QIAGEN Supplementary Protocol

Design of HaploPrep™ probes for automated haplotype-specific extraction using the EZ1® HaploPrep Kit

The EZ1 HaploPrep Kit uses haplotype-specific extraction (HSE) to physically separate diploid genomic DNA into its haploid components, based on allele-distinguishing single nucleotide polymorphisms (SNPs). By separating the alleles, the EZ1 HaploPrep Kit enables individual analysis of each allele by routine DNA typing methods that are currently used on standard diploid DNA.

This protocol provides information on the design of locus-specific HaploPrep probes, enabling the generic use of the EZ1 HaploPrep Kit.

IMPORTANT: Please read the handbook supplied with the EZ1 HaploPrep Kit, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. The EZ1 HaploPrep Kit is For Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Procedure

Principle of probe design

HSE probes represent oligonucleotides similar to PCR primers 15–22 nucleotides in length. They cover one or more allele-distinguishing SNPs and are complementary to the targeted allele sequence. For their design, general oligonucleotide design guidelines as well as specific requirements regarding probe positioning in relation to the distinguishing SNP, and possibly necessary nucleotide modifications should be considered, as specified in the following procedure.

1. **Select probe region.**
   - Identify regions with allele differences, based on initial typing results (e.g., sequencing data, presenting heterozygous positions in diploid DNA).
   - Choose sequence section containing one or preferably more than one allele-distinguishing SNP.
   - Avoid sequence sections with homology to other, nontargeted loci.

2. Position probe in relation to SNP. Position allele-distinguishing SNP within probe sequence as follows:

- One distinguishing SNP: 3' terminus -1, or alternatively right at 3' terminus
  Example 1:

  Allele 1: . . . GAC CGG AAC ACA CAG ATC T**A**C AAG . . .

  Allele 2: . . . GAC CGG AAC ACA CAG ATC T**T**C AAG . . .

  Probe for allele 2: CGG AAC ACA CAG ATC T**T**C

  Alternatively: CGG AAC ACA CAG ATC T**T**

- More than one distinguishing SNP (preferred): position most 3'-bound SNP as described in example 1, so that other SNPs lie in center or next to 5' terminus of probe
  Example 2:

  Allele 1: . . . ACA CAG ATC T**A**C AAG **G**CC CAG GCA . . .

  Allele 2: . . . ACA CAG ATC T**T**C AAG **A**CC **A**A**C** **A**CA . . .

  Probe for allele 2: CAG ATC T**T**C AAG **A**CC **A**A**C** **A**

- Mismatch between probe and nontargeted allele: GG, CC, AA, TT are efficient mismatches; GT mismatches should be avoided (first position represents nucleotide in probe, second position represents nucleotide on complementary strand of nontargeted allele; see example 3 below); for other combinations, see Ayyaddevara et al. (1).
- Probe orientation: Choice of probe in reverse instead of forward orientation may lead to better mismatch. Choose reverse orientation with CA mismatch in order to avoid GT mismatch with forward orientation.
  Example 3:

  Allele 1 forward: . . .TAC AAG CGC CAG GCA CAG **A**CT GAC . . .

  Allele 2 forward: . . .TAC AAG CGC CAG GCA CAG **G**CT GAC . . .

  Probe for allele 2: AAG CGC CAG GCA CAG **G**C GT mismatch

  Allele 1 reverse: . . . CAG GCT CAC TCG GTC AG**T** CTG TGC . . .

  Allele 2 reverse: . . . CAG GCT CAC TCG GTC AG**C** CTG TGC . . .

  Probe for allele 2: G GCT CAC TCG GTC AG**C** C CA mismatch

3. General oligonucleotide design

- Length: 15–22 nucleotides
- GC content: 30–70%
- Melting temperature (T_m): 50–58°C (as calculated with simplified formula for estimating T_m: T_m = 2°C x (A+T) + 4°C x (G+C))
- Sequences that should be avoided: hairpin structures, self-dimer formation, stretches of 4 or more G or C

4. Nucleotide modifications (optional)

- Handling of other, nondistinguishing SNPs: If probe positioning does not allow choice of sequence without occasionally occurring nondistinguishing SNPs, consider nucleotide replacement with inosine (dITP, pairs indiscriminately with A, C, and T).
- Improvement option: In case of insufficient allele separation consider LNA-modification of mismatch position (to increase binding of probe to target; please note: LNA modifications are not possible for 3'-terminal mismatch position).

5. Ordering and use of probes

- Order oligonucleotides in salt-free quality (e.g., from IDT in LabReady format, i.e., adjusted to 100 µM with TE buffer*).
- Use probe at 100 µM concentration according to the EZ1 HaploPrep procedure, and follow protocol as described in *EZ1 HaploPrep Handbook*.

References

1. Ayyadevara, S., Thaden, J.J., and Shmookler Reis, R.J. (2000) Discrimination of Primer 3'-Nucleotide Mismatch by *Taq* DNA Polymerase during Polymerase Chain Reaction. Anal. Biochem. **284**, 11-18.

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Technical assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding probe design, the EZ1 HaploPrep Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see below or visit www.qiagen.com).

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HSE technology is developed under a collaboration of QIAGEN and Generation Biotech. Patents pending US patent 20010031467 and EU patent 00984206.3.

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