The mutation load in region 1 was greater than that observed in region 2 (Figure 3).

Histones 3.1 (*HIST1H3B*) and 3.3 (*H3F3A*) were analysed by Sanger sequencing. The region encompassing codons Lys28 and Gly35, commonly mutated in paediatric high-grade gliomas, was assessed using the current Reference Sequences, NP_003528.1 (*HIST1H3B*) and NP_002098.1 (*H3F3A*). Historically these codons have often been referred to as Lys27 and Gly34 in the literature. A c.83A>T missense mutation was detected in the *H3F3A* gene in

region 1 resulting in the protein change p.Lys28Met (Figure 3). This mutation was not detected in region 2 allowing for the limit of detection of Sanger sequencing of approximately 20%. Region 1 showed the presence of two variants in the *HIST1H3B* gene, c.174G>A and c.267G>A. These variants are predicted to result in synonymous polymorphisms; p.(Ser58Ser) and p.(Ala89Ala) respectively. These two variants were not detected in the DNA extracted from region 2. The p.Ala89Ala variant has been reported in a small number of healthy individuals in the dbSNP database, rs139461801 (NCBI). The other variant, p.Ser58Ser has not been reported in any of the on-line databases. The presence of this polymorphism in region 1 but not region 2 may either represent LOH in region 2 or somatic variants in region 1.

We have described an unusual malignant brain tumour with areas that had histopathological features consistent with both glioblastoma and AT/RT. This morphological variability was reflected in striking genetic heterogeneity (summarised in Table 1). Uniquely, this tumour has a combination of mutations in *SMARCB1*, *H3F3A* and *BRAF*. We detected the *SMARCB1* mutation at the highest levels in the parts of the tumour that showed histological features of an ATRT (i.e. INI1 loss by immunohistochemistry). In contrast, we only found mutations in the *H3F3A* gene in the parts that showed retained INI1 staining. The V600E *BRAF* mutation was present in both regions examined but was present at a higher level in the INI1-retained region. The mutation in *SMARCB1* is novel and is predicted to generate a truncated protein.

To the best of our knowledge this pattern of morphology with matched genetic heterogeneity has not been previously described. It raises a number of diagnostic possibilities. The first is that this is a rhabdoid glioblastoma (R-GBM). R-GBM is a rare subtype of GBM, which may be morphologically indistinguishable from AT/RT. INI-1 staining is usually retained, but can be focal, or the level of expression can be low in the rhabdoid cells[4] but mutations in the *SMARCB1* gene have not been seen [5].

The second possibility is that this is an example of an ATRT arising from a pre-existing tumour. The development of ATRT-like tumours has been rarely described in the context of other, often low grade, tumours[6-8].

Finally, our findings suggest the alternative explanation that the two components of this tumour have evolved out of a single precursor lesion, which lacked mutations in *SMARCB1* or *H3F3A* but may have had a mutation in *BRAF*. Out of that precursor lesion, one component developed a mutation in *SMARCB1* and one component developed a mutation in *H3F3A*. This heterogeneity has important implications for the mechanism of tumour evolution. In addition, it has implications for diagnosis, as sequencing of single regions may not identify the spectrum of mutations in the tumour.

Figure Legends

Figure 1 Coronal FLAIR (left hand panel) and post-contrast coronal T1-weighted images (right hand panel) showing a well-defined, heterogeneous mass centred in the left mesial temporal region with some internal haemorrhage and rim and basal nodular enhancement.

Figure 2 Histological images of the tumour including areas resembling a high-grade astrocytoma (Region 1) with pseudo-palisading necrosis (A) with retained INI-1 staining (C) and areas containing rhabdoid cells (B) with loss of INI-1 staining (D) (Region 2). A few small collections of tumour cells in both regions express GFAP but most tumour cells were negative (E-Region 1, F-Region 2). Scale bars: 50 μ m.

Figure 3 Sequencing of *SMARCB1* (A), *BRAF* (B) and *H3F3A* (C). The top panels show region 1, the middle panels region 2 and the lower panel show a wild type control. A *SMARCB1* mutation is present in region 2 and at low levels in region 1. The *BRAF* mutation is present in both samples but is at a higher level in region 1. The *H3F3A* mutation is present only in region 1. The arrows show the site of the mutations.

Table 1: A summary of the main genetic findings in the two regions of the tumour.

Conflict of interest

The authors have no conflict of interest to declare.

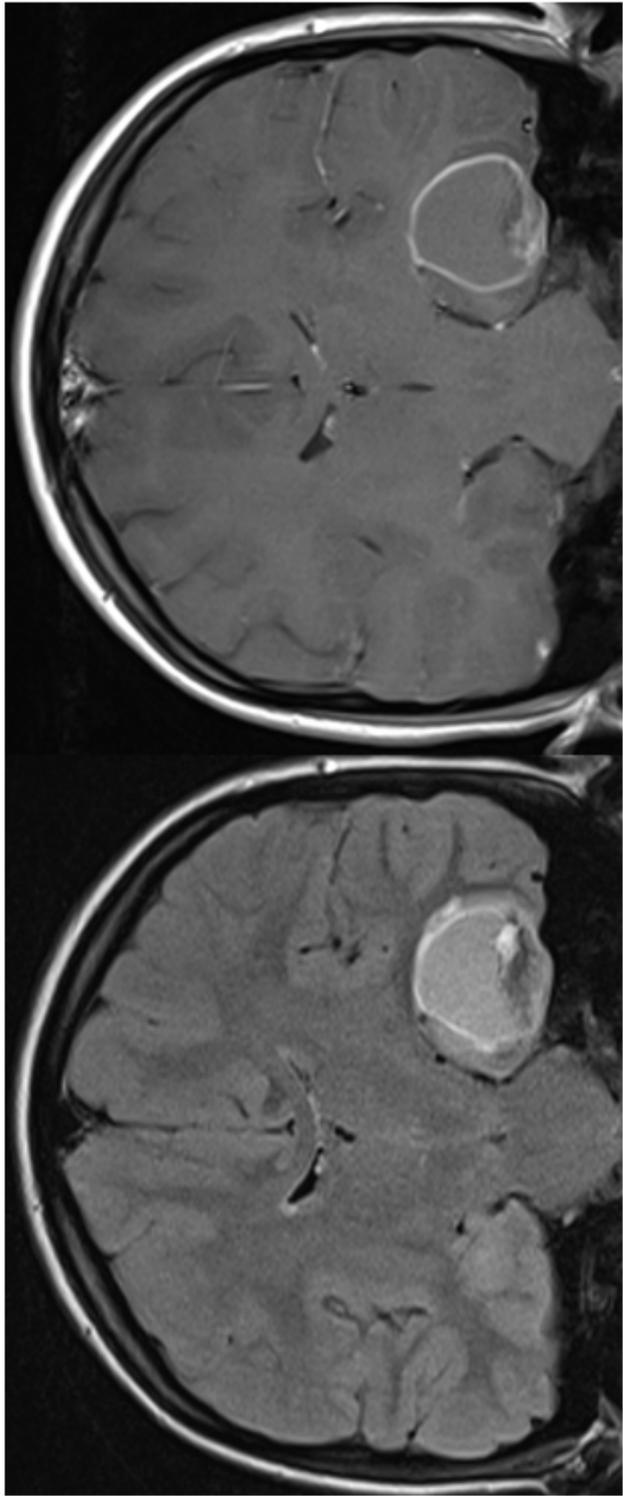
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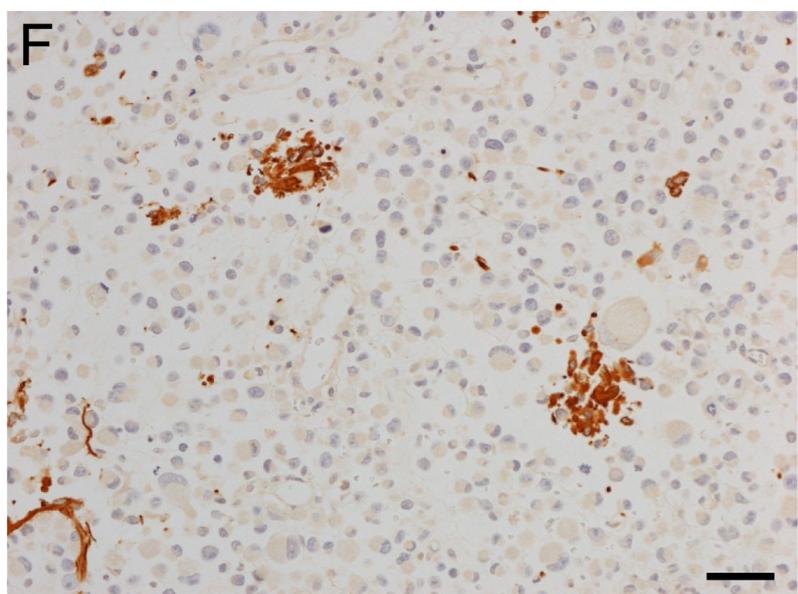
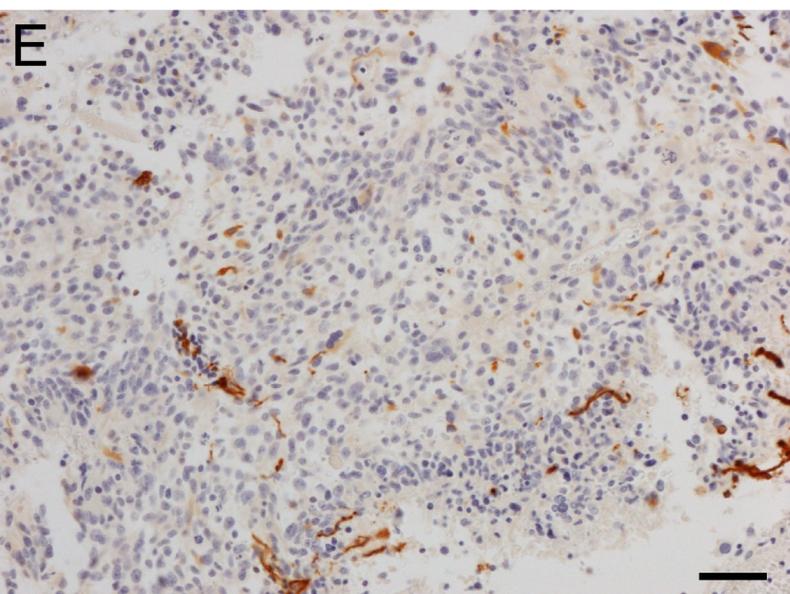
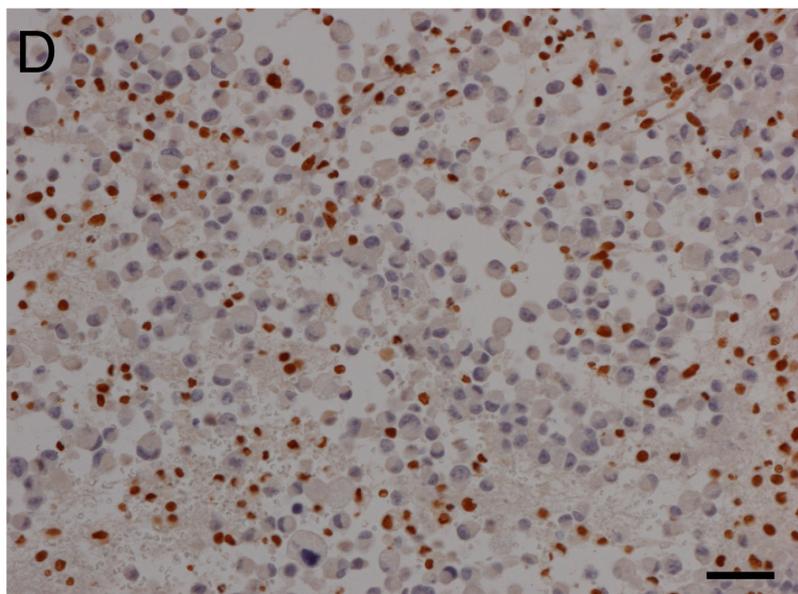
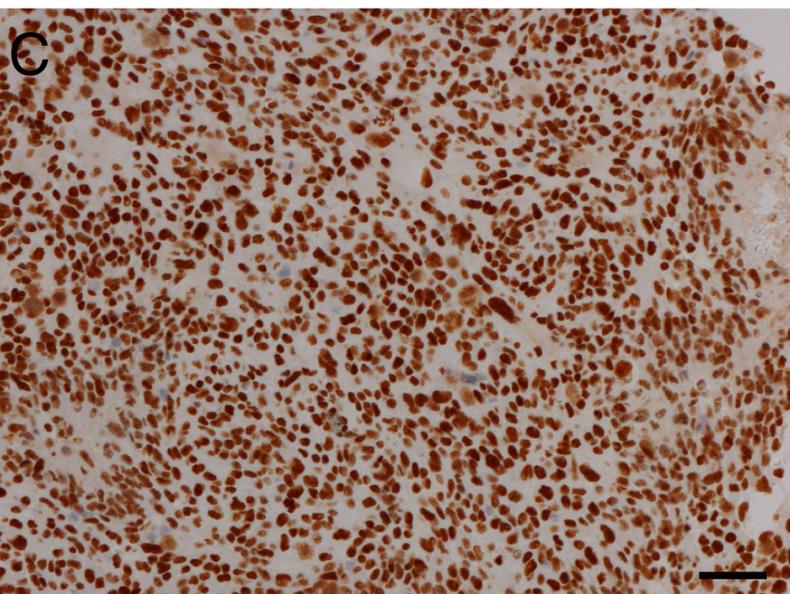
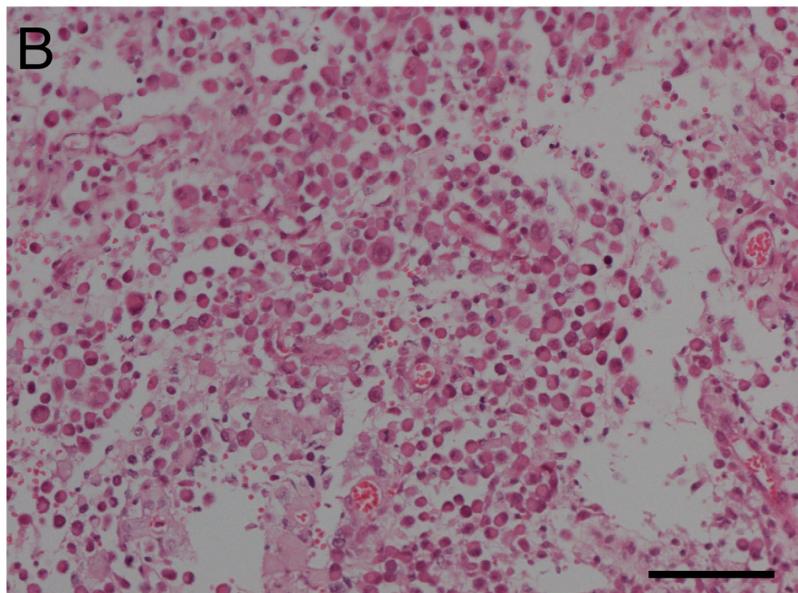
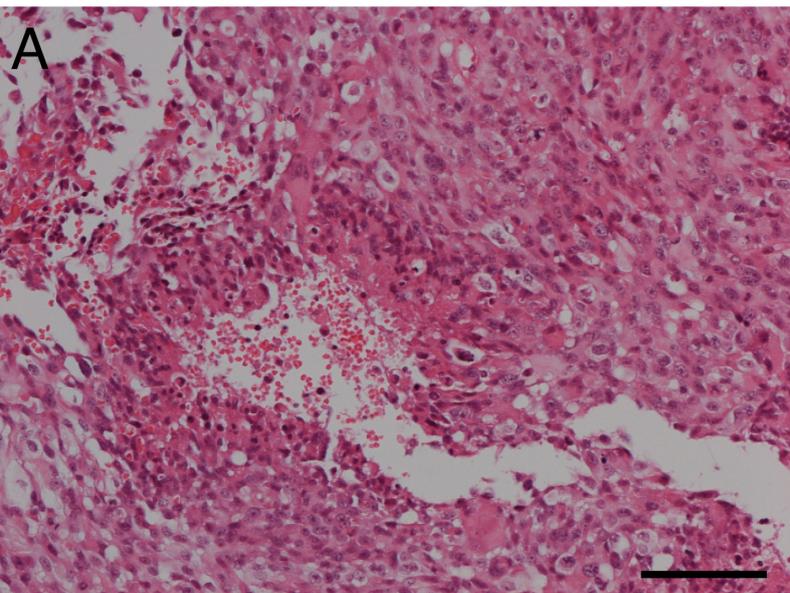
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	Region 1	Region 2
INI1 immunohistochemistry	Mostly retained	Mostly lost
<i>SMARCB1</i> insertion	Present at very low levels	Present at high levels
<i>SMARCB1</i> LOH	Present	Absent
V600E <i>BRAF</i> mutation	Present	Present (at lower levels than region 1)
<i>H3F3A</i> mutation	Present	Absent
<i>HIST1H3B</i> polymorphisms	Present	Absent

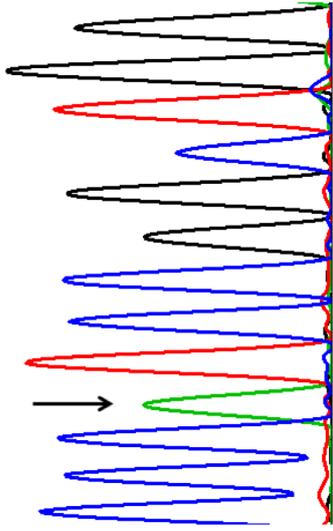
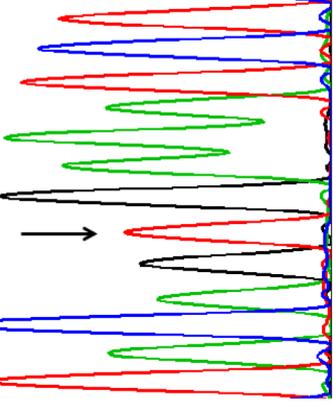
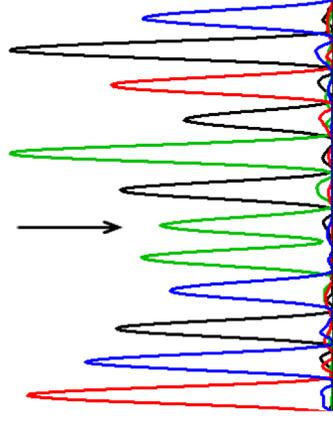




H3F3A

BRAF

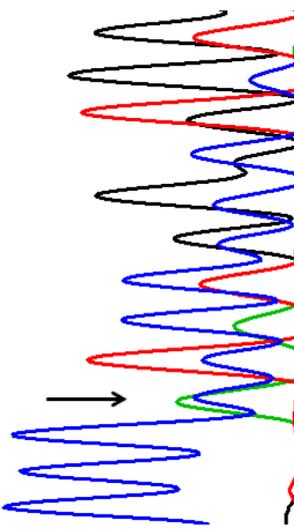
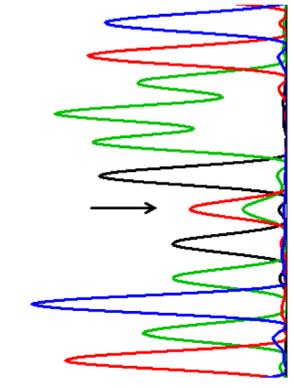
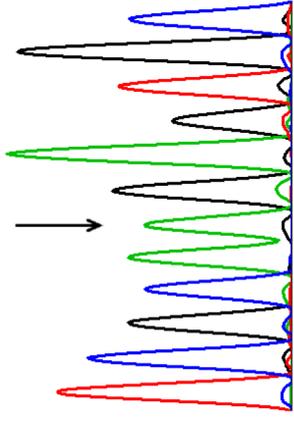
SMARCB1



Wild type

Wild type

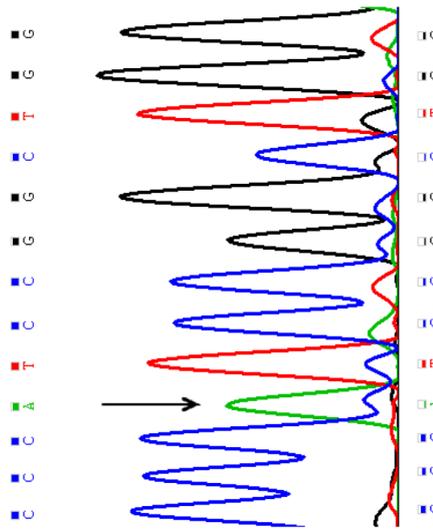
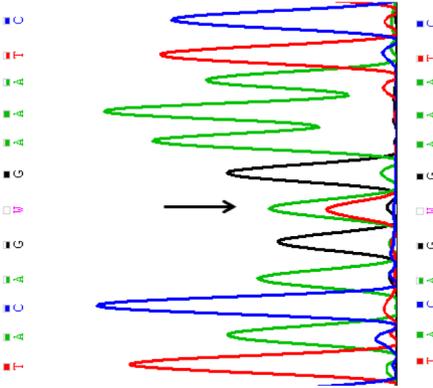
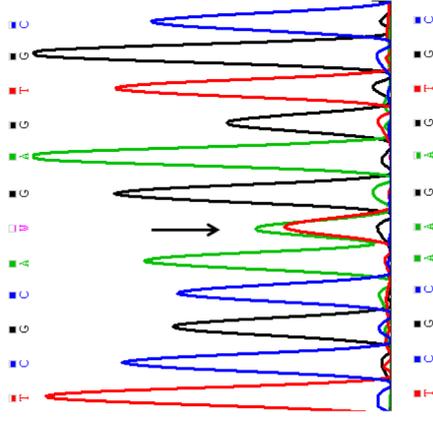
Wild type



Region 2

Region 2

Region 2



Region 1

Region 1

Region 1

C

B

A