Van Andel Institute
Laboratory of Microarray Technology
2007

Procedure 3.4 – Hauenstein Parkinson's Center Exon PCR and Sequencing

Date: ___________________ **Experiment ID: 3.4-**yyyymmdd

Technician: ___________________

Project: ___________________

Client Signature: ___________________

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PCR Amplification of Patient DNA Exons

1. **Locate and thaw appropriate patient DNA from Freezer.**
2. **Record patient Ids in an Excel spreadsheet and save as**

   StandardOperatingProcedures_2007/Procedure_Reports/Procedure_3.4/3.4-yyyymmdd_patientIds

   ____

3. **Prepare the following PCR master mix by multiplying the volumes by n.5, where n is the number of samples being amplified.**

Water	**90.1**	**ul**	___________
Buffer (10X)	**10.7**	**ul**	
MgCl^2 (50mM)	**5.36**	**ul**	
dNTP Mix (25mM each)	**1.07**	**ul**	___________
Forward Primer (1mM)	**0.11**	**ul**	___________
Reverse Primer (1mM)	**0.11**	**ul**	___________
Taq DNA Polymerase (5U/ul)	**0.54**	**ul**	___________

4. **Aliquot PCR master mix into PCR plates (97 ul)** ___________________
5. **Inoculate PCR reactions with 3ul template DNA (30-100 ng).**
6. **Place plate in thermocycler and run *PARKIN* program.**

PCR Parkin Program			
Step	**Description**	**Temperature (°C)**	**Time (s)**
1	Denature	94	120
2	Denature	94	30
3	Anneal	55	30
4	Extend	72	60
5	Go to Step 2 (34 times)		
6	End		

Purification of PCR Products (Qiagen QIAquick 96 PCR Purification Kit)

1. **Prepare QIAvac 96 and 96-well filter plate.** ______________________

   1.1 Place the waste tray in the base.
   1.2 Place QIAvac top plate on the base.
   1.3 Attach the manifold to a vacuum source.
   1.4 Seal unused wells of filter plate with foil tape.
   1.5 Place filter plate on QIAvac top plate.

2. **Add 3 volumes BufferPM to the filter plate wells.**
   Add sample to filter plate wells in corresponding positions.
   Mix by pipetting.

3. **Switch on the vacuum until all liquid has passed through the membrane of the filter plate.**

4. **Wash bound filter plate wells.**

   4.1 Pipette 850ul BufferPE into wells.
   4.2 Vacuum until all liquid has passed through the membrane.
   4.3 Repeat 4.1 and 4.2

5. **Allow filter plate to dry under vacuum for 10 additional minutes.**

6. **Remove the filter plate and the top plate in one piece.**

7. **Remove waste tray and replace with plate base and a new PCR plate.** ______________________

8. **Place filter plate and top plate onto the base assembly.**

9. **Elute PCR products by pipetting 80ul nuclease free water into the wells. Incubate at room temperature for one minute.** ______________________

10. **Apply vacuum for 5 minutes.**

11. **Seal plate containing purified DNA with foil tape and store at -20C.**

<u>Submission of PCR Products For Sequencing</u>

1. **Quantitate PCR products using the NanoDrop as described in appendix 7.1**

2. **Combine 200-400ng of DNA with 3pmol of reverse primer in a volume less than 8ul.**

3. **Submit to the sequencing core facility by placing samples in freezer box and filling out the sequencing request log on door of freezer.**

<u>Sequence Analysis</u>

1. **Sequence information is delivered in the form of .cdt files.**

2. **Open the .cdt file with Chromas and *Export As Fasta*.**

3. **Obtain identity score for the sequence from NCBI Blast.
Identity scores of greater than 95% are considered accurate.
Samples yielding identity scores of <95% should be re-amplified.**

4. **Align Sequences.**

   4.1 Place all sequences for alignment in a common folder.

   4.2 Open Sequencher software.

   4.3 Click <File/Import/Folder of Sequences> and select the appropriate folder.

   4.4 Import consensus sequence (from *ensembl*) for the exon being aligned.

      4.4.1 Click <Sequence/Create New Sequence/
      4.4.2 A dialog box will appear and ask for a sequence name.
      4.4.3 Name sequence Exon_x
      4.4.4 Click <OK>
      4.4.5 Paste ensembl consensus sequence into the window that opens.
      4.4.6 Close window and click <Record as Experimental Data>

   4.5 Select all files and click *Assemble Automatically*

   4.6 When the assembly is complete, click on the contig that is created.

   4.7 The contig will illustrate the sequence alignment of all sequences including the consensus sequence. This information is used to extract sequence variations for each exon as compared to the consensus sequence.