Supplementary Methods

Single strand break LM-PCR protocol for Biomek 2000

Place Plate containing DNA in 2µl at position B6 (plate 1) and plate for labeling reaction at A2 (plate 2). All master mixes are placed at chilled position A6 and all wash buffers for beads at B5.

Initial configuration – (see supplementary Fig. 1)
//ADJUST SAMPLE NUMBER FOR LM-PCR IN EDIT - PATTERN
Reset Tip Rack at A3 to A1 (0 tips used)\
Reset Tip Rack at B3 to A1 (0 tips used)
Reset Tip Rack at A4 to A1 (0 tips used)
Reset Tip Rack at B4 to A1 (0 tips used)
Reset Tip Rack at A5 to A1 (0 tips used)
User Function: MessageSender, Wait

//PRIMER EXTENSION MIX
Pipette 3.00µl from A6 to B6 (Plate 1) using P20 – P20
Pipette 15µl from A6 to B6 using p200L – P250
Gripper move A2, (Plate 2) to A1
Gripper move A1, (Plate 2) to A2

//PRIMER EXTENSION
User Function: MessageSender, wait
User Function: MessageSender, wait
User Function: MessageSender, wait
Gripper move B6 (Plate 1) to A1
Execute bioscript: HomePCT200
User Function: MessageSender, wait
User Function: MessageSender, wait
User Function: MessageSender, wait
Gripper move A2 (Plate 2) to B6
Gripper move A1 (Plate 1) to A2

//LIGATION
Pipette 7.21µl from A6 to A2 using P20 – P20
Gripper move A2 (plate 1) to A1
Execute bioscript: HomePCT200
User Function: MessageSender, wait
User Function: MessageSender, wait
User Function: MessageSender, wait

NOTES

- PCR lid open

- Extension master mix
- Oil

- Engaging gripper prior to hot start

- PCR lid close
- heating block
- PCR lid open

- safe positioning of gripper
- PCR lid close
- primer extension PCR
- PCR lid open

- ligation mix

- safe positioning of gripper
- PCR lid close
- ligation PCR
- PCR lid open

//BINDING OF BEADS

Mix Xµl at A6 using P200L – P250	- Resuspending prewashed magnetic beads
Pipette 12.5µl from A6 to A1 using P200L – P250	- Add magnetic bead to sample
Pause 20 minutes	
Mix 20µl at A1 using P200L - P250	- suspend beads in sample
Pause 20 minutes	
Mix 20µl at A1 using P200L - P250	- suspend beads in sample
Pause 20 minutes	
Gripper move A1, to A2	
System pause 2.30 minutes	
Pipette 60µl from A2 to B5 using P200L - P250	- to waste
Pipette 50µl from B5 to A2 using P200L - P250	- 2X binding buffer
System pause 2.30 minutes	
Pipette 60µl from A2 to B5 using P200L - P250	- to waste
Pipette 50µl from B5 to A2 using P200L - P250	- 1X TE
System pause 2.30 minutes	
Pipette 60µl from A2 to B5 using P200L - P250	- to waste
Pipette 50µl from B5 to A2 using P200L -P250	- 1X TE
System pause 2.30 minutes	
Pipette 60µl from A2 to B5 using P200L - P250	- to waste
Pipette 10µl from B5 to A2 using P200L - P250	- 0.1X TE
Pipette 15µl from A6 to A2 using P200L – P250	- oil

//DENATURING

Gripper move A2 to A1	
Execute bioscript: HomePCT200	- safe positioning of gripper
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- denaturing PCR
User Function: MessageSender, wait	- PCR lid open
Gripper move B6 (plate 2) to A2	
Gripper move A1 to B6	

//AMPLIFICATION

Pipette 40µl from A6 to B6 using P200L –P250	- amplification master mix
Gripper move A2 to A1	- engaging gripper for hot start
Gripper move A1 to A2	
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- Hot start
User Function: MessageSender, wait	- PCR lid open
Gripper move B6 to A1	
Execute bioscript: HomePCT200	- safe positioning of gripper

User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- amplification PCR
User Function: MessageSender, wait	- PCR lid open
Gripper move A2 to A6 (Plate 2)	
//labeling	
Gripper move A1 to A2	
Pipette 4µl from A6 to B6 (Plate 2) using P20 –P20	- labeling master mix
Pipette 5µl from A2 to B6 using P20 – P20	- add sample from plate 1
Pipette 15µl from A6 to B6 using P200L – P250	- add oil
Gripper move A2 to A1	- engaging gripper for hot start
Gripper move A1 to A2	
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- Hot start
User Function: MessageSender, wait	- PCR lid open
Gripper move B6 to A1	
Execute bioscript: HomePCT200	- safe positioning of gripper
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- labeling PCR
User Function: MessageSender, wait	- PCR lid open
//LABELLING REACTION CLEAN UP	
Mix Xµl at A6 using P200L – P250	- resuspending CleanSeq beads
Pipette 10µl from A6 to A1 using P20 - P20	- add CleanSeq
Pipette 40µl from B5 to A1 using P200L – P250	- add 85% ethanol
Pause system 60 seconds	
Gripper move A1 to A2	- transfer plate to magnet
Pause system 5.00 minutes	
Pipette 90µl from A2 to B5	- remove all liquid to waste
Pipette 100µl from B5 to A2	- wash with 85% ethanol
Pause system 60 seconds	
Pipette 110µl from A2 to B5	- remove all liquid to waste
Pause system 10 minutes	- evaporation of residual ethanol
Gripper move A2 to A1	
Pipette 40µl from A6 to A1	- resuspend CleanSeq in CEQ Sample Loading Solution
Pause system 5 minutes	
Gripper move A1 to A2	- separate beads from SLS
Pipette 35µl from A2 to A2	- move separated SLS to clean wells on plate

Gripper move A2 to A1

Execute bioscript: HomePCT200
User Function: MessageSender, wait
END

- store labeled reactions in dark until ready to load on CEQ
- safe positioning of gripper
- PCR lid close

Double strand break LM-PCR protocol for Biomek 2000

Place Plate containing DNA in 5µl at position B6 (plate 1) and plate for labeling reaction at A2 (plate 2). All master mixes are placed at chilled position A6 and all wash buffers for beads at B5.

Initial configuration – (see supplementary Fig. 1)
//ADJUST SAMPLE NUMBER FOR LM-PCR IN EDIT - PATTERN
Reset Tip Rack at A3 to A1 (0 tips used)\
Reset Tip Rack at B3 to A1 (0 tips used)
Reset Tip Rack at A4 to A1 (0 tips used)
Reset Tip Rack at B4 to A1 (0 tips used)
Reset Tip Rack at A5 to A1 (0 tips used)
User Function: MessageSender, Wait

//PRIMER EXTENSION MIX
Pipette 25.00µl from A6 to B6 (Plate 1) using P20 – P20
Pipette 15µl from A6 to B6 using P200L – P250
Gripper move A2, (Plate 2) to A1
Gripper move A1, (Plate 2) to A2

//PRIMER EXTENSION
User Function: MessageSender, wait
User Function: MessageSender, wait
User Function: MessageSender, wait
Gripper move B6 (Plate 1) to A1
Execute bioscript: HomePCT200
User Function: MessageSender, wait
User Function: MessageSender, wait
User Function: MessageSender, wait
Gripper move A2 (Plate 2) to B6
Gripper move A1 (Plate 1) to A2

NOTES

- PCR lid open

- Extension master mix
- Oil

- Engaging gripper prior to hot start

- PCR lid close
- heating block
- PCR lid open

- safe positioning of gripper
- PCR lid close
- primer extension PCR
- PCR lid open

BINDING OF BEADS

Mix Xµl at A6 using P200L – P250	- Resuspending prewashed magnetic beads
Pipette 30µl from A6 to A1 using P200L – P250	- Add magnetic bead to sample
Pause 20 minutes	
Mix 50µl at A1 using P200L - P250	- suspend beads in sample
Pause 20 minutes	
Mix 50µl at A1 using P200L - P250	- suspend beads in sample
Pause 20 minutes	
Gripper move A1, to A2	
System pause 2.30 minutes	
Pipette 80µl from A2 to B5 using P200L - P250	- to waste
Pipette 50µl from B5 to A2 using P200L - P250	- 2X binding buffer
System pause 2.30 minutes	
Pipette 60µl from A2 to B5 using P200L - P250	- to waste
Pipette 50µl from B5 to A2 using P200L - P250	- 1X TE
System pause 2.30 minutes	
Pipette 60µl from A2 to B5 using P200L - P250	- to waste
Pipette 50µl from B5 to A2 using P200L -P250	- 1X TE
System pause 2.30 minutes	
Pipette 60µl from A2 to B5 using P200L - P250	- to waste
Pipette 10µl from B5 to A2 using P200L - P250	- 0.1X TE
Pipette 15µl from A6 to A2 using P200L – P250	- oil

//DENATURING

Gripper move A2 to A1	
Execute bioscript: HomePCT200	- safe positioning of gripper
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- denaturing PCR
User Function: MessageSender, wait	- PCR lid open
Gripper move B6 (plate 2) to A2	
Gripper move A1 to B6	

//AMPLIFICATION

Pipette 40µl from A6 to B6 using P200L –P250	- amplification master mix
Gripper move A2 to A1	- engaging gripper for hot start
Gripper move A1 to A2	
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- Hot start
User Function: MessageSender, wait	- PCR lid open
Gripper move B6 to A1	
Execute bioscript: HomePCT200	- safe positioning of gripper

User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- amplification PCR
User Function: MessageSender, wait	- PCR lid open
Gripper move A2 to A6 (Plate 2)	
//labeling	
Gripper move A1 to A2	
Pipette 4µl from A6 to B6 (Plate 2) using P20 –P20	- labeling master mix
Pipette 5µl from A2 to B6 using P20 – P20	- add sample from plate 1
Pipette 15µl from A6 to B6 using P200L – P250	- add oil
Gripper move A2 to A1	- engaging gripper for hot start
Gripper move A1 to A2	
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- Hot start
User Function: MessageSender, wait	- PCR lid open
Gripper move B6 to A1	
Execute bioscript: HomePCT200	- safe positioning of gripper
User Function: MessageSender, wait	- PCR lid close
User Function: MessageSender, wait	- labeling PCR
User Function: MessageSender, wait	- PCR lid open
//LABELLING REACTION CLEAN UP	
Mix Xµl at A6 using P200L – P250	- resuspending CleanSeq beads
Pipette 10µl from A6 to A1 using P20 - P20	- add CleanSeq
Pipette 40µl from B5 to A1 using P200L – P250	- add 85% ethanol
Pause system 60 seconds	
Gripper move A1 to A2	- transfer plate to magnet
Pause system 5.00 minutes	
Pipette 90µl from A2 to B5	- remove all liquid to waste
Pipette 100µl from B5 to A2	- wash with 85% ethanol
Pause system 60 seconds	
Pipette 110µl from A2 to B5	- remove all liquid to waste
Pause system 10 minutes	- evaporation of residual ethanol
Gripper move A2 to A1	
Pipette 40µl from A6 to A1	- resuspend CleanSeq in CEQ Sample Loading Solution (if running a control reaction labeled with a different colour, then resuspend each in 20µl and combine to run together)

Pause system 5 minutes
Gripper move A1 to A2
Pipette 35µl from A2 to A2

Gripper move A2 to A1

Execute bioscript: HomePCT200
User Function: MessageSender, wait
END

- separate beads from SLS
- move separated SLS to clean wells on plate
- store labeled reactions in dark until ready to load on CEQ
- safe positioning of gripper
- PCR lid close

LM-PCR primer optimization protocol for Biomek 2000

Place Plate containing complete primer extension mix directly into PCR machine (plate 1) and plate for labeling reaction at A2 (plate 2). All master mixes are placed at chilled position A6 and all wash buffers for beads at B5.

Initial configuration – (see supplementary Fig. 1)
//ADJUST SAMPLE NUMBER FOR LM-PCR IN EDIT - PATTERN

NOTES

//PRIMER EXTENSION
Start PCR machine program manually

- Biomeck 2000 software can not control the temperature gradient, this must b set manually.

START BIOMEK program
Reset Tip Rack at A3 to A1 (0 tips used)\
Reset Tip Rack at B3 to A1 (0 tips used)
Reset Tip Rack at A4 to A1 (0 tips used)
Reset Tip Rack at B4 to A1 (0 tips used)
Reset Tip Rack at A5 to A1 (0 tips used)
Pause system 60 minutes

- Allows time for primer extension program to finish

User Function: MessageSender, wait
Gripper move A2 (Plate 2) to B6
Gripper move A1 (Plate 1) to A2
Rest of program as in Single strand break LM-PCR protocol

- PCR lid open