

Pre-screen Modelling – explanatory notes

Sequencing Strategy

- 1. Sequencing orientations - primary screen [S]**

Number of sequencing orientations used for primary screening using a direct sequencing strategy. Since most laboratories now use fluorescent dye terminator Sanger sequencing, this will be [S=2] (i.e. forward and reverse) except in situations where a satisfactory sensitivity is achieved using sequencing in a single orientation only.
- 2. Sequencing orientations – secondary screen [C_{seq}]**

Number of sequencing orientations used for confirmation reactions. When bi-directional sequencing is used for the primary screen it may be considered sufficient to carry out this confirmation in a single orientation only (i.e. $C_{seq} = 1$).
- 3. False positive rate [P_{seq}]**

False positive rates for sequencing reaction (seq) expressed as a proportion of the total number of reactions carried out.
- 4. Failure rate [F_{seq}]**

Failure rates for sequencing reaction (seq) expressed as a proportion of the total number of reactions carried out.

Pre-Screen Strategy

- 5. Sequencing orientations – secondary screen [C_{ps}]**

Number of sequencing orientations used for confirmation reactions. However, when using a pre-screen method it is not possible to know if a detected sequence variant will be detected in one or both orientations. For this reason confirmatory sequencing generally needs to be carried out in both directions (i.e. $C_{ps} = 2$).
- 6. Polymorphic rate [N]**

The proportion of fragments in a particular screen that are likely to contain non-pathogenic sequence variants (polymorphisms).
- 7. False positive rate [P_{ps}]**

False positive rates for pre-screen reaction (ps) expressed as a proportion of the total number of reactions carried out.
- 8. Failure rate [F_{ps}]**

Failure rates for pre-screen (ps) expressed as a proportion of the total number of reactions carried out.

Test Variables

9. Pick-up rate (per patient)

Proportion of patients in which pathogenic mutation is likely to be found.

10. Number of exons

Number of fragments required to screen a single patient for the gene in question.

This value is only used to calculate the specific advantage value (13) and is not used to generate the general output.

11. Relative Value pre-screen [V]

Relative value of a single pre-screen reaction w.r.t a single sequencing reaction. 'Value' can be considered in terms of time alone, overall cost including work load and consumables or any other suitable measurement. A value of 2 represents a test that is twice as good as sequencing in terms of whatever is being measured (eg twice as fast, half the cost).

This value is only used to calculate the specific advantage value (13) and is not used to generate the general output.

12. Expected detection rate [D]

This value is calculated from the number of fragments in the proposed screen (10) and the expected pick-up rate per patient (9) and should not be adjusted. It is the rate, per fragment, at which true mutations are expected to be found. This is a function of For example a screen involving 50 fragments per patient with an expected pick-up rate of 50% would have a detection rate of $1/(50/0.5)=0.01$ (table 1).

Absolute values

This section would generally be used to calculate costing (the descriptions below reflect this) but could equally be used to time per test.

13. Value of single pre-screen reaction

This is the cost of a single reaction using the pre-screen method

14. Batch size

The number of different samples (patients) tested in a batch. For example to clear a backlog it is easier and quicker to test one fragment per plate. If patients need to be tested as they come in a batch size of 1 may be more appropriate. Obviously this would require a great deal more work as control test will need to be run in every batch.

15. Number of controls

The number of controls required for each batch of samples. For example water, normal and positive controls may be required.

Outputs

a. **Advantage of Pre-screen [A]**

This is the overall value of the whole pre-screen strategy w.r.t. the defined sequencing strategy (as distinct from relative value of the pre-screen, which relates to a single reaction in isolation). This value is calculated using the specific number of exons and relative value of pre-screen defined.

b. **Value of overall screen (per patient)**

The absolute value (usually used for costing) of a complete screen for one patient using the defined pre-screen strategy.

c. **Value per mutation detected (ps)**

The absolute value (usually used for costing) of detecting a single mutation using the defined pre-screen strategy.

d. **Value of sequencing equivalent (per patient)**

The absolute value (usually used for costing) of a complete screen for one patient using the defined sequencing strategy.

e. **Value per mutation detected (seq)**

The absolute value (usually used for costing) of detecting a single mutation using the defined sequencing strategy.

f. **General Output**

This is intended to give an overview of how the number of exons and the relative value of the pre-screen affect the advantage value. Each pair of these two variables defines a single comparison with a single advantage value. These advantage values form a surface which is divided into coloured zones according to value.