Form for the evaluation of a genetic test for NHS Service Gene Dossier/Additional Provider

Submitting laboratory: Salisbury RGC

1. Disorder/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website) If this submission is for a panel test please complete appendix 1 listing all of the conditions included using approved OMIM name, symbol and OMIM number.
NF1 and other disorders of the Ras/MAPK pathway - see appendix 1
2. OMIM number for disorder/condition If a panel test – see 1. above
See appendix 1
3a. Disorder/condition – please provide, in laymen's terms, a brief (2-5 sentences) description of how the disorder(s) affect individuals and prognosis.
Rasopathies are a spectrum of disorders caused by mutations in a gene in the RAS/MAPK pathway. Rasopathy disorders have overlapping but variable features, which commonly include short stature, heart defects, developmental delays/intellectual disability, vision and hearing problems, skin problems and characteristic facial features. In some cases, there is also an increased risk of developing tumours or cancer.
3b. Disorder/condition – if required please expand on the description of the disorder provided in answer to Q3a.
Neurofibromatosis 1 (NF1) is a heritable condition the symptoms of which include (but are not limited to): multiple cutaneous neurofibromas, nodules on the iris (Lisch nodules), optic gliomas, malignant peripheral nerve sheath tumours, 25% frequency of autism, 40% frequency of learning difficulties and skeletal complications such as pseudoarthrosis and scoliosis. Similarly mutations in other genes in the RAS/MAPK pathway give rise to a range of developmental disorders collectively referred to as the RASopathies. Patients suffering from these syndromes have similar symptoms which include heart abnormalities, characteristic facial features, cutaneous malformations, gastrointestinal malfunction, failure to thrive and a spectrum of mental retardation. In many RASopathies patients can develop early onset benign and malignant tumours.
4. Disorder/condition – mode of inheritance If this submission is for a panel test, please complete the mode of inheritance for each condition in the table in appendix 1.
See appendix 1
5. Gene – approved name(s) and symbol as published on HGNC database (alternative names will be listed on the UKGTN website) If this submission is for a panel test please complete appendix 1 listing all of the genes included using approved HGNC name, symbol, number and OMIM number.
See appendix 1
6a. OMIM number(s) for gene(s) If a panel test – see 5. above
See appendix 1
6b. HGNC number(s) for gene(s) If a panel test – see 5. above
See appendix 1

7a. Gene – description(s)

If this submission is for a panel test, please provide total number of genes.

Total number of genes = 14

7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic)

(n/a for panel tests)

n/a

7c. GenU band that this test is assigned to for index case testing.

G

8. Mutational spectrum for which you test including details of known common mutations

(n/a for panel tests)

If this application is for a panel test to be used for different clinical phenotypes and/or various sub panel tests – please contact the team for advice before completing a Gene Dossier

n/a

9a. Technical method(s) – please describe the test.

Probes for the gene panel will be selected from the Illumina Trusight One Clinical Exome Panel. Library preparation will use Nextera Rapid Capture Custom Enrichment (Illumina) for sequencing on an Illumina MiSeq.

The technical method can de divided into a number of steps as outlined below:

1. Tagmentation of input DNA
2. Indexing PCR using primers targeted to universal sequences added in step 1
3. Product pooling
4. Hybridisation and capture
5. Post capture amplification
6. Library QC
7. Sequencing (Illumina MiSeq / NextSeq)
8. Read alignment
9. Indel realignment
10. Base quality score recalibration
11. Variant calling
12. Variant filtering
13. Variant annotation

Samples are identified via a pair of 8bp identity sequences, one appended to each end of the finished library constructs by an automated assay. All samples and reagents are bar-coded for tracking and recording.

Data analysis is performed using a bespoke pipeline following recommendations from the Broad Institute, who provides the *de facto* tools and advice for Illumina sequencing analysis. Briefly, paired-end reads are globally aligned to the GRCh37 reference genome and filtered to exclude amplification duplicates based on identical mapping positions. For inclusion, a minimum mapping quality score (MQ) of 40 is used and a read pair's best alignment score must be at least three times greater than its second best score.

Secondly, reads mapped near known or suspected indels are re-aligned to minimise the number of mismatching bases across all reads. Thereafter, the base quality scores are recalibrated to better reflect their true likelihood of error. Recalibration is achieved by analysing co-variation such as reported base quality, cycle and sequence context associated with non-dbSNP138 reference mismatches. Finally, variants are identified and given a probability of error using a Bayesian approach within locally assembled regions followed by filtering and annotation.

An excel output is generated showing gaps in the horizontal coverage for core genes and any sequence variants (excluding known polymorphisms) that require confirmation, i.e. any region in a core gene not

covered >=x30 and all detected variants to be reported are tested/confirmed by a second test.

Filtering for clinical significance and categorisation of variants for the purpose of reporting is performed in a semi-automated process with the aid of disease-specific databases (in-house and online) and Alamut software.

9b. For panel tests, please specify the strategy for dealing with gaps in coverage.

Reportable diagnostic coverage is defined as read depth greater than or equal to 30x on a per base basis. In general the minimum region of interest comprises all coding exons +/- 10bp of intronic sequence to include the consensus splice sites.

Mean coverage for each gene calculated from the sub-panel verification data is given in the appendix.

For “core” genes all horizontal coverage gaps in relation to the minimum region of interest are filled in on a run by run basis to provide horizontal coverage greater than or equal to that provided by the previous conventional methodology.

Gap filling to ensure horizontal coverage is currently performed by Sanger sequencing. From 2015 we will transfer to a PCR based genotyping assay to amplify specific fragments and sequence on an Illumina MiSeq.

For non-core genes, no additional work is done to increase coverage above that provided by the panel test. Percentage coverage of the minimum region of interest is calculated per run for each gene analysed and indicated on the report.

9c. Does the test include MLPA?

(For panel tests, please provide this information in appendix 1)

Yes for genes NF1, SPRED and RASA1. No gene specific MLPA kits are available for the other genes in this panel.

9d. If NGS is used, does the lab adhere to the Association of Clinical Genetic Science Best Practice Guidelines for NGS?

Yes.

10. Is the assay to be provided by the lab or is it to be outsourced to another provider?

If to be outsourced, please provide the name of the laboratory and a copy of their ISO certificate or their CPA number.

Provided by the lab.

11. Validation process

Please explain how this test has been validated for use in your laboratory or submit your internal validation documentation. If this submission is for a panel test, please provide a summary of evidence of:

i) instrument and pipeline validation, and

ii) panel verification for the test

Please submit as appendices to the Gene Dossier (these will be included in the published Gene Dossier available on the website).

Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

(1) Validation.

The full internal validation document is attached as a separate document – validation doc1.

The Illumina TruSightOne sequencing panel (P/N FC-141-1006 and FC-141-1007) is a hybridisation based capture kit that covers 4813 genes designed for preparation of targeted libraries for next generation sequencing on Illumina platforms. The kit and / or sub-panels derived from the kit will be used for mutation scanning in a variety of different disease conditions.

Validation of the Illumina TruSightOne sequencing panel was performed for NGS mutation scanning to calculate estimates of general sensitivity and specificity; individual sub-panels will be verified against the

performance specifications defined by this validation before diagnostic use. The methodology was validated for the detection of both single nucleotide variations and small indels.

Three sequencing libraries were prepared according to the manufacturer's published protocol for the TruSightOne sequencing panel (Part # 15046431 Rev. A October 2013). Each library preparation included one cell line DNA sample (CEPH/UTAH PEDIGREE 146, sample NA12878) pooled with two other genomic DNA samples and sequenced on a separate MiSeq V3 2x150 sequencing run. Data analysis was performed using a bespoke bio-informatics pipeline described in section 9a.

There was complete concordance for all variants detected in the genomic DNA samples compared with those expected form previous analysis of specific genes (BRCA1 and BRCA2). Detection rate was determined by comparing high confidence variation calls available from Genome in a bottle (GIAB platinum project) for the cell line sample NA12878 with calls made in the validation runs (data from ftp://ftp-trace.ncbi.nih.gov/giab/ftp/technical/platinum_genomes/IlluminaPlatinumGenomes_v7.0/merged_platinum/NA12878.vcf) . No depth cut-off was used to determine failed regions in the validation runs. Therefore gold standard calls include variants in regions not covered by the validation.

Results:

Table1. Validation across full TruSight One sequencing Panel

Variant Type	Gold standard[1,2]	Run 1 (A6257)	Run 2 (A63A6)	Run 3 (A639R)	True positive[3]	False negative[3,4]	Detection rate	95% confidence interval
Heterozygous SNVs	4,850	4,706	4,721	4,651	4,693	157	96.8%	96.3% - 97.2%
Homozygous SNVs	2,917	2,895	2,896	2,880	2,890	27	99.1%	98.7% - 99.3%
Heterozygous Indels	277	255	256	248	253	24	91.3%	88.0% - 93.9%
Homozygous Indels	149	145	142	143	143	6	96.2%	92.2% - 98.2%

1 Gold standard calls from ftp://ftp-trace.ncbi.nih.gov/giab/ftp/technical/platinum_genomes/IlluminaPlatinumGenomes_v7.0/merged_platinum/NA12878.vcf
2 No depth cutoff used to determine failed regions in the validation runs. Therefore gold standard calls include variants in regions not covered by the validation.
3 Totals for true positives and false negatives taken as mean values over 3 biological replicates (runs 1, 2 and 3)
4 False negatives include116 variants not detected in any of the 3 validation runs

Specificity: 97-98% (95%CI)

Repeatability: 97.8-98.4% (95% CI)

Therefore, the library preparation and sequencing methodologies and the data analysis pipeline have been shown to perform to a level of accuracy above the preferred threshold stipulated by ACGS guidelines of > 95% sensitivity with 95% Confidence Intervals. Therefore this process is validated for use in the WRGL for the purpose of mutation scanning for constitutional variants in samples extracted form peripheral blood or similar material.

(2) Verification.

All 4813 genes in the Illumina TruSight One are covered by the validation described above.

Verification was performed using the same library preparation, sequencing and data analysis methods as the full validation. The proportion of bases covered >x30 over the region of interest for each gene is shown in appendix 1. Variant calling concordance of the sub-panel region of interest and with the TrusightOne validation is 97.8-99.1% (95%CI). Therefore, the performance of the genes in this sub-panel has been verified to have at least the same quality metrics and coverage (both horizontal and vertical) over the region of interest to that achieved in the full validation.

The intended purpose of the validation was to determine the sensitivity of the technology over the full range of genes on the TruSight One panel, which includes all the genes in the derived sub-panel defined here. A real variant could be of any type and located anywhere and as such may indeed not be detectable, however it is not possible to determine the sensitivity for all possible variants – rather an 'estimate' must be made based on the representative selection available. Consequently all validations are estimated extrapolations of how accurate the test is likely to be in practice. The sensitivity calculations for this panel are based on a very large number of data points and thus take into account as much variation in the ability to detect all types of sequence variations as possible.

Whilst the full range of variant types was not actually seen in this particular gene set there is no reason to suspect that they would not behave as modelled by the large number of variants used for the validation. It is the number of variants tested in the validation that provides confidence that this is the case. We have performed a conventional gene by gene verification for BRCA1 and BRCA2 and for these genes performance and coverage in the derived sub-panel were equivalent to the full validation. From extensive previous testing, the mutation spectrum for Neurofibromatosis Type 1 and Noonan syndrome is comprised of single nucleotide changes (exonic or splicing) and indels. Large deletions will still be screened by MLPA for genes where the relevant kit is available. Deep intronic mutations are not detectable using conventional DNA testing or using a Clinical Exome.

The purpose of verification is to show that the particular region of interest is likely to perform as per the overall validation and not to determine sensitivity for particular variants in the specific region of interest. The addition of one or two variants in these particular genes does not add to the statistical power to determine sensitivity for the region of interest. The best surrogate marker for performance equivalence to the validation is equivalent read coverage in the region of interest. We have therefore based the verification of this panel on whether or not we were able to achieve robust read coverage for the region of interest. Verification was performed using the same library preparation, sequencing and data analysis methods as the full validation; the proportion of bases covered >x30 over the region of interest for each gene during the verification run is shown in appendix 1.

The approach of a large scale technical validation to determine sensitivity followed by verification of derivative sub-panels against the parameters defined in the validation was approved during a CPA inspection of the WRGL's NGS service in December 2014.

To verify that the NGS technology and bioinformatic pipeline would perform as well as the validation, we have run a total of 32 retrospective patients. Each patient had at least one (pathogenic) sequence change and most had a number of other neutral sequence changes and/or a variant of unknown clinical significance that had been previously identified by conventional mutation scanning.

In 20 patients screened for BRCA1 and BRCA2, there were a total of 121 heterozygous sequence variants: 12 intel's (size range 1 to 11bp) and 109 single base substitutions (including nonsense mutations and splice site mutations). A further 12 patients were screened for a range of different genes: (NF1, PTPN11, RAF1, FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2 and MYH11). These patients had a total of three intel's (size range 2 to 4 bop) and 22 SNPS.

All 146 sequence variants (15 intel's and 131 SNPS) were successfully identified (sensitivity with 95% CI >97%). Therefore the NGS technology is verified to detect a range of mutation types across a wide range of genes

12a. Are you providing this test already?

Yes, on an individual gene basis for the genes NF1, SPRED1, PTPN11, SOS1, RAF1 and SHOC2

12b (i). If yes, how many reports have you produced?

NF1 and SPRED1 - 391 (full gene screen).

PTPN11, SOS1, RAF1 and SHOC2 - 53 (sequence analysis for common mutations in Noonan and LEOPARD syndromes)

12b (ii). Number of reports mutation positive?

NF1 and SPRED1 – 276

PTPN11, SOS1, RAF1 and SHOC2 – 12

12b(iii). Number of reports mutation negative?

NF1 and SPRED1 – 115

PTPN11, SOS1, RAF1 and SHOC2 – 41

12b(iv). Please provide the time period in which these reports have been produced and whether in a research or a full clinical diagnostic setting.

Testing for NF1 and SPRED1 commenced in 2009 and for Noonan syndrome in 2010. Both are in a full diagnostic setting.

Report numbers are taken from April 2012 to March 2013 in a full clinical diagnostic setting.

As of January 2015, around 900 index cases have been reported for *NF1/SPRED1* and around 100 for Noonan syndrome

13a. Is there specialised local clinical/research expertise for this disorder?

Yes Dr Diana Baralle and Specialist Nurse Practitioner Carolyn Redman.

13b. If yes, please provide details

Dr Diana Baralle and Specialist Nurse Practitioner Carolyn Redman. Dr Baralle is a Consultant Clinical Geneticist at the Wessex Clinical Genetics Service in Southampton, NF1 specialist within the Wessex region and has extensive expertise in the diagnosis and management of families with NF1 and other Rasopathies. Carolyn Redman maintains and curates the regional neurofibromatosis patient register and provides support and advice to the families. Dr Baralle also has an academic post at SUH and considerable research experience in the field of NF1 and diagnostic testing

14. Based on experience what will be the national (UK wide) activity, per annum, for:

NF1/SPRED :
The national activity is estimated to be approximately 700 index cases per annum.
The national activity for testing family members is estimated to be 150 cases per year.

For other RASopathies:
Estimate approximately 550 index cases per annum, however it may be more than this given that Estimate for RASopathies (excluding NF1) with prevalence of approx. 1 in 1,500 in UK, expect national activity for testing family members to be approx. 150-200 cases per year

15. If your laboratory does not have capacity to provide the full national need please suggest how the national requirement may be met.

For example, are you aware of any other labs (UKGTN members or otherwise) offering this test to NHS patients on a local area basis only? This question has been included in order to gauge if there could be any issues in equity of access for NHS patients. If you are unable to answer this question please write “unknown”.

Theoretically our laboratory has the capacity to provide national testing for patients who present with NF1 and other RASopathies, however other laboratories in the UK currently offer genes and gene panels which overlap with this panel therefore we expect a to test only a proportion of cases.

16. If using this form as an Additional Provider application, please explain why you wish to provide this test as it is already available from another provider.

n/a

EPIDEMIOLOGY

17a. Estimated prevalence of conditions in the general UK population

Prevalence is total number of persons with the condition(s) in a defined population at a specific time.
Please identify the information on which this is based.
For panel tests, please provide estimates for the conditions grouped by phenotypes being tested.

NF1/SPRED = approx. 1 in 3,000

Other RASopathies = approx. 1 in 1,500

17b. Estimated annual incidence of conditions in the general UK population

Incidence is total number of new cases in a year in a defined population.
Please identify the information on which this is based.
For panel tests, please provide for groups of conditions.

NF1/SPRED = approx. 1 in 3,500 births.

Other RASopathies = approx. 1 in 5000 births

18. Estimated gene frequency (Carrier frequency or allele frequency)

Please identify the information on which this is based.
n/a for panel tests.

n/a

19. Estimated penetrance of the condition. Please identify the information on which this is based

n/a for panel tests

n/a

20. Estimated prevalence of conditions in the population of people that will be tested.

n/a for panel tests.

n/a

INTENDED USE (Please use the questions in Annex A to inform your answers)

21. Please tick either yes or no for each clinical purpose listed.

Panel Tests: a panel test would not be used for pre symptomatic testing, carrier testing and pre natal testing as the familial mutation would already be known in this case and the full panel would not be required.

Diagnosis	☒ **Yes**	☐ **No**
Treatment	☒ **Yes**	☐ **No**
Prognosis & management	☒ **Yes**	☐ **No**
Presymptomatic testing (n/a for Panel Tests)	☐ **Yes**	☐ **No**
Carrier testing for family members (n/a for Panel Tests)	☐ **Yes**	☐ **No**
Prenatal testing (n/a for Panel Tests)	☐ **Yes**	☐ **No**

TEST CHARACTERISTICS

22. Analytical sensitivity and specificity

This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up.

Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

Sensitivity: >99% (95% CI) overall, >99% (95% CI) for SNVs and 95.7-98.4% (95% CI) for indels

Specificity: 97-98% (95%CI)

23. Clinical sensitivity and specificity of test in target population

The *clinical sensitivity* of a test is the probability of a positive test result when condition is known to be present; the *clinical specificity* is the probability of a negative test result when disorder is known to be absent. The denominator in this case is the number with the disorder (for sensitivity) or the number without condition (for specificity).

Please provide the best estimate. UKGTN will request actual data after two years service.

Clinical sensitivity:-

NF1/SPRED1 = ~ 80%

Noonan = ~70%

LEOPARD = 95%

Costello = 85%

Specificity: - The phenotypes are fully penetrant therefore we would not expect clinically unaffected individuals to carry mutations in these genes so clinical specificity will approach 100%.

24. Clinical validity (positive and negative predictive value in the target population)

The *clinical validity* of a genetic test is a measure of how well the test predicts the presence or absence of the phenotype, clinical condition or predisposition. It is measured by its *positive predictive value* (the probability of getting the condition given a positive test) and *negative predictive value* (the probability of not getting the condition given a negative test).

Not currently requested for panel tests

n/a

25. Testing pathway for tests where more than one gene is to be tested sequentially

Please include your testing strategy if more than one gene will be tested and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram. This will be added to the published Testing Criteria.

n/a for panel tests

n/a

CLINICAL UTILITY

26. How will the test change the management of the patient and/or alter clinical outcome? Please describe associated benefits for patients and family members. If there are any cost savings AFTER the diagnosis, please detail them here.

Identification of a causative mutation in an index case:

Identifying a specific Rasopathy disorder can be difficult from clinical features alone, especially in the first year of life, therefore molecular testing is essential as prompt diagnosis allows appropriate genetic counselling and more effective patient management.

The main advantage of this panel test is to avoid sequential genetic testing in some cases and to facilitate the diagnosis of the rarer phenotypes.

Molecular laboratory testing provides a number of significant benefits, including a reduction in the need for further clinical investigation for example, there is a list of recommended surveillance and annual investigations for every person suspected of having NF1, if we confirm that they are not affected or use this panel test as further evidence that they are not affected we will save them from annual visits to Paediatrics/GP and investigations, for example diagnosis of SPRED1 will allow them to be discharged.

For undiagnosed cases of NF1 or Noonan spectrum disorders further investigations to try to aid clinical diagnosis will not be required eg brain MRI, x-rays, eye examinations, echocardiograms, audiology, etc, depending on the presenting phenotypes.

A positive laboratory diagnosis can also inform decisions about preventative screening, surgery and clinical management; and in future provide the option of personalised treatment. A major benefit of a positive molecular test in the index case is to enable cascade screening of relatives and facilitate prenatal diagnosis. This will also enable at risk relatives to make informed personal and reproductive choices.

Identification of the causative mutation in asymptomatic family members:

These conditions are fully penetrant, but genetic testing will be useful for those with mild or ambiguous phenotypes.

Exclusion of the causative mutation in asymptomatic family members:

The risk of developing symptoms in relatives (e.g. new born babies) who have not inherited the familial mutation is expected to be very low. These individuals will not require follow up**.**

27. If this test was not available, what would be the consequences for patients and family members?

Patients would be unable to have appropriate surveillance and clinical management in a timely manner. Diagnostic uncertainty would involve numerous testing procedures. Genetic diagnosis is the only way to confirm a clinical diagnosis and to distinguish the different Rasopathy phenotypes.

28. Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test), please state the added advantage of the molecular test.

NF1 is usually diagnosed on clinical features., although molecular testing may be necessary to confirm the diagnosis and to distinguish between different causative genes / syndromes.

29a. What unexpected findings could this test show? For example, lung cancer susceptibility when testing for congenital cataract because ERCC6 gene (primarily associated with lung cancer) is included in a panel test for congenital cataract.

None known

29b. Please list any genes where the main phenotype associated with that gene is unrelated to the phenotype being tested by the panel.

None

30. If testing highlights a condition that is very different from that being tested for, please outline your strategy for dealing with this situation.

n/a

31. If a panel test, is this replacing an existing panel/multi gene test and/or other tests currently carried out through UKGTN using Sanger sequencing? If so, please provide details below.

Yes, this panel will replace previous NF1, SPRED1, PTPN11, SOS1, RAF1 and SHOC2 testing in our laboratory.

The NF1 and SPRED1 genes are currently tested using a CSCE (confirmation sensitive capillary electrophoresis) pre-screen and sequence changes are confirmed and characterised by conventional Sanger sequencing. The PTPN11, SOS1, RAF1 and SHOC2 genes are screened by direct Sanger sequencing.

32. Please describe any specific ethical, legal or social issues with this particular test.

None

IS IT A REASONABLE COST TO THE PUBLIC?

33. In order to establish the potential costs/savings that could be realised in the diagnostic care pathway, please list the tests/procedures that would be required in the index case to make a diagnosis if this genetic test was not available.

	Type of test	Cost (£)
Costs and type of imaging procedures		
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)		
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		

34. Based on the expected annual activity of index cases (Q14), please calculate the estimated annual savings/investments based on information provided in Q33.

Number of index cases expected annually	(a)
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b)
Total annual costs pre genetic test	(a) x (b) = (c)
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case = (d)
Additional savings/investment for 100% positive rate for index cases	(d) – (c) = (e)
Percentage of index cases estimated to be negative	(f)
Number of index cases estimated to be negative	(f) x number of index cases = (g)
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h)
Total costs for tests for index patient activity	(e) + (h) = (i)
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j)
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k)
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l)
Additional costs/savings for all activity expected in a year	(i) + (j) or (i) + (l) Cost neutral

35. REAL LIFE CASE STUDY
Please provide a case study that illustrates the benefits of this test

Index case presented with café au lait spots and freckling aged 14, his mother and brother also had café au lait spots and freckling (thereby fulfilling the NIH criteria for clinical diagnosis of NF1). He was referred for NF1 and SPRED1 testing which showed the presence of a SPRED1 nonsense mutation.

Cascade screening showed the presence of this mutation in the mother and brother. Based on the clinical diagnosis of NF1, this patient would have undergone surveillance for NF1-associated malignancies – however given the genetic test result, this surveillance is now not required and the family can be reassured that they are not at increased risk of malignancy or other age dependent complications.

UKGTN Testing Criteria

Test name: **Neurofibromatosis type I, Legius Syndrome and Noonan Spectrum Disorders 14 Gene Panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Paediatric Neurologist	
Consultant Paediatric Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Two of the following: • Short stature • Characteristic Cardiac defects • Noonan facies **OR**	
Multiple café au lait macules **AND** additionally one of the following: • Short stature • Characteristic Cardiac defect	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Neurofibromatosis type I & Legius Syndrome 2 Gene Panel**	
Approved name and symbol of disorder/condition(s): Legius syndrome Neurofibromatosis type I	**OMIM number(s):** 611431 162200
Approved name and symbol of gene(s): NF1 SPRED1	**OMIM number(s):** 613113 609291

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Paediatrician	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Multiple café au lait macules	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Noonan Spectrum Disorders 12 Gene Panel**	

Approved name and symbol of disease/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer: **Title/Position:**	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you**
Consultant Clinical Geneticist	
Consultant Paediatric Cardiologist	
Fetal Medicine consultant – appropriate for the pre-screen but not the panel	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Suspected clinical diagnosis of Noonan syndrome or RAS/MAPK-related disorder	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample

Appendix 1
Genes in panel test and associated conditions.

All rows highlighted in yellow is where the gene was being fully analysed in the context of a single separate UKGTN test at time of submission of the Gene Dossier

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
Panel 1: All 14 Genes listed below for: Neurofibromatosis 1, Legius Syndrome and Noonan Spectrum Disorders 14 Gene Panel									
Panel 2: Neurofibromatosis 1 & Legius Syndrome 2 Gene Panel									
NF1	7765	613113	Neurofibromatosis 1	AD	162200	Wallace, M. R.,et al Science 249: 181-186, 1990.	mean coverage 95%	Yes	gap filling will ensure 100% coverage
SPRED1	20249	609291	Legius	AD	611431	Brems, H., et al Nature Genet. 39: 1120-1126, 2007.	mean coverage 92%	YES	gap filling will ensure 100% coverage
Panel 3: Noonan Spectrum Disorders 12 Gene Panel									
PTPN11	9644	176876	Noonan 1, Leopard 1	AD	163950 151100	Tartaglia, M. et al. Nature Genet. 29: 465-468, 2001. Digilio, M. C. et al. Am. J. Hum. Genet. 71: 389-394, 2002	mean coverage 95%	No	gap filling will ensure 100% coverage
SOS1	11187	182530	Noonan 4, Gingival fibromatosis 1	AD	610733 135300	Roberts, A. E. et al, Nature Genet. 39: 70-74, 2007. Hart, T. C. et al. Am. J. Hum. Genet. 70: 943-954, 2002.	mean coverage 95%	No	
RAF1	9829	164760	Noonan 5, Leopard 2	AD	611553 611554	Razzaque, M. A. et al Nature Genet. 39: 1013-1017, 2007. Pandit, B et al Nature Genet. 39: 1007-1012, 2007.	mean coverage 99%	No	
KRAS	6407	190070	Noonan 3, Cardio-facio-cutaneous 2	AD	609942 615278	Schubbert, S. et al Nature Genet. 38: 331-336, 2006. Niihori, T et al Nature	mean coverage 100%	No	

						Genet. 38: 294-296, 2006.			
RASA1	9871	139150	Capillary malformation-arteriovenous malformation	AD	608354	Eerola, I., et al . J. Hum. Genet. 73: 1240-1249, 2003.	mean coverage 97%	Yes	
HRAS	5173	190020	Costello	AD	218040	Gripp, K.et al Am. J. Med. Genet. 140A: 1-7, 2006.	mean coverage 86%	No	
BRAF	1097	164757	Cardio-facio-cutaneous 1	AD	115150	Rodriguez-Viciana, P., Science 311: 1287-1290, 2006.	mean coverage 97%	No	
MAP2K1	6840	176872	Cardio-facio-cutaneous 3	AD	615279	Rodriguez-Viciana, P., Science 311: 1287-1290, 2006.	mean coverage 95%	No	
MAP2K2	6842	601263	Cardio-facio-cutaneous 4	AD	615280	Rodriguez-Viciana, P., Science 311: 1287-1290, 2006.	mean coverage 93%	No	
SHOC2	15454	602775	Noonan-like with loose anagen hair	AD	607721	Cordeddu, V., et al Nature Genet. 41: 1022-1026, 2009.	mean coverage 99%	No	100% coverage of exon 1
CBL	1541	165360	Noonan like syndrome with or without juvenile meylomonocytric leukemia	AD	613563	Martinelli, S., et al. Am. J. Hum. Genet. 87: 250-257, 2010.	mean coverage 93%	No	
NRAS	7989	164790	Noonan syndrome 6	AD	613224	Cirstea IC, et al. Nature Genet 42: 27-29. 2010	mean coverage 100%	No	