

Certificate of Probe Design Process

Xmer[®] technology

The DualChip[®] probe design addresses two goals:

- 1) Sensitivity and specificity
- 2) Reliability and reproducibility

These high quality probes are generated by optimized probe selection and verification procedures, combined with demanding quality control of the generated probes. With a length of 200-400 nucleotides, optimized selection, synthesis and quality control procedures, the Xmer technology provides the user with highly specific DNA microarray probes.

The probe design is based on a selected RefSeq sequence, and is optimized in several *in silico* and *in situ* steps. Each step of probe design and validation is characterized by an extensive quality control mechanism. The entire process includes an *in silico* probe design (Fig.1) as a first step and an *in situ* (Fig.2) probe design as a second step.

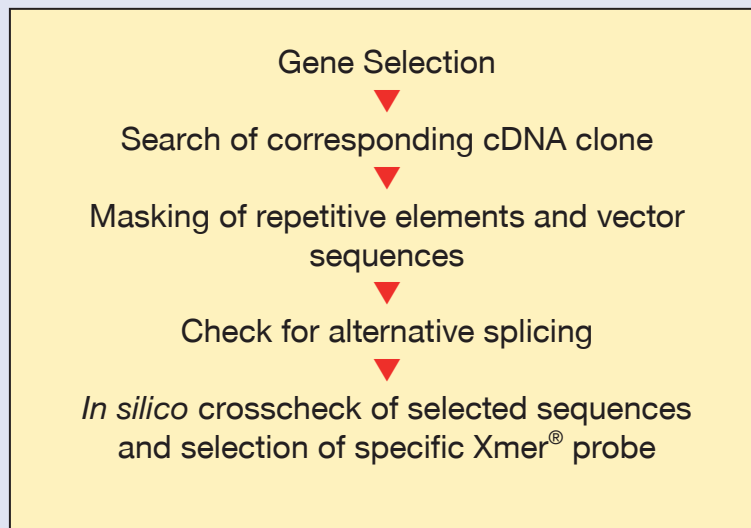


Fig.1: *In silico* probe design

After the *in silico* probe design each selected Xmer probe fulfills the following criteria:

- Specificity is checked by blasting the selected sequences against all sequences available in public databases.
- Each gene is checked for the presence of possible splice variants. In some cases isoforms of a selected gene are represented by one specific Xmer, since probes are chosen in the consensus region.
- Distance to 3'-Poly(A) tail is optimized in order to ensure that reverse transcription efficiency is sufficient to cover the region where the capture probe is located.

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Following the *in silico* approach, an experimental (*in situ*) approach is used to validate the probe design predictions.

1) Individual cross-check:

During this process, antisense DNA molecules corresponding to a certain Xmer probe of the DualChip microarray are produced and labeled. To confirm the Xmer identity and hybridization ability, the labeled antisense strands are hybridized against the entire set of probes.

2) Final specificity test:

Ten biotinylated antisense strands are used in parallel for a hybridization experiment against the entire set of probes; these probes are spotted on the final DualChips as triplicates within the array.

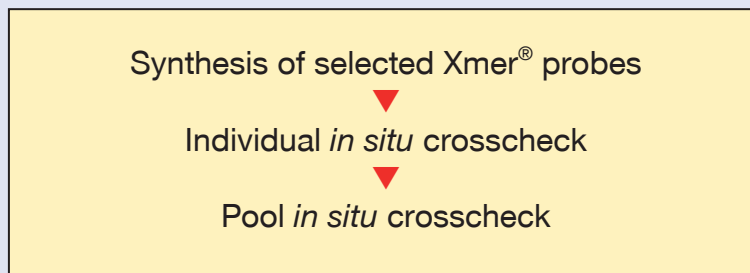


Fig.2: *In situ* probe confirmation

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