CLAIMS

1. A method of identifying a sub-pool of organisms, or reproductive parts thereof, of a predefined species, wherein the sub-pool comprises an organism of a predefined species carrying one or more predetermined mutation(s) in nucleotide(s) of interest [NOI(s)], in a target sequence, said method comprising the steps of:

   a) Providing a pool of organisms of said species, or reproductive parts thereof, representing a plurality of genotypes;

   b) Dividing said pool into one or more sub-pools of organisms, or reproductive parts thereof, wherein each sub-pool comprises more than one individual organism or reproductive parts thereof of each genotype, wherein all progeny of one organism, or reproductive part thereof, from said pool is comprised within one sub-pool;

   c) Randomly dividing each sub-pool into parts in a manner such that each part in theory comprises organisms or reproductive parts thereof representing each genotype of the sub-pool,

   d) Preparing genomic DNA (gDNA) samples from one of said parts of each sub-pool and storing the remainder of organisms or reproductive parts thereof under conditions maintaining the reproductive potential;

   e) Performing a plurality of PCR amplifications, each comprising the gDNA sample from one sub-pool, one or more set(s) of primers each set flanking a target sequence and PCR reagents, thereby amplifying the target sequence(s);

   f) Detecting PCR amplification product(s) comprising one or more target sequence(s) comprising the mutation(s) in the NOI(s), thereby identifying sub-pool(s) comprising said mutation(s).

2. The method according to claim 1, wherein in the range of 10 to 50% of the organisms or parts thereof of each sub-pool is used for preparing the gDNA sample.

3. The method according to any one of the preceding claims, wherein the PCR amplification(s) of step e) is (are) performed by a method comprising the following steps:

- Preparing one or more PCR amplifications comprising the gDNA sample, one or more set(s) of primers each set flanking a target sequence and PCR reagents;
- Partitioning said PCR amplification(s) into a plurality of spatially separated compartments;
- Performing PCR amplification(s);
- Detecting PCR amplification products.

4. The method according to any one of the preceding claims, wherein said pool of organisms is prepared by subjecting the organisms of said species, or reproductive parts thereof, to random mutagenesis.

5. The method according to any one of the preceding claims, wherein the organism pool comprises at least 500,000 organisms, or reproductive parts thereof, with different genotypes.

6. The method according to any one of the preceding claims, wherein each sub-pool comprises at least 100 organisms or reproductive parts thereof with different genotypes.

7. The method according to any one of the preceding claims, wherein the majority of organisms or reproductive parts thereof of the same genotype are contained within the same sub-pool.

8. The method according to any one of the preceding claims, wherein the methods comprise a step of reproduction of the organisms, or reproductive parts thereof, within the pool, and wherein said step of reproducing is performed simultaneously with, or subsequent to, step b) of dividing the organisms into sub-pools.

9. The method according to any one of the preceding claims, wherein the species is a plant, and steps a) and b) of said method comprise the steps of:

- Providing a plurality of seeds of a plant;
- Subjecting said seeds to random mutagenesis, thereby obtaining seeds of generation M0;
- Growing said seeds of generation M0 into mature plants, and obtaining seeds from said mature plants, wherein said seeds are seeds of generation M1;

- Optionally repeating the previous step X times to obtain plants comprising seeds of generation M(1+X);
- Obtaining seeds of either generation M1 or M(1+X) from said mature plants, thereby obtaining a pool of seeds;
- Dividing said pool of the various steps into sub-pools, wherein all seeds from a given mature plant are placed into the same sub-pool.

10. The method according to any one of preceding claims, wherein the species is a plant, and the method further comprises the following steps:
    - Providing a sub-pool comprising a plurality of seeds of a plant, wherein said sub-pool comprises one or several mutation(s) of the NOI(s);
    - Dividing the seeds of said sub-pool into secondary sub-pools, each comprising in the range of 1 to 100 seeds;
    - Obtaining a sample from each seed of the secondary sub-pools – in a manner leaving the seeds sufficiently intact to develop into a plant – and combining all samples from all seeds of each secondary sub-pool;
    - Preparing a gDNA sample from said combined samples

    thereby identifying a secondary sub-pool comprising a plant carrying the mutation(s) in the NOI(s).

11. The method according to any one of claims 1 to 8, wherein the species is a unicellular organism, and steps a) and b) of said method comprise the steps of:
    - Providing a plurality of unicellular organisms;
    - Subjecting said organisms to random mutagenesis;
    - Dividing the mutagenized organisms into sub-pools;
    - Subjecting each sub-pool to a step of reproduction.

12. The method according to claims 1 to 8 and 11, wherein the species is a unicellular species, and the method further comprises the following steps:
    - Providing a sub-pool comprising the mutation(s) in one or several the NOI(s);
    - Reproducing some, or all of the organisms, of said sub-pool in a clonal manner to obtain clonal cultures;
    - Combining a fraction of organisms from a plurality of said clonal cultures to obtain secondary sub-pools

thereby identifying a secondary sub-pool comprising an organism carrying the mutation(s) in the NOI(s).

13. The method according to claim 12, wherein each secondary sub-pool comprises fractions of in the range of 10 to 100 clonal cultures.

14. The method according to any one of the preceding claims, wherein the method further comprises a step of identifying a super-pool comprising one or more mutation(s) in the NOI(s), wherein the super-pool is a group of sub-pools.

15. The method according to claim 14, wherein the method further comprises the following steps performed after step d):
    - Obtaining a fraction of each gDNA sample from each sub-pool;
    - Combining a plurality of fractions into super-pools, thereby obtaining gDNA super-pools comprising gDNA samples from a plurality of sub-pools, wherein gDNA from each sub-pool is only present in one super-pool;
    - Performing a plurality of PCR amplifications, each comprising a gDNA sample super-pool, wherein each PCR amplification comprises a plurality of compartmentalised PCR amplifications, each comprising part of said gDNA sample, one or more set(s) of primers each set flanking a target sequence and PCR reagents, thereby amplifying the target sequence(s);
    - Detecting PCR amplification product(s) comprising one or more target sequence(s) comprising the mutation(s) in the NOI(s), thereby identifying super-pool(s) comprising said mutation.

16. The method according to claim 15, wherein the PCR amplification(s) comprising a gDNA sample super-pool is (are) performed by a method comprising the following steps:
    - Preparing a PCR amplification comprising the gDNA sample, one or more set(s) of primer(s) each set flanking a target sequence and PCR reagents;
    - Partitioning said PCR amplification(s) into a plurality of spatially separated compartments, wherein each compartment has an average volume in the range of 0.1 to 10 pL;
    - Performing a PCR amplification;
    - Detecting PCR amplification products.

17. The method according to any one of claims 1 to 8, 11 to 16, wherein the species is a unicellular organism, for example yeast.

18. The method according to any one of claims 1 to 10, 14 to 16, wherein the species is a plant, for example a flowering plant.

19. The method according to any one of claims 1 to 10, 14 to 16, wherein the species is a cereal, for example barley.

20. The method according to any one of the preceding claims, wherein the mutation is a substitution of a single nucleotide.