Flanking Region PCR Reaction

Introduction

Previously cut flanking regions will be added to the AddGene HTT sequence to complete exon 1.

Materials

- Amplified AddGene HTT sequence
- Cut flanking regions
- Primers for flanking regions
- Polymerase

Procedure

Set-up PCR

1. Mix equal molar amounts of flaniking region DNA and AddGene HTT DNA at concentrations (1-500 ng) sufficient for initial PCR.
2. Add primers at standard PCR conccentrations (0.2-0.5 uM).
3. Intially denature dna at 95˚C for 10 min (**with no polymerase**)
4. Add Taq Polymerase.
5. Cycle (20x) at:

PCR Amplification Parameters

	A	B	C
1	Tempature	Time	Cycle name
2	95˚C	30-60 s	Melt
3	Tm - 5˚C (https://tmcalculator.neb.com/)	30-60 s	Anneal
4	72˚C	1 min / kb	Elongate

6. End cycle as follows

End Cycle Parameters

	A	B	C
1	72˚C	10 min	Final Elongation
2	4˚C	Indefinitly	Hold Chilled

7. Gel Verify the amplification

8. Store the DNA at 4˚C