Whole Genome Shotgun Library Protocol

Purpose

This procedure is intended for the amplification of small quantities of genomic DNA usng a whole genome shotgun library approach in preparation for downstream cloning and sequencing applications.

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Citing Protocol: please contact M. Henn for information regarding citing this protocol (mhenn[at]broadinstitute.org)

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Construction of "Standard" WGS libraries
- Process Overview -

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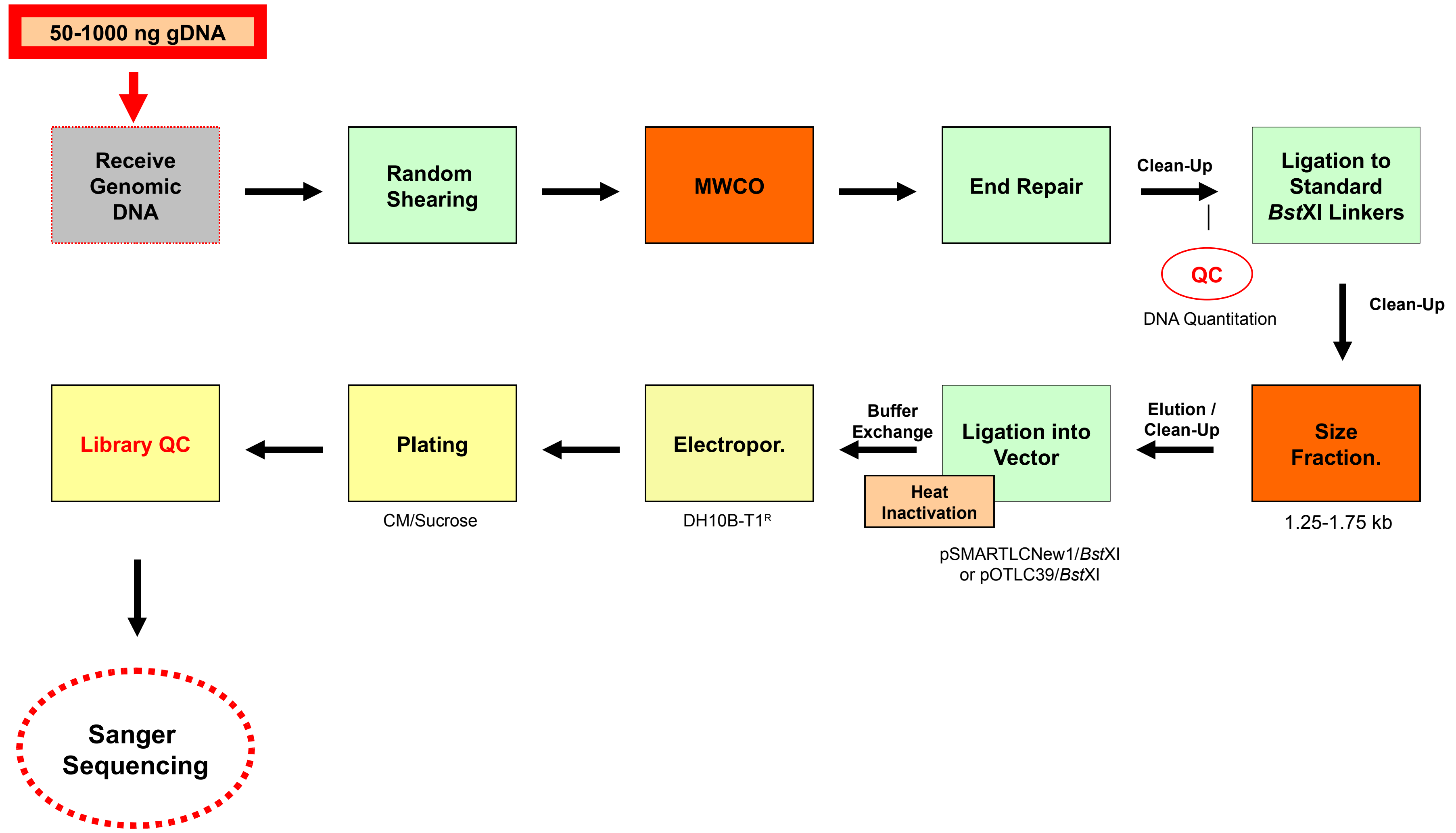

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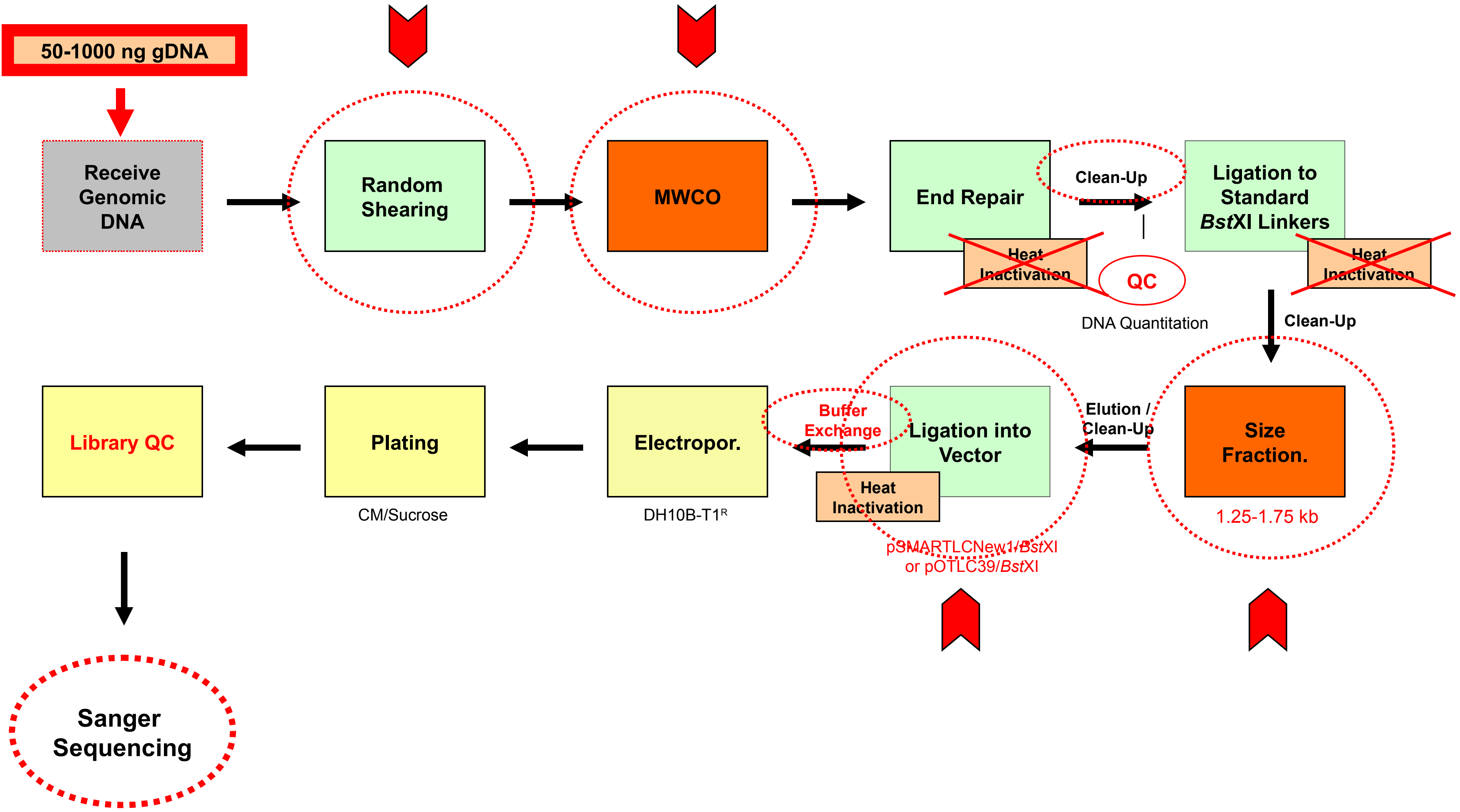
Construction of “Standard” WGS libraries

- Steps modified from standard process to optimize for small sample inputs-



Construction of “Standard” WGS libraries
- Protocol Adjustments -

Optimizations:

- The original DNA starting amount of 10-20 ug is reduced to 50-500 ng.
- The High Frequency Adaptive Focused Acoustics (AFA) technology from Covaris is employed to reproducibly shear gDNA to the desired size range. The enclosed environment prevents sample loss and cross contamination of samples due to carry-over from sample to sample.
- To eliminate the potential risk of generating chimeric DNA molecules during the linker ligation step, sheared DNA fragments of low molecular weight ($\leq$ 200-250 bp) are removed by utilization of the Solid Phase Reversible Immobilization (SPRI) technology.
- All standard protocol steps requiring elevated temperatures are either omitted or performed at high salt concentration (0.3 M NaCl) to stabilize DNA fragments with high AT content and to prevent DNA degradation (i.e. sample loss) due to denaturation.
- QIAGEN's MinElute spin columns with binding and elution properties suitable for small amounts of DNA and small volumes are utilized in all enzymatic reaction clean-up steps.
- To increase stability of AT-rich sequences, DNA fragments are ligated into a low-copy-number version of our standard cloning/sequencing vector.

Construction of WGS Libraries

- Process Map (Page 1) -

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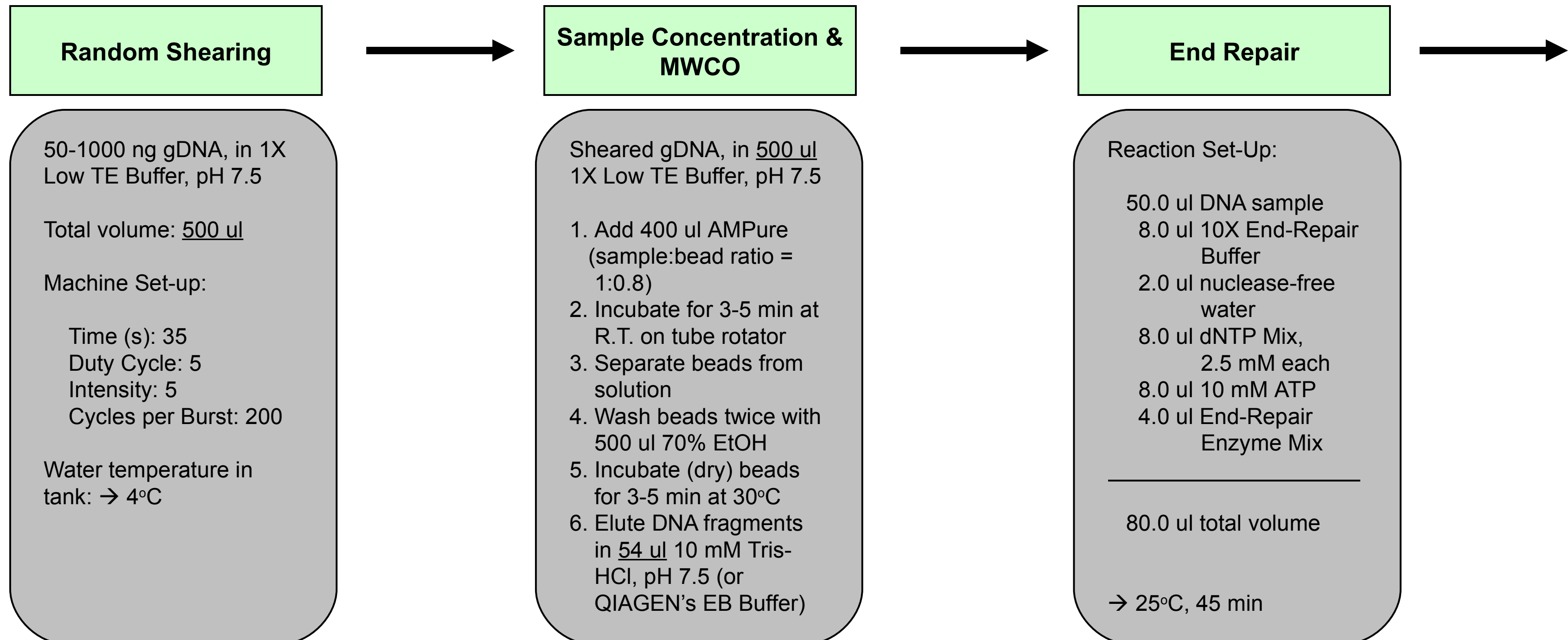

- Covaris S2 Adaptive Focused Acoustics Instrument

- Agencourt AMPure 60 ml Kit (Agencourt Cat # 000130)
- Dynal MPC-S Magnetic Particle Concentrator (Invitrogen Cat # 120-20D)

- End-It DNA End-Repair Kit (Epicentre, Cat # ER0720)
- Nuclease-free water (Promega, Cat # P1193)

Covaris AFA technology is utilized to reproducibly shear nanogram amounts of gDNA to the desired size range (35-40% between 1 and 2 kb).
The enclosed environment prevents sample loss and cross contamination of samples due to carry-over from sample to sample.

To eliminate the potential risk of generating chimeric DNA molecules during the linker ligation step, DNA fragments of low molecular weight ($\leq$ 200-250 bp) are removed by utilization of the SPRI technology (sample:bead ratio = 1:0.8).

In the presence of 1 mM ATP and 0.25 mM dNTPs, T4 DNA Polymerase and T4 Polynucleotide Kinase convert damaged DNA to blunt-end, 5'-phosphorylatyed DNA (33 mM Tris- acetate, pH 7.8, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT).

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Construction of WGS Libraries

- Process Map (Page 2) -

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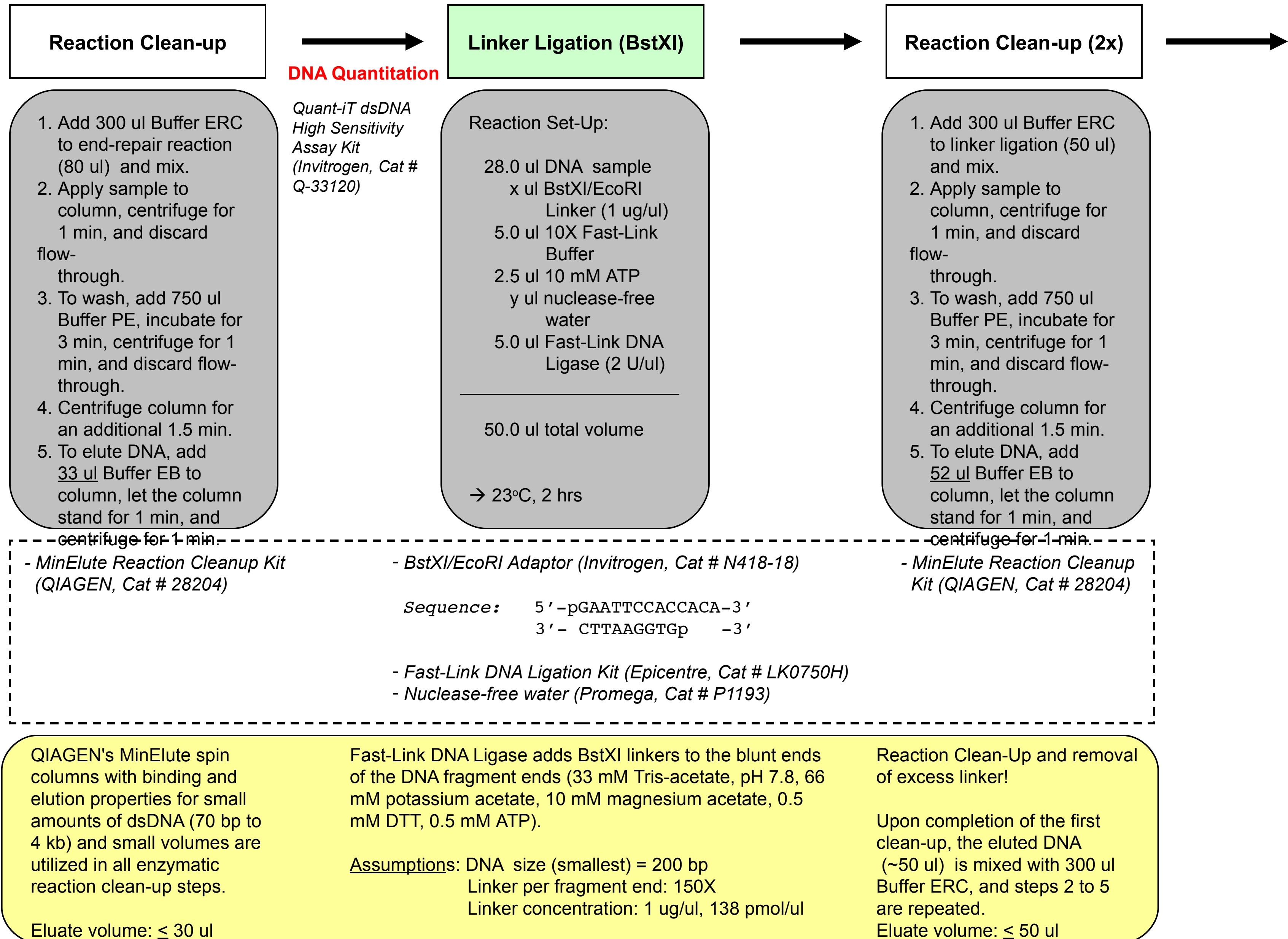

Construction of WGS Libraries
- Process Map (Page 3) -

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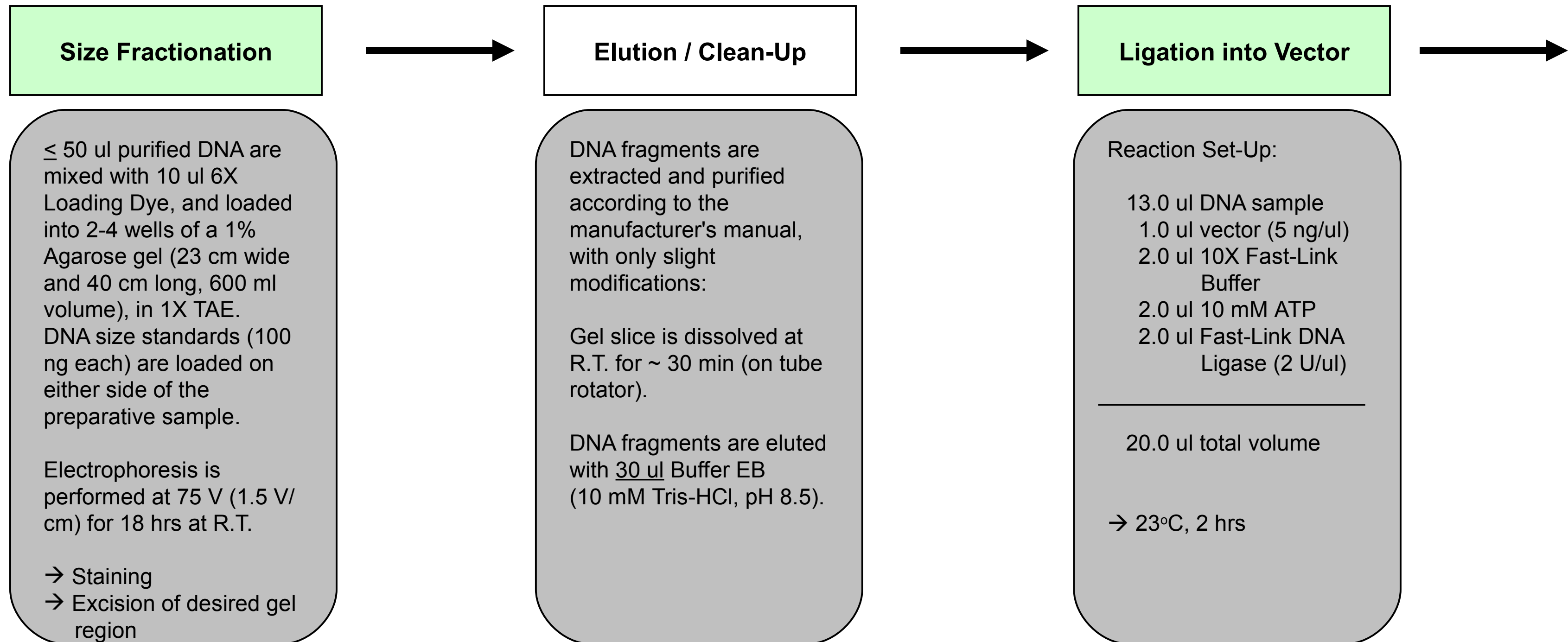

- *SeaKem GTG Agarose (Cambrex, Cat # 50074)*
- *Blue/Orange Loading Dye, 6X (Promega, Cat # G1881)*
- *1 kb DNA Ladder (Invitrogen, Cat # 15615-016)*
- *250 bp DNA Ladder (Invitrogen, Cat # 10596-013)*
- *SYBR Green I Nucleic Acid Gel Stain (Molecular Probes, Cat # 40412, 10,000X concentrate in DMSO)*

- *QIAquick Gel Extraction Kit (QIAGEN, Cat # 28704)*

- *Fast-Link DNA Ligation Kit (Epicentre, Cat # LK0750H)*

Size fractionation and removal of excess linker!

Only the analytical parts of the gels are stained with SYBR Green, and then visualized using a Typhoon variable mode imager. The stained analytical gel parts and the unstained preparative gel part are then reassembled, and a slice containing the 1250-1750 bp fraction is excised from the unstained preparative portion of the gel.

The Gel Extraction Kit is used in combination with a vacuum manifold.

Eluate volume: ≤ 28 ul

Fast-Link DNA Ligase covalently links DNA fragments with linearized vector molecules (33 mM Tris-acetate, pH 7.8, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, 1 mM ATP).

Fragment used:
Maximal amount possible (in 20 ul rxn vol)

Vectors (low-copy-number) used:
pOTLC39/BstXI (1715 bp, 5 ng = 0.0045 pmol)
pSMARTLCNew/BstXI (1860 bp, 5 ng = 0.004 pmol)

Construction of WGS Libraries

- Process Map (Page 4) -

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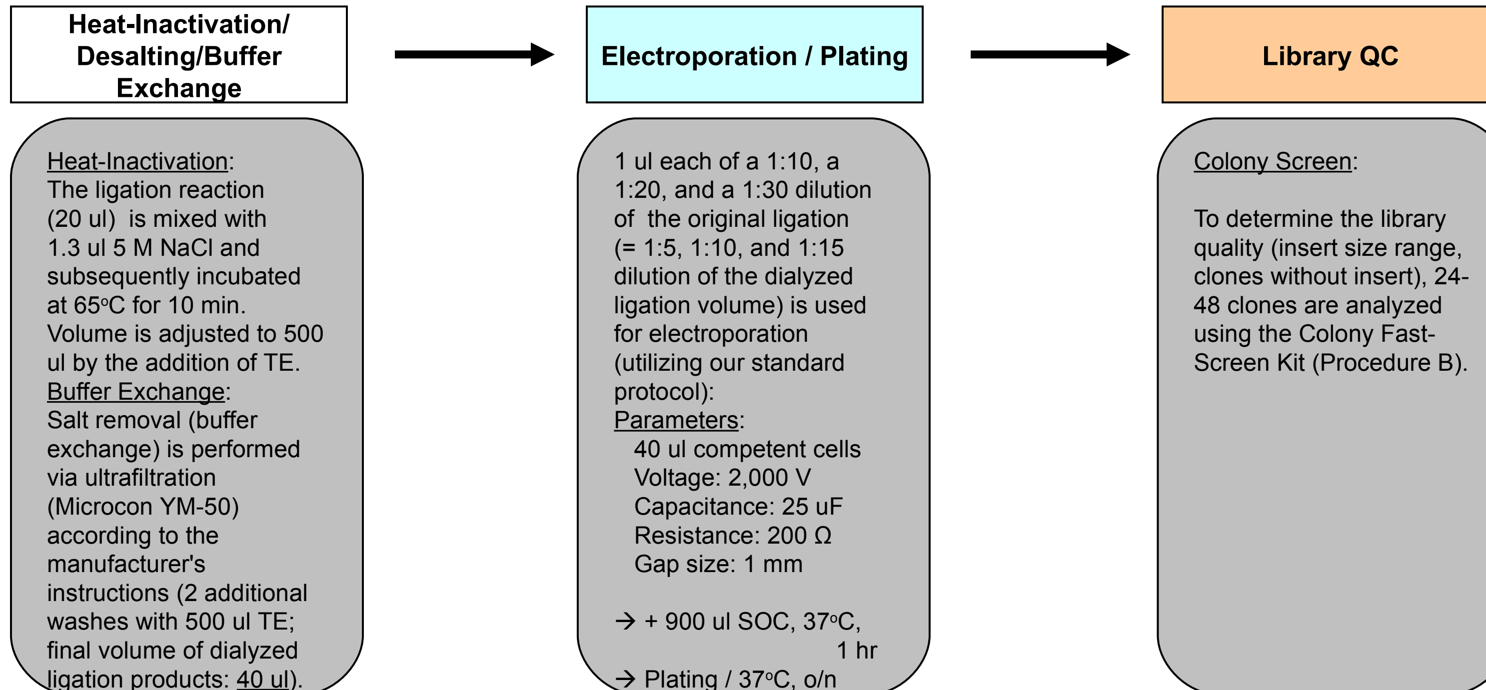

- *Microcon YM-100 Centrifugal Filter Units (Millipore, Cat # 42413)*
- *1X Low TE Buffer, pH 7.5*

- *ElectroMAX DH10B-T1[R] Electrocompetent Cells (Invitrogen, Cat # 12033-101)*
- *SOC Medium (Invitrogen, Cat # 15544-034)*
- *LB/Chloramphenicol/Sucrose Agar Plates (25 mg/ml Chloramphenicol, 5% Sucrose)*
- *Gene Pulser Cuvettes, 0.1 cm gap (Bio-Rad, Cat # 165-2089)*
- *GenePulser Xcell System (Bio-Rad, Cat # 165-2660)*

- *Colony Fast-Screen (Size Screen) Kit (Epicentre, Cat # FS08250*
- *Supercoiled DNA Ladder (Invitrogen, Cat # 15622-012)*

Heat-inactivation of Ligase is performed at high salt concentration (0.3 M NaCl) to prevent DNA degradation and to stabilize DNA fragments with high AT content.
The final volume of the dialyzed ligation is 40 ul, representing a 1:2 fold dilution of the original ligation reaction.

3 different ligation dilutions are electroporated to determine the optimal plating conditions (colony density critical for picking efficiency).

For each library that has passed the lab QC, 384-768 clones (1-2 384-well plates) are sequenced with Forward and Reverse Sequencing Primer each (= test sequencing).