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

Submitting laboratory: Leeds 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.

Genes associated with hereditary forms of cancer

2. OMIM number for disorder/condition

If a panel test – see 1. above

3a. Disorder/condition – please provide, in laymen's terms, a brief (2-5 sentences) description of how the disorder(s) affect individuals and prognosis.

Characteristics of hereditary cancers include;

- most are autosomal dominant, first-degree relatives are at 50% risk
- earlier age of onset than is typical,
- multiple primary cancers in an individual,
- clustering of rare cancers,
- those that do not have the familial mutation have the general population risk for cancer.

3b. Disorder/condition – if required please expand on the description of the disorder provided in answer to Q3a.

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.

Panel test - 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.

Panel test - See appendix 1

6a. OMIM number(s) for gene(s)

If a panel test – see 5. above

Panel test - See appendix 1

6b. HGNC number(s) for gene(s)

If a panel test – see 5. above

Panel test - See appendix 1

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7a. Gene – description(s) If this submission is for a panel test, please provide total number of genes.
Total number of genes: 82
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-H awaiting review of NGS GenU classification system
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.
Analysis of the entire coding regions and exon/intron boundaries of the above cancer genes is performed by clonal sequencing. Target enrichment is achieved by hybridisation using a Custom Design Agilent reagent. Libraries are prepared using Sure Select protocols according to the manufacturers instructions and sequencing is performed on a HiSeq2500. Libraries are loaded on one lane of a flow cell for cluster generation and then sequenced on the HiSeq, according to the manufacturer's protocol. Raw data from the HiSeq is converted and analysed in three steps – alignment (BWA), variant calling (GATKLite) and variant annotation (Alamut-ht). Mutation reports and coverage information are assessed in bespoke excel workbooks. Data from specific genes are analysed according to the clinical referral. A minimum depth of coverage of 50x is considered the laboratories quality standard threshold, with 100% vertical coverage expected for all genes within the panel. Variants are filtered according to frequency taking into consideration the mode of inheritance of the genes being analysed. Once filtered the data are scored. Variants considered of clinical utility are confirmed by Sanger sequence analysis. Sequence variants of no or unlikely clinical significance are omitted from the reported results.
9b. For panel tests, please specify the strategy for dealing with gaps in coverage.
In validation of this particular test, horizontal gaps in sequence or reduced coverage has not been observed. Should reduced horizontal coverage be observed gaps would be filled by Sanger sequencing if requested. If the laboratories vertical coverage standard is not met the test would be repeated as the sensitivity of at least 96% could not be guaranteed.
9c. Does the test include MLPA? **(For panel tests, please provide this information in appendix 1)**
Yes – for certain genes
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.**
Assay provided in-house
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:

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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.

Instrument (HiSeq2000) and bioinformatics pipeline was validated on the cancer gene panel, enabling a comparison to be made between sensitivity of existing pipelines (Sanger sequence analysis and NGS analysis based on enrichment by long range PCR (see validation documentation attached and section 22 and Appendix 3). In summary, 97 patients with 480 known variants (101 unique) were analysed. All the variants were detected by this method. This indicates a sensitivity of at least 95% (with 95% confidence interval).

12a. Are you providing this test already?

Total of 82 patients reported to date.

V1.0 phase of SureSelect reagent (36 hereditary cancer genes – included in this application) = 63 patients

V2.0 phase of reagent (83 hereditary cancer genes – full gene list described in this application) = 19 patients

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

Total = 82

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

Mutation +ve = 12 likely pathogenic/ pathogenic variants detected (UV class 4&5)

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

Mutation –ve = 70 normal reports (UV class 1, 2, 3)

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.

April 2013 to date (July 2014)

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

Yes. Some components of the test are already provided by a different method –

a) Enrichment of sequences by long range PCR followed by next generation sequencing is used for analysis of

i) APC & MUTYH
ii) MSH2, MLH1, MSH6
iii) BRCA1 & BRCA2
iv) TP53
v) SDHB, SDHC, SDHD, SDHAF2, MAX, TMEM127, PRKR1A, RET, VHL

b) The following genes are amplified by PCR and Sanger sequenced

i) CDKN2A &CDK4
ii) MEN1
iii) HOXB13
iv) PMS2

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13b. If yes, please provide details
Dr Julian Adlard – Local Clinical Genetics Department – special interest cancer genetics Dr Alison Kraus - Local Clinical Genetics Department - special interest cancer genetics Leeds Institute of Cancer Pathology - Dr Julia Newton-Bishop – malignant melanoma, Prof Phil Quirke – gastrointestinal cancer, breast cancer, head and neck and lymphomas and ovarian cancer
14. Based on experience what will be the national (UK wide) activity, per annum, for:
Index cases – Diagnostic hereditary cancer tests performed locally in 2012/2013 = 1880 (to note not national activity)
Family members where mutation is known – Familial tests performed locally in 2012/2013 = 137 (to note not national activity) Referral rates to the laboratory increase on average by 10-12% each 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".
Our lab does not have the capacity to meet the national need. Many laboratories offer analysis of hereditary cancer genes across the UK for their local population. However, to our knowledge no lab is currently registered to offer a comprehensive panel of caner genes from which genes may be selected according to clinical need.
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

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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.

Prevalence figures for all cancers in general are high. Office for National Statistics (ONS) figures 2008/2010 indicate a prevalence of >300,000 cases in the UK, which includes figures on all cancers combined. However, the use of this test is expected for patients who are assessed through a Clinical Genetics Service as having a high (>=10%) probability of pathogenic mutation detection from the test, and for whom a multiple gene panel test is assessed as appropriate. Only a minority of all cancer/tumour cases will meet these criteria, which will be similar to current demand for standard genetic testing.

Cancer Research UK provides prevalence data for several common cancers. Defined as the number of people who have received a diagnosis of cancer and who are still alive at a specific time point, the figures represent both patients which will have been cured and others which have not. These prevalence data therefore, reflect the incidence of cancer and its associated survival pattern. The most prevalent types of cancer are those with relatively high incidence rates and good prognosis. In the UK the most prevalent cancer in males is prostate cancer and in females is breast cancer.

See table below for a summary of all malignant neoplasms diagnosed between 1997 and 2006 and registered by the cancer registries of England, Scotland, Wales and Northern Ireland. For that period 1, 5 and 10 year prevalence figures by sex, for all cancers are shown excluding non-melanoma skin cancer.

	Year 1 prevalence (1997)	5 Year prevalence (2001)	10 year prevalence (2006)
Male	98,726	339,971	507,840
Female	101,522	382,030	622,875
Persons	200,522	722,001	1,130,715

Ref: http://www.cancerresearchuk.org/health-professional/cancer-statistics/survival/all-cancers-combined

Using the 10 year prevalence (2006) statistics, the following prevalence figures have been published by CRUK for specific cancers (somatic and hereditary) described in the panel tests offered in this application. This enables the relative prevalence of the major cancers to be compared.

UK population size 2006 = 60.4 million

Panel	Males 10 year prevalence (2006)	Females 10 year prevalence (2006)
All cancers	507,840	622,875
Breast	1732	296,037
Ovarian	N/A	25,066
Bowel	78,483	65,075
Kidney	16,468	10,035
Pheo & PGL	Not reported in these data	
Pituitary, parathyroid, hypercalcemia, MEN1	Not reported in these data	
Neurofibromatosis, NF1	Not reported in these data	
Skin cancer, melanoma	24,617	34,530
Uterine Cancer	N/A	38,667
Pancreatic cancer	2,205	2,144

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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.

Incidence figures for all cancers in general are high (see below). However, the use of this test is expected for patients who are assessed through a Clinical Genetics Service as having a high (>=10%) probability of pathogenic mutation detection from the test, and for whom a multiple gene panel test is assessed as appropriate. Only a minority of all cancer/tumour cases will meet these criteria, which will be similar to current demand for standard genetic testing.

Panel	Male incidence	Female Incidence	General Incidence estimates
All cancers	430.5	375.4	(1)
Breast		125.8	(1)
Ovarian	N/A	16.6	(1)
Bowel	58.4	37.1	(1)
Kidney	13.6	7.4	(1)
Pheo & PGL			The incidence of PGL/PHEO in the general population is estimated to be 1 per 100,000 per year (2) VHL disease; ~1/36,000-45,500 live births (3,4). PHEO/PGL present in ~10-20% of familial cases of VHL dependent on disease sub-type MEN2 (RET); ~1/30,000 (3). PHEO/PGL present in ~50% of familial cases of MEN2A and MEN2B PHEO/PGL related to SDHB, SDHC, SDHD and SDHAF2; not precisely known. The incidence of these tumours appears to be ~1/100,000-300,000 per year (3,5) Carney complex (PRKAR1A); 500 cases worldwide (NIH-Mayo Clinic USA, Cochin Centre France) (6) TMEM127, MAX – unknown.
Pituitary, parathyroid, hypercalcemia, MEN1			1 in 30,000 (1)
Neurofibromatosis, NF1			1 in 3000 births (1)
Skin cancer, melanoma	16.6	17.0	The incidence of melanoma in the UK is approximately 10 cases per 100, 000 per annum (5) Less than 1-2% of melanoma cases are thought to be attributable to mutations of the melanoma susceptibility genes (CDKN2A and CDK4) (7)
Uterine Cancer	N/A	19.1	(1)
Pancreatic cancer	10.9	8.5	(1)

Estimated UK population 2008-2010 ~62-63 million (Office National statistics)

1 Age-standardised incidence rates for common cancers, United Kingdom, 2008−2010 per 100, 000. Source: Office for National Statistics – includes all cancers somatic & hereditary

2. Orphanet

3. GeneReviews,

4. OMIM

5. Sosipatros et al., Curr Opin Oncol 2007, 19:24

6. Roberts et al. (1992) UK Guidelines for the management of cutaneous melanoma. British Journal of Dermatology 2002 **146** 7-17.

7 Melanoma Genetic Consortium Consensus Statement

Estimated UK population Incidence for hereditary breast and ovarian cancer.

NICE Guidelines for Familial Breast Cancer update June 2013 provide incidence data for England as 56,000 cases of breast cancer in women per year and 366 cases in men. For ovarian cancer this is 7100 cases per year. Of these figures, those that have a high risk inherited component are 2800 cases (5%) of breast cancer in women; 18 cases of breast cancer in men (~5%) and 1000 cases of ovarian cancer

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(14%) (NICE Clinical Guideline 164).
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	**x Yes**	**☐ No**
Treatment	**x Yes**	**☐ No**
Prognosis & management	**x 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**

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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.

Summary of validation evidence	
Sensitivity of method	96.41% (100-3.59) 95% confident that the false negative rate is below 3.59%. All class 4 and 5 variants are confirmed by Sanger Sequencing
Specificity of method	Out of 484 variant calls, 4 variant calls were found to be false positive. See Watson et al., 2013 Human Mutation DOI:10.1002/humu.22490
Number of unique variants detected in parallel study with LR-PCR NGS data	101 (total 480)
Mutation spectrum	Missense, nonsense, splice (up to +/-20bp intronic sequence analysed), small insertion/ deletion/duplication mutations all detected as part of validation exercise. (maximum size of deletion/duplication detected to date = 31-40bp)

Validation process

Method:

Target enrichment via Agilent Sureselect Custom design

Library preparation for clonal sequencing (SureSelect)

For detailed protocol information see Laboratory SOP DN258 (can be provided if required)

Libraries are loaded on one lane of a flow cell for cluster generation and then sequenced on the Illumina HiSEQ2000, according to the manufacturer's protocol.

Sequence analysis

Parallel testing

- 49 Validation patients
- 97 (49+48) patients analysed in total
- 480 sequence variants identified
- 101 unique variants detected
- 100% concordance with clonal sequencing by long PCR

Test sensitivity

Data

Total number of known variants by cancer chip sequencing: 480

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Total number confirmed by clonal sequencing: 480

Number of unique variants (*i.e. each variant counted once only*) by 'clonal' (Sanger sequencing) re-tested by cancer chip sequencing: 101
Total number confirmed by cancer chip sequencing: 101

Analysis
Determined by binomial confidence interval method (see http://statpages.org/confint.html). Using 95% confidence interval (2.5% in each tail).

1. Total number of variants

For a total of 480 variants, binomial distribution predicts 95% confidence for the following upper and lower limits:

Number of missed variants	Estimated proportion	Lower limit	Upper limit
3	0.0062	0.0013	0.0182
2	0.0042	0.0005	0.015
1	0.0021	0.0001	0.0116
0	0	0.0000	0.0077

i.e. since no variants have been missed, we can be 95% confident that the false negative rate is below 0.77%.
However, it may be unrealistic to assume all variants are equally detectable, so considering each variant once only is a more cautious approach.

2. Number of unique variants

The most cautious approach to validating clonal sequencing sensitivity would be to test a different variant each time, since re-testing a variant which is already know to be detectable is of limited value. Therefore, a repeat analysis counting only the total number of unique variants (101) is shown below:

Number of missed variants	Estimated proportion	Lower limit	Upper limit
3	0.0297	0.0062	0.0844
2	0.0198	0.0024	0.0697
1	0.0099	0.0002	0.0539
0	0	0	0.0359

i.e. since no variants have been missed, we can be 95% confident that the false negative rate is below 3.59%.

Standard assumptions apply including:

- Quality standards for validation work are the same for subsequent patient tests.
- Testing criteria and methods remain unchanged – or modifications do not have an impact on sensitivity.
- Samples tested (or the range of variants tested in validation) are representative of patients (or variants) that will be tested.

Ref:

Watson et al., 2013. Robust diagnostic genetic testing using solution capture enrichment and a novel variant-filtering interface Human Mutation DOI: 10.1002/humu.22490

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.

	Disease	
Test result	present	absent
+ve	a	b

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-ve	c	b

Clinical sensitivity = a/(a+c)

From analysis of 47 patients with various phenotypes, 9 probably pathogenic variants were detected in 9 patients during validation process (These represented 9 out of 101 unique variants)

9/47 = 19%

Clinical specificity = unknown - no individuals without the condition have been tested by this method.

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.

Historically, clinical care pathways have recommended strategic genetic testing according to defined clinical indicators for various cancers. We have collated a number of genes routinely offered for genetic diagnosis for specific inherited cancer syndromes, allowing potential simultaneous analysis of multiple genes in a single test according to the observed phenotype/family history. The cost of the package of genes is significantly cheaper than the total cost of testing individual genes, or small groups of genes in a stepwise approach. This strategy will be particularly applicable where the clinical phenotype/family history suggests that a genetic predisposition is likely, but plausible genetic causes include multiple genes.

For several inherited cancer syndromes, pathways already exist for structured testing of small panels of genes e.g. PHEO/PGL syndromes, HNPCC. However, this test gives the potential for one-stop testing to assess a much wider range of potential genetic explanations for any family history suspicious of a cancer/tumour syndrome.

Combined molecular analysis of multiple candidate genes, will offer a test with greater sensitivity and allow earlier confirmation of any diagnosis. Standard approaches requiring multiple different tests are more expensive and lengthy and may require multiple patient contacts, increasing anxiety. A ‘one stop’ testing approach will reduce clinical and administration time, in turn reducing costs to the NHS whilst improving efficiency. The greater likelihood of identifying associated pathogenic mutations will be of potential significant value to the index case, to family members, and to the NHS.

Identification of a germ line mutation will give a better indication of the risks to the index patient and any relatives undergoing a subsequent positive predictive test. This will allow better targeted cancer screening, and/or risk-reducing strategies. Family members undergoing a negative predictive test may be spared additional screening, other unnecessary interventions and anxiety. There are potential cost-savings for the NHS in terms of reducing the costs of treatment of cancers (which can be extremely

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high), and reducing unnecessary lifetime screening programmes.

Examples of typical surveillance methods/ treatment approaches and estimated costs

Method	Average Cost (National average)	Source
Counselling session	£49.84	Familial Breast cancer: - Full cost effectiveness evidence and review & reports (January 2013 Draft for consultation NICE)
Outpatient attendance	£108	NHS ref costs 2011
Day case	£693	NHS ref costs 2011
Colonoscopy and examination (1, 3, 5 yr surveillance dependent on risk)	£205	Report for the NHS Bowel Cancer Screening Programme Jan 2011 ScHARR
Sigmoidoscopy with biopsy an therapy	£195	Report for the NHS Bowel Cancer Screening Programme Jan 2011 ScHARR
Cost of treating screening complications (bowel) – perforation, hospitalised bleeding	Range from £264 to £2164	Report for the NHS Bowel Cancer Screening Programme Jan 2011 ScHARR
CT scan	£121	DOH National audit office HC822 30/3/2011
MRI scan	£216	NHS ref costs 2011. DOH National audit office HC822 30/3/2011
Radiotherapy session	£123	DOH National audit office HC822 30/3/2011
Mammography	£93.0	Familial Breast cancer: - Full cost effectiveness evidence and review & reports (January 2013 Draft for consultation NICE)

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

Advances in technology are allowing testing of multiple gene panels at reduced financial cost. If this test was not available, patients and family members would continue to be offered tests which are highly targeted to particular cancer phenotypes, but likely to miss important genetic diagnoses; some patients would continue to be offered sequential testing of different genes, leading to delays in completion of testing, increased costs and anxiety.

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.

Family history of cancer meeting specified criteria, or a particular phenotype may in some cases allow a clinical diagnosis of a cancer syndrome in an affected patient. However, molecular diagnosis is required to allow a more definitive confirmation of the diagnosis, associated risks, and also to allow predictive genetic testing for apparently unaffected relatives. Biochemical screening (e.g. catecholamine monitoring for PHEO), or other tests (e.g. mammography; colonoscopy) can be diagnostic. However, molecular testing is usually required for prediction where a germline mutation can be identified, and cannot generally be replaced by other forms of test.

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.

In addition to their reported role in specific cancer phenotypes the following genes are listed with phenotypes outside the spectrum of cancer:-

SMAD4 - aortopathy, primary pulmonary hypertension, Myhre syndrome (growth mental deficiency syndrome)

KIF1B - Charcot-Marie-Tooth disease, type 2A1.
KIT - Piebaldism.

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MITF –albinism and sensorineural deafness compatible with Tietz syndrome (MIM 103500). MITF gene mutations account for 20% of Waardenburg syndrome (WS) type II. These data, together with the wide spectrum of mutant alleles reported in mi mice (which have pigmentary disorders), suggest that MITF could be regarded as a candidate gene in various pigmentation disorders in man

POLD1 - Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome (MDPL) is an autosomal dominant systemic disorder characterized by prominent loss of subcutaneous fat, a characteristic facial appearance, and metabolic abnormalities including insulin resistance and diabetes mellitus. Sensorineural deafness occurs late in the first or second decades of life

PTCH1 - Mutations of this gene have been associated holoprosencephaly

PTEN – Mutations have also been associated with macrocephaly/autism syndrome characterised by:- Macrocephaly, postnatal, Biparietal narrowing, Broad forehead, Long philtrum, Autism, Global developmental delay, Obesity, Frontal bossing, Short nose, Depressed nasal bridge

SDHA – Mutations are reported in cases of cardiomyopathy, Leigh syndrome and mitochondrial respiratory chain complex II deficiency

PIK3CA – is associated with CLOVE syndrome

SMARCB1 – Mutations have been associated with mental retardation

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

Genes are listed where the primary phenotype described in OMIM is not obviously cancer related.

PRSS1 – Pancreatitis – however, pancreatitis may be the first signal of pancreatic neoplasia

PRSS2 – Pancreatitis protective against

SPINK1 – Pancreatitis, fibrocalculous pancreatic diabetes

CTRC – Pancreatitis

PHOX2B – Central hypoventilation syndrome, with or without Hirshprung disease. Previous reports linking germline mutations to neuroblastoma risk

SPRED1 – Legius syndrome. Differential diagnosis of some features of NF1

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

Panels have been created according to the observed phenotype to minimise identification of findings very different from what is being tested for. Panel 11 contains a selection of genes which the clinician may 'cherry pick' as appropriate and represent genes that are associated with phenotypes distinct from the other recommended panels. Serious consideration must be given to ensuring informed consent is provided and documented so that the patient is aware that for their specific test that, a risk factor other than one relating to cancer or presenting family history may be identified in some cases. It is not intended to offer this testing at present to referrers who are not consultants in clinical genetics services

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.

In the short term we do not propose that this panel test will replace other tests carried out by Sanger sequencing or by other NGS approaches as it will only be cost effective for referrals where two or more tests are appropriate. However, in the future, as testing costs decrease for the custom SureSelect reagent it is anticipated that this mode of testing will replace the majority of existing single gene/ small panel test work streams.

We plan to offer selection of genes/sub-panels to be analysed and reported according to the phenotype/family history, rather than offering to report results from all possible genes on the panel. We believe this is an appropriate staged approach. It is possible that a full panel test could be offered in the future if there was evidence to indicate this as being safe and appropriate. It is also possible that testing could be requested in the future by secondary care clinicians e.g. oncologists if it is shown in studies (e.g. Mainstreaming Cancer Genetics) that this can be done with appropriate training.

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

None, provided informed consent is ensured and documented.

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.

Yes. The value of familial breast cancer screening and bowel cancer screening has been already reviewed in the following documents and has been shown to be cost effective to particular subgroups of patients (*refs (1) Cost effectiveness evidence review. Familial Breast Cancer: Classification and care of women at risk of familial breast cancer and management of breast cancer and related risks in people with a family history of breast cancer. Update of clinical guideline 14 and 41. 2013 (2) National Collaborating Centre for Cancer. Re-appraisal of the options for colorectal cancer screening. Report for the NHS Bowel Cancer Screening Programme Jan 2011, ScHARR University of Sheffield, Cairns et al., 2010 Guidelines for colorectal caner screening and surveillance in moderate and high risk groups (update for 2002) Gut 2010;59:666-690)*.The same argument will apply for the provision of screening for the other hereditary cancer genes included in this panel.

In cases where a pathogenic cancer gene mutation is detected in an index case, cascade testing can be made available to at risk relatives. In mutation negative cases the cost of lifetime surveillance will be reduced. In the case of screening of index cases surveillance methods may also be tailored taking into account to affected status, carrier probability and mutation status For example in breast cancer where MRI (£216) and mammography (£93) are common surveillance methods the following patient management models are reported i.e.

1. Unaffected, BRCA +ve – annual MRI
2. Unaffected, risk at least 30%, BRCA unknown – annual MRI scans
3. Unaffected, risk below 30%, BRCA unknown - annual mammography
4. Unaffected, BRCA -ve -no surveillance
5. Affected, BRCA +ve - annual MRI,
6. Affected individual, BRCA –ve/unknown - annual mammography.

	Type of test	**Cost (£)**
Costs and type of imaging procedures	Eg, MRI Mammography CT scan Colonoscopy	£216 £93 £121 £205
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)		**n/a**

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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 (multi-panel) genetic test	(a) x (b) = (c)
Total annual costs to provide (multi-panel) 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) -£98,500 (saving for adopting multi-panel test)
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

Example case of cost benefit of panel screening

Patient:

- 48 year-old man with multiple gastric polyps and primary cholangiocarcinoma
- Family history - paternal grandfather had cancer of the rectum in his 30s, paternal great aunt had colorectal cancer at age 47 years

Referred for molecular analysis of

1. MLH1, MSH2, MSH6 Nov 2012– tested negative, VUS detected in MSH6– cost £530
2. APC and MUTYH Dec 2012– tested negative – cost £530
3. Cancer panel then available in-house, MLH1, MSH2, MSH6, APC, MUTYH, STK11, SMAD4, BMPR1A and CDH1 requested April 2013 – patient tested +ve for pathogenic mutation in SMAD4 – cost £860

- Mutations in the genes BMPR1A and SMAD4 are associated with Juvenile Polyposis Syndrome (JPS). JPS may never have been considered in this family given the relatively late age at

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presentation

- Proband's son (age 24) was then screened 2 months (Aug 2013) later and found to have the familial SMAD4 mutation. Two yearly screening with colonoscopy and OGD recommended.
- 15-22% of those with JPS and a SMAD4 mutation have Hereditary Haemorrhagic Telangiectasia (HHT), appropriate advice therefore given

Cost of molecular analyses

Total cost of genetic diagnostic analysis in index case = £1920

Cost of panel test = £860

Saving which could have been made on molecular testing if panel test had been available as first line test = £1060

Time taken to perform molecular investigations

Initial diagnostic analysis of index case requested = October 2012

Date of final molecular diagnosis = April 2013

Time elapsed until molecular diagnosis = 10 months ~200 days

Time which could have been saved if panel test first line test with 40day TAT = 160 days

TESTING CRITERIA

36. Please only complete this question if there is previously approved Testing Criteria.
Please contact the UKGTN office if you are unsure whether testing criteria is available.

36a. Do you agree with the previously approved Testing Criteria?

Agree with previously approved testing criteria for:

- Breast/Ovarian familial cancer
- Familial pheochromocytoma and paraganglioma
- Malignant melanoma
- Fanconi anaemia.

Testing Criteria needs to be developed for:

- Familial Bowel Cancer including HNPCC phenotype & Polyposis 14 Gene Panel
- Familial Pancreatic 8 Gene Panel
- Familial Renal Cancer 10 Gene Panel
- Familial Uterine Cancer 9 Gene Panel
- Neurofibromatosis, Schwannomas and Café au Lait 5 Gene Panel
- Familial Pituitary Cancer, Parathyroid & Hypercalcemia 7 Gene Panel

36b. If you do not agree, please provide revised Testing Criteria on the Testing Criteria form and explain below the reasons for the changes.

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UKGTN Testing Criteria

Test name: **Familial Breast/Ovarian Cancer 13 Gene Panel (option A)**	
Approved name and symbol of disorder/condition(s): See website listing	**OMIM number(s):**
Approved name and symbol of gene(s): See website listing	**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/Registered Genetic Counsellor	
OR named Multi-Disciplinary Team clinician: Consultant Oncologist Consultant Gynaecologist Consultant Breast Surgeon	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Woman with breast cancer who has ONE of the following: 1. Bilateral invasive ductal breast cancer and both cancers diagnosed <40 years 2. Grade 3 triple negative breast cancer diagnosed <40 years or <50 if family history unclear or unknown 3. Non- mucinous epithelial ovarian cancer 4. A first-degree relative* with breast cancer and both diagnosed <40 years 5. A first-degree relative* with a histologically confirmed non-mucinous epithelial ovarian cancer 6. A family history with a pathology adjusted Manchester score greater than or equal to 15	
OR Woman with ovarian cancer who has: 1. Histology consistent with a high-grade serous epithelial carcinoma OR 2. A family history with a pathology adjusted Manchester score greater than or equal to 15	
OR Man with a BRCA-related (prostate, breast or pancreas) cancer who has: 1. A family history with a Manchester score greater than or equal to 15	

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OR Affected individual with Ashkenazi Jewish/ Polish ancestry who has: 1. Female breast cancer diagnosed <50 or a male *BRCA*-related cancer (founder mutation screen)	
OR Unaffected individuals: **Referrals only accepted from Consultant Clinical Geneticist or Registered Genetic Counsellor** 1. Unaffected individual who has a family history with a Manchester score greater than or equal to 20 *AND* a first-degree relative with breast/ ovarian/prostate/ pancreatic cancer where there are no affected relatives available for testing (ovarian cancer and cancer in first-degree relative should be confirmed) 2. Unaffected individual with Ashkenazi Jewish/ Polish ancestry who has a first-degree relative with female breast cancer diagnosed <50 or a male *BRCA*-related cancer and a Manchester score greater than or equal to 10 (founder mutation screen[$]) *Or a second-degree relative via a father [$]This should only be done if testing cannot be performed in an affected relative.	

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.

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UKGTN Testing Criteria

Test name: **Fanconi Anaemia 16 Gene Panel**	
Approved name and symbol of disease/condition(s): See website listing	**OMIM number(s):**
Approved name and symbol of gene(s): See website listing	**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 Haematologist	

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 clinical symptoms: • Thumb deformities/absent thumbs/radial aplasia • Pancytopenia/aplastic anemia **AND** other causes excluded • Low birth weight/small stature • Multiple Congenital Anomalies suggestive of Fanconi phenotype (eg. in thumbs, forearms, skeletal system, eyes, kidneys / urinary tract, ear, heart, gastrointestinal system, oral cavity, central nervous system)	

Additional information:
Combinations of features are particularly strong indicators for testing

- Radial ray and thumb anomalies (all)
- Failure to thrive/short stature (consider)
- Combination of Café au Lait (CAL) patches and hypopigmented macules
- VACTERL association (FA in ~5% VACTERL pts)
- Intracranial medulloblastoma/renal Wilms (consider FA in <5yr olds)
- Oropharangeal/anogenital small cell carcinoma in <45yr olds (if no other risk factors)
- Sibling with FA
- Excessive chemo/radiotherapy toxicity

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Continued over page……

- Blood dyscrasias:
  - All children and adults <45yrs with otherwise unexplained macrocytosis, and/or thrombocytopenia, and/or neutropenia, and/or pancytopenia, and/or MDS, and or aplastic anaemia
  - Children and young adults with AML (and ALL) (consider FA)

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.

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UKGTN Testing Criteria

Test name: **Familial Pheochromocytoma and Paraganglioma 11 Gene Panel (option A)**	
Approved name and symbol of disorder/condition(s): See website listing **Approved name and symbol of gene(s):** See website listing	
Patient name:	
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 Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
One of the following:	
PGL in neck or elsewhere	
PHEO with family history of PGL	
PHEO <45 yrs of age	
PHEO over 45yrs of age*	
PHEO with other syndromic features# e.g. MEN2, VHL – **specify**	
Malignant PHEO over 45yrs of age	
Bilateral PHEO / Multiple tumours	
Confirmation of affected status in a family with known mutation	
At risk family members where familial mutation is known.	

- indicates testing for genes specific to stated syndrome
*****Indicates testing for only *TMEM127* and *SDHB* as patients with a later age of diagnosis are more likely to have mutations in these genes than mutations in the other genes (Yao *et al* 2010, Jafri *et al*, 2012)

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.

Approval date: Sept 2014 Submitting laboratory: Leeds RGC

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UKGTN Testing Criteria

Test name: **Familial Melanoma 3 Gene Panel**	
Approved name and symbol of disorder/condition(s): See website listing	**OMIM number(s):**
Approved name and symbol of gene(s): See website listing	**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.**
Clinical Geneticists	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Affected individuals in families with 3 or more cases with melanoma **OR**	
Affected individual in families with 2 cases of melanoma in first degree relatives with multiple primary melanoma in at least one case **OR**	
Affected individual in families with at least one invasive melanoma and two or more other diagnoses of invasive melanoma and/or pancreatic cancer among first- or second-degree relatives on the same side of the family.	

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.

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Appendix 1

Genes in panel test and associated conditions (please expand table if required).

All rows highlighted yellow show genes where there is currently a single separate UKGTN test for that gene.

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 (from analysis of 15 independent cases)	MLPA	Comments
AIP aryl hydrocarbon receptor interacting protein	**358**	**605555**	Pituitary adenoma, ACTH-secreting Pituitary adenoma, growth hormone-secreting Pituitary adenoma, prolactin-secreting	**AD**	**219090** **102200** **600634**	Pituitary cancer, parathyroid/hypercalcaemia See GeneReviews Gene already listed on UKGTN	100		
AKT1 v-akt murine thymoma viral oncogene homolog 1	**391**	**164730**	Breast cancer, somatic Colorectal cancer, somatic Cowden syndrome 6 Ovarian cancer, somatic Proteus syndrome, somatic	**AD**	**114480** **114500** **615109** **167000** **176920**	Am J Hum Genet 2013 92:72-80 Germline PIK3CA and AKT1 mutations in Cowden and Cowden-like syndromes	100		
ALK anaplastic lymphoma receptor tyrosine kinase	**427**	**15590**	Neuroblastoma, susceptibility to, 3	**AD**	**613014**	Eu J Hum Genet 2012 20:291-7 ALK germline mutations in patients with neuroblastoma: a rare and weakly penetrant syndrome.	100		
APC adenomatous polyposis coli	**583**	**611731**	Adenoma, periampullary, somatic Adenomatous polyposis coli Brain tumor-polyposis syndrome 2 Colorectal cancer, somatic Desmoid disease, hereditary Gardner syndrome Gastric cancer, somatic Hepatoblastoma, somatic	**AD**	**175100** **114500** **135290** **613659** **114550**	See GeneReviews Gene already listed on UKGTN	100	Yes	
ATM ataxia telangiectasia mutated	**795**	**607585**	Ataxia-telangiectasia {Breast cancer, susceptibility t	**AR** **AD**	**208900** **114480**	See GeneReviews Gene already listed on UKGTN Rustgi et al., 2014 Familial pancreatic cancer: genetic advances. Genes & Dev 28:1-7 Apostolou & Fostira 2013. Hereditary breast cancer. The era of new susceptibility genes. BioMed Research	100		~3% of breast cancer

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						International 747318			
BAP1 BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)	**950**	**603089**	Tumor predisposition syndrome	**AD**	**614327**	Cheung et al. 2013 Cancer Genet Further evidence for germline BAP1 mutations predisposing to melanoma and malignant mesothelioma	100		Melanoma, RCC, Mesothelioma BAP1 contains binding domains for BRCA1 and BARD1
BARD1 BRCA1 associated RING domain 1	**952**	**601593**	{Breast cancer, susceptibility to	**AD**	**114480**	See OMIM entry	100		Interacts with N-terminal region of BRCA1. Shares homology with N-terminal RING motif and C-terminal BRCT domain. Possible association with aggressive high-risk neuroblastoma
BMPR1A bone morphogenetic protein receptor, type IA	**1076**	**601299**	Juvenile polyposis syndrome, infantile form Polyposis syndrome, hereditary mixed, 2 Polyposis, juvenile intestinal	**AD**	**174900** **610069**	See GeneReviews Gene already listed on UKGTN	100	Yes	Mutations in BMPR1A, SMAD4 and PTEN are responsible for juvenile polypo syndrome, juvenile intestinal polyposis and Cowdens disease respectively
BRCA1 breast cancer 1, early onset	**1100**	**113705**	Breast-ovarian cancer, familial, 1 {Pancreatic cancer, susceptibility to, 4}	**AD**	**604370** **614320**	See GeneReviews Gene already listed on UKGTN	100	Yes	Mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers
BRCA2 breast cancer 2, early onset	**1101**	**600185**	Fanconi anemia, complementation group D1 Pancreatic cancer Prostate cancer Wilms tumor {Breast cancer, male, susceptibility to} {Breast-ovarian cancer, familial, 2} {Glioblastoma 3} {Medulloblastoma {Pre-B-cell acute lymphoblastic leukemia}	**AR –Fanconi anemia** **AD**	**605724** **613347** **176807** **194070** **114480** **612555** **613029** **155255**	See GeneReviews Gene already listed on UKGTN	100	Yes	
BRIP1 BRCA1 interacting protein C-terminal helicase 1	**20473**	**605882**	Breast cancer, early-onset Fanconi anemia, complementation group J	**AD** **AR**	**114480** **609054**	Gene already listed on UKGTN Apostolou & Fostira 2013. Hereditary breast cancer. The era of new susceptibility genes. BioMed Research International 747318	100		Interacts with BRCT repeats of BRCA1
CASR calcium-sensing receptor	**1514**	**601199**	Hypercalciuric hypercalcemia Hyperparathyroidism, neonatal Hypocalcemia, autosomal dominant Hypocalcemia, autosomal dominant, with Bartter syndrome Hypocalciuric hypercalcemia, type I {Calcium, serum level of} {Epilepsy idiopathic generalized, susceptibility to, 8}	**AR** **AD**	**239200** **601198** **145980** **612899**	Marx 2011 Hyperparathyroid genes: sequences reveal answers and questions. Endocr Pract 17 Suppl 3:18-27 Gene already listed on UKGTN	100		
CDC73 cell division cycle 73	**16783**	**607393**	Hyperparathyroidism, familial primary Hyperparathyroidism-jaw tumor syndrome	**AD**	**145000** **145001** **608266**	Gene already listed on UKGTN	100		Phenotype related to defects in MEN1 and CASR

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			Parathyroid adenoma with cystic changes Parathyroid carcinoma						
CDH1 cadherin 1, type 1, E-cadherin (epithelial)	**1748**	**192090**	Endometrial carcinoma, somatic Gastric cancer, familial diffuse, with or without cleft lip and/or palate Ovarian carcinoma, somatic {Breast cancer, lobular} {Prostate cancer, susceptibility to}	**AD**	**608089** **137215** **167000** **114480** **176807**	Gene already listed on UKGTN	100		CDH1 gene alterations in ~46% of hereditary GC, 3.8% were large deletions.
CDK4 cyclin-dependent kinase 4	**1773**	**123829**	{Melanoma, cutaneous malignant, 3}	**AD**	**609048**	Gene already listed on UKGTN	100		
CDKN1B cyclin-dependent kinase inhibitor 1B (p27, Kip1)	**1785**	**600778**	Multiple endocrine neoplasia, type IV		**610755**	Gene already listed on UKGTN	100		MEN1 and MEN2A overlap Association of SNP -79C/T (rs34330) and prostate cancer – age of diagnosis <65 Biallelic loss reported in association with overgrowth and severe DD with autism.
CDKN2A (p16) cyclin-dependent kinase inhibitor 2A	**1787**	**600160**	Melanoma and neural system tumor syndrome Orolaryngeal cancer, multiple Pancreatic cancer/melanoma syndrome {Melanoma, cutaneous malignant, 2}	**AD**	**155755** **606719** **155601**	Gene already listed on UKGTN	100	Yes	The CDKN2A gene produces 2 major proteins: p16(INK4), which is a cyclin-dependent kinase inhibitor, and p14(ARF), which binds the p53-stabilizing protein MDM2.1 rs10811661 T allele is a risk factor in diabetes
CDKN2A (p14) cyclin-dependent kinase inhibitor 2A	**1787**	**600160**				Gene already listed on UKGTN	100		
CHEK2 checkpoint kinase 2	**16627**	**604373**	Li-Fraumeni syndrome 2 Osteosarcoma, somatic {Breast and colorectal cancer, susceptibility to} {Breast cancer, susceptibility to} Prostate cancer, familial, susceptibility to}	**AD**	**609265** **259500** **114480** **176807**	Apostolou & Fostira 2013. Hereditary breast cancer. The era of new susceptibility genes. BioMed Research International 747318	100		1100delC disease causing mutation – has a freq of 1.1% in healthy individuals and 5.1% in individuals with breast cancer derived from 718 families that did not carry mutations in BRCA1 or BRCA2, including 13.5% of individuals from families with male breast cancer. They estimated that the CHEK2*1100delC variant results in an approximately 2-fold increase of breast cancer risk in women and a 10-fold increase of risk in men.
CTRC chymotrypsin C (caldecrin)	**2523**	**601405**	{Pancreatitis, chronic, susceptibility to}	**AD**	**167800**	Rustgi et al., 2014 Familial pancreatic cancer: genetic advances. Genes & Dev 28:1-7	100		The DICER1 gene, a member of the ribonuclease III (RNaseIII) family, is involved in the generation of microRNAs (miRNAs), which modulate gene expression at the posttranscriptional level
DICER1 dicer 1, ribonuclease type III	**17098**	**606241**	Goiter, multinodular 1, with or without Sertoli-Leydig cell tumors Pleuropulmonary blastoma Rhabdomyosarcoma, embryonal, 2	**AD**	**138800** **601200** **180295**	Bahubeshi et al., 2011 miRNA processing and human cancer:DICER cuts the mustard Sci Transi Med 3(111):111ps46	100		
ERCC4 excision repair cross-complementing rodent repair deficiency, complementation group 4	**3436**	**133520**	Fanconi anemia, complementation group Q Xeroderma pigmentosum, group F Xeroderma pigmentosum, type F/Cockayne syndrome XFE progeroid syndrome	**AR**	**615272** **278760** **610965**	Gene dossier for cytogenetic test already approved	100		FANCQ – Fanconi anemia
FANCA Fanconi anemia, complementation group A	**3582**	**607139**	Fanconi anemia, complementation group A	**AR**	**227650**		100		Mutations in this gene are the most common cause of Fanconi anemia
FANCB	**3583**	**300515**	Fanconi anemia, complementation group B	**X-linked**	**300514**		100		X-inactivation studies in the mother and maternal grandmother of the

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Fanconi anemia, complementation group B				**recessive**					proband fetus showed 100% skewing of X inactivation, a feature consistently found in females heterozygous for FANCB mutations
FANCC Fanconi anemia, complementation group C	**3584**	**613899**	Fanconi anemia, complementation group C	**AR**	**227645**		100		
FANCD2 Fanconi anemia, complementation group D2	**3585**	**613984**	Fanconi anemia, complementation group D2	**AR**	**227646**		100		
FANCE Fanconi anemia, complementation group E	**3586**	**613976**	Fanconi anemia, complementation group E	**AR**	**600901**		100		
FANCF Fanconi anemia, complementation group F	**3587**	**613897**	Fanconi anemia, complementation group F	**AR**	**603467**		100		
FANCG Fanconi anemia, complementation group G100	**3588**	**602956**	Fanconi anemia, complementation group G	**AR**	**614082**		100		
FANCI Fanconi anemia, complementation group I	**25568**	**611360**	Fanconi anemia, complementation group I	**AR**	**609053**		100		
FANCL Fanconi anemia, complementation group L	**20748**	**608111**	Fanconi anemia, complementation group L	**AR**	**614083**		100		
FANCM Fanconi anemia, complementation group M	**23168**	**609644**	Fanconi anemia, complementation group M	**AR**	**614087**		100		
FH fumarate hydratase	**3700**	**136850**	Leiomyomatosis and renal cell cancer	**AD**	**150800**	See OMIM entry See GeneReviews Gene already listed on UKGTN	100		Autosomal recessive Fumarase deficiency MIM 606812
FLCN folliculin	**27310**	**607273**	Birt-Hogg-Dube syndrome Colorectal cancer, somatic Pneumothorax, primary spontaneous Renal carcinoma, chromophobe, somatic	**AD**	**135150** **114500** **173600** **144700**	Gene already listed on UKGTN	100		Birt-Hogg-Dube syndrome is a rare inherited genodermatosis characterized by hair follicle hamartomas, kidney tumors, and spontaneous pneumothorax
GALNT12 UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12 (GalNAc-T12)	**19877**	**610290**	Colorectal cancer, susceptibility to, 1}	**AD**	**608812**	Clarke et al., 2012 Inherited deletion variants in GALNT12 are associated with CRC susceptibility. Hum Mutat 33:1056-8 Guda et al., 2009 PNAS 106:12921-5	100		
HOXB13 homeobox B13	**5111**	**604607**	{Prostate cancer, hereditary, 9}	**AD**	**610997**	Akbari et al., 2013 Cancer Epidemiol 37:424-7	100		p.G84E associated with increased risk to prostated cancer. The carrier rate of the G84E mutation was increased by a factor of approximately 20 in 5,083 unrelated subjects of European descent who had prostate cancer, with the mutation found in 72 subjects (1.4%), as compared with 1 in 1,401 control subjects (0.1%) (P = 8.5 x 10(-7)). The mutation was significantly more common in men with early-onset, familial prostate cancer (3.1%) than in those with late-onset, nonfamilial prostate cancer (0.6%) (P = 2.0 x 10(-6))
KIF1B	**16636**	**605995**	Pheochromocytoma {Neuroblastoma, susceptibility	**AD**	**171300** **256700**	See GeneReviews	100		Charcot-Marie-Tooth disease, type 2A1 MIM 118210

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kinesin family member 1B			to, 1}						
KIT v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	**6342**	**164920**	**Gastrointestinal stromal tumor, familial** Germ cell tumors Leukemia, acute myeloid	**AD**	**606764** **273300** **601626**	See GeneReviews	100		Mast cell disease MIM 154800, Piebaldism 172800
MAX MYC associated factor X	**6913**	**154950**	Pheochromocytoma, susceptibility to}	**AD**	**171300**	Gene already listed on UKGTN	100		
MEN1 multiple endocrine neoplasia I	**7010**	**613733**	Adrenal adenoma, somatic Angiofibroma, somatic Carcinoid tumor of lung Lipoma, somatic **Multiple endocrine neoplasia 1** Parathyroid adenoma, somatic	**AD**	**131100**	Marx 2011 Hyperparathyroid genes: sequences reveal answers and questions. Endocr Pract 17 Suppl 3:18-27 Gene already listed on UKGTN	100		
MET met proto-oncogene	**7029**	**164860**	Hepatocellular carcinoma, childhood type Renal cell carcinoma, papillary, 1, familial and somatic	**AD**	**114550** **605074**	Gene already listed on UKGTN	100		TP53 PRCC MIM 179755
MITF microphthalmia-associated transcription factor	**7105**	**156845**	Melanoma, cutaneous malignant, susceptibility to, 8}	**AD**	**614456**	Ghiorzo et al., 2013 Pigment Cell Melanoma Research 26:259-62	100		Tietz albinism-deafness syndrome MIM 103500, Waardenburg syndrome, type 2A MIM 193510, Waardenburg syndrome/ocular albinism, digenic MIM 614456 Associated with RCC also
MLH1 mutL homolog 1	**7127**	**120436**	Colorectal cancer, hereditary nonpolyposis, type 2 Mismatch repair cancer syndrome Muir-Torre syndrome	**AD**	**609310** **276300** **158320**	Gene already listed on UKGTN	100	Yes	
MSH2 mutS homolog 2	**7325**	**609309**	Colorectal cancer, hereditary nonpolyposis, type 1 Mismatch repair cancer syndrome Muir-Torre syndrome	**AD**	**120435** **276300** **158320**	Gene already listed on UKGTN	100	Yes	
MSH6 mutS homolog 6	**7329**	**600678**	Colorectal cancer, hereditary nonpolyposis, type 5 Endometrial cancer, familial Mismatch repair cancer syndrome	**AD**	**614350** **608089** **276300**	Gene already listed on UKGTN	100	Yes	
MUTYH mutY homolog	**7527**	**604933**	Adenomas, multiple colorectal Colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas Gastric cancer, somatic	**AR**	**608456** **132600** **613659**	Gene already listed on UKGTN	100	Yes	Possible predisposing factor to lung cancer
NDUFA13 NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13	**17194**	**609435**	Thyroid carcinoma, Hurthle cell}	**AD**	**607464**	See OMIM	100		
NF1 neurofibromin 1	**7765**	**613113**	Leukemia, juvenile myelomonocytic Melanoma, desmoplastic neurotrophic Neurofibromatosis, familial spinal Neurofibromatosis, type 1 Neurofibromatosis-Noonan syndrome	**AD**	**607785** **613113** **162210** **162200** **601321**	Gene already listed on UKGTN	100		Watson syndrome MIM 613113
NF2 neurofibromin 2 (merlin)	**7773**	**607379**	Meningioma, NF2-related, somatic Neurofibromatosis, type 2 Schwannomatosis	**AD**	**607174** **101000** **162091**	Gene already listed on UKGTN	100		

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PALB2 partner and localizer of BRCA2	**26144**	**610355**	Fanconi anemia, complementation group N Breast cancer, susceptibility to} {Pancreatic cancer, susceptibility to, 3}	**AR** –Fanconi anemia **AD**	**610832** **114480** **613348**	Apostolou & Fostira 2013. Hereditary breast cancer. The era of new susceptibility genes. BioMed Research International 747318	100		
PDGFRA platelet-derived growth factor receptor, alpha polypeptide	**8803**	**173490**	Gastrointestinal stromal tumor, somatic Hypereosinophilic syndrome, idiopathic, resistant to imatinib	**AD**	**606764** **607685**	Agarwal 2009 Hematol Oncol Clin North Am 23:1-13	100		Germline mutations in GIST
PHOX2B paired-like homeobox 2b	**9143**	**603851**	Central hypoventilation syndrome, congenital, with or without Hirschsprung disease Neuroblastoma with Hirschsprung disease Neuroblastoma, susceptibility to, 2}	**AD**	**209880** **613013**	Gene already listed on UKGTN Bourdeaut et al. 2005 Cancer Lett 228:51-8	100		
PIK3CA phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	**8975**	**171834**	Breast cancer, somatic CLOVE syndrome, somatic Colorectal cancer, somatic Cowden syndrome 5 Gastric cancer, somatic Hepatocellular carcinoma, somatic Keratosis, seborrheic, somatic Megalencephaly-capillary malformation-polymicrogyria syndrome, somatic Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome, somatic Nevus, epidermal, somatic Nonsmall cell lung cancer, somatic Ovarian cancer, somatic	**AD**	**114480** **612918** **114500** **615108** **613659** **114550** **182000** **602501** **603387** **162900** **211980** **167000**	Gene already listed on UKGTN Am J Hum Genet 2013 92:72-80 Germline PIK3CA and AKT1 mutations in Cowden and Cowden-like syndromes	100		
PMS2 PMS2 postmeiotic segregation increased 2 (S. cerevisiae)	**9122**	**600259**	Colorectal cancer, hereditary nonpolyposis, type 4 Mismatch repair cancer syndrome	**AD** **AR**	**614337** **276300**	See GeneReviews Gene already listed on UKGTN	100	Yes	
POLD1 polymerase (DNA directed), delta 1, catalytic subunit	**9175**	**174761**	{Colorectal cancer, susceptibility to, 10}	**AD**	**612591**	Briggs and Tomlinson 2013. J Pathology 230:148-53	100		Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome MIM 615381
POLE polymerase (DNA directed), epsilon, catalytic subuni	**9177**	**174762**	{Colorectal cancer, susceptibility to, 12}	**AD**	**615083**	Briggs and Tomlinson 2013. J Pathology 230:148-53	100		AR FILS syndrome MIM 615139
PRKAR1A protein kinase, cAMP-dependent, regulatory, type I, alpha	**9388**	**188830**	Carney complex, type 1 Myxoma, intracardiac Thyroid carcinoma, papillary, somatic	**AD**	**160980** **255960** **188550**	Gene already listed on UKGTN	100		AD Acrodysostosis 1, with or without hormone resistance MIM 101800. Pigmented nodular adrenocortical disease, primary, 1 MIM 610489 Overlap with MEN syndromes, PJS
PRSS1 protease, serine, 1 (trypsin 1)	**9475**	**276000**	Pancreatitis, hereditary	**AD**	**167800**	Gene already listed on UKGTN	100		Trypsinogen deficiency MIM 614044
PRSS2 protease, serine, 2 (trypsin 2)	**9483**	**601564**	{Pancreatitis, chronic, protection against}		**167800**	See GeneReviews for PRSS1 Masson et al., 2008 Clin Gastroenterol Hepatol. 2008;6:82–8 (copy number variants associated with pancreatitis)	50		Variant of the PRSS2 gene, gly191 to arg (G191R), that was protective against the development of chronic pancreatitis
PTCH1 patched 1	**9585**	**601309**	Basal cell carcinoma, somatic Basal cell nevus syndrome	**AD**	**605462** **109400**	Gene already listed on UKGTN	100		Holoprosencephaly-7 MIM 610828

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PTEN phosphatase and tensin homolog	**9588**	**601728**	Cowden syndrome 1 Endometrial carcinoma, somatic Malignant melanoma, somatic PTEN hamartoma tumor syndrome Squamous cell carcinoma, head and neck, somatic Thyroid carcinoma, follicular, somatic Glioma susceptibility 2 Meningioma Prostate cancer, somatic	**AD**	**158350** **608089** **155600** **275355** **188470** **613028** **607174** **176807**	Gene already listed on UKGTN	100		Bannayan-Riley-Ruvalcaba syndrome MIM 153480 Lhermitte-Duclos syndrome MIM 158350 Macrocephaly/autism syndrome MIM 605309 VATER association with macrocephaly and ventriculomegaly MIM 276950
RAD51C RAD51 paralog C	**9820**	**602774**	Fanconi anemia, complementation group O {Breast-ovarian cancer, familial, susceptibility to, 3}	**AR** **–Fanconi anemia** **AD**	**613390** **613399**	Pennington and Swisher 2012. Gynecol Oncol 124:347-53 Somyajit et al., 2010 Carcinogenesis 31:2031-8	100		
RB1 retinoblastoma 1	**9884**	**614041**	Bladder cancer, somatic Osteosarcoma, somatic Retinoblastoma Retinoblastoma, trilateral Small cell cancer of the lung, somatic	**AD**	**109800** **259500** **180200** **182280**	Gene already listed on UKGTN	100		
RET ret proto-oncogene	**9967**	**164761**	Central hypoventilation syndrome, congenital Medullary thyroid carcinoma Multiple endocrine neoplasia IIA Multiple endocrine neoplasia IIB Pheochromocytoma Renal agenesis {Hirschsprung disease, susceptibility to, 1}	**AD**	**209880** **155240** **171400** **162300** **171300** **191830** **142623**	Marx 2011. Hyperparathyroid genes: sequences reveal answers and questions. Endocr Pract 17 Suppl 3:18-27 Gene already listed on UKGTN	100		
RHBDF2 rhomboid 5 homolog 2 (Drosophila)	**20788**	**614404**	Tylosis with esophageal cancer	**AD**	**148500**	Saarinen et al., 2012 .Fam Cancer 11:525-8	100		
SDHA succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	**10680**	**600857**	Paragangliomas 5		**614165**	Gene already listed on UKGTN	100	Yes	Cardiomyopathy, dilated, 1GG, MIM613642, Leigh syndrome MIM 256000, Mitochondrial respiratory chain complex II deficiency MIM 252011
SDHAF2 succinate dehydrogenase complex assembly factor 2	**26034**	**613019**	Paragangliomas 2		**601650**		100		
SDHB succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	**10681**	**185470**	Cowden syndrome 2 Gastrointestinal stromal tumor Paraganglioma and gastric stromal sarcoma Paragangliomas 4 Pheochromocytoma		**612359** **606764** **606864** **115310** **171300**		100	Yes	
SDHC succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa	**10682**	**602413**	Gastrointestinal stromal tumor Paraganglioma and gastric stromal sarcoma Paragangliomas 3		**606764** **606864** **605373**		100	Yes	
SDHD succinate dehydrogenase complex, subunit D, integral membrane protein	**10683**	**602690**	Carcinoid tumors, intestinal Cowden syndrome 3 Merkel cell carcinoma, somatic Paraganglioma and gastric stromal sarcoma Paragangliomas 1, with or without deafness Pheochromocytoma		**114900** **615106** **606864** **168000** **171300**		100	Yes	
SLX4	**23845**	**613278**	Fanconi anemia, complementation group P	**AR**	**613951**	De Garibay et al., 2013 Eur J Hun	100		

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SLX4 structure-specific endonuclease subunit						Genet 21(8)883-6			
SMAD4 SMAD family member 4	**6770**	**600993**	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome Pancreatic cancer, somatic Polyposis, juvenile intestinal	**AD**	**175050** **260350** **174900**	Gene already listed on UKGTN	100	Yes	Myhre syndrome MIM 139210
SMARCB1 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	**11103**	**601607**	Rhabdoid predisposition syndrome 1 Rhabdoid tumors, somatic	**AD**	**609322** **609322**	Gene already listed on UKGTN	100		Mental retardation, autosomal dominant 15 MIM 614608
SMARCE1 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	**11109**	**603111**	{Meningioma, familial, susceptibility to}	**AD**	**607174**	Smith et a., 2013 Nat Genet 45(3):295-8	100		
SPINK1 serine peptidase inhibitor, Kazal type	**11244**	**167790**	Pancreatitis, hereditary Tropical calcific pancreatitis {Fibrocalculous pancreatic diabetes, susceptibility to}	**AR/AD**	**167800** **608189**	Gene already listed on UKGTN	100		
SPRED1 sprouty-related, EVH1 domain containing 1	**20249**	**609291**	Legius syndrome	**AD**	**611431**	Gene already listed on UKGTN	100		Café-au-lait spots, associated with NF1
STK11 serine/threonine kinase 11	**11389**	**602216**	Melanoma, malignant, somatic Pancreatic cancer Peutz-Jeghers syndrome Testicular tumor, somatic	**AD**	**260350** **175200** **273300**	Gene already listed on UKGTN	100	Yes	
SUFU suppressor of fused homolog (Drosophila)	**16466**	**607035**	Medulloblastoma, desmoplastic Meningioma, familial, susceptibility to}	**AD**	**155255** **607174**	Bourdeaut et al., 2014 Pediatr Blood Cancer 61(2):383-6 Aavikko et al., 2012 Am J Hum Genet 91(3):530-6	100		Basal Cell Nevus syndrome overlap
TMEM127 ransmembrane protein 127	**26038**	**613403**	Pheochromocytoma, susceptibility to}	**AD**	**171300**	Gene already listed on UKGTN	100		
TP53 tumor protein p53	**11998**	**191170**	Adrenal cortical carcinoma Breast cancer Choroid plexus papilloma Colorectal cancer Hepatocellular carcinoma Li-Fraumeni syndrome Nasopharyngeal carcinoma Osteosarcoma Pancreatic cancer {Basal cell carcinoma 7} {Glioma susceptibility 1}	**AD**	**202300** **114480** **260500** **114500** **151623** **607107** **259500** **260350** **614740** **137800**	Gene already listed on UKGTN	100	Yes	
VHL von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase	**12687**	**608537**	Erythrocytosis, familial, 2 Hemangioblastoma, cerebellar, somatic Pheochromocytoma Renal cell carcinoma, somatic von Hippel-Lindau syndrome	**AD**	**263400** **171300** **144700** **193300**	Gene already listed on UKGTN	100	Yes	
WT1 Wilms tumor 1	**12796**	**607102**	Denys-Drash syndrome Frasier syndrome Meacham syndrome Mesothelioma, somatic Nephrotic syndrome, type 4 Wilms tumor, type 1	**AD**	**194080** **136680** **608978** **156240** **256370** **194070**	Gene already listed on UKGTN – Denys-Drash Syndrome, Frasier syndrome	100		

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Appendix 3 – Additional evidence for Section 11 – Validation process

i) HiSEQ equipment validation

Equipment Validation Form

- **Name of Equipment: HiSEQ 2500, Illumina cBOT**
- **Undertaken by: RLR**
- **Description: For use for 'Cancer Chip' and Exome analyses – Agilent Sureselect**
- **Supplier: Illumina**
- **Identified Need: To obtain adequate read depth/ coverage for high throughput sequence analysis of multiple genes**

- **Proposed location: Clinical Sciences Building – Translational Unit**
- **Limitations: Run for both University and NHS Departments**
- **Manufacturers Instructions enclosed : Located in CSB Level 6 Translational Unit and detailed in SOP DN257**
- **Date Risk Assessment completed: In progress – awaiting decision from Trust/ University H&S departments regarding formal procedure for waste reagent disposal**
- **Standard Operating Procedure required: Yes**
  If Yes: Index code DN257
- **Training sign off sheet completed: Yes (Machine exclusively operated by Translational unit staff at present)**

Specification
Poor | **Below Average** | **Satisfactory** | **Good** | **Excellent** | **Not applicable**
Comments
Run mode: *HiSeq high-output*: Read length 2 x100, 7 sample lanes and one control lane, run time 11 days (Total reads 375,000,000; Output bp/per lane 37,500, 000,000; Output GB/lane 37.50, GB/flowcell 300.00) – cluster generation on cBOT prior to sequencing
Run mode*: HiSeq Rapid*: Read length 2 x100, four lanes (2 flow cells with 2 lanes each), run time ~27 hrs (Total reads 300, 000,000; Output bp/per lane 30,000,000,000; Output GB/lane 30.00, GB/flowcell 60.00) – cluster generation performed onboard the HiSEQ
Sequencing meets required read depth requirements, redundancy in read depth pooling up to 12 patients per run – 'cancer chip'. For whole exome analysis 5/6 patients are pooled per run.
Scope of coverage at required read depth (50X – cancer chip) greater than MiSEQ, allowing greater flexibility for NHS service i.e multi-gene panels, whole exome sequencing
Cancer Chip – validation run in high-ouput mode

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Design
Poor | **Below Average** | **Satisfactory** | **Good** | **Excellent** | **Not applicable**
Comments
Enables in-house development of multi-gene panel testing at a reduced cost feasible

Overall ability to meet identified need
Poor | **Below Average** | **Satisfactory** | **Good** | **Excellent** | **Not applicable**
Comments – As above

Compatibility with associated equipment
Poor | **Below Average** | **Satisfactory** | **Good** | **Excellent** | **Not applicable**
Comments
Uses Agilent Sureselect protocol – not currently compatible with Spri-TE library prep method.
Appropriate equipment required mostly already in place as used for provision of other NGS services, although automation of some steps of the Agilent protocol not currently possible, but under development

Efficiency in Operation
Poor | **Below Average** | **Satisfactory** | **Good** | **Excellent** | **Not applicable**
Comments – Not applicable for our department as we do not perform the sequence runs.
Have had no significant delays in run scheduling due to maintenance issues in a year of running the NGS sequencing services on this platform.
Validation of Procedure required i.e. use of equipment on samples Yes
If Yes, please describe below. – see accompanying document – Cancer Chip Validation form dated 18/04/2013

Durability and Robustness
Poor | **Below Average** | **Satisfactory** | **Good** | **Excellent** | **Not applicable**
Comments
Have had no significant delays in run scheduling during development phase due to technical issues.

Ease of Operation (including controls/switches) N/A

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Poor	Below Average	Satisfactory	Good	Excellent	Not applicable
Comments					

Weight/Manual N/A					
Poor	**Below Average**	**Satisfactory**	**Good**	**Excellent**	**Not applicable**
Comments					

Summary Disadvantages
The platform is shared between the university and NHS departments and run by the Translational Unit, run scheduling therefore has to be negotiated with the Translational Unit. However, experience with using the MiSeq platform has never been an issue with shared use. As referral rates increase, we will be better placed to organise effective batching and shared usage.

Summary Advantages
Capacity for greater sequence coverage – enables laboratory to develop multi-gene panel tests and exome analysis pipeline. Will reduce testing costs and number of ‘sends’ to external labs for testing and increase scope of testing that the lab can provide.

Summary Modifications
To be updated by Translational Unit should modifications be made to platform or biofinformatics pipeline. Meets our needs in its current form.

General Comments
Machine, software and sequencing chemistry details
TruSeq SBS kits v3.0/Rapid SBS v1.0
Illumina PhiX Control V3.0
Runs HiSEQ control Software V2.0.5.
See also DN257, HiSeq 2500 System User Guide Part 15035786 Rev.A, cBOT Unser guide Part 15006165 RevK, CASAVA v1.8.2 User Guide Part 15011196 RevC

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1. **Final Validation after 4 weeks operation**

See Cancer Chip validation documentation for batches run in the first month of HiSEQ operation – 100% concordance observed for all confirmations conducted (performed by Sanger sequencing of the appropriate amplicon and analysed on the ABI3730 Genetic analyser using data collection V3.0, Sequence analysis software V5.2 and Mutation surveyor V3.2.) and for samples run in parallel on MiSEQ platform analysed for specific genes.

2. **List of documents attached (evidence)**

Cancer Chip validation form
I:\Next Generation Sequencing\Cancer chip\Cancer chip cases – details cases tested in parallel with existing NGS Long PCR services
I:\Next Generation Sequencing\Exome & cancer chip referrals and panel ref – details patients where first line test will be cancer chip analysis or exome (not yet formally validated pipeline)

Management Team

Risk assessment checked date: Risk assessment in preparation – awaiting clarification from University H&S department regarding waste disposal.

Date introduced into service: From 18/04/2012

Management team member: Signature Date

ii) Bioinformatics validation

CANCER CHIP VALIDATION – DATA ANALYSIS

Files received from Translational Unit for analysis

All located Q:\DNA\Cancer Chip data

- **121024_cancerChipTargets.gff – coordinates of ROIs for each gene; used to assess coverage and for variant calling (generated by CW)**
- **0360101_Covered.bed – coordinates of ROIs from Agilent**
- For each sample:
    - **Alamut-HT report ('technical report')**
    - **bam & bai files (alignments)**

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- **per base coverage file & sample interval summary files**

- **analysis log files**

NB each sample in the validation cohort has been analysed using two different aligners – Novalign and BWA – and the corresponding analysis pipelines. **As it is anticipated that all future diagnostic samples will be processed using BWA, validation samples will be reported using BWA results** (with Novalign output checked in parallel for validation purposes).

Reference sequences

Reference sequences chosen based on those currently used in laboratory, or from RefSeqGene. A list of transcripts used and corresponding Ensembl transcript can be found in **Transcript list** tab in **Cancer chip transcript list.xls**
gff file (v2 and v2.b) checked by HL/NC against Ensembl transcript exon coordinates

- all coding regions on gene list covered with **the exception of CDKN2A (not to report)**

- see **gff file check tab** in I:\Next Generation Sequencing\Cancer chip\Development**Cancer chip transcript list.xls**

Coverage check

For each sample a sample interval is generated (based on gff file) which lists each region of interest (target) by genomic coordinate, the average coverage, and the % of bases within that region which don't exceed a given threshold. **For diagnostic reporting, coverage of 50x is required**. Data can be filtered using Excel for those targets where <100% of bases are above 50. The filtered data can then be pasted into a new tab for scoring.
e.g. Q:\DNA\Cancer Chip data\BWA\Batch 1\coverage\121211_L1_Val09_12_03973_TTAGGC_781_925a
For the purposes of validation, those regions falling below 50x are annotated with the chromosomal location and gene affected. If a result is not required (i.e. gene analysis not requested by referring clinician) coverage is scored OK. If a result is required, coverage is scored 'to check' and any regions can be interrogated by viewing in the IGV (V2.3) browser [Broad Institute]. The per base coverage file can also be analysed to assess exact coverage or to interrogate results. Sanger sequencing can be instigated if applicable, or alternatively no result generated for that gene.
For all validation samples, every base analysed has been covered to 50x, with the exception of PRSS2. This gene may be unsuitable for analysis using this SureSelect protocol – Clinical team to be advised.

Technical report (variant list) generation

Excel scoring spreadsheet templates exist for both Novalign and BWA generated data.
For diagnostic reporting, BWA results are used.

1. Paste Alamut-HT report (e.g. Q:\DNA\Cancer Chip data\BWA\Batch 1\alamut-ht alamut_ann_alltrans file – open with Excel) into the first tab **All.** This list of variant calls includes all those on the Cancer Chip panel, and annotated against **multiple transcript** reference sequences.

2. In the **Filtering** tab, data is condensed to include fewer columns than on the original Alamut-HT. From here, data can be filtered by transcript to produce a variant list corresponding to the gene(s) requested for analysis. To do this, first deselect all transcripts in Column B then add in all those required. This will generate a fitered variant list which can then be copied into the final tab **Scoring.**

3. For validation purposes, any result generated by long PCR/NextGene analysis is pasted into the **NextGene result** column. This will facilitate comparison of data generated through each pipeline to assess test sensitivity (calculated by the number of unique concordant variant calls)

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4. In the **Score** column, results can be recorded as Poly / Artefact / UV / Mutation

5. The Sample file identifier i.e. RUNDATE_LX_Val12_12_01234_CGATGT_123_456_789 should be pasted into the sheet (**Column S**) and the file saved using this identifier.

Scoring

Scoring spreadsheet

For Cancer Chip validation samples, data is recorded and scoring sheets generated from the **Cancer Chip scoring sheet (Q:\DNA\Cancer Chip data\BWA).** This spreadsheet includes details of all patients within the validation panel, a record of the genes requested, and individual mutation reports for each patient. The technical reports are pasted directly into the mutation report template. The lab number and patient name will automatically be generated in the patient details box.

Class 1 Polymorphisms

The spreadsheet also contains a tab of all Class 1 polymorphisms which will not be reported. For those genes tested for using NextGene, the list has been generated from existing NGS panel templates (therefore a NextGene result is required for lookup; otherwise variants may require manual inspection). For new Cancer Chip genes, the Class 1 list has been generated based on frequency data (>2%). Any polymorphism identified will be highlighted in the Poly (NG) or Poly (CC) column as appropriate.

Scoring

Mutation reports for each patient are printed off, the variant scores checked and a final result generated for each gene requested (original Cancer Chip request form should be referred to). If necessary the IGV browser can be used to inspect any regions where apparent artefacts are called, for example at homopolymer tracts.

Any Sanger confirmations required for Class 4 or Class 5 variants should be listed.

Checking

For validation samples a check is required to ensure the correct transcripts have been identified, as this has been done manually. To do this, the transcript filtered list should be regenerated and checked that the list matches that on the printed mutation report – this can be done by checking the first and final vcf quality scores and by ensuring the genes listed are the same. If this is OK, tick ‘correct genes selected’.

Coverage excel files should also be checked to ensure all regions requested are sequenced at 50x. If OK, tick ‘Coverage ok’

Results for each gene requested should then be checked and initialled.

Reporting

Samples are activated for disease: **Cancer Gene Panel;** test: **Cancer gene sequencing.**

Results can be input for whole panel sequencing (**Cancer gene seq**) or for individual genes as and when required.

Templates exist on Shire for reporting Cancer Chip results.

iii) Panel verification

See Watson et al., 2013. Robust diagnostic genetic testing using solution capture enrichment and a novel variant-filtering interface Human Mutation DOI: 10.1002/humu.22490

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