Pre-examination Process (for labs)
Validation of test - I

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Pre-examination Process (for labs)

- How to choose a strategy ?
- Strategies for mutation detection

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PART I

- Strategies for linkage analysis
- Validation

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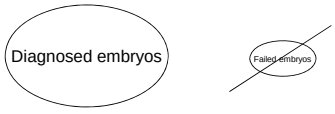
How to choose a strategy ?

- Fast (transfer day 3-5)

- Sensitive : single cell PCR

- Powerful :



- Distinguish affected / unaffected embryos

⇒ Never transfer an affected embryo

Mutation Detection : direct diagnosis

1. Flanking PCR (fluorescent PCR = golden standard)

2. Mutation detection

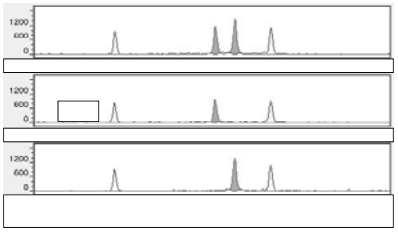
- ✓ small insertions / deletions
- ✓ large insertions / deletions
- ✓ triplets
- ✓ substitutions

Strategy depends on the mutation

Small deletion / insertion (n bp)

PCR ⇨ Migration ⇨ Results :
Normal : normal size
Insertion : normal size + n
Deletion : normal size - n

Example : cystic fibrosis ΔF508



ΔF508/F508

ΔF508/ΔF508

F508/F508

Large deletion / insertion

PCR ⇨ Migration ⇨ Results :
Normal : normal size
Insertion : no amplification
Deletion : no amplification

- No heterozygous detection (normal allele amplified)
- If contamination : possible transfer of an affected embryo
- no difference between deleted/inserted alleles and amplification failure

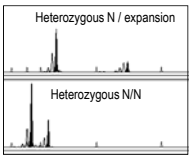
Not recommended

Triplet expansion

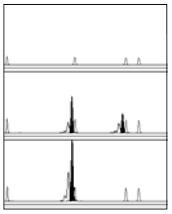
PCR ⇨ Migration ⇨

Results :
Normal : normal range allele
Small expansion : expanded allele
Large expansion : no amplification

Examples : Huntington



Fragile X



♂ mutation

♀ Heterozygous N/N

♂ normal

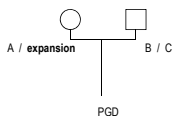
Triplet expansion :

Single cell PCR : only normal alleles amplified

- Precycle workup : informativity testing on parental and proband DNA
- PGD restricted to informative couples

Precycle workup :

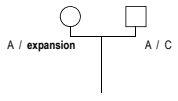
Example : autosomal dominant disease : **informative**



Genotypes for Embryos	A / C	A / B	B / **expansion**	C / **expansion**
PCR results	A / C	A / B	B	C
Embryo Status	Unaffected	Unaffected	Affected	Affected
Transfer	YES	YES	NO	NO

Precycle workup :

Example : autosomal dominant disease : **half-informative**



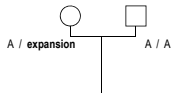
PGD

Genotypes for Embryos	A / C	A / A	A / expansion	C / expansion
PCR results	A / C	A	A	C
Embryo Status	Unaffected	Unaffected	Affected	Affected
Transfer	YES	NO	NO	NO

⇒ **50% unaffected embryos not transferable**

Precycle workup :

Example : autosomal dominant disease : **not informative**



PGD with direct diagnosis

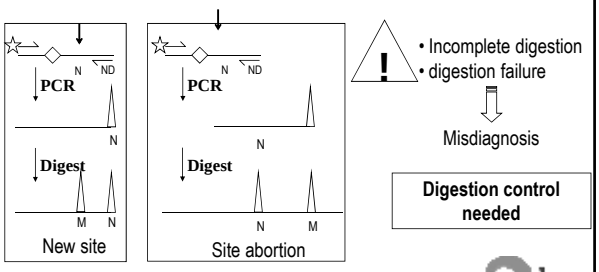
Genotypes for Embryos	A / A	A / A	A / expansion	A / expansion
PCR results	A	A	A	A
Embryo Status	Unaffected	Unaffected	Affected	Affected
Transfer	NO	NO	NON	NO

⇒ **No PGD**

Substitutions

Modification of a restriction site

PCR → Digest → Migration → Results :
Normal : normal size
Mutation : modified size



! • Incomplete digestion
• digestion failure
⇩
Misdiagnosis

Digestion control needed

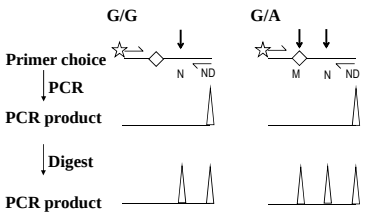
Modification of a restriction site

How to control digestion ?

- Co-amplification with a fragment showing the same restriction site.
- Co-digestion of 2 PCR products (control and blastomere) labelled with different fluorochromes.
- primers designed so that an internal digestion control is present in the fluorescent PCR fragment : Double digest.

Double digest

gcacagagctgagaaacattgaccacactagcctctgctttttagaatttcccaagatttaggctgtttatctatgttgcccaaatgaataacgtgttttttggagaaactctgtcacccttttcattcccagGGGT
ACTTTCATTGAAT**TCCGA**AATGGGATGTTAAACGTGTCCCCTATTGGAAGAAGCTGCAGCCAAGAAGAACGCATTGAGTTCTA
CGAACTCGATAAAgtacgtctt**ctga**aaatatctttggtgaatggctgggtttatggaaataagatat



No need for an external digest control

Mini-sequencing

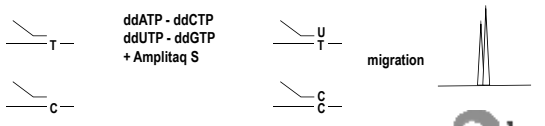
PCR ⇨ Mini-sequencing ⇨ Migration ⇨ Results :
Normal : normal nucleotide
Mutation : nucleotide change

Principle :

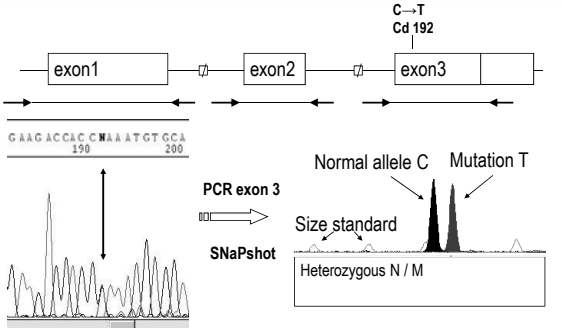
primer extension (1 nucleotide added)

ddATP, ddCTP, ddUTP, ddGTP : specific labelling

fragment labelled according to the added nucleotide



Example :VHL - mutation P192L (exon 2)



Allele specific amplification

- ARMS : amplification refractory mutation system

PCR ⇨ Migration ⇨ Results :
specific : amplification
Not specific : no amplification

- High specificity required (primer design)
- If amplification of Normal allele : no difference between affected embryos and amplication failure
- If amplification of Mutation : misdiagnosis if amplification failure in affected embryos

⇨ **Control needed**

Allele specific amplification : primer design

sequence TCT.GCT.ACA.AAC.TTT.TGT.CTT.A

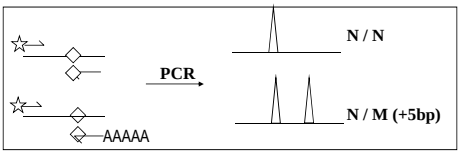
- 3' end : allele specific

TCT.GCT.ACA.AAC.TTT.TGT.CTT.**A**

- before 3' end (3 to 6 nt) : add 1 mismach

primer TCT.GCT.ACA.AAC.TTT.TGT C**G**T.A

D-ARMS : double ARMS (double allele specific amplification)



Reverse primers

- DNA ~~~~~~~~~~~~~~~~~~~~.CTT.A
- ex6. ~~~~~~~~~~~~~~~~~~~~.CGT.A
- ex6M AAAA~~~~~~~~~~~~~~~~~~~~.GTT.G

Normal and mutated alleles are amplified

Conclusion

- **Many mutation detection tests**
- **Strategy depends on the mutation**
- **If mutation detection only : misdiagnosis risk in case of AOF, ADO or contamination**

⇒ **Golden standard : Mutiplex PCR combining**
-mutation detection and linkage
- or linkage with several markers

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- **Strategies for linkage analysis**
- **Validation**

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