**Supplemental Data**

**Table e-1. Mutant allele ratios detected by ddPCR**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **MUT ratios** | **ACD** | **MUT copy number\*** | **WT copy number\*** | **MUT drop number** | **WT drop number** | **FA (%)** |
| 5% | 13301 | 101 | 1988 | 57 | 1078 | 4.84 |
| 5% | 14283 | 107.4 | 2020 | 65 | 1177 | 5.04 |
| 5% | 13909 | 122.2 | 1978 | 72 | 1121 | 5.82 |
| 1% | 13898 | 28.8 | 2120 | 17 | 1195 | 1.34 |
| 1% | 15121 | 17.12 | 2220 | 11 | 1363 | 0.77 |
| 1% | 14610 | 22.6 | 2260 | 14 | 1338 | 0.99 |
| 0.5% | 15357 | 15.32 | 2400 | 10 | 1487 | 0.64 |
| 0.5% | 10132 | 9.3 | 2140 | 4 | 878 | 0.43 |
| 0.5% | 12103 | 9.72 | 2300 | 5 | 1131 | 0.42 |
| 0.25% | 12386 | 9.5 | 2220 | 5 | 1117 | 0.42 |
| 0.25% | 10773 | 8.74 | 2480 | 4 | 1077 | 0.35 |
| 0.25% | 14033 | 5.04 | 2260 | 3 | 1283 | 0.22 |
| 0.1% | 10942 | 0 | 2320 | 0 | 1028 | / |
| 0.1% | 14568 | 4.84 | 2420 | 3 | 1426 | 0.2 |
| 0.1% | 13595 | 3.46 | 2340 | 2 | 1283 | 0.15 |

\*Calculated droplet number using QuantaSoft; MUT: mutant, WT: wild-type; ACD: accepted droplet; FA: fractional abundance

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**Figure e-1. Imaging and molecular evaluation of Case 2 with definite leptomeningeal angiomatosis**

**A** Pre-contrast T1-weighted axial MRI scan showing left cerebral atrophy, most prominent in the posterior cortex. **B** Identification of the wildtype *GNAQ* allele in green (present in brain and blood) and mutant *GNAQ* R183Q allele (in blue) in brain-but not blood-derived DNA. Droplets without DNA template are grey. Y-axis, amplitude of fluorescent signal. WT, wildtype *GNAQ* probe. MUT, mutant *GNAQ* R183Q probe.

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**Figure e-2. Limit of detection for ddPCR assay**

Limit of detection of the ddPCR assay was established by serially diluting mutant samples into wild-type DNA in order to obtain different mutant/(mutant + wild-type) ratios: 5, 1, 0.5, 0.25 and 0.1%. An amplitude of 4000 was set as the positive mutant droplets threshold. Mutant allele at a frequency ≥ 0.25% could be consistently detected, while detection of mutant allele at 0.1% could be achieved in only 2 of 3 wells.

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**Figure e-3. Molecular evaluation of Case 4 with definite leptomeningeal angiomatosis**

Identification of the wildtype *GNAQ* allele in green (present in brain and blood), and mutant *GNAQ* R183Q allele in blue in brain but not blood derived DNA. Droplets without DNA template are grey. Y-axis, amplitude of fluorescent signal. WT, wildtype *GNAQ* probe. MUT, mutant *GNAQ* R183Q probe. ****

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**Figure e-4. Droplet Digital PCR of Case 1 – 2D plot for Brain and Blood**

Droplet digital PCR read-out in 2D plot showing results for brain (**A**) and blood (**B**) derived DNA. Droplets positive for *GNAQ* R183Q allele are blue (in brain only), droplets positive for wild-type probe are green, droplets without DNA template grey, and droplets containing multiple DNA templates orange. Axes, amplitude of fluorescent signal.

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**Figure e-5. Droplet Digital PCR of Case 2 – 2D plot for Brain and Blood**

Droplet digital PCR read-out in 2D plot showing results for brain (**A**) and blood (**B**) derived DNA. Droplets positive for *GNAQ* R183Q allele are blue (in brain only), droplets positive for wild-type probe are green, droplets without DNA template grey, and droplets containing multiple DNA templates orange. Axes, amplitude of fluorescent signal.

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**Figure e-6. Droplet Digital PCR of Case 3 – 2D plot for Brain**

Droplet digital PCR read-out in 2D plot showing results for brain derived DNA. Droplets positive for *GNAQ* R183Q allele are blue, droplets positive for wild-type probe are green, and droplets without DNA template grey. Axes, amplitude of fluorescent signal.

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**Figure e-7. Droplet Digital PCR of Case 4 – 2D plot for Brain and Blood**

Droplet digital PCR read-out in 2D plot showing results for brain (**A**) and blood (**B**) derived DNA. Droplets positive for *GNAQ* R183Q allele are blue (in brain only), droplets positive for wild-type probe are green, and droplets without DNA template grey. Axes, amplitude of fluorescent signal.