Appendices
Appendix I

Colchicine – 0.2μg/μl

Hypotonic solution
0.449g of KCL and 0.04g of trisodium citrate in 100ml of distilled water, maintained at 37°C.

Fixative
Acetic acid : methanol – 1:3

Giemsa stock solution
0.2M Na$_2$HPO$_4$

Working Giemsa water
80ml Giemsa stock in 880ml distilled water and pH was maintained to 6.8 with 0.1 citric acid

Phosphate buffer
KH$_2$PO$_4$ (9.073g/litre) and Na$_2$HPO$_4$ (11.8g/litre) in 1:1 ratio

Saline – 0.9% NaCl

Trypsin – 3% in 0.9% NaCl

Giemsa stain
760mg Giemsa powder in 50ml glycerol and 50ml methanol, incubated at 60°C for 3-4 hours.

Appendix II

Solution A (For 1 litre)
Sucrose – 109.5g
1M MgCl$_2$ – 5ml
Triton-X 100 – 10ml
Maintained the volume upto 1 litre, autoclaved and stored at 4°C.

Solution B (For 100ml)
1M Tris-Cl – 40ml
0.5M EDTA (pH 8.0) – 12ml
1M NaCl – 15ml
Maintained the volume to 95ml, autoclaved and 5ml of 20% SDS added to make volume 100ml after cooling. Stored at room temperature.

Solution C
5M Sodium perchlorate, autoclaved and stored at room temp.
Appendix III

Solution I (for 10 ml) stored at 4°C
1 M Glucose – 0.5 ml
1M Tris-Cl (pH-8.0) – 0.25 ml
0.5 M EDTA (pH-8.0) – 0.2 ml
MQ – 9.05 ml

Solution II (for 10 ml) stored at RT
10N NaOH – 0.2 ml
20% SDS – 0.5 ml
MQ – 9.3 ml

Solution III (for 10 ml) stored at 4°C
5M Patassium acetate – 6 ml
Glacial acetic acid – 1.15 ml
MQ – 2.85 ml

Appendix IV

1X TE buffer pH-8.0
10mM Tris-Cl pH8.0
1mM EDTA pH8.0

Appendix V

50X Tris-Acetate-EDTA (TAE) buffer (For 1 litre)
Tris base – 242g
Glacial acetic acid - 57.1ml
0.5M EDTA pH8.0 – 100ml
Appendix VI

**10X PCR buffer**
100mM Tris-Cl pH 9.0
500mM KCl
15mM mgCl$_2$
0.1% Gelatine

Appendix VII

**Master Mix I**
125mM EDTA pH 8.0 - 2μl
Deionised water - 10μl

**Master Mix II**
Absolute alcohol - 50μl
3M NaOAc pH 4.6 - 2μl
## Appendix VIII

### Patient Questionnaire

**Title of the research:** Molecular Genetic Analysis of Congenital Limb Malformations

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Date</th>
<th>Sporadic/Familial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Address:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contact No.:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients name</th>
<th>Sex:</th>
<th>Age:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Father’s name</th>
<th>Mother’s name:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Description of Limb anomalies:**

<table>
<thead>
<tr>
<th></th>
<th>Humerous/Femur</th>
<th>Radioulna/Tibiafibula</th>
<th>Carpel/Tarsels</th>
<th>Metacarpels/Metatarsels</th>
<th>Digits/Phalanges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left hand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right hand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Clinical Diagnosis**

:……………………………………………………………………………….(Nonsyndromic/syndromic)

<table>
<thead>
<tr>
<th>Features</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fore Limb Defect</td>
<td></td>
</tr>
<tr>
<td>Hind Limb Defect</td>
<td></td>
</tr>
<tr>
<td>CLP/CP</td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
</tr>
<tr>
<td>Teeth</td>
<td></td>
</tr>
<tr>
<td>Nail</td>
<td></td>
</tr>
<tr>
<td>Mammary Gland</td>
<td></td>
</tr>
<tr>
<td>Lachrymal</td>
<td></td>
</tr>
<tr>
<td>Ankyloblepharon</td>
<td></td>
</tr>
<tr>
<td>Lip Pits</td>
<td></td>
</tr>
<tr>
<td>Pterygium</td>
<td></td>
</tr>
<tr>
<td>Ear</td>
<td></td>
</tr>
<tr>
<td>Freckling</td>
<td></td>
</tr>
<tr>
<td>Hallux valgus</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other features/Information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Pedigree:**
Appendix IX

Certificate of Consent

I……………………………………………….. have been advised to undergo molecular genetic/chromosomal test for myself/my family members by my consultant doctor for the analyses of the suspected genetic defect. I here give consent to provide the blood/other tissue (…………) sample of myself and my family members for the said test. I also give consent to provide the photographs and other information of myself and my family members. I understand that the results obtained will be disclosed to me and to my consultant only. I also agree that the photographs as well as the results obtained can be used for scientific analyses, presentation and publication under the condition of strict confidentiality and anonymity.

Signature of patient/guardian:……………………………………………………………….. Date:………………

Relationship of the guardian with the patient:…………………………………………………… Date:………………

Full signature of the researcher:……………………………………………………………………. Date:………………

Consent for Storage and Future Use of Unused Samples

(According to WHO Preprint)

If any of the (TYPE OF SAMPLE i.e. blood, tissue) I have provided for this research project is unused or leftover when the project is completed (Tick one choice from each of the following boxes)

Consent for Storage and Future Use of Unused Samples:

☐ I wish my [blood] sample to be destroyed immediately.
☐ I want my [blood] sample to be destroyed after _____ years.
☐ I give permission for my [blood] sample to be stored indefinitely

AND (if the sample is to be stored)

☐ I give permission for my [blood] sample to be stored and used in future research but only on the same subject as the current research project. [Molecular Genetic Exploration of Congenital Limb Malformations and Limb Development]
☐ I give my permission for my [blood] sample to be stored and used in future research of any type which has been properly approved
☐ I give permission for my [blood] sample to be stored and used in future research except for research about [Molecular Genetic Exploration of Congenital Limb Malformations and Limb Development]

AND

☐ I want my identity to be removed from my [blood] sample.
☐ I want my identity to be kept with my [blood] sample.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily to have my samples stored in the manner and for the purpose indicated above.

Name of Participant………………….. Signature………………….. Date…………………..

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Thumb print of participant………………….. Name of witness………………….. Signature………………….. Date…………………..

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. DNA will be isolated from his or her peripheral blood sample and stored.
2. Genotyping and other Molecular Biology procedures will be performed on his sample.
3. Data will be analyzed and can be published and/or presented.
4. His/her identity will be kept confidential.
5. He/she can withdraw from being participated in this study at any time.

I confirm that the participant was given an opportunity to ask questions about the nature and manner of storage of the samples, and all the questions asked by the participant have been answered correctly and to the best of my ability.

I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent………………….. Signature………………….. Date…………………..
Appendix X

Certificate of Ethical clearance

Faculty of Science  
Banaras Hindu University  
Varanasi-221005

No. F.Sc./Ethical/2013-2014/2  
April 30, 2013

Dr. Akhtar Ali  
Principal Investigator  
& Assistant Professor  
Centre for Genetic Disorders  
Faculty of Science  
Banaras Hindu University

Dear Sir,

The Ethical Committee meeting of Science Faculty, BHU was held on April 30, 2013  
at 3.30 PM at Centre for Genetic Disorders for ethical clearance of Project/Ph. D  
synthesis.

| Project Title - Molecular Genetic Exploration of Congenital Limb Malformations  
| and Limb Development |
| Ethical Observation - |
| Remarks: The above project has been approved by the ethical committee |

This is for your information and necessary action at your end.

Parimal Das  
(Parimal Das)  
MEMBER SECRETARY  
& COORDINATOR

Yours sincerely

(Prof. J.K. Agrawal)  
CHAIRPERSON OF THE ETHICAL COMMITTEE